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Address Information
Plant Breeders Union of Turkey
Adakale Street, No.: 22/12 Kızılay, 06420 Cankaya/Ankara - TURKEY
Phone: +90 312 433 3065-66 Fax: +90 312 433 3006
Email: bisab@bisab.org.tr • info@ekinjournal.com

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Next Generation Breeding in Potato

Muhammad Abu Bakar ZIA Muhammad NAEEM Ufuk DEMIREL Mehmet Emin CALISKAN*

Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, 51240 Niğde, Turkey

* Corresponding author e-mail: caliskanme@ohu.edu.tr

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ABSTRACT

Potato is the world's number one non-grain commodity and ranks at fourth position after maize, rice and wheat. As a species, potato is very docile to cell culture, it also contains an extended history of applications in the field of bio-technology for the improvement of crops. The genomic insurgency from the recent past has significantly enhanced the overall knowhow of the genetic structure of all the crops. Crop genome sequences has totally reformed our view and understanding for genome association and genome development. Increased knowledge in markers along with the advanced phenotyping, genotyping by sequencing, genomewide association studies added a new way for determining marker-trait associations that can withstand genome based breeding programs. Accessibility of sequencing of genomic data has permitted editing of genome (localized mutagenesis), for obtaining sequences of gene that is anticipated by the breeders. To develop some genetic maps, markers application and genomics in the field of potato breeding these genetic characteristics have also assigned the tasks to the breeders. Many strategies are formulated to describe the potato loci, (contender) genes and alleles, and association of genotype with the phenotype are also stated. This review demonstrates how next generation phenotyping, genome-wide association studies and genome editing tools can be used to modify tools to genomics for the need of potato breeders to transform potato improvement.

Keywords: Next generation phenotyping, Genomewide association studies, genome editing, potato.

Introduction

Potato (*Solanum tuberosum* L.) is a vital crop and occupies 4th place in production among other food commodities behind maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aesitvum* L.) worldwide (FAO, 2014). Potato is tetraploid and highly heterozygous crop which has the major importance for food, feed and the industrial use. It was cultivated on an area of 130.000 ha having the production of 4.175 million tons in Turkey (TUİK 2015). Central Anatolia including Niğde portions almost 60% potato production in this regard. Potato has a wide range of production in the country, and has an important role in Turkish agricultural sector.

The cultivated European potato *Solanum tuberosum* ssp. *tuberosum* is autotetraploid (2n=4x=48), which means that they have four alleles per locus. Homologous chromosomes pair at random during meiosis (Milbourne *et al.* 2007). Moreover, there are tuber-bearing varieties under cultivation that are non-tuberosum types ranging from diploid to hexaploidy Van den Berg and Jacobs (2007). Potatoes are outbreeding plants. Therefore, they obtain a high level of heterozygosity and are prone to inbreeding depression, making it difficult to obtain homozygous lines. The heterozygosity in commercial cultivars is preserved by the clonal propagation of tubers (Milbourne *et al.* 2007; The Potato Genome Sequencing Consortium 2011).

From start, potato has been nominated and reared in production areas for advanced echelons of locally adapted to several environmental conditions. This effect was gained in relatively short time because of the potato's highly diversified genetics, allowing the

selection and identification of the genotypes that are high performing in different type of environments. This type of genetic heterogeneity is a result of both out-breeding habit and the chromosomal changes that takes places in the chromosomes of the species (Slater *et al.* 2014a). Notwithstanding to all, hereditary advancement in multifaceted characters, like crop produce, is very sluggish to nearly absent (Jansky 2009), particularly at the time at which we associate it with different crops like as maize, wheat, and rice (Fischer and Edmeades 2010).

Presently, there are 7.25 billion people that exists on earth, and it is estimated that the population of world can get up to 70 million per annum for the next 40 years. It is expected that the population of the world will approximately be 9.2 billion by year 2050, Attentiveness of carbon dioxide (Co_2) and the ozone will reach upto 550 ppm and 60 ppm, correspondingly and the climate is going to be warm by 2˚C as at present (Jaggard *et al.* 2010). It is anticipated by that time that about 90% of this world's population will be living in continents like Asia, Africa, and Latin America (FAO 2012, Silva 2014). To cope with this dramatically increasing population and hunger, plant breeders have been working very hard to increase the food production. Recently, plant breeding has switched from an entirely phenotypic-dependent procedure to have an improved dependence on genotype-based selection up to some level of extent (Varshney *et al.* 2014).

Considerable research is being carried out for the improvement of genomic possessions to increase the potato breeding as compared to the other crops, that concluded in an estimated potato genome (Potato Genome Sequencing Consortium 2011). All the work on genomic level has enabled the discovery of over 39,000 genes, together with many genes that regulates a biotic rinsing confrontation in potato (Bakker *et al.* 2011; Jupe *et al.* 2012; Jupe *et al.* 2013) and the quantitative trait loci (QTLs) for improving the traits for the quality of the plants (D'hoop *et al.* 2014; Uitdewilligen *et al.* 2013). Though, QTLs of huge result are improbable, however these characters show practical heights of heritability for some traits like yield (Slater *et al.* 2014b). The traits like yield and some others that are related to that are used to arise genome estimated breeding estimates for the traits that are being affected by genome selection (Meuwissen *et al.* 2001), applicable for the crop like potato, provided with huge number of SNPs that have been discovered with the help of sequencing of genomes (Uitdewilligen *et al.* 2013). Genome selection is being applied effectively for several plants and animal expansion plans (Crossa *et al.* 2010; Daetwyler *et al.* 2010a; Grattapaglia *et al.* 2011; Lin *et al.* 2014; Resende *et al.* 2012; Riedelsheimer *et al.* 2012; VanRaden *et al.* 2009; Wiggans *et al.* 2011; Wolc *et al.* 2011, 2015). DNA sequencing have become increased visibility and extremely low pricing with the progress in recent developments for next generation sequencing (NGS) techniques. To sightsee the associations within genetic and phenotype range many possibilities are being opened with a tenacity that had never achieved earlier just because of these developments in the field of science. This review is about the discussion for the possible enforcement of next generation breeding techniques in potato, including the genotyping by sequencing (GBS), genomic selection (GS), genome wide association studies (GWAS), genome editing, next generation phenotyping, and their overview, advantages disadvantages and some future prospective of all the next generation approaches.

Genotyping by sequencing

Current progresses in the field of next generation sequences (NGS) helped reduced cost for the sequencing of DNA to the great extent. Therefore, sequencing by genotyping is used to produce a reliable high-throughput data for highly diversified and large number of genome species and samples (Elshire *et al.* 2011). GBS creates many SNPs for genetic examinations and genotyping (Beissinger *et al.* 2013). Main mechanism includes reduced price, limited handling of the samples, less PCR and decontamination procedures, no size fractionation, no reference limits, acceptance to scale-up as well as reliable barcoding (Davey *et al.* 2011).

GBS was basically designed for increased occurrence determination in association studies in crops like maize and, similarly restriction site associated DNA (RAD), has been stretched to the degree of species having an intricate genome. GBS has commonly been implemented in multiple crops to evaluate the breeding and mapping population ranging from 10-100s of 1000s of SNP markers being technically very simple, highly precise, GBS is suitable for the studies like population studies, germplasm characterization, plant genetics and breeding in highly diversified crops, (Poland *et al.* 2012 a).

Application of Genotyping by Sequencing

Genotyping by sequencing has become a supreme podium for the studies which range from single gene to the whole genome (Poland and Rife 2012). In the

field of plant breeding GBS has the most valuable applications. It offers a quick and cost-effective means for the genotyping among the breeding populations, letting plant breeders to contrivance genome wide association studies, genome diversity studies, analysis of genetic linkage, discovery of molecular markers and genomic selection (GS).

As genomewide association studies require hundreds of thousands to loads of markers to produce adequate data besides exposure, hence with the emergence of such NGS technologies, there is an obvious improvement in the marker resolutions as compared to the earlier resolutions and technologies (Edwards and Batley 2010). To evaluate and map the numerous interested characters in breeding running programs, recently genotyping by sequencing through the next generation sequencing approach was used to re-sequence assemblies of recombinant inbred lines (RILs) (Deschamps *et al.* 2012). Maize, wheat, barley, rice, potato and cassava and many other crops have been improved by genotyping by sequencing for the effective, reduced price and vast gauges of sequencing of genome (Poland and Rife 2012; van Poecke *et al.* 2013). The main purpose for the implementation of GBS is the expansion of molecular markers for the whole genome with inreased absorption with reduced charge (Heffner *et al.* 2009, 2010; Jannink *et al.* 2010).

An inclusive 2,815 maize genotyping concurrences exposed 681,257 SNP markers that are banquet all over the whole genomic region, from that few SNPs are connected with recognized contender genes that are responsible for kernel color, sweetness, and flowering time (Romay *et al.* 2013). A set of 205,614 SNPs have been recognized so far subsequently re-sequencing 31 soybean genotypes (Lam *et al.*, 2010). Across 83 tetraploid potato cultivars, 12.4 gigabases of increased value sequence information were produced and plotted with the potato genome of reference that is 2.1 Mb. In addition, a mean different concentration of 1 SNP/24 bp in exon regions and 1 SNP/15 bp in intron regions was observed across 83 potato cultivars (Uitdewilligen *et al.* 2013).

Related to conventional Marker assisted selection, Genomic selection is an innovative method which cartels both phenotype and pedigree data with molecular markers to upsurge precision on genotypic morals in different breeding programs (Heffner *et al.* 2009). Conjectural as well as functional studies on GS disclosed great aspect to increased development of new cultivar. GS over the GBS method attitudes to be a vital element to the conventional crop development and we can move genomic- assisted breeding to the commercial crops that have a large and complicated genome which is a vital property of this techniques (Poland and Rife, 2012).

Genotyping by Sequencing has become a convincing tool for the studies that are being held on genetic diversity in the crops (Fu and Peterson, 2011; Lu *et al.*, 2013; Fu *et al.*, 2014). For instance, Fu and Peterson (2011) operated with Roche 454 GS FLX Titanium expertise with lessened genomic illustration and increased bioinformatic tactics for examination of the collection of 16 various barley landraces, revealed 2,578 contigs, and 3,980 SNPs, and established a main topographical separation in the gene pool of sown barley. SNP detection etiquette to improve the analysis of diversity for 540 different plants of switchgrass tested from different 66 inhabitants and exposed edifying designs of genetic association with the deference to their ploidy level, geographic spread and ecotype was established a network-based by Lu *et al.* (2013). Gene diversity of 24 various accessions of mustard yellow, in which around 1.2 million reads with sequence (total about 392 million nucleotides) were produced, 512 contigs, and 828 SNPs were recognized by using genotyping by Sequencing etiquette (Fu *et al.*, 2014). 26.1% of total distinction exist in cultivar, breeding lines and landrace, and 24.7% among black-seeded and yellow-seeded germplasm was revealed by variety examination of these yellow mustard SNPs.

Genotyping by Sequencing is an outstanding stage for the implementation of plant breeding even if there are no reference genome sequences or without the earlier polymorphic DNA invention through integration of genotyping the huge populations and some molecular markers. Examination with the help of genetics and molecular marker expansion of rapeseed, lupin, lettuce, switchgrass, soybean, and maize has been shown to be suited to genotyping by sequencing approach (Bus *et al.*, 2012; Truong *et al.*, 2012; Yang *et al.*, 2012; Lu *et al.*, 2013; Sonah *et al.*, 2013).

Potato Breeding and Genomic Selection

Breeding of potato is a difficult task, as ~40 of the characters are inspected during the development of a fresh variety (Gebhardt 2013). These characters can be divided into different classes like indulgence to biotic stresses, abiotic stresses, yield-related traits and tuber quality features (Slater *et al.* 2014a). Information about genetics of each character and extent of ecological effect of the target traits is significant and will affect the preference of method

to be selected for selecting the advanced genotypes and the phenotypes. Some of the characters are dealt by only one gene nevertheless some are being controlled by numerous complex characters (Slater *et al.* 2014a). Potato breeding is much difficult and stimulating as compared to the other plants, not only because there are more market-specific traits that are also thought while dealing with breeding of potato but also because potato is extremely autotetraploid and heterozygous in nature. Target traits can be pretentious mainly with the environment in which they are grown, that can vary like meaningfully including yield, tuber number, tuber size, specific gravity, and processing quality (Jansky 2009). As a result, a conventional breeding plan involves selection of genotypes across several clonal peers in addition to many suitable sites for a variety of required characters, which can take the time over 10 years (Jansky 2009).

Recently, considerable developments have been done to understand the heredities of potato to expand breeding for brisker inherited gain. A conservative breeding scheme involves creation of a huge inhabitants, before employing phenotypic repeated selections over several peers, by the use of development for selection burdens for minimizing inhabitant extent although simultaneously swelling quantity under assessment for each genotype (Bradshaw and Mackay 1994; Jansky 2009).

Several improvements have been made in potato conventional breeding to enhance the yield and efficiency utilizing the minimum resources. The use of molecular markers offers the chance for the progress of breeding meaningfully with the reduction of both extent and prices in breeding cycle. Marker-assisted selection (MAS) can select the characters many years former in any breeding program than by using it practically in conventional breeding program. Marker assisted selection (MAS) can become useful technique for the characters like qualitative ones that are being administered by foremost genes but it may also be significant as well as for the characteristics like quantitative ones, if the QTLs with an increased significance donated to the known characteristic. There are very less reports for their profitable potato breeding programs used, though, a considerable quantity of markers that are related to the genetic factor for significant characters are recognized (Dalla Rizza *et al.* 2006; Ortega and Lopez-Vizcon 2012; Ottoman *et al.* 2009; Schultz *et al.* 2012). The breeders that are working with potato to accept marker assisted selection, compared to conventional screening the use of the markers must be low in cost, as it is exposed to become the case for the control for the screening of pest and disease confrontation. (Slater *et al.* 2013).

Marker assisted selection can also be applied economically to the second generation (Slater *et al.* 2013) then at the same time the results can be premeditated for many compound (Slater *et al.* 2014b), the combination of both approaches could help to reduce the cycle for breeding purposes from more than 10 years until 4 years (Slater *et al.* 2014a). Such milestones can really speed up the breeding period and therefore upsurges genetic increase over conventional breeding methods in a very less time. MAS is also used mutually with the help of biased selection index, this is going to give guarantee for the expansion that is made within calculated characters. Additional decreases in the life span could only be probable with the help of selection through genomic strategy.

Genomic selection is different than marker assisted selection as it equally scrutinizes whole molecular marker data and can therefore restrain whole genetic alteration, while MAS only incarcerates a limited number QTLs variance. Moreover, GS do not practice specific complications that are related to GWAS as well as quantitative trait locus studies, like the embellish of marker results (Beavis 1998). As the achievements in the potato genome arrangements and the detection of many SNPs that are present in the whole genome (Uitdewilligen *et al.* 2013), appliance of GS for potato can be vigorously estimated in coming years, even with the heterozygous nature of potato genome (Slater *et al.* 2014a) will comprise few conscientious policies.

Genomic selection necessitates a significant quantity of markers that are vast all over the whole genome of potato. There are some studies that have discussed the same thing, with the following studies growing the sum of markers recognized throughout the genome in advancement for the genome-wide marker maps (Bonierbale *et al.* 1988; Dong *et al.* 2000; Gebhardt *et al.* 1991, 1989; Milbourne *et al.* 1998; Tanksley *et al.* 1992). The procedure dominated for the growth of a thick inherited linkage map populated with 10 thousand of amplified fragment length polymorphic markers (Van Os *et al.* 2006), which was used for the development of a map that assisted in the gathering the sequence potato genome.

GS in plants has received much attention and evaluations were performed recently in species such as maize (Guo *et al.* 2012; Zhao *et al.* 2012), wheat (Lado*et al.* 2013), sugar beet (Würschum *et al.* 2013) and trees (Resende *et al.* 2012abc). Heffner *et al.*

2011b performed some trials on genomic selection both in biparental populaces and transversely among several relatives in the program of breeding (Heffner *et al.* 2011a). Rutkoski *et al.* have performed some trials on GS for the development of stem rust confrontation in plants and provided a review (Rutkoski *et al.* 2010), approaches to trust the absent statistics deprived of arranged indications and work showing genomic selection for the resistance against fusarium head blight (Rutkoski *et al.* 2013). GS also provided some help for gene theory that the many variants that are affecting maize flowering time are clustered in a few common loci has been provided by the current widespread mapping exertions for time to flowering in maize (Buckler *et al.* 2009) (Table 1.)

The genome sequence achievement has permitted the knowing of several SNPs with somewhat sequenced genotypes which are associated to that (Uitdewilligen *et al.* 2013). We can use the specific SNPs as a compactly linked molecular markers set. The frequency of these SNP in potato has been predicted and its around 1 in 24 bp in the exons (Uitdewilligen *et al.* 2013), which illustrates degree for the arrangement range in potato. Arrangement of the potato sequenced genome provided a chance in the formation of an 8303-featured chip with SNPs (Felcher *et al.* 2012; Hamilton *et al.* 2011). Implementing genomic selection in plants is inadequate, possibly due to the restricted number assayed markers, as SNPs not segregating under examination population, or over problems of polyploid calling of SNP that's why SNPs chip in various other classes have measured a considerably huge percentage of impracticable statistics only because of missing data (Jan *et al.* 2016)

Hitches for SNPs bunch calling are the polyploids, primarily of the genotypic classes that are heterozygous, have been somewhat talked over the growth of custom packages of softwares (Voorrips *et al.* 2011) that software now allows the infinium calling (Illumina, San Diego, CA) for genotypic classes of five different classes data (Pembleton *et al.* 2013). GS also offers a suggestion for re-sequencing for the transfer for a huge amount of molecular genetic markers within no time.

Even though the SNP chips permits credentials of genes through GWAS with a large effect through, they may not detect the perfectly by the spectrum of frequency of alleles (i.e., ascertainment bias) and that's why might not be able to detect some of the related properties too. Genotyping-by-sequencing (GBS) methods can soon outdate single nucleotide polymorphism (SNP) chip systems potentially, that could deliver SNP profiles genome-wide by relatively low price (Elshire *et al.* 2011; Xu *et al.* 2012). To attain this all, main problems confronted with methods of GBS presently are the quantity of properties examined, the size of absent information that must be remunerated, and amount of dominant types of marker that are involved in the information.

Main benefit of a huge quantity of SNPs, that these offer an experience to the entire genome, ensuring that available LD have all the QTLs present within at least one marker and thus having the mainstream of genetic change. The crops with slow LD decay, but many for the crops with rapid LD decay would involve thousands of markers. (Xu *et al.* 2012). Calus *et al.* (2008) anticipated that 0.25 of LD amongst the markers that are adjacent was enough for thriving imitation studies for the genomic selection. For potato, LD with large number has been revealed to be at distances of less than 1 cM and with so quick decay to less than 0.2 at inter-marker distances greater than 1 cM (D'hoop *et al.* 2010).

Meuwissen *et al.* (2001) anticipated genomic selection (GS), which was thought to resolve the glitches that are connected to marker assisted selection of composite traits. In different ways, this technique also applies to the molecular markers. Dissimilar with MAS, within markers for GS are not being used for the finding of a specific trait. In genomic selection, increased density marker management is needed with at least one marker to have all QTL in LD. Effects of markers and haplotypes throughout genome is used to estimate genomic estimated breeding value (GEBV) of breeding population for a single line using all the inclusive data on all probable loci.

GS of superior positions are easily accepted in any of the breeding population. For the empowerment of the successful genomic selection, a recognized population trial population should be preferred. Population must be the result from bi-parental cross as well as it should essentially be the characteristic of candidates to be selected in the breeding program in which genomic selection is going to be implemented (Heffner *et al.* 2009). Trial populace essentially be genotyped always with the huge markers number. Captivating the considerations of the minimum sequence price, finest is the execution of GBS that will yield increased value of polymorphisms. Sequence of the two collections of genotype and phenotype data, is random and can be performed side by side. One can start "training" molecular markers when both phenotypic and genotypic data are organized (Zhong S. *et al.* 2009).

Explanation of the reference genome of potato, including 39,000 protein coding genes, has created

lots of opportunities to identify candidate genes in regions associated with a trait of interest rapidly. For instance, detection of both StSP6A gene that helps in initiation of tubers in the crops (Navarro *et al.*, 2011) and the StCDF1 gene accountable in maturity of plants (Kloosterman *et al.*, 2013) was significantly assisted by sequencing of genome (Table 1)

Apparent rewards of genomic selection over outdated marker assisted selection have been effectively confirmed in breeding of animals (Hayes and Goddard 2010). The quick development in genotyping systems with increased yielding SNPs and sequencing technologies are allowing creation and confirmation of lots of markers, providing a "careful hopefulness" in the coming days for the successful application of GS in plant breeding.

Genome Wide Association Studies

Genome-wide association studies (GWAS) are extensively being applied in many crops as well as in potatoes to study complex traits in diversity and breeding populations. The association of phenotypic trait values with segregating alleles of molecular markers in a mapping population is referred to as QTL mapping. The intend of QTL mapping is to detect genomic regions that explain phenotypic variation in a trait of interest and the subsequent identification of potential causal genes in that region. QTL are regions on the chromosomes which are physically linked to a molecular marker allele. The QTL and the marker allele are inherited together to the next generation. Principal genes of a quantitative trait, which has a wide distribution of phenotypes, can be located on all chromosomes (Gebhardt *et al.* 2005). For linkage analysis, several types of mapping populations are suitable (Collard *et al.* 2005). After establishing the mapping population, it is genotyped with segregating molecular markers and phenotyped for the quantitative characteristic of our own interest. A linkage map is produced from the molecular marker data and QTL are detected by marker trait association.

QTL mapping in potato is mainly carried out on diploid level. This is due to the heterozygous nature of the potato plants. Many QTL studies deal with resistances to biotic stresses like *Phytophthora infestans* (Li *et al.* 1998), root cyst nematodes (Kreike *et al.*, 1994) and abiotic stresses (e.g. drought tolerance: Anithakumari *et al.* 2011). Furthermore, yield- and quality-related traits were studied with QTL mapping, such as specific gravity (Freyre and Douches, 1994), starch content and yield (Schafer-Pregl *et al.* 1998), cold-sweetening (Menendez *et al.* 2002) and enzymatic discoloration (Werij *et al.*, 2007).

Although QTL mapping in tetraploid potato is not as straight-forward as in diploid potato, there are successful examples, such as the resistance studies for late blight (Bradshaw *et al.* 1998; Li *et al.* 1998; Meyer *et al.* 1998). Bradshaw *et al.* (2008) mapped 16 QTL for yield, agronomic and tuber quality traits in a tetraploid full-sub family mapping population. More examples were reviewed by Milbourne *et al.* (2007) and Van Eck (2007).

Alternatively, to the family-based linkage mapping approach, association mapping is a method to detect marker-trait relations in a given population of individuals that are related through ancestry. The method takes advantage of historical meiotic recombination and linkage disequilibrium (Flint-Garcia *et al.* 2003). It was first established in the study of complex inherited diseases in human populations, where it is not feasible to establish segregating mapping populations from crosses (Gebhardt *et al.* 2004). For AM, a populace consisting of a diverse germplasm including cultivars, breeding clones and landraces is assembled and phenotyped for the complex traits of interest. Molecular markers are then analyzed in the population and marker-trait associations between phenotypic and genetic variation are detected. In the case of candidate gene association mapping, the molecular markers are obtained from knowledge-based candidates, whereas markers for genome-wide association mapping randomly cover all chromosomes in high density.

Association mapping are based on linkage disequilibrium (LD). Non-random association of two alleles in any population is described as LD (Flint-Garcia *et al.* 2003). This is the case for loci that are near each other sharing the same chromosome (linkage). However, LD can also occur between alleles on different chromosomes (Flint-Garcia *et al.* 2003). There are different opinions regarding the extend of LD in tetraploid potato. D'hoop *et al.* (2010) reported 5 cM for genome-wide LD. Stich *et al.* (2013) suggest a linkage decay within 275 bp. Association mapping is an application of LD (Soto-Cerda and Cloutier, 2012), where the associated marker and the quantitative trait locus are in LD or physically linked in the ideal case (Gebhardt, 2013).

The genotypes of a potato population are a collection of individuals that are related by descent (Gebhardt *et al.* 2005). As a result, there is a possible bias towards relatedness in the statistical analysis, which means that a trait of interest can, for example, be linked to a gene pool or a geographic origin (Flint-

Garcia *et al.* 2003). The information about the degree of relatedness between genotypes in the mapping population plays a critical role in association mapping in order to avoid false positives. While a marker may not be linked to a QTL, there is a significant risk of finding a considerable association only based on the genetic relatedness between individuals (Pritchard *et al.* 2000).

There are several options to assess population structure in potato based on genetic markers. The two options arising from a factor analysis approach are principal coordinate (D'hoop *et al.* 2010; Pajerowska-Mukhtar *et al.* 2009; Urbany *et al.* 2011) and principal component analyses (D'hoop *et al.*, 2010), where genotyping information from molecular marker data is processed. In another approach, the marker data are analyzed by Bayesian clustering, implemented in the software Structure (Pritchard *et al.* 2000) and is being applied in the field of potato research in several studies (D'hoop *et al.* 2010; Li *et al.* 2008; Pajerowska-Mukhtar *et al.* 2009; Simko, 2004; Simko *et al.* 2006). Further options for population structure assessment are Analysis of Molecular Variance (AMOVA) and hierarchical clustering (D'hoop *et al.* 2010).

AM is useful for the detection of genetic difference that narrate variations in the complex traits in plants, as for example in corn (Wilson *et al.* 2004), wheat (Breseghello and Sorrells 2006), barley (Cockram *et al.* 2008) rice (Huang *et al.* 2012), perennial ryegrass (Skot *et al.* 2005), Arabidopsis (Aranzana *et al.* 2005), rapeseed and sugar beet (Stich and Melchinger 2009). There are many studies that have been conducted by using association mapping and considerable results have also been found. The genetic architectures of time to flower, leaf orientation, size of leaf, and resistance to disease traits in maize were separated by implementing linkage mapping and genomewide association mapping jointly in the nested association mapping panel, and numerous associated candidate genes were recognized (Buckler *et al.* 2009; Kump *et al.* 2011; Poland *et al.* 2011; Tian *et al.* 2011). GWAS now a day is being performed with many plant species including rice, foxtail millet, maize and sorghum (Huang *et al.* 2010, 2012; Kump *et al.* 2011; Jia *et al.* 2013; Li *et al.* 2013; Morris *et al.* 2012; Zhao *et al.* 2011). 1,083 sown O. sativa ssp. *indica* and O. *sativa* ssp. *japonica* varieties and 446 wild rice accessions (*Oryza rufipogon*) were gathered and with the help of low genome coverage was sequenced (Huang *et al.* 2012). Characterization of the alleles associated with 10 grain-related traits and flowering time was conducted with the help of GWAS to use the inclusive information set of almost 1.3 million SNPs next to the high-concentration haplotype map of the rice genome was built using data accusation (Table 2). Gebhardt *et al.* (2004) first published the example of association mapping in tetraploid potato germplasm, who worked on an assembled collection of 600 potato cultivars for the detection of markers associated with the late blight resistance and maturity based on historic recombination events. Later on, studies on association mapping was carried out that were based on candidate genes responsible for resistance against *Verticillium dahliae* (Simko *et al.* 2004) and *Phytophthora infestans* (Malosetti *et al.* 2007; Pajerowska-Mukhtar *et al.* 2009). More examples on association based studies are yield and tuber quality related traits such as tuber starch content, tuber yield, starch yield and chip quality (Fischer *et al.* 2013; Li *et al.* 2008, 2005). Likewise, Urbany *et al.* 2011, studied tuber bruising susceptibility, tuber shape and plant maturity were studied by AM in potato (Tetraploid) (Table 2).

By GWAS, a broader way of looking at marker-trait associations (MTA) is possible. D'hoop *et al.* (2008) gave a first ever example of this achievement, although the number of markers used in the study was considerably low. Another example for genome-wide association mapping in a small genotype panel was illustrated by Uitdewilligen *et al.* (2013).

According to Flint-Garcia *et al.* (2003), there are three main benefits of AM over linkage mapping. Firstly, mapping tenacity of AM is better due to the higher number of meiotic events, whereas linkage mapping generally looks at the recombination in a single meiotic generation (Gebhardt, 2007). However, when working with potatoes, this is not such a considerable advantage, as Gebhardt *et al.* (2004) found that only relatively few meiotic generations separate individual genotypes. This is likely due to the clonal propagation of potato whereby the meiotic generation is conserved.

Secondly, a high number of alleles can be detected with association mapping. In a segregation population, the maximum number of different alleles possibly detected at one locus in the offspring of a diploid linkage mapping population are four and eight in a tetraploid linkage mapping population. In an assembled population of 200 tetraploid genotypes, the theoretical maximum number of different alleles at one locus is 800. Because of a reduced statistical power, marker-trait associations of very rare alleles are not likely to be detected. Therefore, association mapping is mainly suitable for the detection of

common variants (Flint-Garcia *et al.* 2003).

Thirdly, the markers can be applied right away in breeding programs. Detected markers are directly and broadly applicable when the mapping population consists of appropriate breeding material (Li *et al.* 2013; Stich and Melchinger 2010).

Results from AM are influenced by various reasons like population structure, relationship among parents (kinship), selection history etc., that can lead to the detection of false positives among markers and the QTLs.

Improving Potato Through Editing of Genome

Genome Editing Tools

Targeted gene alteration known as 'genome editing' results in the generation of new allelic variants in the genome of cultivated species (Barabaschi *et al.* 2016). Editing genome using SSNs (Sequence Specific Nucleases) offers a resourceful substitute to routined genetic engineering (i.e., extracellular DNA manipulation, transgenesis, cisgenesis, GMO). Major advancement in SSNs technology is rapidly becoming a next generation tool for robust genetic improvement and breeding of crop species. To date, three most widely used SSNs have been developed for genome editing, including ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-Like Effector Nucleases) and CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR- associated proteins (Cas9) system. Among SSNs, CRISPR-Cas9 system is RNA-guided (gRNA or sgRNA) approach to target DNA sequence. The system depends upon the Cas-proteins endonuclease activity and high sequence specificity of crRNAs (CRISPR RNAs) to induce double-stranded breaks in DNA, adjacent to PAM (Protospacer Adjacent Motif) sequence. It received much attention and used widely, due to, its multiplexing capability, user-friendly, cost-effectiveness, and efficient way of producing target-specific constructs. (Xiong *et al.* 2015; Wang *et al.* 2015; Andersson *et al.* 2016; Butler and Douches 2016; Barabaschi *et al.* 2016; Khatodia *et al.*, 2016; Schiml and Puchta 2016). Site directed mutagenesis (SDM) and gene silencing evolved as potent concepts in plant research and breeding, to study the function of gene and to develop cultivars with improved traits (Quetier 2016). It depends on transient action of SSNs to induce double-strand breaks (DSBs) at specific genomic sites. The DSB causes targeted mutations and repaired endogenously, either through NHEJ (non-homologous end joining) or via HR (homologous recombination) (Butler *et al.* 2015; Araki and Ishii 2015). The NHEJ pathway is error-prone and efficiently yields small insertions and/or deletions (InDels) at specific locus, without use of exogenous DNA (Zhang *et al.* 2013). It is being widely accepted in plants to induce mutations and targeted gene knock-outs. A few studies have revealed that NHEJ-mediated indels can confer disease resistance in wheat (hexaploid specie) without the need to use a transgene (Li *et al.* 2012; Shan *et al.* 2013; Wang *et al.* 2014). In contrast, the homology-directed repair (HDR) way can familiarize a required DNA sequence or gene into a targeted site, subjected to the length of exogenous DNA, which is carried to the plant cells together with the nucleases (Butler and Douches 2016; Ding *et al.* 2016). HDR may results in gene stacking and allelic substitutions (Knoll *et al.* 2014).

Precise genome editing may face shortcomings in terms of off-target mutations. Multiple gene targeting ability of CRISPR/Cas9 may result in hybridization of gRNA to DNA sequence having mismatch bases, and can thus cause off-target mutations (Lee *et al.* 2016). The off-target effects can be minimized by careful selection of composition and structure of gRNA. Since, CRISPR/Cas9 system is based on nucleotide (RNA)-nucleotide (DNA) interaction, one can design the target sequence in more predictable way as compared to ZFNs and TALENs. Furthermore, the availability of accurate genome sequence information proves to be helpful in precise determination of target site (Brabaschi *et al.* 2016).

Genome Editing in Potato via Sequence Specific Nucleases (SSNS)

Recent reports on the genome editing of major crops of economic importance, including tomato (*Solanum lycopersicum*), soybean (*Glycine max*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*) and potato (*Solanum tuberosum*), have shown high efficiency of SSN platforms for site directed precise mutagenesis (indels) of desired gene for resolute modification (Table 4). Among the above-mentioned crops, the breeding of potato using genome editing tools is of great importance. Potato is autotetraploid, so the formation of new cultivar according to the routined breeding practices is very slow and intricate, due to tetrasomic inheritance and increased heterozygosity in nature (Muthoni *et al.* 2015). Genetic modification (GM), by stable integration of genetic material, has been used widely in research and breeding of potato, for a long time (Barrell *et al.* 2013). But there are some limitations

for commercialization of the developed GM plants in Europe or elsewhere. New breeding techniques such as gene knock-outs via site-directed mutagenesis (SDM) using SSNs, (where no recombinant DNA is introduced/maintained in plant chromosomes i.e., NHEJ resulting in small indels) has shown promising approach and not considered as GMOs (Araki and Ishii 2015). Moreover, efficient gene transformation and availability of genome sequence of potato made it an ultimate aspirant for genome editing system (The Potato Genome Sequencing Consortium, 2011). Here, we discuss the use of sequence specific nucleases i.e., mainly TALENs and CRISPR/Cas9 in potato breeding to target specific locus for SDM and gene silencing, without the introduction of exogenous DNA.

Cold storage of potatoes causes cold induced sweetening (CIS), which may leads to the accumulation of reducing sugars in tubers. When processed at high temperature, it accumulates acrylamide content in French-fries and chips, which are unacceptable to consumers due to its bitter taste and being carcinogenic (Dale and Bradshaw 2003). *VInv* (vacuolar invertase) gene plays a critical role in the production of reducing sugars in cold stored tubers (Kumar *et al*.2004). RNAi mediated gene knock-down of *VInv*, significantly reduces CIS (Zhu *et al.* 2014), but being transgenic, subject to de-regulation before commercialization. Clasen *et al.* (2016) designed TALENs targeting *VInv* gene in tetraploid potato cultivar, Ranger Russet. Protoplast transformation was done to introduce TALEN encoding plasmids. TALEN-mediated mutagenesis of *VInv* without stable integration of plasmid DNA was investigated. The later point, is of great significance in clonally propagated plants such as potato in which homozygosity can neither be achieved through selfing, nor the genetic cross proves to be successful to eliminate integrated TALEN reagents. The results revealed that only 3% (18 out of 600 regenerated plants) contained targeted mutations. Moreover, only 5 plants out of 600 (0.83%), were detected with all four *VInv* alleles mutated. Interestingly, PCR with designed primers demonstrated, 7 events (0.33%) out of 18 mutant plants, having complete "knock-out" and were also "TALEN free". Furthermore, *VInv* knock-out events having no integrated TALEN, were propagated in green-house trials and harvested tubers can be stored accordingly to probe the CIS effects. In addition to Ranger Russet, three other commercial varieties (Atlantic, Russet Burbank and Shepody) were also selected for targeted *VInv* gene knock-out in all alleles. Mutation frequency across all four varieties ranged from 2% to 15.9%. So, TALENs can be sought as useful genome editing tool for SDM (Clasen *et al.* 2016) and targeted production of healthy and safe tubers without integrated SSN reagents (Sawai *et al.* 2014).

α-solanine and α-chaconine are naturally occurring steroidal glycoalkaloids (SGAs) in potato. A 20mg/100g fresh weight of tubers is the current safety limit of SGAs in edible tubers (Ginzberg *et al.* 2009). High concentration in green tubers and sprouts may cause toxicity, thus inadequate for human consumption. Studies revealed that *SSR2* enzyme plays a crucial role in cholesterol biosynthesis pathway (precursor), which induces the production of toxic SGAs in potato. *StSSR2* disrupted or *StSSR2* silenced potatoes by targeted gene knock down, achieved through TALENs curtailed the level of SGAs in tubers. Interestingly, the *StSSR2* TALEN-transformants obtained after targeted genome editing at all four loci in tetraploid potato, had no remaining intact alleles (Fig. 1). Thus, *StSSR2*-knockout potato deprived of transgene will be obtained by segregation after self-crossing the transformants (Sawai *et al.* 2014). TALEN platform can thus be employed in breeding potatoes for desired low SGAs content.

Similar SSN platform was employed for SDM in tetraploid potato (cv. Desiree) to knock out ALS (acetolactate synthase) gene through transient expression of TALENs in protoplasts. Although, targeted mutation in calli and regenerated shoots were 11-13% and 10%, respectively. However, gDNA sequencing of calli and plantlets confirmed no full knock out *ALS* mutants (Nicolia *et al.* 2015). Therefore, limited efficiency of targeted mutagenesis by TALENs and some off-target mutations, advocate the use of other potentially efficient SSNs (e.g., CRISPER/Cas9).

CRISPR/Cas9 is accounted for efficient site directed mutagenesis and gene silencing in potato (Butler *et al.* 2015; Wang *et al.* 2015; Andersson *et al.* 2016). In a study, CRISPR/Cas9 was designed with two sgRNAs to target *StALS1* gene (responsible for herbicide resistance) in *Solanum tuberosum* (Butler *et al.* 2015). Generation and inheritance of targeted mutation in calli and primary events of both diploid and tetraploid (*cv*.Desiree) potato genotypes were investigated, in combination with two T-DNA vectors (conventional 35S and modified geminivirus LSL). CRISPR/Cas reagents were delivered via., *Agrobacterium* to analyze transient expression in calli and generation of primary events. Modified enrichment PCR detected targeted mutations in calli of both diploid and tetraploid genotypes. Furthermore,

transformed calli were regenerated to determine the targeted mutations in primary events. On the basis of number of ALS alleles, 3-60% individual events have targeted mutations, whereas, 0-29% possesses targeted mutations above threshold level (Butler *et al.* 2015). No wonder, these percentages were higher as compared to the previous studies using TALENs for gene knock-out indicating its efficacy (Clasen *et al.* 2015; Nicolia *et al.* 2015). The results further indicate the transient expression of CRISPR/Cas reagents in primary events, without integration of geminivirus LSL T-DNA. Voytas and Gao (2014) were also of the view that transient delivery of sequence specific nucleases (SSNs) such as Cas9, using viral vectors, do not result in integration of vector into the plant genomes and effectively employed in targeted plant breeding. Similar outcomes were recorded in protoplast mediated transformation of TALENs to bring about mutations without integration. This is extremely important in polyploidy species in which crossing cannot remove SSN reagents. In order to determine the germline inheritance, one diploid and two tetraploid primary events were screened for Cas9 free progeny along with targeted mutations. Selfing was done in tetraploid mutant events, while diploid event was crossed with self compatible diploid line. Transmission of targeted mutations in three different populations ranged from 87% to 100% indicating high efficacy of targeted mutations in primary events using CRISPR/Cas9 genome editing tool. Cas9-free progeny along with desired mutagenesis suggest that these progenies could be used for further study or commercial development (Butler *et al.* 2015).

Likewise, CRISPR/Cas9 plasmid construct was transformed via-Agrobacterium in double haploid potato cultivar targeting *StIAA2* gene. Kloosterman *et al.* (2006) cloned and analyzed this gene and revealed that it encodes for Aux/IAA protein in potato. Monoallelic and biallelic homozygous mutants with targeted knock-out of *StIAA2* gene was obtained in T1 generation, confirmed through PCR results. Moreover, no off-target mutations were observed, which ascertains the efficiency of CRISPR/Cas9 over other SSN platforms (Wang *et al.* 2015). These findings were in line with those obtained by Butler *et al.* (2015).

CRISPR/Cas9 transient expression in protoplasts of tetraploid potato cultivar was examined to target Granule Bound Starch Synthase (*GBSS*) gene. Since, it is responsible for amylose synthesis, therefore, silencing of *GBSS* gene functionality will yield waxy potato (amylopectin rich potatoes). Three different regions of this gene were targeted by CRISPR/Cas9 construct and regenerated shoots showed a mutation frequency of 2% to 12% in at least one allele. While, frequency of multiple mutated alleles was found to be up to 67%. Non-homologous end joining (NHEJ) after double stranded break in DNA may result in small indels of 1bp to 10bp in most mutations. A PCR based HRFA (high resolution fragment analysis) was carried out to identify the multiple mutated lines upto a resolution of 1bp. Phenotypic studies of starch also confirmed full knock-out of *GBSS* in all four-allele mutated lines (Andersson *et al.* 2016). Conclusively, CRISPR/Cas9 transient expression would be desirable for novel potato germplasm development, with targeted gene knock-outs without any stable integration of DNA.

Genome Editing: A Paradigm in Potato Breeding

Genome editing has entered a new era. The ability to prompt specific mutations through SSNs would enable direct modification/introduction of related agronomic traits into elite lines for breeding. NHEJ repair pathway i.e., indels, forced the regulatory authorities to amend current regulations about GM crops. Although, traditional breeding of potato done at tetraploid level and vegetatively propagated, yet diploid breeding is getting popular in public and private sector. Recombinant inbred lines (RILs) developed after diploid breeding substantiate to be a potent tool for genome editing in potato. Genome editing reagents can be used to modify self-compatible and inbred lines and following modifications can be fixed by selfing. So, development of diploid, self-compatible germplasm is indeed the next generation approach for gene editing in potato (Butler *et al.* 2016; Barabaschi *et al.* 2016).

Next Generation Phenotyping of Potato Need of high-throughput/next generation phenotyping

Novelty in crop improvement techniques is incumbent for plant breeders, geneticists, biotechnologists and agronomists to fulfill world food production demands and counter the prodigious biotic and abiotic stress conditions (Godfray *et al.* 2010; Mittler and Blumwald 2010; Sankaran *et al.* 2015). Over the past 20 years, a significant improvement in genetic technologies (Marker-Assisted Selection (MAS), Next Generation Sequencing (NGS), Genomic Selection (GS))and functional genomics has boost up the knowledge of plant genomes, but the capability to exploit available

genomic tools to their full potential are now limited by the ability to phenotype (Araus and Cairns 2014).Current approaches to phenotyping are slow, laborious, expensive and often destructive and allows the use of only a few sensors at a time (Furbank and Tester 2011; Cobb *et al.* 2013; Fiorani and Schurr 2013; Virlet *et al.* 2016). Since 2010, 'phenomics' and rapid high-throughput crop phenotyping methods evolved as next generation approach which significantly contributes to plant breeding (Furbank and Tester 2011; Walter *et al.* 2012; Dhondt *et al.* 2013; Fiorani and Schurr 2013; Cobb *et al.* 2013; Araus and Cairns 2014; Prashar and Jones 2014). The selection efficiency and plant performance over the years is greatly influenced by environmental factors. The environmental variations can be assessed efficiently by high-throughput phenotyping methods than current practices, thereby increasing selection efficiency (Sankaran *et al.* 2015; Virlet *et al.* 2016). Rapid and inexpensive genomic information is the outcome of advances in high-throughput genotyping. For phenotyping of thousands and millions of recombinant inbred lines (RILs), low cost, high-throughput genotyping has paved the way for the development of diversity panels and huge mapping populations (Araus and Cairns 2014). Developments in phenotyping are probably crucial to exploit the developments in conventional, transgenic and molecular breeding to ensure for the improvement in crop genetics for future food security.

High-Throughput Techniques and Platforms (HTPPS)

Automation and robotics; novel sensors; imaging (2D, 3D and high resolution) technologies (hardware and software) provide a range of applications for high-throughput phenotyping (HTP) of crops under controlled and field conditions (Kolukisaoglu and Thurow 2010; Fiorani and Schurr 2013; Li *et al.* 2014). The HTPtechniquesinclude the application of visible light imaging for estimation of germination rates, height size morphology and shoot biomass (Berger *et al.* 2010; Golzarian *et al.* 2011), fluorescence sensing for estimating photosynthesis (Baker 2008; Munns *et al.* 2010; Tuberosa 2012), thermal imaging for detecting canopy/leaf temperature and stomatal conductance (Pask and Pietragalla 2012; Li *et al.* 2014), near infrared spectroscopy and hyper-spectral imaging for measuring leaf area index (LAI), carbon isotope discrimination and various physiological changes induced by nutrient and water stress (Van Maarschalkerweerd *et al.* 2013; Monneveux *et al.* 2013), magnetic resonance imaging and X-ray computed tomography for assessment of root system architecture (RSA) (Li *et al.* 2014; Yol *et al.* 2015). Cobb *et al.* (2013) reported the use of various image analysis software programs viz., PlaRoM (Yazdanbakhsh and Fisahn 2009), RootReader2D and 3D (Clark *et al.* 2011 & 2012), Gia-Roots (Galkovskyi *et al.* 2012), LeafAnalyzer(Weight *et al.* 2007), LAMINA (Bylesjo *et al.* 2008), LEAFPROCESSOR (Backhaus *et al.* 2010), TraitMill (Reuzeau *et al.* 2006) and LemnaTec 3D Scanalyzer (Golzarian *et al.* 2011) for high-throughput phenotyping.Various phenotyping platforms have been developed to augment the resolution, accuracy, throughput and precision of phenotyping, including aerial solutions, controlled environment based systems, and several ground/field based-platforms, each having its own pros and cons (Deery *et al.* 2014; Li *et al.* 2014).

HTPP's in Potato

High-throughput phenotyping techniques and platforms (HTPPs) have been employed in a number of crop species such as *Arabidopsis thaliana*, wheat, maize, rice, soybean, beans, legumes, sugar beet, tomato; and potato is no exception.Phenotyping of potato under drought stress conditions have been reported by Monneveux *et al.* (2013) and Wishart *et al.* (2014). Development of high yielding improved potato cultivars, tolerant to biotic and abiotic stress environments required phenotyping of different structural, morphological, physiological, biochemical, molecular and performance related traits. Recently, Phenofab and Keytrack System (KeyGene, The Netherlands) have been developed, that uses multiple imaging systems and thermal sensors including automated plant handling under controlled environment (Laboratory/Glass-house) conditions for quantification of plant growth and functions (Jalink and Van der Schoor 2015; Furbank and Tester 2011). Indeed, HTPP's under controlled conditions allow detailed non-invasive observation and phenotyping of individual plants in potted soil. However, there exists a bottleneck to correlate the phenotyping results obtained from glass-house/green-house with the field conditions. Particularly, in case of Potato which have large canopy size and depict restricted growth and development in pots, it becomes imperative to develop effective automated and non-invasive remote sensing, field high-throughput phenotyping platforms. This approach provides better insights into crop behavior as breeding and genetic analysis for most crop species including potato is usually carried out under natural conditions (Prashar *et al.* 2013). Thus, to address the bottleneck of field

high-throughput phenotyping of potato, field/ground-based HTPPs (often called 'phenomobiles') are often considered superior over controlled environment based platforms since they function directly in the field. Moreover they can be used across multiple sites and have a potential for high temporal and spatial resolution.

Recently, Rothamsted's Field Scanalyzer have been developed which is fixed-site phenotyping platform, fully-automated and high-throughput, carrying multiple imaging sensors for non-invasive monitoring of plant growth, physiology and morphology (Virlet *et al.* 2016). The information obtained from Field Scanalyzer may be utilized, directly by potato breeders to produce new elite germplasm by estimating temporal, spatial and resource integrated traits. Some commonly used traits and non-invasive high-throughput approaches for phenotyping of potato is shown in Table 3. For instance, Prashar *et al.* (2013) estimated traits *viz*., stomatal conductance and canopy temperature by infrared thermography (IRT) in potato (*Solanum tuberosum* L.). Thermal images were taken from a fork-lift (8 m height; covered 9 horizontal plots and 3-4 rows) fitted with ThermaCAM P25 infrared camera (FLIR systems, USA). Thermal images were processed via., ThermaCAM Researcher Pro 2.8 SR-1 software (FLIR systems). The study showed substantial differences in canopy temperature among various potato genotypes even with sufficient water supply. A negative correlation was found between tuber yield and canopy temperature. This information may be used further to associate with SNPs (Single Nucleotide Polymorphisms) for mapping regions that control stomatal conductance and canopy temperature. We can also combine IRT data with carbon isotope signatures (delta C13) to identify water stress tolerant potato genotypes (efficient transpirants). The combination of mapping approach and genotypic responses to water availability will be helpful in breeding genotypes that can conserve water (stomatal closure), but momentarily took advantage of available water. Dammer *et al.* (2016) illustrated the use of camera-sensor based phenotyping positioned on tractor, to monitor green canopy coverage of potato and correlate it with LAI values. It will be helpful in detecting diseases (Late blight of potato) and providing information for disease forecasting models or decision support systems.

Furthermore, there are some key caveats associated with ground-based HTPP's such as soil compaction, high level of supervision, non-simultaneous measurements etc. These limitations can be addressed by using low altitude, high resolution Unmanned Aerial Vehicles (UAVs) integrated with sensors (Thermal, Fluorescence, spectral 3D cameras and LIDAR). The traits mentioned in Table 3 can be estimated by mounted imaging tools and sensors on UAVs (Rotocopters and unmanned helicopters) in potato and several other row and field crops (Sankaran *et al.* 2015). One such example in potato is the use of UAV platform Piper Seneca fitted with NIR (near infrared cameras) and satellite multispectral imaging to study vegetation indices (*SAVI*; Soil Adjusted Vegetation Index and *NDVI*; Normalized Difference Vegetation Index). Since, potato is very sensitive to water stress especially during the late vegetative, tuber initiation and yield formation phase. These vegetation indices monitor vegetation growth and can predict tuber yield (Sivarajan 2011). Despite of the fact that UAV emerged as next generation phenotyping tool, which possess characteristics such as stability, reliability, high resolution, simultaneous field high-throughput phenotyping. Concerns on developing data processing algorithms/tools to convert sensory data into useful phenotyping data for genotypic selection, image blur and geometric distortion corrections, automated feature extraction ability, geo referencing needs to be improved to utilize the full potential of UAV's in phenomics research (Zhang and Kovacs 2012; Sankaran *et al.* 2015). Therefore, with precise, accurate and optimal selection of robust phenotypic tool and platform, we can achieve goal of next generation phenotyping in potato. It also empowers genome-wide association studies (GWAS), high-resolution linkage mapping and for training genomic selection (GS) models in plant improvement.

Perspectives

Plant breeding is a major potential player keeping in view the global climate changes, diminishing land and water resources to address the world-wide food security issue. Whereas the molecular age has laid down the basis of molecular breeding for improving crop productivity, the start of genomic technologies and associated tools has been providing astounding abilities for the plant growth, development and fundamental characters for understanding of molecular basis. The purpose of this article is to unify latest high throughput advances in various fields of biology and conceptualize a technique that could markedly enhance the efficacy of plant breeding particularly in potato. Genotyping by sequencing, genomic selection (GS), genome wide association studies (GWAS), genome editing

and next generation phenotyping techniques are new and latest applications that are being used as next generation selection protocols for crop improvement (both in terms of quality and quantity). The low cost of genotyping by sequencing with a high density of SNP markers makes it a smart approach to inundate the mapping and breeding populations. High density of SNP markers from NGS will be widely applied to MAS, GS and GWAS. It could be foreseen that large crop genomes will be sequenced by the plant breeders/geneticists and high density of genetic linkage maps will be established from breeding populations. Future applications of GWAS, GBS, NGP, genome editing in crop improvement may allow plant breeders to conduct marker assisted selection or genomic selection on a novel germplasm and/or species without prior having any molecular tools. Since, sequence based genotyping is available for the whole range of genomic studies, it will be a vital component in plant breeding and genetics in the upcoming years.

Through the applications of GWAS, GBS, NGP, genome editing or a combination of all the technologies aimed at potato breeding explained in this review, potato can provide an amplified quantity of the food intake that is required for the predicted increase in population over the forthcoming years. Approach to these biotechnological techniques are energetic for countering food security in developing countries.

Table 1. Crop species whom whole genome has been sequenced using different sequencing technologies.

Name	Ploidy level	Genome Size (Mb)	Sequencing technologies	References
Vitis vinifera ssp.sativa (Grapevine)	diploid	504	Sanger paired end /Illumina GA	Velasco *et al*. (2007)
Gossypium raimondii (cotton)	diploid	880	Roche 454 / Illumina GA	http://www.jgi.doe.gov/sequencing/why/gossypium.html
Triticum aestivum ('Chinese Spring' wheat)	Hexaploid	16000	Roche 454	http://www.wheatgenome.org
Solanum tuberosum (potato)	Tetraploid	856	Sanger/454/Illumina 79.2x coverage contig N50: 31,429bp scaffold N50: 1,318,511bp	Xu *et al*. (2011)
Sorghum bicolor genotype BTx623	Tetraploid	730	Contig N50:195.4kbp scaffold N50: 62.4Mbp Sanger, 8.5x coverage WGS	Paterson *et al*. (2009)
Fragaria vesca (Woodland Strawberry)	diploid	240	Rohe 454 /Illumina GA/ABI SOLiD	Shulaev *et al*. (2011)
Zea Mays Maize	diploid	2,300	contig N50 40kbp scaffold N50: 76kbp Sanger, 4-6x coverage per BAC	Schnable *et al*.(2009)

Table 2. Association studies that are carried out in different plants including potato.

Crop	Mating System	LD extent	Mapped Traits	References
Potato	Selfing	0.3-1, 3cM	Resistance to wilt disease, bacterial blight, Phytophtora, and potato quality (tubershape, flesh color, under water weight,maturity, tuber starch, tuber yield etc)	Gupta *et al*. 2004, 2005, 2014; Ravel *et al*. 2006, Simko *et al*. 2004 and malosetti *et al*. 2007.
Maize	Outcrossing	200-2000bp, 3-500kb, 4-41cM	Plant height, Flowering time, endosperm color, starch production, maysin and chlorogenic acid accumulation, cell wall digestability, forage quality and oleic acid level.	Stich *et al*. 2006; Remington *et al*. 2001; Tenaillon *et al*. 2001; Thornsberry *et al*. 2001, Thornsberry et al. 2007; Stich *et al*. 2005; Guillet-Claude *et al*. 2004; Palaisa et al. 2003; wilson *et al*. 2004; Andersen *et al*. 2005; Szalma *et al*. 2005; Lubberstedt *et al*. 2005; Belo *et al*. 2008.
Rice	Selfing	5-500kb, 50-225cM	Plant height, heading date, flag leaf length and width, tiller number, stem diameter, panicle length, grain length width, grain thickness, 1000-grain weight,width and lenth of milled rice grain.	Zhang *et al*. 2005; Agrama *et al*. 2007, Iwata *et al*. 2007; Mather *et al*. 2007; Rakshit *et al*. 2007; Agrama *et al*. 2008; Garris *et al*. 2003.
Soyabean	Selfing	10-50cM	Seed protein content	Zhu *et al*. 2003.
Hexaploid Wheat	Selfing	<1-10cM	Kernel size and milling, high molecular weight glutenin and blotch resistance	Tommasini *et al*., 2007; Breseghello *et al*. 2006; Ravel *et al*. 2006; Chao *et al* 2007.
Barley	Selfing	10-5-cM, 98-500kb, 300bp	Yield, yield stability, heading date, flowering time, plant height, rachilla length, resistance to mildew and leaf rust.	Chapman *et al*., 2003; Kraakman *et al*. 2004; Kraakman *et al*. 2006; Caldwell *et al*. 2006; Malysheva-Otto *et al*. 2006; Morrell *et al*. 2005; Igartua *et al*. 1999; Ivandic *et al*. 2003.

Table 3: Some commonly used traits and non-invasive high-throughput approaches for phenotyping of potato (*Solanum tuberosum* L.)

Sr. No.	Traits	Devices/Sensors/Imaging techniques/Softwares	Advantages	Limitations	References
1.	Canopy Temperature	Thermal Imaging by Thermal Infrared thermometers	Reliable for high-throughput crop temperature phenotyping under water stress conditions in potato; Potentialinformation aboutleaf and canopytranspiration andheat dissipation	Time of day (morning readings are usually lower due to lower incident of solar radiation and air temperature); Adjustment of proper measurement angle; Difficult to separate soil temperature from plant temperature in sparse canopies; Sound physics based result interpretation required.	Roth and Goyne (2004); Grant *et al*. (2007); Biskup et al. (2007); O'Shaughnessy *et al*. (2011); Li *et al*. (2014)
2.	Stomatal conductance	Infrared thermography (IRT)/Near infrared cameras *Plant Eye; Field scan (Semi-automated and Automated HTPP)	Measured gas traffic and transpiration at cellular level; Easy, rapid and nondestructive method of screening for stomatal behavior; evaluate large population trials for genetic analysis.	Measurements influenced by humidity, time and other factors; Imaging sensor calibration and atmospheric correction are often required.	Munns *et al*. (2010); Pask and Pietragalla (2012); Fiorani and Schurr (2013); Prashar *et al*. (2013) *(https://phenospex.com/blog/field-phenotyping/)
3.	Chlorophyll fluorescence	Hyper spectral-radiometers/ Imaging sensors	Determined the influence of various genes and environmental conditions on photosynthetic efficiency (photosystem- II); Crucial to provide information about dissipation of excess light energy.	Several inaccurate measurements may occur due to changes of fluorescence during estimation of the PSII operating efficiency, Handling of large amount of data generated, expensive	(http://www.plantphenomics.org); Furbank and Tester (2011); Monneveux *et al*. (2013) Yol *et al*. (2015)
4.	Chlorophyll content	Multi-spectral imaging/ Digital imaging/SPAD meter	Positive correlation between root dry mass, tuber yield and chlorosis.	Limitations in terms of solar angle, measurement time, leaf position, leaf surface status, calibration errors in chlorophyll meter.	Songsri et al. (2009); Vollmann *et al*. (2011); Moghaddam *et al*. (2011); Monneveux *et al*. (2013)

Continuing table 3

Sr. No.	Traits	Devices/Sensors/Imaging techniques/Softwares	Advantages	Limitations	References
5.	Leaf Area / Leaf area Index	Digital camera imaging using appropriate softwares/ Near Infrared cameras (*camera sensor consisted of a 3-chip MS2100 CCD camera) laser scanners or LIDAR LAMINA SOFTWARE (Automated and semi-automated image analysis)/ LEAF PROCESSOR (Novel tool leaf shape analysis)	Quick, efficient, easy manipulation of physiological data; High 2D and 3D accuracy; Shoot and canopy models enabled,Quantification of leaf size and shape	Synchronization with GPS and encoder position systems needed for geo-referencing, personal constraints in access, storage, analysis and management of imaging data.	Bylesjo¨ et al. (2008); Backhaus *et al*. (2010); Cobb *et al*. (2013); Fiorani and Schurr (2013); Yol *et al*. (2015); *Dammer *et al*. (2016)
6.	Carbon isotope discrimination	Near Infrared Reflectance Spectroscopy (NIRS)	Determined the amount of C^{13} used by photosynthetic activity, positively correlated with stomatal conductance and drought tolerance in potato clones; development of genotypes with improved WUE.	Complicated, need of data transformation.	Ferrio et al. (2007); Monneveux *et al*. (2013); Yol *et al*. (2015)
7.	Root dynamics	PlaRoM- root extension profiling software (noninvasive video image technique)/3D digital imaging system and RootReader 3D/Gia Root (semi-automated 2D)/ X-ray CT (3D)	Allow non-destructive imaging, analysis and automatic phenotyping of RSA.	Controlled environment, understanding complex software packages, X-ray source effects for imaging time series to be evaluated	Tracy *et al*. (2010); Clark *et al*. (2011); Galkovskyi *et al*. (2012); Yazdanbakhsh and Fisahn(2012); Monneveux *et al*. (2013); Yol *et al*. (2015)

Acronyms; 2D (Two dimensional), 3D (Three Dimensional), GPS (Geo Positioning System),HTPP (High *Throughput Phenotyping Platform*), IRT (Infrared Thermography), LAMINA software (*Leaf Shape Determination*),LIDAR (*Light detection and Ranging*), PlaRoM (*PLAntROot Monitoring platform*), RSA (*Root System Architecture*), WUE (*Water Use Efficiency*), X-ray CT (*X-ray Computed Tomography*).

Table. 4.Examples of genome editing mediated gene modifications in major crops.

Crop species	Target Locus/gene	Genome-Editing Technique	Type of Modification	Frequency of mutation (%)	Off-Target Mutation	Purpose/Targeted Modification	References
Potato (*Solanum tuberosum*)	*St SSR2*	TALEN	MultialleleicIndels	-	Yes	Targeted breeding for low cholesterol and SGAs (steroidal glycoalkaloids) levels.	Sawai *et al.* (2014)
	ALS	TALEN	Indels	10%	N. D	SDM as tool in Plant Breeding	Nicolia *et al.* (2015)
	St ALS1	CRISPR-Cas 9	Indels	3%-60%	No	Herbicide resistance	Butler *et al.* (2015)
	StIAA2	CRISPR-Cas 9	Monoalleleic and bialleleic homozygous mutants Indels	83%	No	Functional studies of uncharacteized genes in potato (double haploid potato cultivar)	Kloosterman *et al.* (2006); Wang *et al.* (2015)
	GBSS	CRISPR-Cas 9	MultiallelicIndels	67% (multiple alleles); 2%-12% (one allele)	N. D	Amylopectin rich potato; Waxy Potato	Andersson *et al.* (2016)
	Vlnv	TALEN	MultiallelicIndels (1-4)	2% - 15.9%	Yes (3 or 4 bases mismatch)	Minimize accumulation of reducing sugars and also acrylamide levels; Targeted reduction of CIS	Clasen *et al.* (2016)
Tomato (*Solanum lycopersicum*)	*PROCERA*	TALEN	Biallelicindel	2.5%	N. D	Negative regulator of GA, mutants were tall, slender with light green vegetation	Lor *et al.* (2014)
	RIN	CRISPR-Cas 9	Indels (1 base insertion and deletion of upto 3 bases)	0%-100%	No	Regulate fruit ripening	Ito *et al.* (2015)

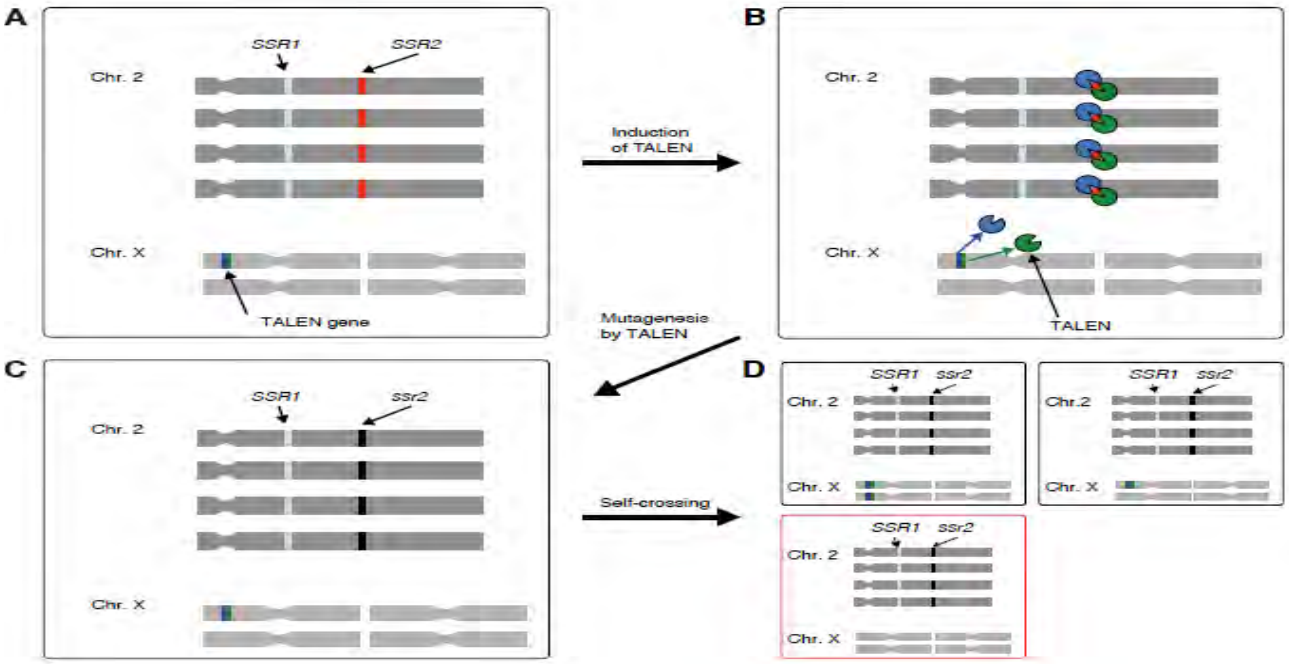
Continuing table 4

Crop species	Target Locus/gene	Genome-Editing Technique	Type of Modification	Frequency of mutation (%)	Off-Target Mutation	Purpose/Targeted Modification	References
Soybean (*Glycine max*)	*FAD2*	TALEN	Biallelicindel	33.3%	No	Improved soybean oil (poly-unsaturated fats) quality	Haun *et al.* (2014)
	GFP	CRISPR-Cas 9	Biallelicindel	78%-95%	Yes (2 loci out of 11)	Modification of soybean genes for improved agronomic and physiological traits.	Jacobs *et al.* (2015)
Wheat (*Triticum aestivum*)	*TaMLO*	CRISPR-Cas 9	Indel	28.5%	N. D	Adaptive Immune system against Powdery mildew	Shan *et al.* (2013)
Rice (*Oryza sativa*)	*OsBADH2, OsCKX2*	TALEN	Biallelicindel	12.5%, 3.4%	N. D	Selective removal of gene clusters, detect intergenic regions	Shan *et al.* (2013)
	OsBEL	CRISPR-Cas 9	Biallelicindel	2.2%	No	Herbicide resistant approach	Xu *et al.* (2014)
Maize (*Zea mays*)	*ZmIPK1*	ZFN	Inserting PAT gene	3.4% - 22.1%	No	Herbicide-tolerant plant	Kim *et al.* (2011)

N.D; Not Determined

Abbreviations: CIS (Cold-induced Sweetening); *GBSS* (Granule-Bound Starch Synthase);*SSR2* (Sterol Side chain Reductase 2);*ALS1* (Acetolactate Synthase1); *Vlnv* (Vacuolar Invertase Gene); *IAA* (Indole Acetic Acid); *SDM* (Site Directed Mutagenesis); GA (Gibberellic Acid); *RIN* (Ripening Inhibitor); *FAD2* (Fatty acid Desaturase 2); *GFP* (Green Flourescent Protein); *BEL* (Bentazon Sensitive Lethal)

Figure 1. TALEN induced *SSR2* Knock-out in *Solanum tuberosum* L. (Picture courtesy: Sawai *et al.* 2014)



A) Insertion of TALEN expression cassette, unlinked to St *SSR2* target site (Red) (B) TALEN construct (Green&Blue) targeting St *SSR2* loci. (C) Mutations at all four St *SSR2* loci in tetraploid potato genome without modification of St *SSR1*(light blue) (D)St *SSR2* knockout potato without integration of transgene achieved by segregation after selfing the transformants.

References

Agrama HA and Eizenga GC (2008). "Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of Oryza sativa and its wild relatives," Euphytica, vol. 160, no. 3, pp. 339-355.

Agrama HA, Eizenga GC, and Yan W (2007). "Association mapping of yield and its components in rice cultivars," Molecular Breeding, vol. 19, no. 4, pp. 341-356.

Andersen JR, SchragT, Melchinger AE, Zein I, and Lubberstedt T (2005). "Validation of Dwarf8 polymorphisms associated with flowering time in elite European inbred lines of maize (*Zea mays* L.)," Theoretical and Applied Genetics, vol.111, no. 2, pp. 206-217.

Andersen JR, Zein I and Wenzel G (2007). "High levels of linkage disequilibrium and associations with forage qualityat a Phenylalanine Ammonia-Lyase locus in European maize (*Zea mays* L.) inbreds," Theoretical and Applied Genetics, vol.114, no. 2, pp. 307-319.

Andersson M, Turesson H, Nicolia A, Fält AS, Samuelsson M and Hofvander P (2016). Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Reports, 1-12.

Anithakumari AM, Dolstra O, Vosman B, Visser RGF, and Van der Linden CG (2011). In vitro screening and QTL analysis for drought tolerance in diploid potato. Euphytica, 181(3):357-369.

Araki M and Ishii T (2015). Towards social acceptance of plant breeding by genome editing. Trends in plant science, 20(3): 145-149.

Aranzana MJ, Kim S, Zhao K, Bakker E, Horton M, Jakob K, Lister C, Molitor J, Shindo C, Tang C, Toomajian C, Traw B, Zheng H, Bergelson J, Dean C, Marjoram P and Nordborg M (2005). Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. PLOS Genetics, 1: e60.

Araus JL and Cairns JE (2014). Field high-throughput phenotyping: the new crop breeding frontier. Trends in Plant Science, 19(1):52-61.

Backhaus A,Kuwabara A, Bauch M, Monk N, Sanguinetti G and Fleming A (2010). LEAFPROCESSOR: a new leaf phenotyping tool using contour bending energy and shape cluster analysis. New Phytol 187:251-261.

Baker NR (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59: 89-113.

Bakker E, Borm T, Prins P, van der Vossen E, Uenk G, Arens M (2011). A genome-wide genetic map of NB-LRR disease resistance loci in potato. Theor. Appl. Genet. 123:493-508. doi:10.1007/s00122-011-1602-z.

Barabaschi D, Tondelli A, Desiderio F, Volante A,Vaccino P, Valè G and Cattivelli L(2016). Next generation breeding. Plant Science, 242:3-13.

Barrell PJ, Meiyalaghan S, Jacobs JME and Conner AJ (2013). Applications of biotechnology and genomics in potato improvement. Plant Biotechnol J. 11:907-920.

Beavis WD (1998). QTL analysis: Power, precision, and accuracy. In: A.C. Paterson, editor, Molecular dissection of complex traits. CRC Press, New York. p. 145-162.

Beissinger TM, Hirsch CN, Sekhon RS, Foerster JM, Johnson J M, Muttoni G (2013).Marker density and read depth for genotyping populations using genotyping-by-sequencing. Genetics 193, 1073-1081.doi: 10.1534/genetics.112.147710

Belo A, Zheng P, Luck S (2008). Whole genome scan detects an allelic variant of fad2 associated with increased oleic acid levels in maize. Molecular Genetics and Genomics, vol. 279, no. 1, pp. 1-10.

Berger B, Parent B and Tester M (2010). High-throughput shoot imaging to study drought responses. J. Exp. Bot. 61: 3519-3528.

Biskup B, Scharr H Schurr U and Rascher U (2007). A stereo imaging system for measuring structural parameters of plant canopies. Plant Cell Environ. 2007, 30, 1299-1308.

Bonierbale MW, Plaisted RL and Tanksley SD (1988). RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103.

Bradshaw JE and Mackay GR (1994). Breeding strategies for clonally propagated potatoes. In: J.E. Bradshaw and G.R. Mackay, editors, Potato genetics. CAB International, Wallingford. p. 467-497.

Bradshaw JE, Hackett CA, Meyer RC, Milbourne D, McNicol JW, Phillips MS, and Waugh R

(1998). Identification of AFLP and SSR markers associated with quantitative resistance to Globodera pallida (Stone) in tetraploid potato (*Solanum tuberosum* subsp. tuberosum) with a view to marker-assisted selection. Theoretical and applied genetics, 97(1-2):202-210.

Bradshaw JE, Hackett CA, Pande B, Waugh R, and Bryan GJ (2008). QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp. tuberosum). Theoretical and Applied Genetics, 116(2):193-211.

Breseghello F and Sorrells ME (2006). "Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars," Genetics, vol. 172, no. 2, pp. 1165-1177.

Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ (2009). The genetic architecture of maize flowering time. Science 325:714-18.

Bus A, Hecht J, Huettel B, Reinhardt R,and Stich B (2012). High-throughput polymorphism detection and genotyping in Brassica napus using next-generation RAD sequencing. BMC Genomics 13:281. doi:10.1186/1471-2164-13-281.

Butler NM and Douches DS (2016). Sequence-Specific Nucleases for Genetic Improvement of Potato. American Journal of Potato Research, 1-18.

Butler NM, Atkins PA, Voytas DF and Douches DS (2015). Generation and inheritance of targeted mutations in potato (*Solanum tuberosum* L.) using the CRISPR/Cas system. PloS one, 10(12): e0144591.

Bylesjo M, Segura V, Soolanayakanahally RY, Rae AM, Trygg J, Gustafsson P, Jansson S, Street NR (2008). LAMINA: a tool for rapid quantification of leaf size and shape parameters. BMC Plant Biol 8:82.

Caldwell KS, Russell J, Langridge P and Powell W (2006). "Extreme population-dependent linkage disequilibrium detected in an inbreeding plant species, *Hordeum vulgare*," Genetics, vol. 172, no. 1, pp. 557-567.

Calus MPL, Meuwissen THE, de Roos APW, and Veerkamp RF (2008). Accuracy of genomic selection using different methods to define haplotypes. Genetics 178:553-561. doi:10.1534/genetics.107.080838.

Chao S, Zhang W, Dubcovsky J and Sorrells M (2007). "Evaluatio of genetic diversity and genome-wide linkage disequilibriumamong U.S. wheat (*Triticum aestivum* L.) germplas representing different market classes," Crop Science, vol. 47, no. 3, pp. 1018-1030.

Chapman JM, Cooper JD, Todd JA, and Clayton DG (2003). "Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power," Human Heredity, vol. 56, no. 1-3, pp. 18-31.

Clark RT, Famoso AN, Zhao K, Shaff JE, Craft EJ, Bustamante CD, McCouch SR, Aneshansley DJ and Kochian LV (2012). Highthroughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant Cell Environ. doi. doi:10.1111/j.1365-3040.2012.02587x.

Clark RT, MacCurdy RB, Jung JK, Shaff JE, McCouch SR, Aneshansley DJ and Kochian LV (2011). Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiol 156: 455–465.

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F and Coffman A (2016). Improving cold storage and processing traits in potato through targeted gene knockout. Plant biotechnology journal, 14(1): 169-176.

Cobb JN, DeClerck G,Greenberg A, Clark R and McCouch S (2013). Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. Theoretical and Applied Genetics, 126(4): 867-887.

Cockram J, White J, Leigh FJ, Lea VJ, Chiapparino E, Laurie DA, Mackay IJ, Powell W, and O'Sullivan DM (2008). Association mapping of partitioning loci in barley. BMC genetics, 9:16.

Collard B C Y, Jahufer M Z Z, Brouwer J B, and Pang E C K (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142(1-2):169-196.

Crossa J, Campos GI, Perez P, Gianola D, Burgueno J, Araus JL (2010). Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers. Genetics186: 713724.doi:10.1534/genetics.110.118521.

D'hoop B, Keizer PC, Paulo MJ, Visser RF, Eeuwijk F, and Eck H (2014). Identification of agronomically important QTL in tetraploid potato cultivars using

a marker-trait association analysis. Theor. Appl. Genet. 127:731-748. doi:10.1007/s00122-013-2254-y.

D'hoop B, Paulo M, Kowitwanich K, Sengers M, Visser R, van Eck H (2010). Population structure and linkage disequilibrium unravelled in tetraploid potato. Theor. Appl. Genet. 121:1151-1170. doi:10.1007/ s00122-010-1379-5.

Daetwyler HD, Hickey JM, Henshall JM, Dominik S, Gredler B, van der Werf JHJ (2010). Accuracy of estimated genomic breeding values for wool and meat traits in a multi-breed sheep population. Anim. Prod. Sci. 50:1004-1010. doi:10.1071/ AN10096.

Dale MF and Bradshaw JE (2003). Progress in improving processing attributes in potato. Trends Plant Sci. 8: 310-312.

Dalla Rizza M, Vilaro F, Torres D, and Maeso D (2006). Detection of PVY extreme resistance genes in potato germplasm from the Uruguayan breeding program. Am. J. Potato Res. 83:297-304. doi:10.1007/ BF02871590.

Dammer KH, Dworak V and Selbeck J (2016). On-the-go Phenotyping in Field Potatoes Using Camera Vision. Potato Research, 1-15.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM and Blaxter ML (2011).Genome-wide genetic marker discovery and genotyping using next- generation sequencing. Nat.Rev. Genet. 12, 499-510.doi:10.1038/nrg3012.

Deery D, Jimenez-Berni J, Jones H, Sirault X and Furbank R (2014). Proximal remote sensing buggies and potential applications for field-based phenotyping. Agronomy, 4: 349-379.

Deschamps S, Llaca V, and May GD (2012). Genotyping-by-sequencing in plants. Biology 1, 460–483.doi:10.3390/biology1030460.

Dhondt S, Wuyts N and Inzé D (2013). Cell to whole-plant phenotyping: the best is yet to come. Trends Plant Sci. 18: 428-439.

D'hoop BB, Paulo MJ, Kowitwanich K, Sengers M, Visser RGF, Van Eck H J and Van Eeuwijk F (2010). Population structure and linkage disequilibrium unravelled in tetraploid potato. Theoretical and Applied Genetics, 121(6):1151-1170.

Ding Y, Li H, Chen LL and Xie K (2016). Recent Advances in Genome Editing Using CRISPR/ Cas9. Frontiers in plant science, 7.

Dong F, Song J, Naess SK, Helgeson JP, Gebhardt C and Jiang J (2000). Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor. Appl. Genet. 101:1001-1007. doi:10.1007/ s001220051573.

Edwards D and Batley J (2010). Plant genome sequencing:applicationsforcrop improvement. Plant Biotechnol. J. 8, 2-9.doi:10.1111 /j.1467-7652.2009.00459. x.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 123:307-326.

FAO (2014) The State of the World Population Report. By Choice, Not by Chance: Family Planning, Human Rights and Development. United Nations Population Fund, New York.

Felcher KJ, Coombs JJ, Massa AN, Hansey CN, Hamilton JP, Veilleux RE (2012). Integration of two diploid potato linkage maps with the potato genome sequence. PLoS One 7: e36347. doi:10.1371/journal. pone.0036347.

Ferrio JP, Mateo MA, Bort J, Abdalla O, Voltas J and Araus JL (2007). Relationships of grain D13C and D18O with wheat phenology and yield under water- limited conditions. Ann Appl Biol 150:207-215

Fiorani F and Schurr U (2013). Future scenarios for plant phenotyping. Annual review of plant biology, 64: 267-291.

Fiorani F, Schurr U (2013). Future Scenarios for Plant Phenotyping Annu. Rev. Plant Biol. 2013.64:267-291.

Fischer M, Schreiber L, Colby T, Kuckenberg M, Tacke E, Hofferbert HR, Schmidt J, and Gebhardt C (2013). Novel candidate genes inuencing natural variation in potato tuber cold sweetening identified by comparative proteomics and association mapping. BMC Plant Biology, 13:113.

Fischer RA, and Edmeades GO (2010). Breeding and cereal yield progress. Crop Sci. 50: S-85-S-98. doi:10.2135/cropsci2009.10.0564.

Flint-Garcia SA, Thornsberry JM, and Buckler ES (2003). Structure of linkage disequilibrium in plants. Annual Review of Plant Biology, 54(1):357-374.

Freyre R and Douches DS (1994). Development of a model for marker-assisted selection of specific gravity in diploid potato across environments. Crop Science, 34:1361-1368.

Fu YB and Peterson GW (2011). Genetic diversit yanalysis with 454 pyro-sequencing and genomic reduction confirmed the eastern and western division in the cultivated barley genepool. Plant Genome 4, 226-237.doi: 10.3835/plantgenome2011.08.0022.

Fu YB, Cheng B and Peterson GW (2014). Genetic diversity analysis of yellow mustard (*Sinapis alba* L.) germplasm based on genotyping by sequencing. Genet. Resour. Crop Evol. 61, 579-594.doi:10.1007/s10722-013-0058-1.

Furbank R. and Tester M (2011). Phenomics technologies to relieve the phenotyping bottleneck. Trends Plant Sci. 16:635-44.

Galkovskyi, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR and Fang S (2012). GiA Roots: software for the high throughput analysis of plant root system architecture. BMC Plant Biol 12:116.

Garris AJ, McCouch SR, and Kresovich S (2003). Population structure and its effect on haplotype diversity and linkagedisequilibrium surrounding the xa5 locus of rice (*Oryza sativa* L.), Genetics, vol. 165, no. 2, pp. 759-769.

Gebhardt C (2005). Potato genetics: molecular maps and more. In Lorz, H. and Wenzel, G., editors, Biotechnology in Agriculture and Forestry, volume 55, chapter II, pages 215- 227. Springer, Berlin Heidelberg.

Gebhardt C (2013). Bridging the gap between genome analysis and precision breeding in potato. Trends Genet. 29:248-256. doi:10.1016/j tig.2012.11.006.

Gebhardt C, Ballvora A, Walkemeier B, Oberhagemann P and Schuler K (2004). "Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type," Molecular Breeding, vol. 13, no. 1, pp. 93-102.

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U (1991). RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor. Appl. Genet. 83:49-57. doi:10.1007/BF00229225

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H (1989). RFLP analysis and linkage mapping in Solanum tuberosum. Theor. Appl. Genet. 78:65-75. doi:10.1007/BF00299755.

Ginzberg I, Tokuhisa JG and Veilleux RE (2009). Potato steroidal glycoalkaloids: biosynthesis and genetic manipulation. Potato Research, 52(1):1-15.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM and Toulmin C (2010). Food security: the challenge of feeding 9 billion people. Science 327: 812-818.

Golzarian MR, Frick RA, Rajendran K, Berger B, Roy S, Tester M and Lun DS (2011). Accurate inference of shoot biomass from high-throughput images of cereal plants. Plant Methods 7:1-11.

Grant OM, Tronina L, Jones HG and Chaves MM (2007). Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. J. Exp. Bot. 58:815-825.

Grattapaglia D, Vilela Resende MD, Resende MR, Sansaloni CP, Petroli CD, Missiaggia AA (2011). Genomic selection for growth traits in Eucalyptus: Accuracy within and across breeding populations. BMC Proc. 5:1-2. doi:10.1186/1753-6561-5-S7-A1.

Guillet-Claude C, Birolleau-Touchard C, Manicacci D (2004). "Nucleotide diversity of the ZmPox3 maize peroxidasegene: relationship between a MITE insertion in exon 2 and variation in forage maize digestibility," BMC Genetics, vol. 5, article 19, pp. 1-11.

Guo X, Ronhovde K, Yuan L, Yao B, Soundararajan MP, Elthon T, Zhang C, Holding DR (2012) Pyrophosphate-dependent fructose-6-phosphate 1-phosphotransferase induction and attenuation of Hsp gene expression during endosperm modification in quality protein maize. Plant physiology 158: 917-929.

Gupta PK, Rustgi S, and Kulwal PL (2005). "Linkage disequilibrium and association studies in higher plants: present status and future prospects," Plant Molecular Biology, vol. 57, no. 4, pp. 461-485.

Hamilton J, Hansey C, Whitty B, Stoffel K, Massa A, Van Deynze A (2011). Single nucleotide polymorphism discovery in elite North American

potato germplasm. BMC Genomics 12:302. doi:10.1186/1471- 2164-12-302.

Haun W (2014). Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol. J. 12: 934 -940

Hayes B and Goddard M (2010). Genome-wide association and genomic selection in animal breeding. Genome Vol. 53, No. 11, pp. 876-883, ISSN 0831-2796.

Heffner EL, Jannink JL, Iwata H *et al.* (2011b). Genomic selection accuracy for grain quality traits in biparental wheat populations. Crop Sci 51:2597-2606.

Heffner EL, Jannink JL, Sorrells ME (2011a). Genomic selection accuracy using multi-family prediction models in a wheat breeding program. Plant Genome 4:65-75.

Heffner EL, Sorrells ME, and Jannink JL (2009). Genomic selection for crop improvement. Crop Science, Vol. 49, No. 1, pp 1-12.

Heffner EL, Lorenz AJ, Jannink JL, and Sorrells ME (2010). Plant breeding with genomic selection: gain per unit time and cost. Crop Sci. 50, 1681-1690.doi: 10.2135/cropsci2009.11.0662.

http://www.turkstat.gov.tr/PreTabloArama.do 2014/ 2015.

Huang X, Kurata N, Wei X, Wang ZX, Wang A (2012). A map of rice genome variation reveals the origin of cultivated rice. Nature 490:497-501.

Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. Nat. Genet. 42:961-967. doi:10.1038/ng.695.

Huang X, Zhao Y, Wei X, Li C, Wang A (2012). Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nat. Genet. 44:32-39.

Igartua E, Casas AM, Ciudad F, Montoya JL and Romagosa I (1999). "RFLP markers associated with major genes controlling heading date evaluated in a barley germ plasm pool," Heredity, vol. 83, no. 5, pp. 551-559.

Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M and Toki S (2015). CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. Biochemical and Biophysical Research Communications, 467(1): 76-82.

Ivandic V, Thomas WTB, Nevo E, Zhang Z and Forster BP (2003). "Associations of simple sequence repeats with quantitative trait variation including biotic and abiotic stress tolerance in *Hordeum spontaneum*," Plant Breeding, vol. 122, no. 4, pp. 300-304.

Iwata H, Uga Y, Yoshioka Y, Ebana K and Hayashi T (2007). "Bayesian association mapping of multiple quantitative traitloci and its application to the analysis of genetic variation among *Oryza sativa* L. germplasms," Theoretical and Applied Genetics, vol. 114, no. 8, pp. 1437-1449.

Jacobs TB, LaFayette PR, Schmitz RJ and Parrott WA (2015). Targeted genome modifications in soybean with CRISPR/Cas9. BMC biotechnology, 15(1): 1.

Jaggard KW, Qi AM and Ober ES (2010). Possible Changes to Arable Crop Yields by 2050. Philosophical Transactions of the Royal Society B: Biological Sciences, 365, 2835-2851. http://dx.doi.org/10.1098/rstb.2010.0153

Jalink H and Van der Schoor R (2015). Role of Fluorescence Approaches to Understand Functional Traits of Photosynthesis. In Phenomics in Crop Plants: Trends, Options and Limitations (pp. 181-194). Springer India.

Jan HU, Abbadi A, Lucke S, Nichols RA and Snowdon RJ (2016). Genomic prediction of testcross performance in canola (*Brassica napus*). PLoS One 11: e0147769. doi: 10.1371/journal.pone.0147769.

Jannink JL, Lorenz AJ, and IwataH (2010). Genomic selection in plant breeding: from theory to practice. Brief. Funct. Genomics 9, 166-177. doi: 10.1093/bfgp/elq001.

Jansky S (2009). Breeding, genetics and cultivar development. In: J. Singh and L. Kaur, editors, Advances in potato chemistry and technology. Academic Press, New York. p. 27-62.

Jia G, Huang X, Zhi H, Zhao Y, Zhao Q (2013). A haplotypemap of genomic variations and genomewide association studies of agronomic traits in foxtail millet (*Setaria italica*). Nat. Genet. 45:957-61.

Jupe F, Pritchard L, Etherington G, MacKenzie K, Cock P, Wright F (2012). Identification and localisation of the NB-LRR gene family within the potato genome. BMC Genomics 13:75. doi:10.1186/1471-2164-13-75.

Jupe F, Witek K, Verweij W, Śliwka J, Pritchard L, Etherington GJ (2013). Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. Plant J. 76:530-544. doi:10.1111/tpj.12307.

Khatodia S, Bhatotia K, Passricha N, Khurana SMP and Tuteja N (2016) The CRISPR/Cas Genome-Editing Tool: Application in Improvement of Crops. Front. Plant Sci. 7:506. doi: 10.3389/fpls.2016.00506

Kim S and Kim JS (2011). Targeted genome engineering via zinc finger nucleases. Plant biotechnology reports, 5(1): 9-17.

Kloosterman B, Abelenda JA, del Mar Carretero Gomez M, Oortwijn M, de Boer JM, Kowitwanich K, Horvath B.M, van Eck HJ, Smaczniak C, Prat S, Visser RGF and Bachem CWB (2013). Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature, 495, 246-250.

Kloosterman B, Visser RG and Bachem CW (2006). Isolation and characterization of a novel potato auxin/indole-3-acetic acid family member (StIAA2) that is involved in petiole hyponasty and shoot morphogenesis. Plant Physiol Biochem 44:766–775.

Knoll A, Fauser F and Puchta H (2014). DNA recombination in somatic plant cells:mechanisms and evolutionary consequences, Chromosome Res. 22: 191-201.

Kolukisaoglu U and Thurow K (2010). Future and frontiers of automated screening in plant sciences. Plant Sci. 178:476-84.

Kraakman ATW, Martınez F, Mussiraliev B, van Eeuwijk FA and Niks RE (2006). Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars, Molecular Breeding vol. 17, no. 1, pp. 41-58.

Kraakman ATW, Niks RE, Van den Berg RMMM, Stam P and Van Eeuwijk FA (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars, Genetics, vol. 168, no. 1, pp. 435-446.

Kreike CM, De Koning JR, Vinke JH, Van Ooijen JW and Stiekema WJ (1994). Quantitatively-inherited resistance to Globodera pallida is dominated by one major locus in Solanum spegazzinii. Theoretical and Applied Genetics, 88(6-7):764- 9.

Kumar D, Singh BP and Kumar P (2004). An overview of the factors affecting sugar content of potatoes. Ann. Appl. Biol. 145: 247-256.

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat. Genet. 43:163-68.

Lado B, Matus I, Rodríguez A, Inostroza L, Poland J, Belzile F, Pozo A del, Quincke M, Zitzewitz J von (2013). Increased Genomic Prediction Accuracy in Wheat Breeding Through Spatial Adjustment of Field Trial Data. G3 Genes Genomes Genetics: g3.113.007807.

Lam HM, Xu X, Liu X, Chen W, Yang G, Wong FL (2010). Resequencing of 31 wild and cultivated soybean genomesidentifies patterns of genetic diversity and selection. Nat. Genet. 42, 1053-1059.doi:10.1038/ng.715.

Lee CM, Cradick TJ, Fine EJ and Bao G (2016). Nuclease Target Site Selection for Maximizing On-target Activity and Minimizing Off-target Effects in Genome Editing. Molecular Therapy, 24(3): 475-487.

Li H, Peng Z, Yang X, Wang W, Fu J (2013). Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat. Genet. 45:43-50.

Li L, Zhang Q and Huang D (2014). A review of imaging techniques for plant phenotyping. Sensors, 14(11): 20078-20111.

Li T (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat. Biotechnol. 30: 390-392.

Li X, Van Eck H J, Rouppe van der Voort J N A M, Huigen D-J, Stam P and Jacobsen E (1998). Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance to Phytophthora infestans mapped on potato chromosome 4. Theoretical and Applied Genetics, 96(8):1121-1128.

Lin Z, Hayes BJ and Daetwyler HD (2014). Genomic selection in crops, trees and forages: A review. Crop Pasture Sci. 65:1177–1191. doi:10.1071/CP13363.

Lor VS (2014). Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. Plant Physiol. 166: 1288-1291.

Lu F, Lipka AE, Glaubitz J, Elshire R, Cherney JH, Casler MD (2013). Switchgrass genomic diversity, ploidy and evolution: novel insights from a network-basedSNPdiscoveryprotocol. PLoSGenetics 9: e1003215. doi: 10.1371/journal.pgen.1003215.

Lubberstedt T, Zein I and Andersen JR (2005). "Development and application of functional markers in maize," Euphytica, vol. 146, no. 1-2, pp. 101-108.

Malosetti M, van der Linden CG, Vosman B and van Eeuwijk FA (2007). "A mixed-model approach to association mapping using pedigree information with an illustration of resistance to *Phytophthora infestans* in potato," Genetics, vol. 175, no. 2, pp. 879-889.

Malysheva-Otto LV, Ganal MW and Roder MS (2006). "Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.)," BMC Genetics, vol. 7, article 6.

Mather KA, Caicedo AL, Polato NR, Olsen KM, McCouch S and Purugganan MD (2007). "The extent of linkagedisequilibrium in rice (*Oryza sativa* L.)," Genetics, vol. 177, no. 4, pp. 2223-2232.

Menendez CM, Ritter E, Schafer-Pregl R, Walkemeier B, Kalde A, Salamini F, and Gebhardt C (2002). Cold sweetening in diploid potato: mapping quantitative trait loci and candidate genes. Genetics, 162(3):1423- 34.

Meuwissen THE, Hayes BJ and Goddar ME. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829.

Meyer RC, Milbourne D, Hackett CA, Bradshaw JE, McNichol JW, and Waugh R (1998). Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). Molecular and General Genetics, 259:150-160.

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C and Waugh R (1998). Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol. Gen. Genet. 259:233-245. doi:10.1007/s004380050809.

Milbourne D, Pande B and Bryan GJ (2007). Potato. In Kole, C., editor, Genome Mapping and Molecular Breeding in Plants. Pulses, Sugar and Tuber Crops, volume 3, chapter 12, pages 205-236. Springer, Berlin Heidelberg.

Mittler R and Blumwald E (2010). Genetic engineering for modern agriculture: challenges and perspectives. Annu. Rev. Plant Biol. 61: 443-462.

Moghaddam PA, Derafshi MH and Shirzad V (2011). Estimation of single leaf chlorophyll content in sugar beet using machine vision. Turk J Agric for 35:563-568

Monneveux P, Ramírez DA and Pino MT (2013). Drought tolerance in potato (*S. tuberosum* L.): Can we learn from drought tolerance research in cereals? Plant Science, 205:76-86.

Morrell PL, Toleno DM, Lundy KE and Clegg MT (2005). "Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. spontaneum) despite high rates of selffertilization," Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 7, pp. 2442-2447.

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T (2012). Population genomic and genomewide association studies of agro climatic traits in sorghum. Proc. Natl. Acad. Sci. USA 110:453–58.

Munns R, James RA, Sirault XR, Furbank RT and Jones HG (2010). New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. J. Exp. Bot., 3499.

Muthoni J, Kabira J, Shimelis H and Melis R (2015). Tetrasomic inheritance in cultivated potato and implications in conventional breeding. Aust J Crop Sci 9:185-190

Navarro C, Abelenda JA, Cruz-Oro E, Cuellar CA, Tamaki S, Silva J, Shimamoto K and Prat S (2011). Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature, 478, 119-122.

Nicolia A, Proux-Wéra E, Åhman I, Onkokesung N, Andersson M, Andreasson E and Zhu LH (2015). Targeted gene mutation in tetraploid potato through transient TALEN expression in protoplasts. Journal of biotechnology, 204: 17-24.

O'Shaughnessy SA, Hebel, Evett PD Colaizzi (2011). Evaluation of a wireless infrared thermometer with a narrow field of view. Comput Electron Agric. 76:59-68.

Ortega F and Lopez-Vizcon C (2012). Application of molecular marker assisted selection (MAS) for disease resistance in a practical potato breeding programme. Potato Res. 55:1-13. doi:10.1007/s11540-011-9202-5.

Ottoman R, Hane D, Brown C, Yilma S, James S, Mosley A (2009). Validation and implementation of marker-assisted selection (MAS) for PVY resistance (Ryadg gene) in a tetraploid potato breeding program. Am. J. Potato Res. 86:304-314. doi:10.1007/s12230-009- 9084-0.

Pajerowska-Mukhtar KM, Stich B, Achenbach U, Ballvora A, Lubeck J, Strahwald Tacke E, Hofferbert HR, Ilarionova E, Bellin D, Walkemeier B, Basekow R, Kersten B and Gebhardt C (2009). Single Nucleotide Polymorphisms in the Allene Oxide Synthase 2 Gene Are Associated with Field Resistance to Late Blight in Populations of Tetraploid Potato Cultivars. Genetics. 181(3): 1115-1127.

Palaisa KA, Morgante M, Williams M and Rafalski A (2003). "Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci," The Plant Cell, vol. 15, no. 8, pp. 1795-1806.

Pask A and Pietragalla J (2012). Leaf area, green crop area and senescence. In: Pask A, Pietragalla J, Mullan D, Reynolds M (eds) Physiological breeding II: a field guide to wheat phenotyping. CIMMYT, Mexico, pp 58-62.

Paterson A, Bowers J, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E, Maher CA Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L (2009). "The Sorghum bicolor genome and the diversification of grasses" (PDF). Nature. 457(7229): 551 556. Bibcode: 2009 Natur. 457..551P. doi:10.1038/nature07723. PMID 19189423.

Pembleton LW, Cogan NOI and ForsterJW (2013). StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Mol. Ecol. Resour. 13:946-952. doi:10.1111/1755-0998.12129.

po den Berg RG and Jacobs MM (2007). Molecular taxonomy. In Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M. A., MacKerron, D. K., and Ross, H. A., editors, Potato Biology and Biotechnology: Advances and Perspectives, chapter 4.

Poland JA, Bradbury PJ, Buckler ES, Nelson RJ (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc. Natl. Acad. Sci. USA 108:6893-98.

Poland JA, Brown PJ, Sorrells ME and Jannink JL (2012a). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoSONE 7: e32253. doi: 10.1371/journal.pone.0032253.

Poland JA and Rife TW (2012). Genotyping-by-sequencing for plant breeding and genetics. Plant Genome 5, 92–102.doi:10.3835/plantgenome2012.05.0005.

Prashar A and Jones HG (2014). Infra-red thermography as a high-throughput tool for field phenotyping. Agronomy 4: 397-417.

Prashar A, Yildiz J, McNicol JW, Bryan GJ and Jones HG (2013). Infra-red thermography for high throughput field phenotyping in Solanum tuberosum. PLoS One, 8(6): e65816.

Pritchard JK, Stephens M and Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2):945- 959.

Quetier F (2016). The CRISPR-Cas9 technology: closer to the ultimate toolkit for targeted genome editing. Plant Sci 242:65-76

Rakshit S, Rakshit A and Matsumura H (2007). "Large-scale DNA polymorphism study of Oryza sativa and O. rufipogon reveals the origin and divergence of Asian rice," Theoretical and Applied Genetics, vol. 114, no. 4, pp. 731-743.

Ravel C, Praud S, Murigneux A (2006). Identification of Glu-B1-1 as a candidate gene for the quantity of high molecular-weight glutenin in bread wheat (*Triticum aestivum* L.) by means of an association study. Theoretical and Applied Genetics, vol. 112, no. 4, pp. 738-743.

Remington DL, Thornsberry JM, Matsuoka Y (2001). Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 20, pp. 11479-11484.

Resende MDV, Resende MFR, Sansaloni CP, Petroli CD, Mssiaggia AA, Aguiar AM, Abad JM, Takahashi EK, Rosado AM, Faria DA, Pappas GJ, Kilian A, Grattapaglia D (2012c). Genomic selection for growth and wood quality in Eucalyptus: capturing the missing heritability and accelerating breeding for complex traits in forest trees. New Phytol. 194: 116-128.

Resende MFR, Muñoz P, Acosta JJ, Peter GF, Davis JM, Grattapaglia D, Resende MDV, Kirst M (2012a). Accelerating the domestication of trees using genomic selection: accuracy of prediction models across ages and environments. New Phytol. 193: 617-624.

Resende MFR, Munoz P, Resende MDV, Garrick DJ, Fernando RL, Davis JM (2012). Accuracy of genomic selection methods in a standard data set of loblolly pine (*Pinus taeda* L.). Genetics 190:1503– 1510. doi:10.1534/genetics.111.137026.

Resende MFR, Muñoz P, Resende MDV, Garrick DJ, Fernando RL, Davis JM, Jokela EJ, Martin TA, Peter GF, Kirst M (2012b). Accuracy of Genomic Selection Methods in a Standard Data Set of Loblolly Pine (*Pinus taeda* L.). Genetics 190: 1503-1510.

Reuzeau C, Frankard V, Hatzfeld Y,Sanz A, Van Camp W, Lejeune P, De Wilde C, Lievens K, de Wolf J, Vranken E (2006). Traitmill TM: a functional genomics platform for the phenotypic analysis of cereals. Plant Gen Res 4:20.

Riedelsheimer C, Czedik-Eysenberg A, Grieder C, Lisec J, Technow F, Sulpice R, Altmann T, Stitt M, Willmitzer L and Melchinger AE (2012). Genomic and metabolic prediction of complex heterotic traits in hybrid maize. Nat. Genet. 44:217-220. doi:10.1038/ng.1033.

Romay MC, Millard MJ, Glaubitz JC, Peiffer JA, Swarts KL, Casstevens TM (2013). Comprehensive genotyping of the USA national maize inbred seed bank. Genome Biol. 14, R55.doi:10.1186/gb-2013-14-6-r55.

Roth G and Goyne P (2004). Measuring plant water status. In: Dugdale H, Harris G, Neilsen J, Richards J, Roth G, Williams D (eds) WATER pak – a guide for irrigation management in cotton. Cotton Research and Development Corporation, Australia, pp, 157-164.

Rutkoski J, Benson J, Jia Y et al (2012). Evaluation of genomic prediction methods for fusarium head blight resistance in wheat. Plant Genome 5:51-61.

Rutkoski JE, Heffner EL, Sorrells ME (2010). Genomic selection for durable stem rust resistance in wheat. Euphytica 179:161-173.

Rutkoski JE, Poland J, Jannink JL, Sorrells ME (2013). Imputation of unordered markers and the impact on genomic selection accuracy. G3 3:427-439.

Sankaran S, Khot LR, Espinoza CZ, Jarolmasjed S, Sathuvalli VR,Vandemark GJ and Pavek MJ (2015). Low-altitude, high-resolution aerial imaging systems for row and field crop phenotyping: A review. European Journal of Agronomy, 70: 112-123.

Sawai S, Ohyama K, Yasumoto S, Seki H, Sakuma T, Yamamoto T and Muranaka T (2014). Sterol side chain reductase 2 is a key enzyme in the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in potato. The Plant Cell, 26(9): 3763-3774.

Schafer-Pragl R, Ritter E, Concilio L, Hesselbech J, Lovatti L, Walkemeier B, Thelen H, Salamini F, Gebhardt C (1998). Analysis of quantitative trait loci (QTLs) and /quantitative trait allels (QTAs) for potato tuber yield and starch content. Theor. and Appl. Genet 97:834-846.

Schiml S and Puchta S (2016). Revolutionizing plant biology: multiple ways of genome engineering by CRISPR/Cas. Plant Methods, 12:8.

Schnable P, Ware D, Fulton RS (2009). "The B73 Maize Genome: Complexity, Diversity, and Dynamics". Science. 326 (5956): 1112 1115. Bibcode:2009Sci...326.1112S. doi:10.1126/science.1178534. PMID 19965430.

Schultz L, Cogan NOI, McLean K, Dale MFB, Bryan GJ, Forster JW (2012). Evaluation and implementation of a potential diagnostic molecular marker for H1-conferred potato cyst nematode resistance in potato (*Solanum tuberosum* L.). Plant Breed. 131:315–321. doi:10.1111/ j.1439-0523.2012.01949. x.

Shan Q (2013). Rapid and efficient gene modification in rice and Brachy podium using TALENs. Mol. Plant 6: 1365–1368.

Shan Q (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. Nat. Biotechnol. 8: 686-688.

Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Delcher AL, Jaiswal P, Mockaitis K, Liston A, Mane SP, Burns P, Davis TM, Slovin JP, Bassil N, Hellens RP, Evans C, Harkins T,

Kodira C, Desany B, Crasta OR, Jensen RV, Allan AC, Michael TP, Setubal JC, Celton JM, Rees DJ, Williams KP, Holt SH, Ruiz Rojas JJ, Chatterjee M, Liu B, Silva H, Meisel L, Adato A, Filichkin SA, Troggio M, Viola R, Ashman TL, Wang H, Dharmawardhana P, Elser J, Raja R, Priest HD, Bryant DW Jr, Fox SE, Givan SA, Wilhelm LJ, Naithani S, Christoffels A, Salama DY, Carter J, Lopez Girona E, Zdepski A, Wang W, Kerstetter RA, Schwab W, Korban SS, Davik J, Monfort A, Denoyes-Rothan B, Arus P, Mittler R, Flinn B, Aharoni A, Bennetzen JL, Salzberg SL, Dickerman AW, Velasco R, Borodovsky M, Veilleux RE, Folta KM (2011). The genome of woodland strawberry (Fragaria vesca). Nat Genet.43(2):109-16. doi: 10.1038/ng.740.

Silva D, Dias, JC (2014). Guiding Strategies for Breeding Vegetable Cultivars. *Agricultural Sciences*, 5, 9-32. http://dx.doi.org/10.4236/as.2014.51002.

Simko I (2004). One potato, two potato: haplotype association mapping in autotetraploids. Trends in Plant Science, 9(9):441-448.

Simko I, Costanzo S, Haynes KG, Christ BJ and Jones RW (2004). "Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach," Theoretical and Applied Genetics, vol. 108, no. 2, pp. 217-224.

Simko I, Haynes KG and Jones RW (2006). Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. Genetics, 173(4):2237-45.

Sivarajan S (2011). Estimating yield of irrigated potatoes using aerial and satellite remote sensing.

Skot L, Humphreys MO, Armstead I, Heywood S, Skot KP, Sanderson R, Thomas ID, Chorlton KH and Hamilton NRS (2005). An association mapping approach to identify owering time genes in natural populations of *Lolium perenne* (L.). Molecular Breeding, 15(3):233- 245.

Slater AT, Cogan NOI and Forster JW (2013). Cost analysis of the application of marker-assisted selection in potato breeding. Mol. Breed. 32:299-310. doi:10.1007/s11032-013-9871-7.

Slater AT, Cogan NOI, Hayes BJ, Schultz L, Dale MFB, Bryan GJ (2014a). Improving breeding efficiency in potato using molecular and quantitative genetics. Theor. Appl. Genet. 127:2279–2292. doi:10.1007/ s00122-014-2386-8.

Slater AT, Wilson GM, Cogan NOI, Forster JW and Hayes BJ (2014b). Improving the analysis of low heritability complex traits for enhanced genetic gain in potato. Theor. Appl. Genet. 127:809–820. doi:10.1007/ s00122-013-2258-7.

Sonah H, Bastien M, Iquira E, Tardivel A, Legare G, Boyle B (2013). An improved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PLoSONE 8: e54603. doi: 10.1371/journal.pone.0054603.

Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C and Patanothai A (2009). Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric. Water Manag. 96:790–798.

Soto-Cerda BJ and Cloutier S (2012). Association mapping in plant genomes. Genetic Diversity in Plants, chapter 3, pages 29-54.

Stich B and Melchinger AE (2009). Comparison of mixed-model approaches for association mapping in rapeseed, potato, sugar beet, maize and Arabidopsis. BMC Genomics, 10(1):94.

Stich B, Maurer HP, Melchinger AE (2006). Comparison of linkage disequilibrium in elite European maize inbred lines using AFLP and SSR markers. Molecular Breeding, vol. 17, no. 3, pp. 217-226.

Stich B, Melchinger AE, Frisch M, Maurer HP, Heckenberger M and Reif JC (2005). Linkage disequilibrium in European elite maize germplasm investigated with SSRs, Theoretical and Applied Genetics, vol. 111, no. 4, pp. 723–730.

Stich B, Urbany C, Hoffmann P and Gebhardt C (2013). Population structure and linkage disequilibrium in diploid and tetraploid potato revealed by genome-wide high-density genotyping using the SolCAP SNP array. Plant Breeding, 132(6):718-724.

Szalma SJ, Buckler ES IV, Snook ME and McMullen MD (2005). "Association analysis of candidate genes for maysin and chlorogenic acid accumulation in maize silks," Theoretical and Applied Genetics, vol. 110, no. 7, pp. 1324-1333.

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P (1992). High density

molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160.

Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF and Gaut BS (2001). Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. mays L.). Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 16, pp. 9161–9166.

The Potato Genome Sequencing Consortium (2011). Genome sequence and analysis of the tuber crop potato. Nature, 475:189- 195.

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, and Buckler ES (2001). "Dwarf8 polymorphisms associate with variation in flowering time," Nature Genetics, vol. 28, no. 3, pp. 286-289.

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q (2011). Genome-wide association study of leaf architecture in the maize nested association mapping population. Nat. Genet. 43:159–62.

Tommasini L, Schnurbusch T, Fossati D, Mascher F and Keller B (2007). "Association mapping of Stagonospora nodorumblotch resistance in modern European winter wheat varieties," Theoretical and Applied Genetics, vol. 115, no. 5, pp. 697-708.

Tracy SR, Roberts JA, Black CR, McNeill A, Davidson R and Mooney SJ (2010). The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. J Exp Bot 61:311-313

Truong HT, Ramos AM, Yalcin F, de Ruiter M, van der Poel HJ, Huvenaars KH (2012). Sequence-based genotyping for marker discovery and co-dominant scoring in germplasm and populations. PLoSONE 7: e37565. doi: 10.1371/journal.pone.0037565.

Tuberosa R (2012). Phenotyping for drought tolerance of crops in the genomics era.Front. Physiol. 3.

TUIK (2015). http://www.turkstat.gov.tr/PreTabloArama.do

Uitdewilligen JGAML, Wolters AMA, D'hoop BB, Borm TJA, Visser RGF and Van Eck HJ (2013). A next-generation sequencing method for genotyping by-sequencing of highly heterozygous autotetraploid potato. PLOS ONE, 8(5): e62355.

Urbany C, Stich B, Schmidt L, Simon L, Berding H, Junghans H, Niehoff K-H, Braun A, Tacke E, Hoberbert H-R, Lubec J, Strahwald J and Gebhardt C (2011). Association genetics in Solanum tuberosum provides new insights into potato tuber bruising and enzymatic tissue discoloration. BMC Genomics, 12(1):7.

Van Eck HJ (2007). Genetics of morphological and tuber traits. In Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M. A., MacKerron, D. K., and Ross, H. A., editors, Potato Biology and Biotechnology: Advances and Perspectives, chapter 6, pages 91- 115. Elsevier, London.

Van Maarschalkerweerd M, Bro R, Egeboand E, Husted S (2013). Diagnosing latent copper deficiency in intact barley leaves (*Hordeum vulgare*, L.) using near infrared spectroscopy. J. Agric. Food Chem. 61: 10901-10910.

Van Os H, Andrzejewski S, Bakker E, Barrena I, Bryan GJ, Caromel B (2006). Construction of a 10,000-marker ultradense genetic recombination map of potato: Providing a framework for accelerated gene isolation and a genomewide physical map. Genetics 173:1075-1087. doi:10.1534/genetics.106.055871.

Van Poecke RM, Maccaferri M, Tang J, Truong HT, Janssen A, van Orsouw NJ (2013). Sequence-based SNP genotyping in durum wheat. Plant Biotechnol. J. 11, 809-817.doi:10.1111/pbi.12072.

VanRaden PM, Van Tassell CP, Wiggans GR, Sonstegard TS, Schnabel RD, Taylor JF (2009). Invited review: Reliability of genomic predictions for North American Holstein bulls. J. Dairy Sci. 92:16–24. doi:10.3168/jds.2008-1514.

Varshney RK, Terauchi R, McCouch SR (2014). Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding, PLoSBiol. 12 e1001883.

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss D, Pindo M, FitzGerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma T, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Demattè L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Si-Ammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L,

Vandepoele K, Grando SM, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar ST, Fontana P, Gutin A, Van de Peer Y, Salamini F, Viola R (2007). A High Quality Draft Consensus Sequence of the Genome of a Heterozygous Grapevine Variety. PLoS ONE 2(12): e1326. https://doi.org/10.1371/journal.pone.0001326.

Virlet N, Sabermanesh K, Sadeghi-Tehran P and Hawkesford MJ (2016). Field Scanalyzer: An automated robotic field phenotyping platform for detailed crop monitoring. Functional Plant Biology.

Vollmann J, Walter H, Sato T, Schweiger P (2011). Digital image analysis and chlorophyll metering for phenotyping the effects of nodulation in soybean. Comput. Electron Agric. 75:190-195.

Voorrips RE, Gort G and Vosman B (2011). Genotype calling in tetraploid species from bi-allelic marker data using mixture models. BMC Bioinformatics 12:1–11. doi:10.1186/1471-2105-12-172.

Voytas DF and Gao C (2014). Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biol. 12: e1001877

Walter A, Studer BK and olliker R (2012). Advanced phenotyping offers opportunities for improved breeding of forage and turf species. Ann. Bot. 110:1271-79.

Wang S, Zhang S, Wang W, Xiong X, Meng Fand Cui X (2015). Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. Plant Cell Rep, 34(9): 1473-1476.

Wang Y (2014). Simultaneous editing of three homoeo alleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotechnol. 32: 947-951.

Weight C, Parnham D and Waites R (2007). TECHNICAL ADVANCE: LeafAnalyser: a computational method for rapid and large-scale analyses of leaf shape variation. Plant J. 53:578-586.

Werij J, Kloosterman BA, Celis-Gamboa C, de Vos C, America T, Visser RGF and Bachem CWB (2007). Unravelling enzymatic discoloration in potato through a combined approach of candidate genes, QTL, and expression analysis. Theoretical and Applied Genetics, 115(2):245-252.

Wiggans GR, Vanraden PM and Cooper TA (2011). The genomic evaluation system in the United States: Past, present, future. J. Dairy Sci. 94:3202-3211. doi:10.3168/jds.2010-3866.

Wilson LM, Whitt SR, Ibanez AM, Rocheford TR, Goodman MM and Buckler ES (2004). "Dissection of maize kernel composition and starch production by candidate gene association," The Plant Cell, vol. 16, no. 10, pp. 2719-2733.

Wishart J, George TS, Brown LK, White PJ, Ramsay G, Jones H and Gregory PJ (2014). Field phenotyping of potato to assess root and shoot characteristics associated with drought tolerance. Plant and soil, 378(1-2): 351-363.

Wolc A, Stricker C, Arango J, Settar P, Fulton J, O'Sullivan N (2011). Breeding value prediction for production traits in layer chickens using pedigree or genomic relationships in a reduced animal model. Genet. Sel. Evol. 43:5. doi:10.1186/1297-9686-43-5.

Wolc A, Zhao H, Arango J, Settar P, Fulton J, O'Sullivan N (2015). Response and inbreeding from a genomic selection experiment in layer chickens. Genet. Sel. Evol. 47:59. doi:10.1186/s12711-015-0133-5.

Würschum T, Reif JC, Kraft T, Janssen G, Zhao Y (2013). Genomic selection in sugar beet breeding populations. BMC Genet. 14: 85.

Xiong JS, Ding J and Li Y (2015). Genome-editing technologies and their potential application in horticultural crop breeding. Horticulture Research, 2: 15019.

Xu R 2014. Gene targeting using the Agrobacterium tumefaciens-mediated CRISPR-Cas system in rice. Rice 7: 5

Xu X, Xu S, Pan S, Cheng B, Zhang D, Mu P, Ni G Zhang S, Yang R, Li J, Wang G, Orjeda F, Guzman M, Torres R, Lozano O, Ponce D, Martinez GN, De La Cruz SK, Chakrabarti VU, Patil KG, Skryabin BB, Kuznetsov NV, Ravin TV, Kolganova AV, Beletsky AV, Mardanov A, Di Genova DM, Bolser DMA, Martin G and Li Y (2011). "Genome sequence and analysis of the tuber crop potato". Nature. 475 (7355): 189-195. doi:10.1038/nature10158. PMID 21743474.

Xu Y, Lu Y, Xie C, Gao S, Wan J and Prasanna B(2012). Whole-genome strategies for marker-assisted plant breeding. Mol. Breed. 29:833-854. doi:10.1007/s11032-012-9699-6.

Yang H, Tao Y, Zheng Z, Li C, Sweetingham MW, and Howieson JG (2012). Application of next-generation sequencing for rapid marker development in molecular plant breeding:a case study on anthracnose disease resistance

in *Lupinusan gustifolius* L. BMC Genomics 13:318. doi:10.1186/1471-2164-13-318.

Yazdanbakhsh N and Fisahn J (2009). High throughput phenotyping of root growth dynamics, lateral root formation, root architecture and root hair development enabled by PlaRoM. Funct Plant Biol 36:938-946.

Yol E, Toker C and Uzun B (2015). Traits for Phenotyping. In Phenomics in Crop Plants: Trends, Options and Limitations (pp. 11-26). Springer India.

Zhang C, Kovacs JM (2012). The application of small unmanned aerial systems for precision agriculture: a review. Precis. Agric. 13: 693–712.

Zhang N, Xu Y, Akash M, McCouch S and Oard JH (2005). "Identification of candidate markers associated with agronomic traits in rice using discriminant analysis," Theoretical and Applied Genetics, vol. 110, no. 4, pp. 721-729.

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ and Voytas DF (2013). Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiology 161: 20-27.

Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat. Commun. 2:467.

Zhao Y, Gowda M, Liu W, Würschum T, Maurer HP, Longin FH, Ranc N, Reif JC (2012). Accuracy of genomic selection in European maize elite breeding populations. TAG Theor. Appl. Genet. Theor. Angew. Genet. 124: 769-776.

Zhong S, Dekker JCM, Fernando RL and Jannink JL (2009). Factors affecting accuracy from genomic selection in populations derived from multiple inbred lines: A barley case study. Genetics 182:355-364.

Zhu YL, Song QJ and Hyten DL (2003). "Single-nucleotide polymorphisms in soybean," Genetics, vol. 163, no. 3, pp.1123-1134.

ZhuX, Richael C, Chamberlain P, Busse JS, Bussan AJ, Jiang J and Bethke PC (2014). Vacuolar invertase gene silencing in potato (*Solanum tuberosum* L.) improves processing quality by decreasing the frequency of sugar-end defects. PloS One 9: e93381.

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The Morphological Diversity and Fruit Characterization of Turkish Eggplant (*Solanum melongena* L.) Populations

Zuleyha CAKIR[1] Ahmet BALKAYA[1*] Seyma SARIBAS[1] Dilek KANDEMIR[2]

[1] Agriculture Faculty, Horticulture Department, Ondokuz Mayıs University, Samsun, Turkey
[2] Vocational High School of Samsun, Ondokuz Mayıs University, Samsun, Turkey

[*] Corresponding author e-mail: abalkaya@omu.edu.tr

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ABSTRACT

Turkey is one of the most important countries in the world for plant genetic resources and genetic diversity. Genetic resources are characterized by morphological and agronomical traits. There is a need to collect, characterize and evaluate remnants of local populations before they disappear. Morphological characterization is the first stage of the identification and classification of genetic resources. In this study, the aim was to determine the similarities and differences in the morphological variations of the eggplant populations collected from different eco-geographical regions of Turkey. Seventy five populations of eggplant were characterized using standard morphological descriptors specified by the IBPGR. The phenotypic diversity in their fruit characters was also assessed. Cluster and Principal Component Analysis (PCA) was performed to determine the relationships among these accessions and to obtain information on the fruit characteristics for the definition of groups. The principal component analysis showed that the first four principal component axes explained 71.38% of the total multivariate variation. The results demonstrated many differences in fruit traits in the detailed eggplant populations. Clustered into nine groups, a dendrogram was prepared to evaluate the morphological differences among the populations. It revealed a high degree of variation. The results provided information on the diversity, and this identified eggplant genetic resources to be evaluated for the development of new candidate varieties in future breeding activities.

Keywords: *Solanum melongena,* genetic resources, characterization, classification, diversity, Turkey

Introduction

The eggplant (*Solanum melongena* L.) is a very important commercial vegetable crop. It is grown widely throughout tropical zones and in the temperate regions of the world. Eggplant production varies according to the country and continent. China and India are the major producers in the world. The eggplant is one of the most important *Solanaceae* vegetable crops in Turkey. The total eggplant production in Turkey was 827,830 tonnes and was ranked fifth in the world (TUIK 2016). Turkey is important producer country within Europe in terms of eggplant production.

The eggplant was first cultivated in India, which is regarded as the primary centre of origin and diversity (Kumar *et al*., 2013). La Malfa (1990), listed China as the secondary centre of diversification. The eggplant arrived in Europe around 1300, and the eggplant fruits were used as food after the sixteenth century (D'Anna and Sabatino 2013). The introduction of the eggplant to the west was primarily around the Mediterranean region, which is the secondary "domestication region" and covers Turkey, Syria and Persia (Küçük 2003; Daunay *et al*., 2001; Tümbilen 2007). Many local eggplant landraces are found in Turkey. These landraces are grown by producers in almost all regions (Balkaya and Karaagac 2005). These traditional landraces are an

important genetic resource for plant breeders because of their considerable genotypic variation.

Crop improvement to increase productivity has always relied on genetic diversity, and therefore, on the ability of the crop to adapt to soil and climate changes; it is due to this selection process, used by farmers over the years, that most of the biodiversity has been preserved (Schippmann *et al*., 2002). The local populations are genotypes of remarkable intrinsic value; their ability to adapt to their original environment could make them more suited to sustainable horticulture than hybrids and varieties created in different soil and climate conditions, and which often require higher energy inputs (D'Anna and Sabatino 2013). A morphological characterization is the first step in the description and classification of local genetic resources (Smith and Smith 1989). There was a need to characterize the eggplant populations collected so that they could then be used as lines for the development of new varieties.

Morphological identification using conventional descriptors has proved useful for describing and establishing relationships among local eggplant genetic resources in Turkey. Similar collecting studies have also been carried out in different regions of Turkey (Filiz and Özçalabı 1992; Pirinç 1999; Tümbilen 2007; Boyaci *et al*., 2010; Topcu 2014). According to these literatures, similarities and differences were found regarding morphological variations in eggplant genetic resources collected from different eco-geographical regions of Turkey. Conservation and maintenance of these valuable genetic resources are necessary because these populations are important sources of diversity that can be used in future breeding programs (Balkaya and Karaagac 2005).

Evaluation of genetic diversity is important to identify the source of genes for a particular trait within the available germplasm (Quamruzzaman *et al*., 2009; Karim *et al*,. 2016). There has been great morphological diversity observed in several characteristics among eggplant populations. To date, several traits have been used for evaluation of plant diversity. Fruit colour, fruit size, fruit shape and taste are the most noticeable traits, and differences were shown for each eggplant genotype (Daunay *et al*., 2001; Kashyap *et al*., 2003; Prohens *et al*., 2005; Tümbilen *et al*., 2011). The fruit of the eggplant is classified as a non-climacteric berry that can grow to various sizes, and in various shapes and colours depending on the genotype. Fruit colour can vary from white to black with variations in purple, yellow and green. Other variable fruit characteristics for eggplants are the fruit shapes (round, egg shaped, oblong, pear shaped, long and curved) and fruit sizes (Tümbilen 2007). In Turkey, small oblong or rounded fruit types are stuffed or preserved; long cylindrical types are grilled, fried or stuffed and large round or longish oblong types are stewed or fried (Tümbilen *et al*., 2011). The eggplant populations of Turkey showed a high variability in fruit sizes, fruit shape, fruit color and fruit weight (Tümbilen 2007). Unfortunately, this considerable variation is not adequately characterized. To date, there has been no detailed investigation of variations in the fruit traits of eggplant populations in Turkey. Therefore, the aim of this study was to analyse genotypic variations among seventy five populations of eggplant fruits in Turkey. These findings should also help with the selection of core collections and accessions that can be used for eggplant breeding in the near future.

Material and Methods

***Materials*:** This study used a total of seventy five eggplant populations of fruit and/or seeds collected from different regions of Turkey (Table 1, Figure 1). Forty accessions of the *S. melongena* populations were obtained from the USDA-ARS National Germplasm Bank, twenty accessions of the *S. melongena* populations were provided from the Turkish National Seed Gene Bank (AARI) and fifteen accessions of the *S. melongena* populations were collected by Prof. Dr. Ahmet Balkaya, of the Horticulture Department of the Faculty of Agriculture of Ondokuz Mayıs University (Table 1). The genetic material consisted of landraces and native populations maintained by farmers for generations.

Growth conditions: The field component of this study was carried out in the Samsun province in 2016 year. The soil of the experimental area was sandy loam with a pH of 6.5. The seeds of all populations were sown into plug trays containing peat and perlite (in the ratio 3:1) on April 16, 2016. Forty seedlings from each population were field planted at the 4 to 5 true leaf stage at a spacing of 60 × 40 cm on June 13. Soil tests were done before and after planting. Standard fertilization and weed control practices were applied.

Characterization: The plants were harvested manually at full maturity. The harvest period began at the end of July 17 and lasted until the middle of September, because the populations had different maturation periods. The selected fruit characteristics were described according to the IBPGR *Solanum melongena* descriptors list, the characteristics of the genetic material and previous field observations (Table 2).

All fruit characteristics were measured at the normal harvest time, and their scales are presented in Table 2. Fruit characteristic analyses were carried out on 10 fruits from each of the population of 40 plants. Fruit dimensions: length, width and fruit stalk length were all measured. The fruit weight in grams was the mean of a sample of 20 fruits, when fruits were at the optimal maturity stage for fresh consumption. To obtain a better description of the eggplant populations, fruit shape, fruit apex shape, fruit colour, colour distribution and fruit glossiness traits were also recorded.

Data analysis: Statistical analysis was performed using the statistical software package SPSS (21.0 for Windows). For a better overview of diversity in the local eggplant populations, Cluster analysis was also used. Hierarchical cluster analyses were performed using Ward's criteria, minimizing the total sum of the squared distances of objects to the cluster centres. Ward's criteria were preferred because they tend to produce desirable compact clusters (Zewdie and Zeven 1997). In the Principal Component Analysis (PCA) and the load coefficient values which relate the values, those principal components with eigenvalues >1.0 were selected and those characters with load coefficient values >0.6 were considered highly relevant characters cores for principal components (Jeffers 1967; Balkaya *et al*., 2009).

Results and Discussion

Principal Component Analysis (PCA) was used for revealing the general differences between genotypes as numerical values, which indicate the traits that could be used to differentiate between genotypes (Balkaya *et al*., 2010). In this study, a PCA was performed on eggplant populations that considered fruit characteristics that included 4 quantitative and 7 qualitative variables. The principal component axes accounted for 71.38% of the total multivariate variation among the detailed eggplant accessions. The first principal component axis accounted for 34.55% of the variation, whereas the second and third axes accounted for 16.76% and 10.8%, respectively (Table 3). The first three principal component axes explained 62.11% of the variation, suggesting considerable diversity among the fruit characters (Figure 2). In this study, traits with high coefficients in the first, second and the third principal components should be considered more important since these axes explain the biggest share of the total variation. Though clear guidelines do not exist to determine the significance of a character coefficient, one rule of thumb is to treat coefficients >0.6 as having a large enough effect to be considered important (Jeffers 1967; Balkaya *et al*., 2009). Characteristics with high coefficients are: fruit shape (0.87), fruit curvature (0.83), fruit apex shape (-0.82), fruit length (0.78) and fruit diameter (-0.61) for principal component 1; fruit glossiness (0.67) and fruit colour (0.64) for the second principal component, and fruit stalk length (0.66) for the third principal component. These traits are considered to be the most important, since they define the axes which explain 62.11% of the total variation (Table 3, Figure 2). Finally, principal component 4 was mainly related to fruit calyx prickles (0.75). Earlier results indicated that the Turkish eggplant populations could be distinguished by fruit shape, fruit curvature, fruit apex shape, fruit length and fruit diameter, which had the highest coefficients on the first principal component axis.

To better understand the overall diversity of the Turkish eggplant populations, the data were analysed by Cluster analysis that revealed the distribution of genetic diversity and is displayed in Table 4. In this study, cluster analysis grouped the populations into nine clusters. The related dendogram is shown in Figure 3. The means and standard deviations of some of the traits for each cluster are given in Table 4. Among the nine different groups, group F and group H were divided into five subgroups, group E into four subgroups, and groups A, C, D, and group G into three subgroups. Group B and group I were both divided into two subgroups (Figure 3). The nine groups and 30 subgroups can be considered to be distinct germplasm pools. This study shows that there is considerable morphological variability between the eggplant populations sampled. No association was observed for clusters within the collection zone (Table 1, Figure 3).

General fruit characteristics of the investigated Turkish eggplant populations are as follows:

Group A: This group consisted of three subgroups. There were a total of 18 populations in group A. The fruit diameter (8.05 cm) is very large compared to the other groups. Fruits are long, straight and have an ellipsoidal shape (Table 4). The fruit colours were purple to purple black tones. There were either no fruit calyx prickles or just a few levels. The average fruit weight in this group was 382.87 g, higher than all the other groups.

Group B: There were five populations in this group. It included two subgroups: a fruit length of 16.68 cm and a fruit diameter of 6.71 cm, ranking second out of all the groups. All populations have globular and ellipsoidal fruit shapes. The fruit colours

were purple-black to black tones. The fruit colour distribution was very uniform. The fruit stalk length of 7.72 cm was identified as an intermediate value. The average fruit weight in group B was 277.18 g and it was higher than any group except group A (Table 4).

Group C: This group consisted of nine populations. Genotypes in this group were clustered into three subgroups. Fruits in this group had the shortest lengths (16.52 cm). Fruits were globular and ellipsoidal in shape (Table 4). The fruit apex shape was depressed. There were no fruit calyx prickles. In this group, the fruit brightness exhibited was very shiny. The average fruit weight was found to be 241.51 g.

Group D: There were six populations in this group. The fruit lengths of the populations were short. All fruits were straight, ellipsoidal or cylindrically shaped. The fruit apex had a protruding shape. Fruit colours changed according to genotypes and were green, lilac and purple tones. The fruit stalk length was 7.90 cm, and this was identified as an intermediate value. The fruit brightness was identified as very shiny.

Group E: This group included fourteen populations. It had the biggest cluster of genetic groups. The fruits were long, but slightly curved or curved. This group had cylindrical and long cylindrical fruits (Table 4). The fruit apex had a protruding shape. Fruit colours were lilac, purple and purple-black tones. The fruit brightness was found to be shiny or very shiny. The average fruit weight was 225.88 g. The fruit stalk length was 6.93 cm and this value was identified as an intermediate value.

Group F: This group consisted of eleven populations. It had the second biggest cluster of all groups. Genotypes in this group were clustered into five subgroups. The fruit length was 27.27 cm. This value was the longest of all the groups (Table 4). The fruit diameter had the shortest widths (4.05 cm). All fruits had a long cylindrical shape. The fruit had a protruding apex shape. The fruit stalk length was long (8.39 cm) and the fruit colours were purple-black to black tones. The fruit colour distribution was very uniform.

Group G: This group included eight populations. It had the biggest cluster of genetic groups. The fruit length was 24.41 cm. The fruit length of this group was the second longest value of all the groups (Table 4).The fruits were cylindrical or had a long cylindrical curved shaped. The fruit stalk length was the longest (8.61 cm) of all the clusters. The fruit calyx prickles were either absent or only had a few levels. The average fruit weight in this group was 284.80 g and was higher than all the other groups, except for group A.

Group H: There were six populations in this group. The fruit length of the populations was long (21.52 cm). The fruit diameter of this group was the second widest value of all the groups (Table 4). The fruits were cylindrical or long and cylindrical. The fruit had a protruding or rounded apex shape. The fruit stalk length was 7.25 cm and this was identified as an intermediate value. The average fruit weight was 190.36 g and was the lowest among all groups (Table 4).

Group I: This group included three populations. It had the smallest cluster of genetic materials. The fruits were cylindrical or long and cylindrical. The fruit colour tone was lilac. The fruit colour distribution was mottled or striped, and the fruit stalk length was the shortest at 6.93 cm. There were many fruit calyx prickles found on very many levels.

The knowledge of the extent of genetic diversity, and the identification, differentiation and characterization of genotypes and populations, provides an information tool for the detection of duplicates in collections and also delivers better characterization and utilization in breeding (Hornokova *et al*., 2003). The clustering of eggplant genetic resources of Turkey on the dendogram in 9 separate groups resulted from their different morphological structure and special fruit characteristics. This study showed that there is considerable morphological variability because of the introduction of diverse eggplant genetic materials to Turkey from different countries. Cluster groups were not associated with the geographical origins of the eggplant genotypes. There is no clear relationship between clusters and the coastal or inland areas of Turkey (Table 1; Figure 2). In other research, it has been determined that there are many significant morphological differences between local eggplant genetic resources (Tümbilen *et al*., 2011). This absence of association may be a result of continuous conscious and unconscious seed transport by humans. Secondly, it may be a result of previous selection for different uses (Filiz and Özçalabı 1992; Pirinç 1999; Tümbilen 2007; Boyaci *et al*., 2010).

At the end of this study, we have found that genetic diversity within landraces and populations of eggplant is high, including variations in fruit shape, fruit weight, fruit size (length, diameter), fruit glossiness and the colour of fruits. Reliable information on characteristic variability within germplasm collections is very useful to breeders in planning eggplant variety improvement programs.

Conclusion

In conclusion, we have presented some fruit characteristics of eggplant populations grown in Turkey. In addition, the components of the fruit characteristics of *S. melongena* were demonstrated by applying multivariate techniques to the morphological data sets. Fruit traits proved useful in assessing the diversity and relationships of Turkish eggplant genetic resources.The current study revealed considerable diversity in some fruit characteristics of the eggplant populations. The potential use of Turkey's eggplant genotypes as genetic resources in breeding programmes was highlighted for further investigation. In addition all eggplant populations used for this study are also generated as inbred lines for variety breeding programs in another study.

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Figure 1. The view of the diversity fruit size, shape and color for Turkish *Solanum melongena* populations

Figure 2. Scatter plot constructed at the basis the first three principal component axes, which contain 62.2% of total variation

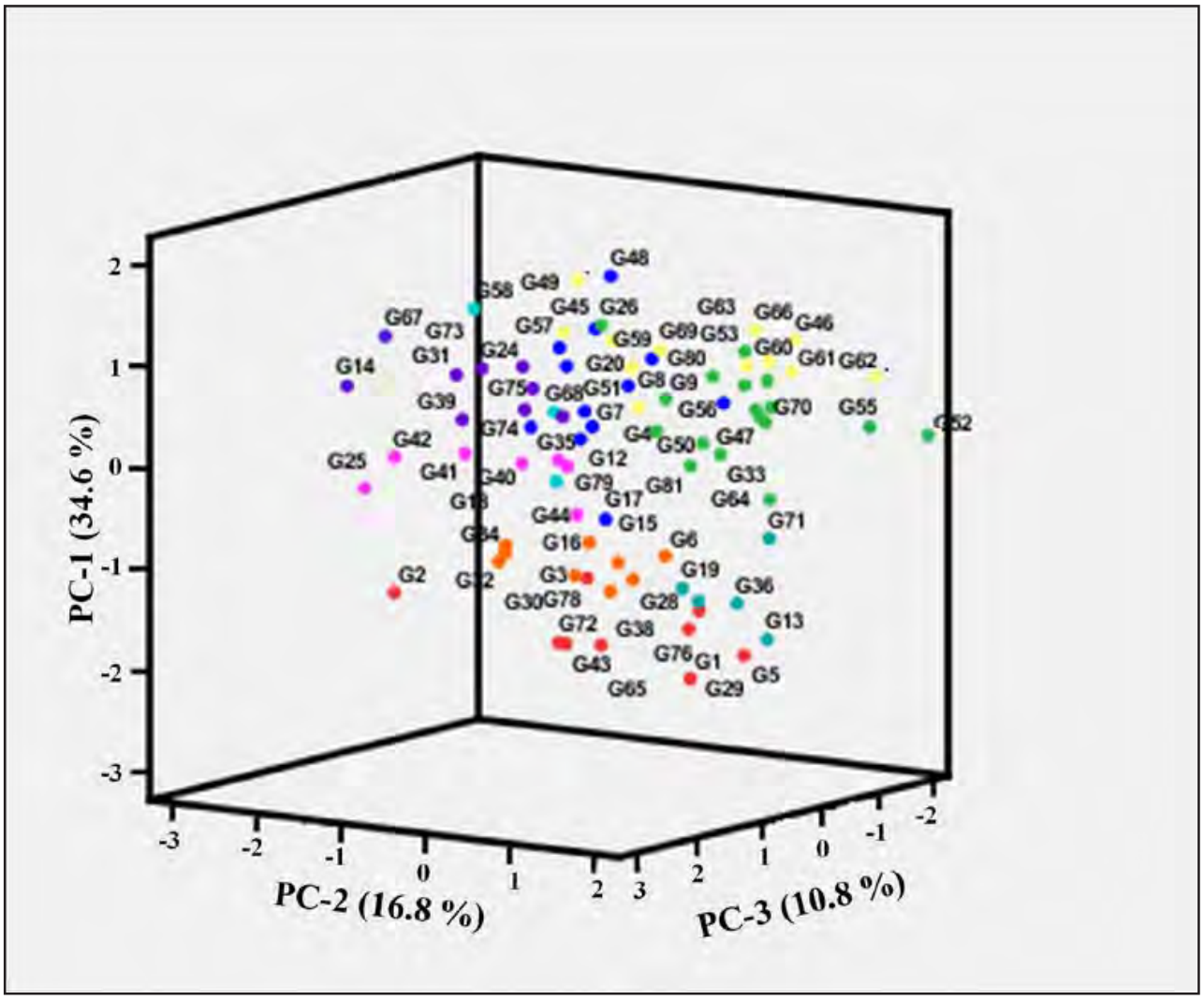

Figure 3. Dendrogram of eggplant populations obtained from cluster analysis of eleven fruit traits

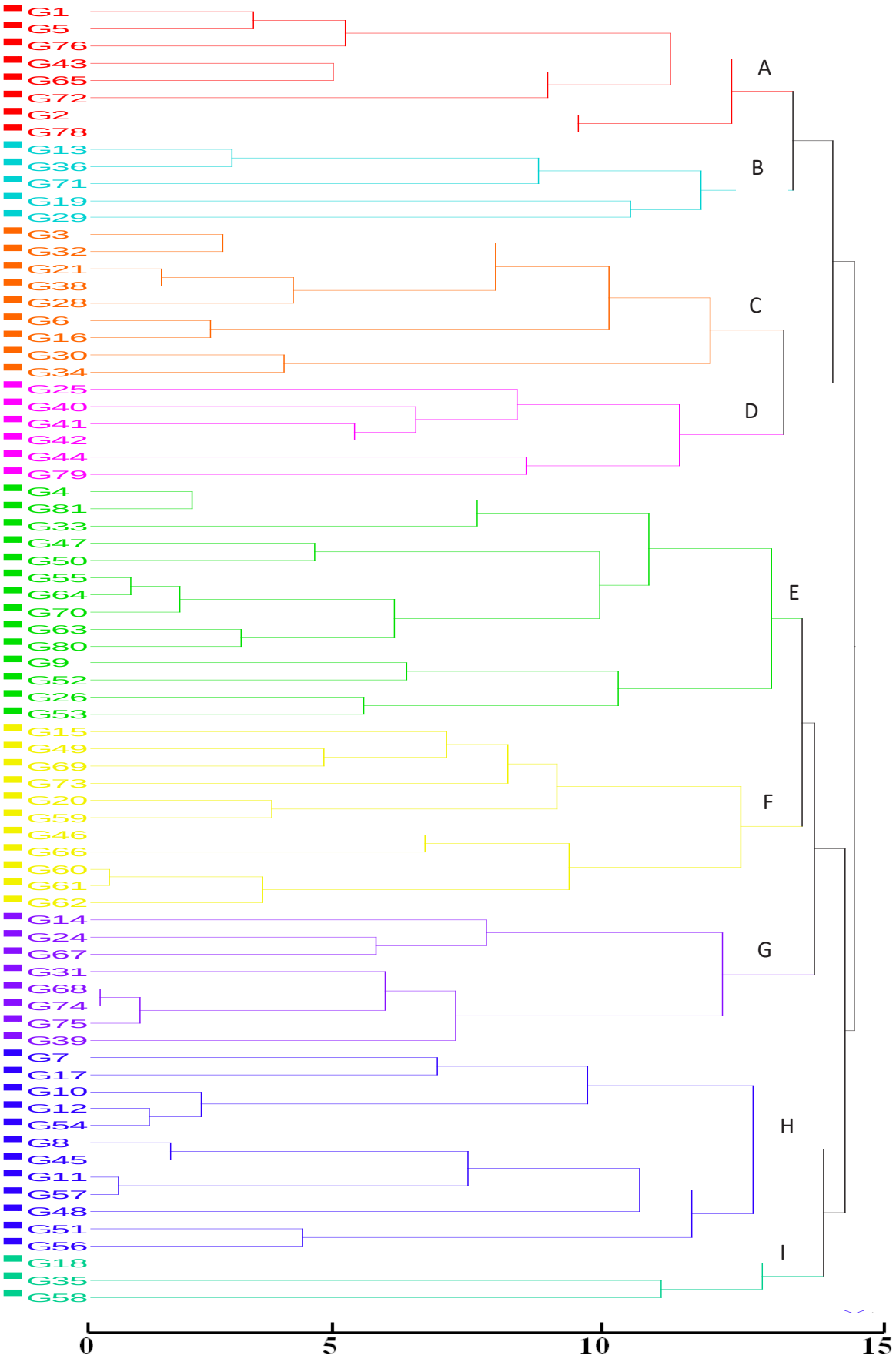

Table 1. Code, accession number and collected sites of *Solanum melongena* in Turkey.

Code	Accession Number	Collected Sites	Code	Accession Number	Collected Sites
G1	PI 166994 01	Hatay/USDA	G43	PI 204630 01	Kayseri
G2	PI 167381 01	Adana/USDA	G44	PI 204731 01	Kayseri
G3	PI 169642 01	Aydın/USDA	G45	TR 61766	Muğla
G4	PI 169644 01	Muğla	G46	TR 55995	Trabzon
G5	PI 169649 01	İzmir	G47	TR 70757	Samsun
G6	PI 169658 01	Kırklareli	G48	TR 70758	Samsun
G7	PI 169667 01	Kocaeli	G49	TR 70756	Amasya
G8	PI 171850 01	Kastamonu	G50	TR 69835	Çorum
G9	PI 171851 01	Samsun	G51	TR 70768	Kastamonu
G10	PI 171853 01	Tokat	G52	TR 70767	Kastamonu
G11	PI 173104 01	Artvin	G53	TR 70766	Sinop
G12	PI 173106 01	Ağrı	G54	TR 68531	Bartın
G13	PI 173111 01	Kahramanmaraş	G55	TR 68532	Bartın
G14	PI 174359 01	Van	G56	TR 68528	Zonguldak
G15	PI 174360 01	Diyarbakır	G57	TR 55678	Giresun
G16	PI 174362 01	Mardin	G58	TR 77307	Edirne
G17	PI 174369 01	Gaziantep	G59	TR 69211	Antalya
G18	PI 174371 01	Gaziantep	G60	TR 75349	Artvin
G19	PI 174373 01	Malatya	G61	TR 70764	Sinop
G20	PI 174374 01	Elazığ	G62	TR 70765	Sinop
G21	PI 175909 01	Balıkesir	G63	TR 75345	Artvin
G24	PI 175913 01	Çorum	G64	TR 70759	Samsun
G25	PI 175914 01	Yozgat	G65	OMU-ZF/BAH	Aydın
G26	PI 175916 01	Kayseri	G66	OMU-ZF/BAH	Aydın
G28	PI 176758 01	Niğde	G67	OMU-ZF/BAH	Manisa, Salihli
G29	PI 176760 01	Konya	G68	OMU-ZF/BAH	Aydın, İncirliova
G30	PI 176761 01	Konya	G69	OMU-ZF/BAH	Aydın
G31	PI 176762 01	Bilecik	G70	OMU-ZF/BAH	Kemer
G32	PI 176763 01	Eskişehir	G71	OMU-ZF/BAH	İzmir, Bayındır
G33	PI 177073 01	Çanakkale	G72	OMU-ZF/BAH	Aydın
G34	PI 177074 01	Kayseri	G73	OMU-ZF/BAH	Diyarbakır
G35	PI 177076 01	Konya	G74	OMU-ZF/BAH	Hatay,Samandağ
G36	PI 179045 01	Tekirdağ	G75	OMU-ZF/BAH	Aydın, Nazilli
G38	PI 179496 01	Bursa	G76	OMU-ZF/BAH	Şanlıurfa, Birecik
G39	PI 179498 01	İstanbul	G78	OMU-ZF/BAH	Mersin, Mut
G40	PI 182299 01	Muş	G79	OMU-ZF/BAH	Bursa
G41	PI 182300 01	Kahramanmaraş	G80	OMU-ZF/BAH	Mersin, Mut
G42	PI 183718 01	Kahramanmaraş			

Table 2. List of morphological traits used in characterization of Turkish eggplant populations.

No	Traits
1	**Fruit length** [**1.** very short (<1 cm), **3.** short (~2 cm), **5.** intermediate (~5 cm), **7.** long (~20 cm), **9.** very long (>30 cm)]
2	**Fruit diameter** [**1.** very small (<1 cm), **3.** small (~3 cm), **5.** intermediate (~5 cm), **7.** large (~10 cm), **9.** very large (>10 cm)]
3	**Fruit curvature** [**1.** none (fruit straight), **3.** slightly curved, **5.** curved, **7.** snack shaped **8.** sickle shaped, **9.** U shaped]
4	**Fruit shape** [**1.** globular, **3.** obovate, **5.** ellipsoid, **7.** cylindrical, **9.** long cylindrical]
5	**Fruit apex shape** (**3.** protruded, **5.** rounded, **7.** depressed]
6	**Fruit colour** [**1.** green, **3.**white, **4.** lilac, **5.** purple, **7.** purple black, **9.** black]
7	**Fruit glossiness** [**3**. dull, **5.** shiny, **7.** very shiny]
8	**Fruit calyx prickles** [**0.** none, **1.** very few (<3), **3.** few (~5), **5.** intermediate (~10), **7.** many (~20), **9.** very many (>30)]
9	**Fruit stalk length** [**1.** very short (<3 cm), **3.** short (~5 cm), **5.** intermediate (5-8 cm), **7.** long (~10 cm), **9.** very long (>10 cm)]
10	**Fruit colour distribution** [**1.** uniform, **3.** mottled, **5.** netted, **7.** striped]
11	**Fruit weight** [**1.** <150 g, **3.** 150-250 g, **5.** 250-350 g, **7.** >350 g]

Table 3. Principal component (PC) coefficients of each fruit trait in Turkish eggplant populations. Proportions of variations are associated with first four PC axes, which correspond to eigenvalues greater than 1.

	PC Axis			
Eigen values	3.80	1.84	1.19	1.02
Explained proportion of variation (%)	34.55	16.76	10.80	9.27
Cumulative proportion of variation (%)	34.55	51.31	62.11	71.38
Traits	**PC1**	**PC2**	**PC3**	**PC4**
Fruit length (cm)	**0.78**	0.77	0.18	0.21
Fruit diameter (cm)	**-0.61**	0.34	0.34	0.18
Fruit curvature	**0.83**	0.24	0.09	0.22
Fruit shape	**0.87**	-0.13	0.06	0.01
Fruit apex shape	**-0.82**	0.26	-0.19	-0.11
Fruit colour	0.41	**0.64**	-0.52	0.01
Fruit glossiness	0.40	**0.67**	-0.21	0.22
Fruit calyx prickles	-0.22	-0.18	-0.42	**0.75**
Fruit stalk length (cm)	0.17	0.38	**0.66**	0.21
Fruit colour distribution	-0.34	-0.50	0.18	0.49
Fruit weight (g)	-0.44	**0.69**	0.16	0.11

Table 4. Average values of the traits used in identify Turkish eggplant populations.

Cluster Groups	Traits*										
	1	2	3	4	5	6	7	8	9	10	11
A	17.32±3.16	8.05±0.98	1	3	5	5,7	5	0,1	7.62±1.82	1,3	382.87±48.55
B	16.68±2.96	6.71±1.35	1	1,5	5	7,9	3,7	3,5	7.72±1.17	1	277.18±51.73
C	16.52±3.30	6.18±0.62	1	1,5	5	1,4,5	3	1	6.72±0.67	1,3	241.51±71.26
D	18.46±2.89	5.49±0.65	1	5,7	3	1,4,5	3	0,1,3	7.90±0.78	1	233.83±55.83
E	23.36±2.08	5.06±0.53	3,5	7,9	3	4,5,9	3,5	1,3,5	6.93±0.60	1	225.88±35.00
F	27.27±4.40	4.05±0.91	3,5	9	3	7,9	5,7	0,1,3	8.39±0.90	1	206.15±28.71
G	24.41±2.18	5.61±0.56	5	7,9	3	1,5,9	3,5	1,3	8.61±0.50	1,3	284.80±55.15
H	21.52±2.90	4.39±0.77	1,3,5	7,9	3,5	1,4,9	3,5	1,3	7.25±0.74	1,3	190.36±39.69
I	19.30±6.67	5.36±0.16	1,5	7,9	3,5	4	3,5	3,5,7	6.93±0.75	3,7	198.23±80.73

* 1. Fruit length (cm), 2. Fruit diameter (cm), 3. Fruit curvature, 4. Fruit shape, 5. Fruit apex shape, 6. Fruit colour, 7. Fruit glossiness, 8. Fruit calyx prickles, 9. Fruit stalk length (cm), 10. Fruit colour distribution, 11. Fruit weight (g)

References

Balkaya A and Karaagac O (2005). Vegetable genetic resources of Turkey. Journal of Vegetable Science, 11(4): 81-102.

Balkaya A, Yanmaz R and Ozbakir M (2009). Evaluation of variation in seed characters in Turkish winter squash (*Cucurbita maxima* Duch.) populations. New Zealand Journal of Crop and Horticultural Science, 37(3): 167-178.

Balkaya A, Ozbakir M and Kurtar E (2010). The phenotypic and fruit characterization of winter squash (*Cucurbita maxima*) populations from

the Black Sea region of Turkey. African Journal of Biotechnogy, 9(2): 152-162.

Boyaci F, Unlu A and Abak K (2010). Screening for resistance to fusarium wilt of some cultivated eggplants and wild Solanum accessions. 28th International Horticultural Congress, 22-27 August, 935, pp. 23-27.

D'Anna F and Sabatino L (2013). Morphological and agronomical characterization of eggplant genetic resources from the Sicily area. Journal of Food, Agriculture & Environment, 11(1): 401-404.

Daunay MC, Lester RN, Gebhardt C, Hennart W, Jahn M and Doganlar S (2001). Genetic resources of eggplant (*Solanum melongena* L.) and allied species: a new challenge for molecular geneticists and eggplant breeders. Nijmegen University Press, Nijmegan, Netherlands pp. 251-274.

Filiz N and Ozçalabi R (1992). Determination of phenological, morphological and pomological characteristics of some aubergine cultivars (in Turkish). I Turkish Horticulture Congress, 13-16 October, Izmir, Turkey.

Hornokova O, Zavodna M, Zakova M, Kraic J, Debre F (2003). Diversity of common bean landraces collected in the western and eastern Carpatien. Czech J. Genet. Plant Breed. 39: 73-83.

Jeffers JNR (1967). Two cases studies in the application of principal component analysis. Appl. Stat. 16: 225-236.

Karim MR, Rahman MM and Quamruzzaman AKM (2016). Genetic divergence in eggplant (*Solanum melongena* L.) genotypes. Bangladesh Journal Agril. Res. 41(3): 433-439.

Kashyap V, Kumar SV, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D and Rajam MV (2003). Biotechnology of eggplant. Scientia Horticulturae, 97: 1-25.

Kumar SR, Arumugam T and Anandakumar CR (2013). Genetic diversity in eggplant. Plant Gene and Trait, 4(2): 4-8.

Küçük SA (2003). *Solanaceae* genetic resources activities in Turkey. National Collections. *Solanaceae* Genetic Resources in Europe. Report of two meetings 21 September 2001, Nijmegen, The Netherlands. 22 May 2003, Skierniwice, Poland.

La Malfa G (1990). Scienza e tecniche delle produzioni vegetali. Orticoltura pp. 793-811.

Pirinç V (1999). Şanlıurfa yerel patlıcan populasyonlarının kendilenmiş hatlarıyla karşılaştırılması üzerine bir araştırma. Harran Üniversitesi Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Yüksek Lisans Tezi, 82 s. (In Turkish)

Prohens J, Blanca JM and Nuez F (2005). Morphological and molecular variation in a collection of eggplants from a secondary centre of diversity: Implications for conservation and breeding. Journal of the American Society for Horticultural Science, 130(1): 54-63.

Quamruzzaman AKM, Rashid MA, Ahmed S and Moniruzzzaman M (2009). Genetic divergence analysis in eggplant (*Solanum melongena* L.). Bangladesh J. Agril. Res. 34(4): 705-712.

Schippmann U, Leaman DJ and Cunningham AB (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: Global trend sand issues. Biodiversity and the Ecosystem approach in Agriculture, Forestry and Fisheries, FAO, Rome, 21 p.

Smith JSC and Smith OS (1989). The description and assessment of distances between inbred lines of maize: the utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica, 34: 151-161.

Topcu V (2014). Kendileme yoluyla saflaştırılmış bazı patlıcan hatlarının morfolojik ve moleküler karakterizasyonu. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Yüksek Lisans Tezi, 82 s. (In Turkish)

TUIK (2016). Turkish Statistical Institute Statistical Data (Accessed on 10.06.2017).

Tümbilen Y (2007). Determination of genetic diversity between eggplant and its wild relatives. Master Thesis. The Graduate School of Engineering and Sciences of İzmir Institute of Technology, Molecular Biology and Genetics, 76 p.

Tümbilen Y, Frary A, Mutlu S and Doganlar S (2011). Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses. International Research Journal of Biotechnology, 2(1): 16-25.

Zewdie Y and Zeven AC (1997). Variation in Yugoslavian hot pepper (*Capsicum annuum* L.) accessions. Euphytica, 97: 81-89.

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Synthetic Wheat: An Indispensable Pre-breeding Source for High Yield and Resistance to Biotic and Abiotic Stresses in Wheat Improvement

Mustafa YILDIRIM[1*] Vehbi ESER[2] Zoltan BEDŐ[3] Seydi Ahmet BAĞCI[4]

Márta MOLNÁR-LÁNG[3] László LÁNG[3]

[1] Kahramanmaraş Sutçu Imam University, Faculty of Agriculture, Kahramanmaras, Turkey
[2] Republic of Turkey Ministry of Food, Agriculture and Livestock, Ankara, Turkey
[3] Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary
[4] Selcuk University, Sarayönü Vocational School, Konya, Turkey

[*] Corresponding author e-mail: myildirimkm@gmail.com

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ABSTRACT

In addition to being the most widely cultivated crop, wheat is also the most ancient cultivated plant species. Today, as in the past, wheat continues to be a crop of strategic importance. Cultivated hexaploid bread wheat (2n=42) consists of three genome groups (AA, BB, and DD), with each genome group further comprising three diploid wild species. Over the past 70 years, the world population has been rapidly increasing, while the area of agricultural lands has remained more or less constant. To be able to feed this continually increasing human population, scientists have begun to investigate the biological origins/roots of wheat, with the aim of achieving higher yield and greater resistance to biotic and abiotic stresses. This was because, based on the studies they performed, they determined that "reconstructing" wheat from its origins was a more effective solution than working with limited and currently available genetic resources. Bread wheat reconstructed by using diploid wild forms is called "synthetic wheat". Synthetic wheat receives certain characteristics from wild forms that render them superior to cultivated wheat. Diploid wild forms bearing the "D" genome (*Aegilopstauschii*) are known to be particularly very resistant to biotic and abiotic stresses. Nowadays, it has become imperative to use synthetic wheat in order to increase genetic variation in breeding programs. To break the "yield per unit area" barrier, to ensure world peace, and to prevent the starvation of children around the world, wheat breeders must place greater emphasis on the production of synthetic wheat.

Keywords: Synthetic wheat, aegilops, D genome, crossing.

Introduction

The origin of cultivated wheat is Southwestern Asia. Archeological findings indicate that the earliest locations of wheat cultivation were regions corresponding to present-day Turkey, Syria, Jordan, and Iraq (Kirtok, 1997). Cultivated wheat first appeared nearly 10,000 years ago through the first hybrids and mutations of Emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) (Yadon *et al*., 2000). It is very probable that the first Emmer wheat was distributed in Southeastern Turkey, in the environs of Diyarbakır's Karacadağ (Heun *et al*., 1997; Luo *et al*., 2007). Throughout history, wheat has been an important cultivated plant for human nutrition; for this reason, it is also the cultivated plant on which the most breeding studies have been performed.

In the year 1900, the world population was nearly 1.6 billion (Chen and Shi, 2013), while world wheat production was at an estimated 90 million tons. Wheat production later reached 147 million tons by 1950

(FAO, 1952), and 586 million tons by 2000 (FAO, 2014). Meanwhile, the world population continued to increase rapidly, as well, reaching 2.5 billion in 1950, and 6.5 billion in 2000 (Bongaarts, 2009). It is estimated that the world population will reach 9.1 billion by 2050 (Atabay *et al*., 2014); therefore, to maintain the ratio of "wheat produced per person" in the year 2000, the world wheat production must be raised to approximately 820 million tons by the year 2050. Considering the effects of climate change and population increase, the general picture for food production and demand in the future appears rather bleak (Figure 1). For this reason, ensuring adequate food supply to the world population of the future - as well as preventing future food wars - necessitates important and bold approaches for increases in food production. Dr. Norman Borlaug, who was awarded the Nobel Peace Prize in *1970,* said that "if you desire peace, cultivate justice, but at the same time cultivate the fields to produce more bread; otherwise there will be no peace" (David, 2013).

Today, increasing the amount of agricultural lands around the world no longer seems possible. Quite the contrary, increasing urbanization and industrialization, as well as many other factors associated with the modern world, are causing a decrease in arable lands (Young, 1999; Cassman *et al*., 2003). In the 1950s, researchers placed emphasis on high-yield varieties to meet the world food demand, laying the foundations for a "green revolution". They were thus able to achieve higher yields, owing mainly to genetic advances that enabled greater yield potential and resistance to biotic and abiotic stresses in wheat varieties (Blum, 1996; Kaya *et al*., 2002; Reynolds and Borlaug, 2006; Rana *et al*., 2013). However, as of today, researchers have already used almost all available genetic resources in the world in studies aiming to develop high yield varieties. Consequently, they have already reached an impassable "ceiling" in terms of the maximum yield potential that can be achieved with current genetic materials. For this reason, to reach higher yields beyond this level, researchers have begun looking for new genetic materials for higher yield potential and greater resistance to biotic and abiotic stresses. Higher yield per unit area will hence be achieved through the efforts and studies of wheat breeders (Bindraban, 1996).

As of today, studies that initially began with the green revolution to develop higher yield varieties have already reached the highest yield level possible with current genetic materials, and the new varieties developed based on these materials (Rana *et al*., 2013). In recent new varieties, the increase achieved in annual yield are in the environs of just 1% (Sayre, 1990). As such, it has become necessary to initiate a new green revolution (Rana *et al*., 2013). Although the maximum yield levels achieved with wheat have gradually steadied and become constant in recent times, higher yields are still possible. The Quran, the holy book of Islam, describes that a single wheat seed can yield 700 seeds (Arslan, 1995). This target seems far beyond what is possible with present-day wheat yield levels.

In recent years, breeders have begun conducting extensive studies for expanding the gradually narrowing genetic basis and materials for wheat (Shiva, 1992) by using wild wheat forms to produce synthetic wheat. Numerous researchers have reported higher resistance to biotic and abiotic stresses in wild form, as well as higher adaptability (Shah *et al*., 1987; Cox, *et al*., 1994; Mujeeb–Kazi and Hettel, 1995; Mujeeb-Kazi, *et al*., 2008; Thompson, and Zwart, 2008). Hexaploid synthetic wheat serves as a genetic bridge for polygenic transfers between wild forms and cultivated bread wheat (Mujeeb-KaziandHettel, 1995; Calderini and Ortiz-Monasterio, 2003a).

Today, synthetic wheat appears to be the strongest candidates to obtain breeding materials that will enable the development of wheat varieties with higher yields and resistance to biotic and abiotic stresses.

What is synthetic wheat?

Synthetic hexaploid wheat is an interspecific hybrid obtained through the hybridization of *Triticum turgidum* ssp. *Dicoccum turgidum* (Emmer) and *Aegilops* groups (Mujeeb-Kazi and Hettel, 1995; Feuillet *et al*., 2008; Trethowan and Van Ginkel, 2009). Hexaploid synthetic wheat serves as a genetic bridge for polygenic transfers between wild forms and cultivated bread wheat (Mujeeb-Kaziand Hettel, 1995; Calderini and Ortiz-Monasterio, 2003b). The female progenitor of cultivated bread wheat is Emmer wheat (Matsuoka and Nasuda, 2004). On the other hand, the male donor for the "D" genome in bread wheat is *Aegilops tauschii* (McFadden and Sears, 1946; Feuillet *et al*., 2008) (Figure 2). Nowadays, the most commonly used diploid (2n=14) for the production of synthetic hexaploid wheat is the *Aegilops tauschii* (synonyms *Aegilops squarrosa* or *Triticum tauschii*) species (William *et al*., 1993; Mujeeb-Kazi and Hettel, 1995). Other diploid groups carrying the "D" genome include the *Triticum cylindricum, Triticum ventricocum, Triticum crassum, Triticum juvenile* and *Triticum syriacum* species (Kimber and Feldman, 1987).During synthetic wheat production, laboratory tissue culture techniques and chromosome doubling are used to ensure the germination of haploid hybrid seeds (Figure2).

The first synthetic wheat production began with the use of the colchicine technique (Sears, 1939) for chromosome doubling (Sears, 1941; Kihara, 1944; Sears, 1944; McFadden and Sears, 1946; Sears, 1955). Researchers have recommended the use of hexaploid synthetic wheat in breeding programs owing to their resistance to biotic and abiotic stresses (Shah *et al*., 1987; Cox *et al*., 1994; Mujeeb-Kazi and Hettel, 1995; Trethowan and Mujeeb-Kazi, 2008) and their yield potential (Villareal *et al*., 1994; Lage *et al*., 2004; Mujeeb-Kazi *et al*., 2008).

Despite studies on synthetic wheat production from the 1940s to the early 1980s, no varieties were presented for farmers' use during this period. The first synthetic wheat-based varieties were presented to farmers for agricultural production during the 1980s (Gill *et al*., 1985). Today, the number of synthetic wheat varieties produced by researchers has exceeded 1000.

We must use synthetic wheat in breeding programs. Why?

The greatest advantages of using synthetic hexaploid wheat as pre-breeding material are as follows: synthetic hexaploid wheat was produced based on a knowledge of their diploid and tetraploid parents' high resistance to biotic and abiotic stresses. Synthetic hexaploid wheat largely preserves these same characteristics, since they receive them from their parents in chromosome sets. Another advantage of synthetic hexaploidis that it is not a genetically modified organism, since it is obtained through the hybridization of wild goat-grass and durum wheat by using traditional methods. This is because synthetic wheat production is performed using natural genomes, without involving any foreign gene transfers.

1. Grain Yield and Yield Components

Primary synthetic wheat tend to have low yields (Trethowan and Van Ginkel, 2009). When primary synthetics are used in breeding programs, the desired level of yield can be attained by performing several backcrossings with the parent wheat (Trethowan and Van Ginkel, 2009; Trethowan and Mujeeb-Kazi, 2008).

Cooper *et al*., (2012) previously reported that using synthetic wheat in hybridization programs by taking into account their number of ears, as well as the number of seeds in each ear, is a more effective approach for developing the grain yield of winter wheat. Synthetic wheat generally preserves their seed weight in different years and different locations (Cooper *et al*., 2012). Furthermore, certain researchers have also observed that the sperm of primary synthetics resulted in wider grains, as well as higher seed weight (Cooper *et al*., 2012; Cooper, 2013). On the other hand, Lage *et al*., (2006) reported a considerable genetic variation among synthetic wheat in terms of grain weight and size.

In studies where synthetic wheat x spring wheat F_1 hybrids were backcrossed with the recurrent parent, researchers reported higher yields for these hybrids than their spring donor parents (Villareal *et al*., 1994; Lage *et al*., 2004; Mujeeb-Kazi *et al*., 2008).Other researchers have reported that the use of synthetic wheat was positively and significantly related with higher grain yield, improved harvest index, improved grain weight, and higher biomass. Mohammad *et al*., (2010) observed that in 33 of their experimental lines, synthetic wheat varieties weighted more by nearly 1000 kernels than all control varieties.

At the end of their two year study,Mujeeb-Kazi and Van Ginkel (2004) reported that the two varieties they produced from synthetic wheat were associated with 20% to 35% higher yields than commercial varieties. Similarly, Van Ginkel and Ogbonnaya (2007) reported that synthetic wheat was associated with 18% to 30% higher yields under heavy rain conditions compared to commercial varieties. According to the findings of other researchers (Del Blanco *et al*., 2001; Ogbonnaya *et al*., 2007), lines produced from synthetic wheat exhibited higher yield levels than their recurrent parents.

2. Grain Quality

A number of researchers have reported that the "D" genome carried by *Triticum tauschii* will enable the improvement of grain quality in wheat (Yueming *et al*., 2003). Similarly, William *et al*., (1993) reported that the superior grain qualities observed in *Ae. tauschii* represented a rich source for increasing the genetic quality/superiority of hexaploid bread wheat. Pfluger *et al*., (2001) described that *Ae. tauschii* showed greater variability compared to bread wheat in terms of gliadin, gluteninand endosperm proteincontent. Lage *et al.,* (2006), on the other hand, reported significant genetic variation between synthetic wheat varieties with respect to the protein content and quality of their grains. Calderini and Ortiz-Monasterio (2003) reported that synthetic wheat possessed higher concentrations of macro and micro elements compared to commercial varieties. In contrast, Trethowan and Van Ginkel (2009) reported that primary synthetic wheat is more likely to have lower grain quality.

3. Resistance to biotic and abiotic stresses

In recent years, the production of synthetic wheat has gained further pace following the successful

results obtained in breeding programs using synthetic hexaploid wheat as pre-breeding materials in order to assess their resistance against biotic and abiotic stresses. Numerous researchers who are concerned about the worldwide effects of climate change have published reports describing the resistance and tolerance of synthetic wheat to various biotic and abiotic stresses (Shah *et al*., 1987; Cox *et al*., 1994; Mujeeb-Kazi and Hettel, 1995; Trethowan and Mujeeb-Kazi, 2008).

Synthetic hexaploid wheat is resistant or tolerant to numerous disease-causing biotic factors. Some of these biotic factors includeleaf rust (Kerber, 1987), tan spots (Siedler *et al*., 1994), stem rust (Marais *et al*., 1994), stripe rust (Ma *et al*., 1995; Assefa and Fehrmann, 2000), karnal bunt (Villareal *et al*., 1996),spot blotch (Mujeeb-Kazi *et al*., 1996; Mujeeb-Kazi and Delgado, 1998), leaf blotch (Arraiano *et al*., 2001), cereal cyst nematodes (Eastwood *et al*., 1991), root lesion nematodes (Thompson *et al*., 1999), powdery mildew (Kong *et al*., 1999), glume blotch (Loughman *et al*., 2001), leaf blight (Mujeeb-Kazi *et al*., 2001), and hessian fly (Tyler and Hatchett, 1983).

Numerous researchers have also reported resistance or tolerance to abiotic stresses, as well as wider adaptability, among the wild forms that constitute the origin of bread wheat (Shah *et al*., 1987; Cox *et al*., 1994; Mujeeb–Kazi and Hettel, 1995; Mujeeb–Kazi *et al*., 2008; Thompson, and Zwart, 2008). Based on an experiment performed on *T. tauschii* lines and drought-resistant hexaploid lines held under low-water conditions, Reddy*et al*. (1996) demonstrated that *T. tauschii* lines were more tolerant. Other researchers determined that synthetic wheat has significant tolerance against salinity (Pritchard *et al*., 2002), drought, waterlogging (Villareal *et al*., 2001), frost at flowering and heat (Van Ginkel and Ogbonnaya, 2007).

Conclusion

Climate change is gradually increasing the average world temperature, while also reducing water resources and causing agricultural lands to become drier. Parallel to these negative developments, the world population is rapidly rising while the area of agricultural/arable lands remain constant. Many scientists believe that the inability to produce enough food to feed the increasing world population will inevitably lead to food wars. In this context, it is imperative to increase yield per unit area by developing varieties that are more resistant to biotic and abiotic stresses.

Until recently, new wheat varieties were developed using available genetic materials that have been used and reused for many years. However, these materials have not allowed researchers to achieve the desirable levels of resistance against the biotic and abiotic stress factors that limit grain/seed yield. To achieve such desirable traits in new varieties, it has become necessary to look at the origins of wheat. Understanding this necessity, researchers have focused in recent years on rediscovering the origins/roots of wheat, and on rebuilding it from a genetic standpoint. A general evaluation of the results obtained by researchers around the world clearly indicate varieties with higher yield potential, as well as tolerance and resistance to biotic and abiotic stresses, can be achieved through the greater use of synthetic hexaploid wheat as pre-breeding materials. To meet world food demand by the year 2050, we mustincrease wheat production by nearly 29% compared to present levels. Synthetic wheat currently represents the best and closest genetic source for developing higher-yield varieties. For this reason, wheat breeders must place greater emphasis on the production of synthetic wheat in order to ensure world peace, and to prevent the starvation of children around the world.

Figure 1. World population and wheat production in the years 1900, 1950, and 2000, and the estimated world population and wheat production for the year 2050.

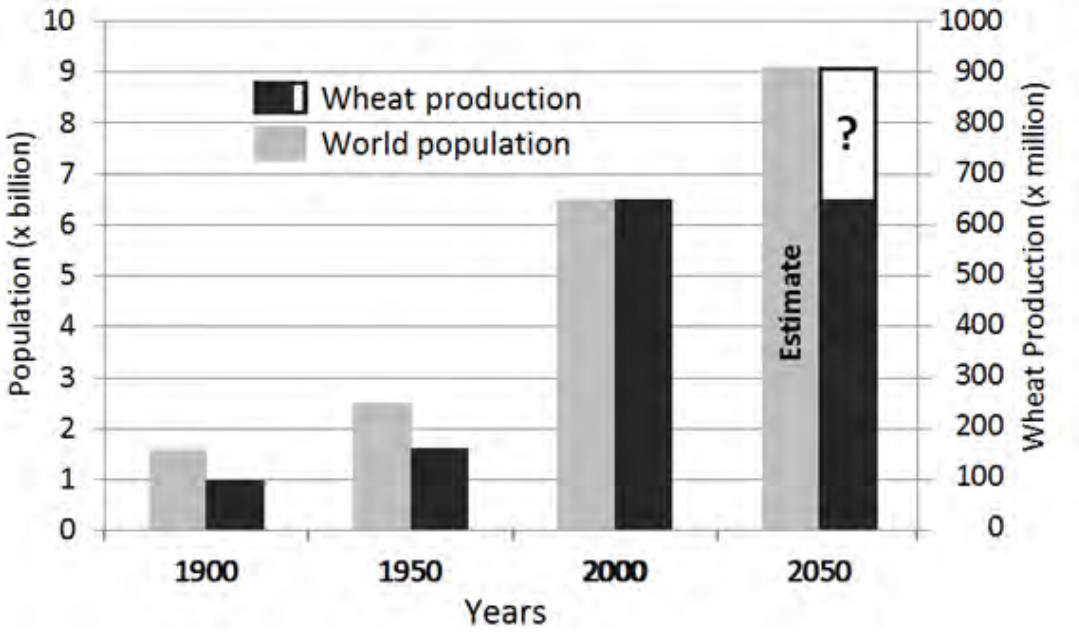
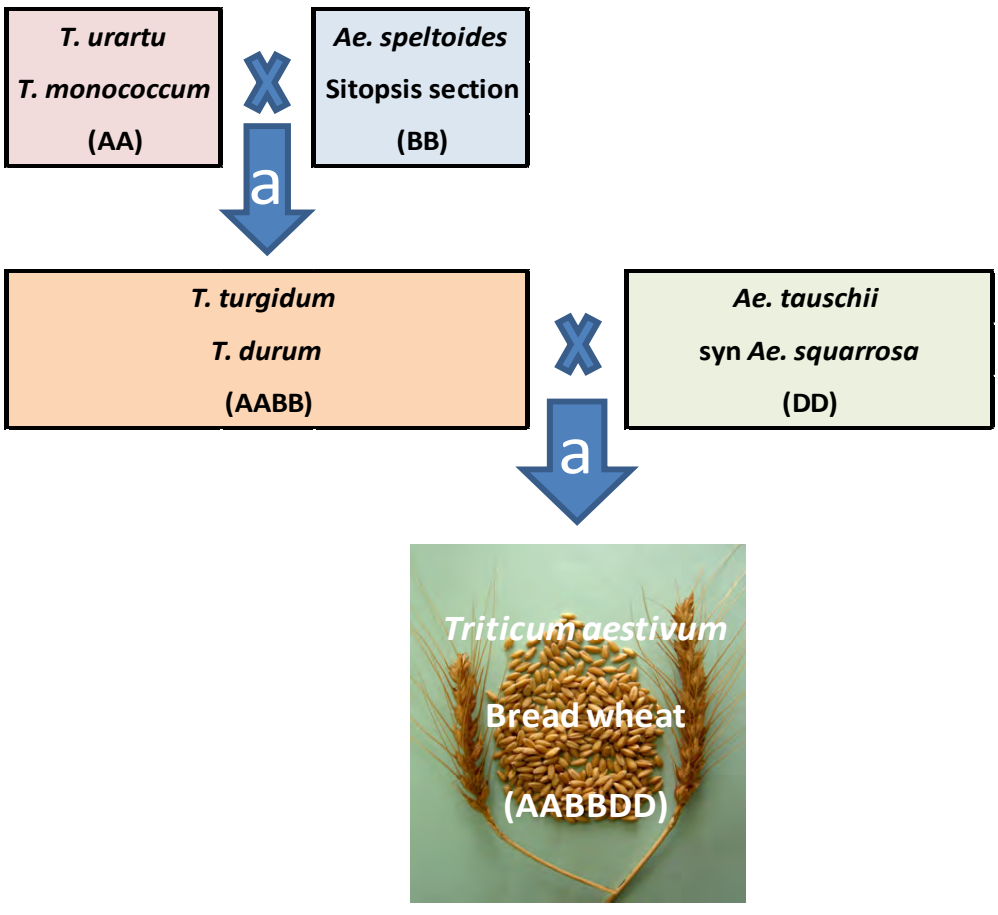


Figure 2. The origin of hexaploid bread wheat. Following each hybridization, fertility was ensured by performing chromosome doubling with the colchicine technique (a).



References

Arraiano LS, Worland AJ, Ellerbrook C and Brown JKM (2001). Chromosomal location of a gene for resistance to S*eptoriatritici* blotch (*Mycosphaerellagraminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor. App. Genet. 103: 758-764.

Arslan A (1995). Bakara Suresi 261. Âyet, Hicretin 9. Yılı Mîlâdî 630. Büyük Kur'an Tefsiri, Okusan Yayıncılık. Cilt: 16, s. 62 (in Turkish).

Assefa S and Fehrmann H (2000). Resistance to wheat leaf rust in *Aegilopstauschii* Coss. and inheritance of resistance in hexaploid wheat. Genet. Res. Crop Evol. 47: 135-140.

Atabay S, Karasu M and Koca C (2014). İklim Değişikliği ve Geleceğimiz. Yıldız Teknik Üniversitesi Mimarlık Fakültesi, Kütüphane ve Dokümantasyon Merkezi Sayı: YTÜ.MF-BK-2014.0884. ISBN: 978-975-461-513-5. P. 1-132. İstanbul (in Turkish).

Eastwood RF, Lagudah ES, Appels R, Hannah M and Kollmorgen JF (1991). *Triticumtauschii*: a novel source of resistance to cereal cyst nematode (*Heteroderaavenae*). Aust. J. Agric. Res. 42: 69-77.

Bindraban PS (1996). Quantitative Understanding of Wheat Growgth and Yield for Identifying Crop Chracteristics to Further Increase. Proceeding of a Workshop Held in Ciudad Obregon, Sonora, Mexico by M. P. Reynolds, S Rajaram and A. McNab, editors. Pages: 230-236.

Blum A (1996). Yield Potantial and Drought Resistance; Are They Mutually Exclusive. Proceeding of a Workshop Held in Ciudad Obregon, Sonora, Mexico by Reynolds MP, Rajaram S and McNab A, editors. Pages: 90-100.

Bongaarts J (2009). Human population growth and the demographic transition. Philosophical Transactions of The Royal Society - B. 364: 2985-2990.

Calderini DF and Ortiz-Monasterio I (2003a). Grain position affects grain macro nutrientand micronutrient concentrations in wheat. Crop Sci. 43:141-151.

Calderini DF and Ortiz-Monasterio I (2003b). Are synthetic hexaploids a means of increasing grain element concentrations in wheat? Euph. 134: 169-178.

Cassman KG, Dobermann A, Walters DT and Yang H (2003). Meeting cereal demand while protecting natural resources and improving environmental quality. Ann. Rev. Environ. Resour. 28: 315-358.

Chen J and Shi H (2013). Do we need construct more dams? Agu Fall Meeting. 9–13 December 2013, Poster. San Francisco-USA.

Cooper JK (2013). Synthetic hexaploid wheat as a source of improvement for winter wheat (*Triticumaestivum*L.) in texas. Texas A&M University.

Cooper JK, Ibrahim AMH, Rudd J, Malla S, Hays DB and Baker J (2012). Increasing hard winter wheat yield potential via synthetic wheat: I. Path-Coefficient Analysis of Yield and Its Components. Crop Sci. 52: 2014-2022.

Cox TS, Raupp WJ and Gill BS (1994). Leaf rust–resistance genes Lr41, Lr42 and Lr43 transferred from *Triticumtauschii* to common wheat. Crop Sci. 34: 339-349.

David M (2013). Dr. Norman Borlaug; "The Man Who Saved a Billion Lives". (15 October 2013) Huffington Post.

Del Blanco IA, Rajaram S and Kronstad WE (2001). Agronomic potential of synthetic hexaploid wheat-derived populations. Crop Sci. 41: 670-676.

FAO (1952). The State of Food and Agriculture: Review and Outlook. Chapter IV - Review and Outlook by Commodities - Wheat. p. 81-86. Rome.

FAO (2014). Wheat Production Quantity. FAOSTAT (http://faostat3.fao.org).

Feuillet C, Langridge P and Waugh R (2008). Cereal breeding takes a walk on the wild side. Trends Genet. 24: 24-32.

Gill BS, Sharma HC, Raupp, WJ, Browder LE, Hatchett JH, Harvey TL, Moseman JG and Waines JG (1985). Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, Hessian fly, and greenbug. Plant Dis. 69: 314-316.

Heun M, Schafer-Preg R, Klanan D, Castagna R, Accerbi, M. Borghi B and Salamini F (1997). Site of Eirkorn Wheat Domestication Identified by DNA Fingerpriting. Sci. 278: 1312-1314.

Kaya Y, Palta C and Taner S (2002). Additive main effects and multiplicative interaction analysis of yield performance in bread wheat genotypes across environment. Turk. J. Agric. For. 26: 275-279.

Kerber ER (1987). Resistance to leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *T. tauschii*. Crop Sci. 27: 204-206.

Kirtok Y (1997). Genel Tarla Bitkileri. Serin ve Sıcak İklim Tahılları. Çukurova Üni. Ziraat Fak. Ders Kitabı. Adana (in Turkish).

Kihara (H) (1944). Discovery of the DD analyser, one of the ancestors of *Triticum vulgare*. Agric. Hort. 19: 889-890.

Kimber G and Feldman M (1987). Wild wheat. An introduction. Special Report 353, College of Agriculture, University of Missouri, Columbia, USA.

Kong L, Dong Y, Jia J, Kong LR, Dong YC and Jia JZ (1999). Location of a powdery mildew resistance gene in Am6, an amphidiploid between *Triticum durum* and *Aegilopstauschii*, and its utilisation. ActaPhyto. Sinica 26: 116-120.

Lage J, Skovmand B and Andersen S (2004). Field evaluation of emmer wheat-derived synthetic hexaploid wheat for resistance to Russian wheat aphid (homoptera: Aphididae). J. Econ. Ento. 97: 1065-1070.

Lage J, Skovmand B, Pena RJ and Andersen SB (2006). Grain quality of emmer wheat derived synthetic hexaploid wheats. Genet. Res. Crop Evol. 53: 955–962.

Loughman R, Lagudah ES, Trottet M, Wilson RE and Mathews A (2001). *Septorianodorum* blotch resistance in Aegilopstauschii and its expression in synthetic amphiploids. Aust. J. Agric. Res. 52: 1393-1402.

Luo M, Yang Z, You F, Kawahara T, Waines J and Dvorak J (2007). The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. Theor. Appl. Genet. 114: 947–959.

Ma H, RP Singh and A Mujeeb-Kazi (1995). Resistance to stripe rust in *T. turgidum*, *T. tauschii*, and their synthetic hexaploids. Euph. 82: 117-124.

Marais GF, Potgieter GF and Roux HS (1994). An assessment of the variation for stem rust resistance in the progeny of a cross involving the *Triticum* species *aestivum, turgidum* and *tauschii*. S.A. J. Plant Soil. 11: 15–19.

Matsuoka Y and Nasuda S (2004). Durum wheat as a candidate for the unknown female progenitor of bread wheat: An empirical study with a highly fertile f-1 hybrid with *Aegilopstauschii* Coss. Theor. Appl. Genet. 109: 1710-1717.

McFadden ES and Sears ER (1946). The origin of *Triticum spelta* and its free–threshing hexaploid relatives. J. Hered. 37: 107–116.

Mohammad F, Abdalla OS, Rajaram S, Yaljarouka A, Khalil SK, Khan NU, Khalil IH and Ahmad AI (2010).Yield Of synthetic-derived bread wheat under varying moisture regimes.Pak. J. Bot. 42: 4103-4112.

Mujeeb-Kazi A, Cano S, Rosas V, Cortes A and Delgado R (2001). Registration of five synthetic hexaploid wheat and seven bread wheat linesresistant to wheat spot blotch. Crop Sci. 4: 1653-1654.

Mujeeb-Kazi A and Delgado R (1998). Bread wheat/D genome synthetic hexaploid derivatives resistant to *Helminthosporiumsativum* spot blotch. P. 297-299. In: *Proc. of the Ninth International Wheat Genet. Symp*., (Ed.): A.E. Slinkard. Vol. 3, Section 6. Univ. Ext. Press, Saskatoon, SK, Canada.

Mujeeb-Kazi A, Gul A, Farooq M, Rizwan S and Ahmad I (2008). Rebirth of synthetic hexaploids with global implications for wheat improvement. Aust. J. Agric. Res. 59: 391-398.

Mujeeb-Kazi A and Hettel GP (1995). Utilizing Wild Grass Biodiversity in Wheat Improvement: 15 Years of Wide Cross Research at CMMYT. Report No: 2. Mexico.

Mujeeb-Kazi A, Rosas V and Roldan S (1996). Conservation of the genetic variation of *T. tauchii* (Coss.) Schmalh. (*Aegilopssquarrosa* auct. non L.) in synthetic hexaploid wheats (*T. tugidum* L. X *T. tauschii*; 2n = 6x = 42, AABBDD) and its potential utilization of wheat improvement. Genet. Res. Crop Evol. 43: 129-134.

Mujeeb-Kazi M and Van Ginkel M (2004). Wild wheat relatives help boost genetic diversity. CIMMYT News.

Ogbonnaya FC, Ye G, Trethowan R, Dreccer F, Lush D, Shepperd J and Van Ginkel M (2007). Yield of synthetic backcross-derived lines in rainfed environments of Australia. Euph. 157: 321–336.

Pfluger LA, D'Ovidio R, Margiotta B, Pena RJ, Mujeeb-Kazi A and Lafiandra D (2001). Characterisation of high- and low-molecular weight glutenin subunits associated to the D genome of *Aegilopstauschii* in a collection of synthetic hexaploid wheats. Theor. Appl. Genet. 103:1293-1301.

Pritchard DJ, Hollington PA, Davies, WP, Gorham JL, Diaz de Leon F and Mujeeb-Kazi A (2002). K+/Na+ discrimination in synthetic hexaploid

wheat lines: Transfer of the trait for K+/Na+ discrimination from *Aegilopstauschii* into a *Triticumturgidium* background. Cereal Res. Com. 30: 261–267.

Rana RM, Bilal M, Rehman SU, Iqbal F and Shah MKN (2013). Synthetıc Wheat; A New Hope for the Hungry World. Asian. J. Agric. Biol. 1: 91-94.

Reddy N, Halloran GM and Nicolas ME (1996). Agronomic assessment of lines derived from a direct cross of wheat with *T. tauschii* L. In: *Proc 8th Assembly of Wheat Breed. Soc. of Australia*, (Eds.) R.A. Richards, C.W. Wrigley, H.M. Rawson, G.J. Rebetzke, J.L. Davidson and R.I.S. Brettell. pp. 24–26. Canberra, Australia.

Reynolds M and Borlaug N (2006). Impacts of breeding on international collaborative wheat improvement. J. Agric. Sci. 144:3-7.

Sayre KD (1990). Improvement of Input-use Efficiency in Irrigated Wheat Production. In: Crop Management Physiology Subprogram of the CIMMYT. Mexico, D.F.

Sears ER (1939). Amphidiploids in the Triticinae induced by colchicine. J. Hered. 30: 3843.

Sears ER (1941). Amphidiploids in the seven-chromosome Triticinae. Mo. Agric. Expt. Sta. Res. Bul. 336.

Sears ER (1944). The amphidiploids *Aegilops cylindrical* X *Triticum durum* and *A. Ventricosa* x *T. durum* and their hybrids with *T.aestivum.* J. Agric. Res. 68: 135-144.

Sears ER (1955). An induced gene transfer from *Aegilops*to *Triticum.* Genet. 40: 595.

Shah S, Gorham J, Forster B and Wyn Jones RG (1987). Salt tolerance in the Triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. J. Exp. Bot. 38: 254-269.

Shiva V (1992). The violence of green revolution: Third world agriculture, ecology and politics. Zed Books.

Siedler H, Obst A, Hsam SLK and Zeller FJ (1994). Evaluation for resistance to *Pyrenophoratritici–repentis* in *Aegilopstauschii* Coss and synthetic hexaploid wheat amphiploids. Genet. Res. Crop Evol. 41: 27-34.

Thompson JP, Brennan PS, Clewett TG, Sheedy JG and Seymour NP (1999). Progress in breeding wheat for tolerance and resistance to root-lesion nematode (Pratylenchusthornei). Aust. Plant Path. 28: 45-52.

Thompson JP and Zwart RS (2008). Synthetic hexaploid wheats for resistance to root-lesion nematodes. Proceeding of the 11th International Wheat Genetics Symposium, 24-29 August2008, Australia, 3: 849-851.

Trethowan R and Mujeeb-Kazi A (2008). Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Sci. 48: 1255-1265.

Trethowan R and Van Ginkel M (2009). Synthetic wheat-an emerging genetic resource. *In*: B. Carver (ed.) Wheat science and trade. Wiley-Blackwell, Ames, IA. p. 369-385.

Tyler JM and Hatchett JH (1983). Temperature influence on expression of resistance to Hessian fly (Diptera: *Cecidomyiidae*) in wheat derived from *Triticumtauschii*. J. Econ. Ento. 76: 323-326.

VanGinkel M and Ogbonnaya F (2007). Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. Field Crops Res. 104: 86-94.

Villareal RL, Mujeeb-Kazi A, Fuentes-Davila G and Rajaram S (1996). Registration of four synthetic hexaploid wheat germplasm lines derived from *T. turgidum x T. tauschii* crosses and resistant to karnal bunt. Crop Sci. 36: 218-220.

Villareal R, Mujeeb-Kazi A, Fuentes-Davila G, Rajaram S and Deltoro E (1994). Resistance to Karnal bunt (*Tilletiaindicamitra*) in synthetic hexaploid wheats derived from *Triticumturgidum.T. tauschii*. Plant Breed. 112: 63–69.

William MDHM, Pena RJ and Mujeeb-Kazi A (1993). Seed protein and isozyme variations in *Triticum-tauschii* (*Aegilopssquarrosa*). Theor. Appl. Genet.87: 257-263.

Yadon SI, Gopher A and Aboo S (2000). The Cradle of Agriculture Science. (Çeviri: Tarımın Kökeni. Bilim Tek. Der., s. 64-65 (in Turkish).

Young A (1999). Is there really spare land? A critique of estimates of available cultivable land in developing countries. Environment, Development and Sustainability 1: 3-18.

Yueming Y, Hsam SLK, Jianzhong Y, Jiang Y and Zeller FJ (2003). Allelic variation of the HMW glutenin subunits in *Aegilopstauschii* accessions detected by sodium dodecyl sulphate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis. Euph. 130: 377-385.

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The Study of the Sum of Active Temperatures Affecting Autumn Bread (*Triticum aestivum* L.) Wheat Under Dry Rainfed Conditions

Atabey A. JAHANGIROV[1*] Ali A. JAHANGIROV[1] Hamid N. HAMIDOV[1] Irada M. HUSEYNOVA[2]

[1] Gobustan Experimental Station of Research Institute of Crop Husbandry
[2] Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, 2A Matbuat Avenue, Baku AZ1073, Azerbaijan

[*] Corresponding author e-mail: a.cahangir@hotmail.com

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ABSTRACT

The sum of active temperatures affecting 21 autumn bread wheat genotypes with contrasting morphophysiological properties during the flowering and grain filling phases after earing has been studied under dry rainfed conditions in 2013-2014, during vegetation periods characterized by different hydrometeorological parameters. The functioning period of flag leaves in Murov 2, Gyrmyzy gul-1 and Zirva 85 genotypes appeared to be longest and they had higher sums of active temperatures (SAT) compared with other varieties during both years. Qyzyl bugda, Layagatli-80 and 12 IWWYT № 6 varieties had quite high higher sums of active temperatures values in 2013, but they showed lower results under unfavorable climatic conditions in 2014. There were a positive correlation between SAT and productivity at $r=0.55^{**}$, and a negative correlation between earing dates and higher sums of active temperatures at $r=-0.41^{**}$ during both years.

Keywords: *Triticum aestivum* L., morphophysiological properties, productivity, dry rainfed conditions.

Introduction

Wheat is the most important food plant for humanity, one of the strategic foods in the world. After the so called “Green Period” the world wheat productivity and production increased by 50% and 38% , respectively. This increase occurred at the expense of developing short varieties and increasing number of productive stems (Aliyev, 2000; Aliyev, 2002a). Whereas, the total biological mass remained almost unchanged. However, productivity increase due to genetic progresses does not seem to meet the rising demands. Currently, substantial achievements have been made in the wheat selection by using morphophysiological parameters. Considering other morphophysiological parameters and different selection approaches is important for the further success (Aliyev *et al*., 2002b; Aliyev, 2012). Most wheat in our country is produced in the regions with dry rainfed conditions, including Daghlig Shirvan’s foothill areas. Little and unevenly distributed annual precipitation is characteristic for this district. Generally, drought begins at the periods close to the beginning of flowering and continues intensively till the end of the grain filling phase.

Currently, some visible morphological parameters (height, leaf and ear sizes, etc.) of plants as well as fast-growing is used as the selection signs and taking into account productivity, researches have been carried out for developing drought tolerance varieties. However, in developing drought tolerant wheat genotypes, along with the morphological parameters, there is a great need in physiological tests which are repeatable and easily performable.

The main purpose of the research was to study the sum of active temperatures affecting plants for characterizing their functioning during the flowering and grain filling phases.

Materials and Methods

The research was carried out in dry rainfed areas of the Daghlig Shirvan district of the Republic of Azerbaijan, during the 2012-2013 and 2013-2014 vegetation years. The planting area is located at 780 m above sea level. The light-brown type soil of the district is carbonated. The amount of humus in the planting layer with pH 8.6 is 1.25-2.17%, digestible nitrogen in 1kg of soil is 42.7 mg, phosphorus 23.4 mg and K_2O in 100 g of soil is 17.5 mg. Multiyear average atmospheric precipitation in the district is 350-400 mm and unevenly distributed during the vegetation period of the autumn wheat. Average annual temperature is 10.2°C. Sometimes temperature in winter is -15-20°C, in summer temperature rises to 30-35°C and relative humidity declines to 25%.

Thirteen autumn bread wheat genotypes with contrasting morphophysiological properties and eight lines chosen from the International Selection Centers for the rainfed districts were used as the study objects. Wheat varieties of tall stature (115-130 cm) - Bezostaya-1, Gizil bugda, Sheki-1, Sonmez were planted under rainfed conditions. Medium-height (110-115 cm) varieties Gobustan and Leyagetli-80 were developed for the rainfed districts. But, they show also good results under irrigated conditions. Short-stature wheat varieties - Aran, Vostorg, Murov-2, Tale-38, Fatima, Girmizi gul-1, Zirve-85 demand more water and are highly productive under watered conditions.

To determine the sum of active temperatures influencing on the plant after the earing phase, chlorophyll amount was measured in flag leaves on different dates using SPAD meter. According to the obtained data the theoretical zero-point for chlorophyll was estimated using the square regression method, where the SPAD value was dependent variable (y) and the sum of active temperatures, measured from the beginning of the earing phase, was independent variable (x). The sum of active temperatures corresponding to this point was used for the assessment of the flag leaf functioning. Moreover, using SPAD values and the same method the day corresponding to the zero-point for chlorophyll was established, which was then used for the assessment of the flag leaf functioning in various genotypes (Çekiç, 2007). The results were analyzed using the JMP 5.0.1 statistical program.

Results and Discussion

Weather conditions during the research are presented in Table 1. As seen from the table during the vegetation period the average annual temperature was higher than multiyear average temperature in both years.

The amount of precipitation was slightly lower during the 2012-2013 vegetation year compared with the multiyear average values. However, it was sufficient for the plant development in spring months and the grain filling period (264.5 mm). During the 2013-2014 vegetation year, 156.7 mm and 91.5 mm less amount of precipitation was observed compared with the multiyear average values found for the plant development in spring months and the grain filling period, respectively. Such changes in weather conditions had an effect on developmental stages of genotypes and finally on the plant productivity. The SPAD values of bread wheat flag leaves are presented in Table2.

As seen in the table the chlorophyll amount decreased to the end of the vegetation, but a higher rate of this decrease was observed in 2013-2014, due to the less precipitaion and higher temperature. The highest chlorophyll amount was observed in the Gyrmyzy gul 1, Bezostaya 1 and Tale 38 genotypes and the lowest in the Gyzyl bugda, Fatima varieties and 12 IWWYT № 6, 12 IWWYT № 17, 4th FEFWSN № 50 lines.

Based on the SPAD values the theoretical zero-point for chlorophyll was estimated using the square regression method and beginning from the earing date, the sum of active temperatures of the corresponding day was found according to the the daily weather data. The chlorophyll amount decline in flag leaves of the genotypes has been presented in Figure 1. As seen in the figure in flag leaves of Murov 2 and Gyrmyzy gul 1 the chlorophyll amount fell to zero after a longer period of time. Zero-point for the chlorophyll amount was achieved faster in flag leaves of the Gyzyl bugda and 12 IWWYT № 6 genotypes. It should be noted that other genotypes, which are not presented in the figure, occupy an intermediate position.

Sum of the active temperatures affecting all the studied genotypes till the end of the earing period was estimated and the results along with the earing date and productivity were presented in Table 3. As seen in the Table average SAT values were 547 and 477°C in 2013 and 2014, respectively. This 70°C decline in the SAT value may be the result of the less precipitation and fast yellowing of the plants observed in 2014. The different responses of the studied genotypes should be also noted. During both years SAT values of the Murov 2, Gyrmyzy gul 1, Zirva 85 genotypes were found to be 663, 577 and 607°C, respectively, which are the highest results among the studied genotypes. Unlike to others, Murov 2 and Zirva 8 are fast-growing genotypes.

High SAT values for the Gyrmyzy gul 1 genotype are attributed to the late yellowing of its leaves in spite of the late earing. So, according to Table 4 the

Murov 2 and Zirva 85 genotypes are fast-growing and the functioning period of their flag leaves are the longest during both years. In spite of the late earing of Gyrmyzy gul 1, the flag leaf was yellowed late and its functioning period was long and thereby this genotype also had high SAT values.

Rather high values of SAT for the Gyzyl bugda, Layagatli 80 and 12 IWWYT № 6 varieties observed during favorable 2013, decreased significantly in unfavorable 2014. This can occur due to the earlier yellowing of their leaves in spite of the earlier earing period compared with other genotypes. So, the table shows that the functioning period of flag leaves of these genotypes during the unfavorable year decreased by 14.3, 14.7 and 16.7 days, respectively.

The dependence between SAT influencing on plants and earing dates and also between SAT and productivity was studied for both years. As seen in figure 2, there is a negative correlation between earing dates and SAT, at r= -0.41.

Thus, the research carried out showed a positive relationship between SAT affecting plants after the earing period and productivity. The obtained results are recommended for the use in the selection of the autumn bread wheat.

Table 1. Amount of precipitation and temperature during the 2012-2013 and 2013-2014 vegetation years

Months	2012-2013		2013-2014		Multiyear average	
	Precip. (mm)	Average temp. °C	Precip. (mm)	Average temp. °C	Precip. (mm)	Average temp. °C
September	23.1	18.0	52.0	19.5	31.0	17.1
October	24.1	14.9	23.2	11.2	45.0	11.2
November	21.6	7.8	11.7	7.2	36.0	6.0
December	28.7	2.2	18.3	-0.7	30.0	1.7
January	27.5	1.0	20.1	0.03	26.0	-0.2
February	48.2	3.2	12.5	-0.23	35.0	0.1
March	28.9	6.2	37.4	4.9	42.0	3.1
April	40.2	9.7	19.8	10.7	47.0	9.2
May	75.2	15.7	23.2	19.5	47.0	14.9
June	20.2	20.9	4.1	21.1	40.0	19.5
Total:	337.7		222.3		379.0	
Average:		9.96		9.32		8.3

Table 2. SPAD values during the 2012-2013 and 2013-2014 vegetation years

N	Varieties	2013						2014						
		08.06	11.06	16.06	18.06	20.06	24.06	19.05	27.05	02.06	06.06	09.06	12.06	16.06
1	Bezostaya 1	51.5	50.9	33.8	22.8	2.5	-	51.4	50.6	45.4	25.6	17.9	-	-
2	Gyzyl bugda	45.8	36.9	6.8	2.0	-	-	49.0	47.8	40.8	14.1	-	-	-
3	Sheki 1	48.2	50.8	32.3	20.2	4.2	-	52.2	50.9	46.5	40.5	35.5	22.5	10.0
4	Sonmez	49.2	51.0	19.3	8.4	-	-	51.0	50.7	46.5	23.2	16.3	10.0	-
5	Aran	49.2	52.4	45.4	27.2	20.3	8.0	49.7	49.6	45.8	40.6	28.1	14.1	6.0
6	Vostorg	50.1	52.8	31.6	8.4	-	-	51.8	52.8	45.6	33.1	20.7	8.5	-
7	Murov 2	49.8	48.6	41.3	31.1	21.1	10.2	48.0	47.4	45.7	40.5	30.9	19.1	10.0
8	Gobustan	47.8	42.5	5.4	-	-	-	49.8	50.6	38.7	19.0	-	-	-
9	Tale 38	50.9	51.0	16.3	3.5	-	-	53.6	45.7	47.1	35.2	12.5	-	-
10	Fatima	46.0	51.1	20.2	4.5	-	-	42.0	51.8	43.3	25.3	10.2	-	-
11	Gyrmyzygul 1	52.6	49.8	42.5	30.1	15.6	5.7	49.0	47.5	48.5	46.5	35.0	22.4	12.0
12	Zirva 85	49.1	45.7	32.5	20.4	6.5	-	49.2	50.6	45.1	35.2	15.6	8.0	-
13	Layagatli 80	48.7	51.8	19.9	4.5	-	-	48.3	50.6	48.2	16.8	-	-	-
14	Ferrigineum 2/19	51.0	48.1	20.3	7.5	-	-	50.3	50.4	46.2	20.2	-	-	-
15	11 IWWYUT № 20	47.6	50.2	24.5	13.2	3.5	-	47.7	48.6	45.2	23.2	-	-	-
16	12 IWWYUT № 6	42.9	30.2	3.5	-	-	-	52.0	49.2	44.0	10.6	-	-	-
17	12 IWWYUT № 8	47.7	49.0	20.5	6.5	-	-	50.1	52.9	46.8	30.9	18.7	-	-
18	12 IIWWYUT № 9	47.7	51.4	13.1	4.3	-	-	47.6	45.6	44.5	21.1	-	-	-
19	12 IWWYUT № 17	44.1	37.3	4.1	-	-	-	46.8	45.7	43.2	18.5	-	-	-
20	7 WONSA № 477	50.1	47.5	25.9	16.3	4.1	-	47.2	48.2	45.8	38.3	27.2	10.5	-
21	4th FEFWSN № 50	40.3	50.0	6.1	2.0	-	-	52.0	46.7	43.9	14.6	-	-	-

Table 3. Earing dates (days from May the 1st), productivity and SAT values for the studied genotypes

№	Genotypes	Earing date		SAT, °C		Productivity, g/m²	
		2013	2014	2013	2014	2013	2014
1	Bezostaya 1	15.7	16.0	507	445	562	421
2	Gyzyl bugda	8.3	13.3	548	428	596	450
3	Shaki 1	16.7	17.0	552	527	572	428
4	Sonmez	9.0	13.0	551	515	678	488
5	Aran	18.0	17.3	550	497	630	440
6	Vostorg	15.0	15.3	511	481	608	409
7	Murov 2	8.3	13.0	663	592	624	378
8	Gobustan	7.0	11.7	529	476	812	501
9	Tale 38	14.0	15.7	494	435	811	449
10	Fatima	11.3	14.0	526	454	723	358
11	Gyrmyzy gul 1	15.0	16.0	577	542	752	468
12	Zirva 85	7.3	12.0	607	531	705	442
13	Layagatli 80	8.0	13.7	551	432	794	560
14	Ferrigenium 2/19	8.3	11.7	560	462	782	437
15	11 IWWYT № 20	10.3	13.0	569	460	717	432
16	12 IWWYT № 6	6.3	12.7	538	418	768	503
17	12 IWWYT № 8	11.0	14.0	531	479	728	475
18	12 IWWYT № 9	13.3	13.7	505	442	642	445
19	12 IWWYT № 17	8.3	14.0	518	430	689	407
20	7 WON-SA №477	12.0	14.0	558	517	723	476
21	4th FEFWSN №50	7.3	13.3	551	450	680	424
	Average	11.0	14.0	547	477	695	447
	and %	9.4	5.14			5.4	8.4
	Minimal important difference (MID)	1.7	1.2			62	62

Table 4. Functioning periods (in days) of flag leaves after earing

N	Variety name	2013	2014	Difference
1	Bezostaya 1	35.4	27.4	8.0
2	Gyzyl bugda	41.0	26.7	14.3
3	Shaki 1	35.4	32.7	2.7
4	Sonmez	40.7	31.5	9.2
5	Aran	38.0	31.0	7.0
6	Vostorg	34.8	29.6	5.2
7	Murov 2	48.6	36.2	12.4
8	Gobustan	40.4	29.5	10.9
9	Tale 38	35.2	27.0	8.2
10	Fatima	38.2	28.0	10.2
11	Gyrmyzy gul 1	40.7	33.5	7.2
12	Zirva 85	44.8	32.3	12.5
13	Layagatli 80	41.7	27.0	14.7
14	FERRIGINEUM2/19	41.5	28.8	12.7
15	11 IWWYT№ 20	41.2	28.7	12.5
16	12 IWWYT№ 6	42.7	26.0	16.7
17	12 IWWYT№ 8	38.5	29.5	9.0
18	12 IWWYT№ 9	36.1	27.9	8.2
19	12 IWWYT№ 17	39.4	26.9	12.5
20	7 WON-SA№ 477	39.5	31.6	7.9
21	4th FEFWSN № 50	41.7	28.0	13.7

Figure 1. A decrease in chlorophyll content of flag leaves of the genotypes in 2014

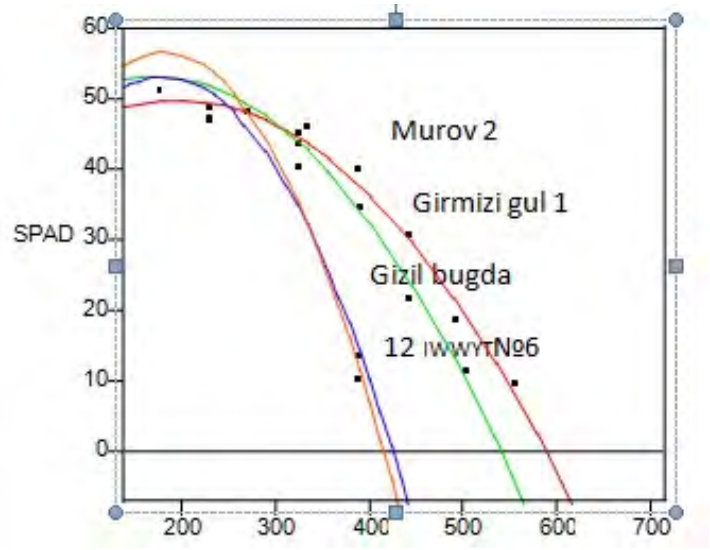


Figure 2. Relationship between earing dates and SAT values

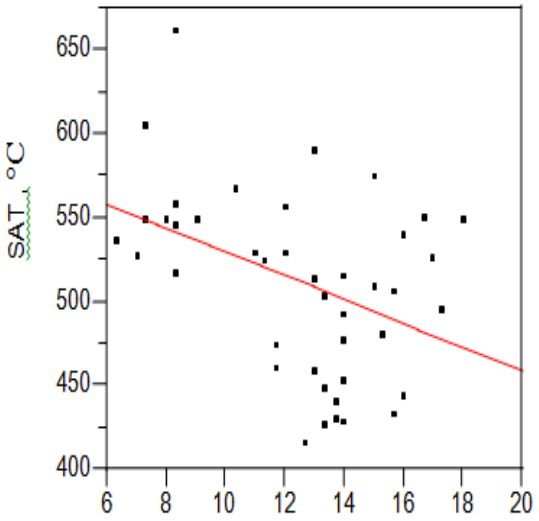
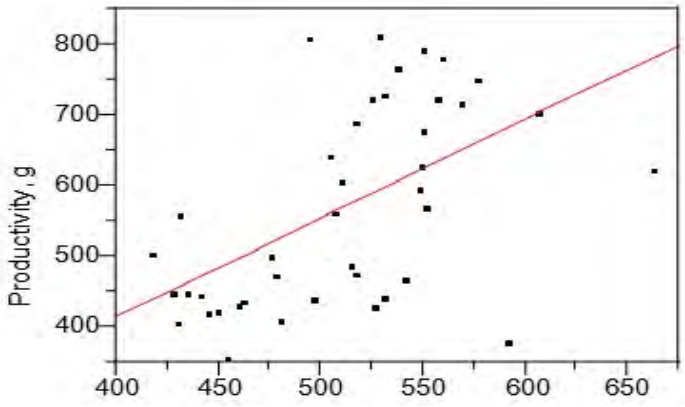


Figure 3. Relationship between SAT and productivity



References

Aliyev JA. Physiological bases of wheat breeding tolerant to water stress. Proceedings of the 6th International Wheat Conference, Budapest, Hungary, 2000. In: Wheat in a Global Environment (Z. Bedo and Lang L., eds.) Kluwer Academic Publisters, Dordrecht-Boston-London 9; p 693-698.

Aliyev JA. Significance of photosynthesis of various organs in the protein synthesis in grains of wheat genotypes under water stress. Proceedings of ANAS (Biol. Sciences) 2002a, №1-6, pp 5-19 (in Russian).

Aliyev JA, Kazibekova EG. Photosynthetic characteristics of the highly productive wheat and the use of the photosynthetic signs for the selection. Proceedings of ANAS (Biol. Sciences) 2002b, №1-6, pp 20-29 (in Russian).

Aliyev JA. Photosynthesis, photorespiration and productivity of wheat and soybean genotypes. Physiol. Plantarum, 2012, 145: 369-383.

Çekiç C. Kurağa dayanıklı buğday (*Triticum aestivum* L.) islahında seleksion kriteri olabilecek fiziolojik parametrelerin araştırılması. Doktora tezi, Ankara, 2007, 114 s.

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Genetic Diversity in Bread Wheat for Heat Tolerance

Satyavir Singh DHANDA **Renu MUNJAL***

Department of Genetics & Plant Breeding, CCS HAU, Hisar

* Corresponding author e-mail: munjalrenu66@gmail.com

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ABSTRACT

Heat stress is a major yield limiting factor of wheat productivity in India. Therefore, development of high temperature tolerant wheat genotype is an important objective of wheat breeding. Significant differences for the years, genotypes and their interactions indicated that the responses of genotypes under heat stress varied not only among themselves, but also over the years. This suggested the need for evaluation of wheat genotypes over a wide range of environments/years for obtaining consistent expression for heat tolerance. There was considerable influence of high temperature stress in both years as grain yield was reduced nearly about 50% under late sown experiment as compared to that of timely sown conditions. Only one genotype, i.e., Raj 3765 have shown significantly high yield under both conditions indicating a complementation of high yield and heat resistance genes. The genotypes DBW 88, HD 2967, HI 1563, HI 1571, NIAW 1951, Raj 4083 and UP 2425 were with significantly high yield under timely sown, while HD 2932, PBW1 75, PBW 373, Raj 3765, UAS 320, WH1124, WH 1142 and WH1164 were promising for grain yield under heat stress and for physiological parameters undertaken. Indices of heat tolerance, i.e., heat response index (HRI) and heat susceptibility index (HSI) were the most promising traits because of their strong associations with heat tolerance parameters, namely, tetrazolium triphenyle chloride test (TTC) and cell membrane stability (CMS), and due to their significance for their mean values for majority of the genotypes under heat stress conditions. In addition, the above traits had a contribution of about 70% of the total variation for heat tolerance as revealed by principal component analysis in the material under study. The diversity and spatial analysis revealed that the contrasting genotypes in terms of heat tolerance and spatial distribution may generate the desirable segregating material for improvement of heat tolerance in bread wheat.

Keywords: Bread wheat, genetic diversity, heat tolerance

Introduction

Globally, high temperature stress is one of the major constraints in wheat productivity. The report of the Intergovernmental Panel on Climatic Change (IPCC) indicate that global mean temperature will rise 0.3 ^{0}C per decade reaching to approximately 1 and 3 ^{0}C above the present value by the years 2025 and 2100, respectively, (Hays *et al*., 2007; Asseng *et al*.,2011; Singh and Dwivedi, 2015). Therefore, the understanding of mechanisms of heat tolerance in view of crop improvement under heat stress has become an important objective. High temperature stress is indicated by increase in temperature above a threshold for a period of time which may cause irreversible damage to plant growth and physiological development (Wahid *et al*., 2007; Sareen *et al*., 2015). High temperature stress is a complicated mechanism comprising heat intensity (temperature degrees days), heat duration and fluctuations in high temperature affecting the key physiological processes, namely, photosynthesis, translocation and assimilate partitioning to the grain. The influence of heat stress is more visible when the temperature increases more than the threshold level influencing fluidity and viability of cell membranes leading to membrane injury and even up to death of the organ. (Schoffl *et al*., 1999).

Heat stress reduces growth and development of plants by disturbing lipid structure and reducing the membrane lipid, resulting in to loss of membrane integrity and irreversible damage of cell membranes

(Gigon *et al*., 2004, Harb *et al*., 2010). Increase in the fluidity of membranes resulting into disintegration of lipid bilayer membranes and membrane stability is considered as one of the reliable parameter of heat tolerance (Blum *et al*., 2001; Magdalena *et al*., 2015). It was also observed that lipid peroxidation resulted in to polyunsaturated precursors with hydrocarbon components, namely, ketones, malondialdehyde etc. (Garg and Manchanda 2009). Malondialdehyde constitutes a three carbon dialdehydes which is highly reactive and formed as result of metabolism of polyunsaturated and arachidonic fatty acid metabolism. (Hameed *et al.,* 2012). Membranes are sensitive to heat stress because the increase in temperatures alter the integrity and functions of membranes resulting into tertiary and quaternary structure of proteins. As the membranes contain proteins in a moving mosaic form and lipids sway between their monolayers and rotate around their axes and carbon bonds with acyl chains. The protein conformation is influence by both increase and decrease in the temperature resulting into unfolding of proteins (Pastore *et al.,* 2007). Membrane thermosability of wheat inbred lines has been utilized as the parameter of heat tolerance (Gupta *et al*., 2013). Genetic variability for membrane thermostability have been reported by various workers (Dhanda and Munjal, 2006;Dhanda and Munjal, 2012; Munjal and Dhanda, 2016) which may be used for improvement of wheat for heat tolerance.

Membrane based damage are mainly observed in crop plants as cell membrane stability for injury in plasma membrane, as chlorophyll fluorescence for injury in thylakoid membrane, and as viability test for stability of mitochondrial membrane (Essemine *et al.,* 2011; Jha *et al*., 2014). These tests are also being used in various crops for quantification for the level of acquired high temperature tolerance in plants (Wahid *et al*, 2007). For example, the 2, 3, 5 triphenyl tetrazolium chloride test is used to measure reduction of TTC by electrons from mitochondrial electron transport chain (Towill and Mazur, 1974). The inhibition of triphenyl tetrazolium chloride reduction is an indicator of respiratory enzyme inactivation or mitochondrial dysfunction which reflects the relative level of cell viability. Lipid peroxidation in membranes is presumed as one of the important cause for alteration of fluidity in membranes due to heat stress on plants (Mirza *et al*., 2013). The extent of membrane fluidity increases with increase in temperature stress and membrane composition. The lipids having comparatively more unsaturated fatty acids with short fatty acid chains or with low sterol content were generally more fluid and had better stability under heat stress (Allakhverdiev *et al*., 2008).

Heat stress has a major impact on chloroplasts, metabolism of carbon, stroma and on thylakoid lamellae during photochemical reactions. (Wang *et al*., 2009). The chloroplasts are the primary site of injury resulting in to distorted structure and organization of thylakoid membranes, reduction and swelling of grana (Rodríguez *et al*., 2005). Thus, the activity of the photosystem II is highly influenced and may even stop under severe high temperature stress (Morales *et al*., 2003). High temperature stress also influences soluble proteins, Rubisco binding proteins, activity of key enzymes involved in photosynthesis, namely, sucrose phosphate synthase, ADP-glucose pyrophosphorylase, and invertase resulting into reduced synthesis of starch and sucrose (Hasanuzzaman *et al*., 2013). Thylakoid membrane is measured in terms of chlorophyll fluorescence and highly susceptible to heat stress. Chlorophyll fluorescence related with photosystem II, reflected the extent of photosynthesis and has become one of the most reliable methods to determine photosynthetic efficiency under heat stress in crop plants (Kornyeyev *et al*., 2003).

During prolonged heat stress conditions, plant indicates the mechanisms which help in adaptations in short-term avoidance of heat stress through transpirational cooling, earlier flowering and other physiological mechanisms. In well irrigated conditions, high transpiration rate may cause to lower the canopy temperature from the ambient temperature up to 6°C (Fitter and Hay, 2002). Dense Root system of plant also contributes towards heat avoidance through obtaining more moisture through deep penetration and wide coverage in the soil (Fisher *et al*., 1982). High temperature also enhances the rate of reproductive development resulting into shorter time to photosynthesis contributing towards grain yield (Xue *et al*., 2004) as plant growth and flowering depend on accumulate degree days in temperature (Penuelas and Fillela, 2001). The short duration genotypes may escape from heat stress at later stages of plant growth, but the genotypes with high grain yield and comparatively longer duration under heat stress may possess the genes conferring protective mechanisms. Thus, identification of heat tolerant genotypes at different stages of plant growth for various traits related to heat stress and may help to combine heat tolerant genes in desired background. Therefore, the objectives of the present investigations are to determine the mean performance and genetic diversity of wheat genotypes for heat tolerance and the relative contribution of the physiological traits under heat stress conditions in wheat bread wheat.

Materials and Methods

Fifty-six genotypes of wheat (*Triticum aestivum* L.) differing in their performance under heat stress were grown under normal (second week of November) and heat stress environments (last week of December) during the years 2012-2013 and 2013-2014 under field conditions at the experimental area of Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar India. In order to create heat stress at anthesis and the reproductive stages, the sowing of the heat stress experiment was delayed by about 5 weeks. The experiments were conducted in a randomized complete block design with three replications for both environments and with a plot size of a 2 rows of 1.5m length with a 20×5 cm spacing within rows and between plants. Data on average of five competitive plants selected randomly from each row were recorded for grain yield per plant, days to 50% heading and physiological parameters as given below. The data for physiological traits was recorded reproductive stage of plant growth after 1st week of April in the heat stress experiment when the maximum day time ambient temperature was above 33°C in both years (Figure 4).

Chlorophyll fluorescence:

The Chlorophyll fluorescence measurements, F0, Fm, and Fv/Fm were taken 4 cm from the base of abaxial surface of flag leaves using a chlorophyll fluorometer, model 0S-30p Opti-Sciences under late sown environments at post anthesis stage. Measurements were taken on five randomly selected plants in each three replications. The fully expanded leaves were first acclimated to the dark for 15 minutes by fixing clips. The dark-adapted samples were continuously irradiated for 1second, provided by an array of 3 light emitting diodes in sensor. The florescence signals were detected as F0, Fm, and Fv/Fm.

Cell membrane stability:

Membrane thermostability was measured by the method of Sullivan (1972), modified later on by Ibrahim and Quick, (2001) was followed. A random sample of flag leaf from 3 plants from each replication was collected at post anthesis stage. At field, each sample was collected in sealed plastic bags and immediately kept in ice boxes. At laboratory all the samples were thoroughly rinsed twice in deionised water. The mid rib of flag leaves were removed gently by hand and about 5 cm portion from central flag leaf area was excised and cut in to 4 equal parts. Leaf was taken in glass test tubes. The test tubes samples were tightly covered with aluminum foil and submerged in water bath (maintained at 45°C) to depth equal to height of water in test tubes for 30-minute time periods after the treatment 10 ml deionised water was added and the test tubes were held overnight at 40°C in refrigerator. The samples were brought to room temperature and conductance was measured with an electrical conductivity meter after calibration with a standardized KCl solution (T1). The test tubes samples were then autoclaved at pressure of 0.10 Mpa for 10 minutes to completely kill plant tissues and release all the electrolytes. The conductance (T2) was measured again after autoclaved. Membrane thermostability was expressed in %. Membrane thermostability was measured by the formula given below.

Membrane thermostability= $1- (T_1/T_2)\times100$

Where

T1 = conductivity reading after heat treatment
T2 = conductivity reading after autoclaving

Canopy temperature depression:

A hand held infrared thermometer (Everest Interscience Inc.), model6110.4ZL, temp range low, USA for rapid indirect determination was used for instantaneous measurement of canopy temperature depression. Canopy temperature depression, the difference between air temperature (Ta) and canopy temperature (Tc), Measurement were taken when infrared thermometer viewed 100 per cent canopy cover and held at an angle of 30°C, approximately 50 cm above the canopy from horizontal and at 1 m distance from the edge of the plot end. Data was recorded between 12:00 hrs to 14:00 hrs. Measurements was taken when the sky was clear and there was little or no wind.

Tetrazolium Triphenyl Chloride Assay:

TTC assay was done following the method of Ibrahim and Quick (2001). Two sets of long flag leaves (3.5 cm) were excised, rinsed in deionized water, and placed singly in a test tube with 0.1 ml of deionized water. Out of two sets, the first set was kept at 25° C for 90 min as control set, and the second set was placed in a water bath at 49°C for 90 min. Immediately following the 25°C and 49°C treatments 10 ml of TTC solution (0.8% TTC in 0.05 M NaPO4 buffer, pH 7.4, and 0.5 ml/l Tween 20) was added per tube and vacuum-infiltrated for 10 min. The leaves were incubated in the TTC solution for 24 h at 25°C in dark. After incubation, the leaves were removed and rinsed with distilled water, placed individually in separate tubes containing 2 ml of 95% ethanol, and submerged for 24 h at 25°C in the dark. The level of acquired high temperature tolerance was determined

by measuring the percentage reduction of TTC to formazan using the following formula.

TTC= (ODh/ ODc)×100.

Where ODh referred to the mean optical density (530 nm) values for the heat-stressed set (49°C for 90 min), and OD referred to the mean optical density for the control set (25°C for 90 min).

Heat susceptibility index for grain yield (HSI): Heat susceptibility index (HSI) was calculated over stress and non-stress environment. The HSI of individual genotype was calculated by the method suggested by Fischer and Maurer (1978) with the following formula:

HSI for grain yield = 1-(Y/YP)/D.

Where, D = 1- (X/XP), Y and YP is grain yield for individual genotypes under heat stress and normal environment, respectively. X and XP represents mean grain yields of all genotypes under heat stress and normal environment, respectively.

Heat Response Index for grain yield (HRI):

The heat tolerance of individual genotype was computed using the formula given by Bidinger *et al*., (1987) as HRI = (Ya- Yest)/SES

Where, Yest and Ya are the estimated yields by regression and actual yields, respectively, and SES is the standard error of the dependent trait, significant positive values of HRI denote heat tolerance, while significant negative values denote heat susceptibility of genotype negative values denote heat susceptibility of genotype.

Statistical Analysis:

Simple correlation coefficients among various traits were calculated by the method given by Snedecor and Cochran (1981). Principal components analysis was performed by the method given by (Everitt and Dunn, 1992). The genotypes were further subjected to cluster analysis by following the method of (Everitt, 1993; Eisen *et al*., 1998). The statistical analysis was performed by using the softwares, namely, OPSTAT and SPSS 19.

Results

Analysis of variance and mean performance

The mean sum of squares due to genotypes, years and for the interactions between genotype and years were significant for almost all the traits under heat stress conditions, but nonsignificant under timely sown conditions except for grain yield (Table 2). Therefore, under timely sown conditions the data for all the traits expect for grain yield was excluded for further analysis. The considerable differences for the genotypes among themselves and over the years existed and to get consistent performance these should be evaluated over some more number of years. The genotype Raj 3765 was significantly higher yielder than overall mean values under both timely (16.03*) and late sown (11.46**) conditions along with other heat stress related traits indicating a desirable combination high yielding and heat tolerant genes in this genotype which may be exploited for obtaining high grain yields under heat stress affected environments (Table 3). On the other hand, the genotypes DBW 88 (17.30**), HD 2967 (17.18**), HI 1571 (18.08), NIAW 1951 (16.12*), Raj 4083 (18.27**), UP 2425 (18.91**), were significantly higher yielder under timely sown conditions and may serve as the good source of high yielding attributes.

The mean values of other genotypes, namely, HD 2932 (12.41**), PBW 373 (10.51*), UAS 320 (11.02*), WH1124 (10.28*), WH 1142 (10.83*) and WH1164 (12.75**) were significantly higher than overall mean for grain yield and one or more heat stress related traits, under heat stress conditions. The better performance of above genotypes may be exploited for incorporation of heat stress tolerant genes in high yielding background. The information from the parental details revealed that the heat tolerance in above genotypes might have been contributed by the renowned stress tolerant varieties like Kauz, Veery, GW 322, *Ae. Squrossa* and Pastor (Table 1). The genotypes HD 3059, NIAW 34, Sonalika, WH 1021, WH 1123, and WH 730 were also significantly superior to the of the physiological traits, namely, mitochondrial viability test TTC, CMS, efficiency of photosystem II (chlorophyll fluorescence) and high rate of transpiration (CTD) under heat stress, but not for grain yield. Such types of material may be specifically used for improvement of heat tolerant traits.

The early heading genotype may escape heat stress particularly in the areas subjected to under late heat stress. Thus, the genotypes DBW 16 (86.00**), HD 2851 (83.00**), HI 1563 (84.72**) MP 3379 (86.00**), NW 5054 (85.39**), NI 5439 (86.06*), PBW 590 (85.00**), PBW 688 (83.33**), UP 2425 (85.61*), WH 1021 (83.67**), WH 147 (82.33**) and WH 730 (85.33**) tended to escape from heat stress as they were among the entries which were significantly earlier in heading than overall mean (90.36±3.69).

Heat sensitivity index (HSI) and heat response index (HRI) are based on relative reduction of grain yield from normal to heat stress environments. HSI provides the relative reduction irrespective of effects of other

variables, while HRI can remove the intervening influence of other traits namely, grain yield potential and escape mechanism due to early heading etc. The genotypes DBW 90, MP 3336, NI 5439, Raj 3765, Sonalika, WH 730, WH 021, WH 1123, WH 1124 and DBW 16 performed significantly better over their mean values for both i.e. HSI and HRI, while, NIAW 34, HPW 251, UP 2845, UP 2843, WH 1080 were better for either of these traits. Thus, based on grain yield, physiological traits and heat tolerance indices the genotypes Raj 3765, NI 5439, WH 730, WH 1021, WH 1123, WH 1124 and WH 1142 may be termed as heat resistant /tolerant, on the other hand the genotypes HD 2985, HD 3043, HD 3090, HUW 667, K 1114, NIAW 1951, PBW 343, Raj 4083, UP 2425, UP 2844, WH 147 and WH 542 showed heat susceptible response.

Correlations

Correlation coefficient analysis revealed that increased grain yield under heat stress conditions was significantly contributed by TTC (0.314*) CMS (0.365**) late heading (0.446**), chlorophyll fluorescence (0.330*), CTD (0.539**), HSI (-0.661**) and HRI (0.668**) (Table 4). This suggested the considerable role of above traits selecting for higher grain yield under heat stress. The positive correlations of TTC with other physiological traits, chlorophyll fluorescence (0.504**), HSI (-0.391**) and HRI (0.394**) indicated that heat resistant genotypes, in general, had better viability of mitochondrial cell viability as well better stability of thylakoid membrane. Days to heading appeared to be independent of physiological traits except with CTD (0.274*) indicating that heat tolerant plant may be of both types, i.e., early and late heading, but about transpiration rate the genotypes with delayed heading had higher rate of transpiration. The HRI appeared to be the most promising trait followed by HSI in terms of association with other traits and as an index of heat tolerance, because this index is free from influences of intervening variables such as escape from heat stress and high yielding genes. The strong association of HSI with HRI (-0.847**) suggested that the HSI was also effective to that of HRI for categorization of genotypes in their response towards heat stress in present set of material.

Principal components

To find out a desirable combination of few important traits required for high grain yield under heat stress conditions and to reduce the redundancy of the variables for simplification the selection process principal component analysis was performed (Table 5). The scree plot of principal components (Figure 1) indicated that first four principal components accumulated for 86.6% of the total variation. The first component accounted the highest contribution (47.25%) in total variation for heat tolerance, followed by the second (19.55%) and third (12.41%). This revealed that HSI (-0.907) followed by HRI (0.877) appeared to the important traits with a contribution of 47.25% of the total variation under heat stress conditions indicated by first component. The second component revealed that the physiological traits, namely, CMS (0.983) and TTC test (0.851) had a contribution of 20% towards total variation. While days to heading (0.963) in third component and chlorophyll fluorescence (0.989) in fourth component were a part of 12.41% and 7.33% of total variation under heat stress. Canopy temperature depression, however was the last contributing trait (1.003) in total variation due to heat stress with a contribution of 6.82%. Thus, HSI followed by HRI, CMS and TTC were the important yield determines of grain yield under heat stress conditions in present study.

Cluster analysis

To find out diversity for heat tolerance all the genotypes were classified into six different clusters (Figure 2). First cluster comprised a group of 6 entries, namely, Sonalika, WH 1179, WH 730, DBW 16, WH 1021 and HP 1744. Majority of these genotypes were significant for one or more physiological traits including heat tolerant resistance/ tolerant indices indicating heat tolerance response. The second cluster consisted of a group of 8 entries having moderate heat tolerant response. Among these the genotypes NW 2036, WH 1124, NIAW 34 and Raj 3765 showed significance for HRI and or HSI in addition to other physiological parameters. The remaining genotypes in this group were also better for one or more parameters of heat stress. The third cluster comprised a group of 11 genotypes with mix responses towards heat stress. The genotypes WH 1123, WH 1164, DBW 90 and HD 2932 indicated significance for one or more heat tolerant traits, while DBW 88, WH 1126, WH 542, HD 3043 and PBW 343 had sensitive type response. Group 4 and 5 comprised majority of heat sensitive genotypes except few genotypes indicating heat tolerant response in one or more characters. In group 6 all the genotypes, in general, had heat sensitive response except NW 2036, HPW 251 and MP 3379 which were significantly better at least one character related to heat tolerance. Thus, heat stress revealed considerable influence on grouping of genotypes, for example, first and second group consisted heat tolerant genotypes, third and fourth groups contained mixed type of genotypes having moderately heat tolerant to heat sensitive genotypes. In fifth and sixth group the response of genotypes was in general heat sensitive.

Scatter plot of genotypes

In addition to the diversity analysis which indicates arbitrary distances within and among the groups of genotypes, the scatter plot of the genotypes further elaborated their diversity in terms of spatial distance (Figure 3). Genotypes plotted on scatter plot according to PC 1 and PC 2 depicting 66.8% of total variation. PC1 constituted 47.25% of the total variation loaded with HSI and HRI involving as major components of heat tolerance, while PC2 represented 19.55% of the total variation loaded with CMS and TTC. the genotypes, Raj 3765, WH 1179, NIAW 34, WH 730, UP 2843, WH 1124, PBW 343 and WH 1021were better for both axis and showed a desirable combination of heat tolerance indices (HSI and HRI) and other heat tolerant traits namely, CMS and TTC as these genotypes had better performance for on both axis, while the genotypes PBW 373, DBW 90, PBW 373 WH 1164, WH 1123, HPW 251, MP 3336, UP 2845 and WH 1142 had better performance for the traits loaded on PC1, i.e., HSI, and HRI. The genotypes DL 153-2, HI 1571, PBW 644, HD 3059 and HP 1744, HD 3059, NW 2036, Sonalika, WH 542 and DBW 16 were better only CMS and TTC loaded on PC 2. This may probably due to their inherent characteristics as most of, many of above genotypes had one or more parent as stress tolerant in their pedigrees, for example, the varieties PRL "S", VE#5 'S', Pastor, Parula, Raj 3765, GW 173, PBW 175, Kauz and *Ae. Sq* (Taus) are the promising stress tolerant varieties of wheat which are one or more parent in the pedigrees of above genotypes (Table 1). The other genotypes which were near to origin or lower position in graph indicated moderately heat tolerant to heat sensitive response.

Discussion

It is now well established that various important cellular functions depend on proper working various membrane related activities, involved in photosynthesis, respiration and cellular activities for nutrient transport. (Vigh *et al*., 1998; Kültz 2005; Vigh *et al*., 2007; Crul *et al*., 2013). Due to high temperature stress the leakage of the plasma membrane causes severe protein denaturation. Lipid peroxidation is also one of the most deleterious processes occur under severe heat stress conditions in the membranes resulting into distortion of fluidity in membranes. The deleterious effects on membrane-based processes may be on plasmalemma, thylakoid membranes and mitochondrial membranes. Heat stress increases the fluidity in the membranes leading to disintegration of the lipid bilayer and proteins. Damage in the membranes is generally taken as a parameter for heat stress tolerance in terms of lipid destruction (Asthir, 2015). Lipid denaturation are mainly formed due to polyunsaturated precursors including some hydrocarbon components, namely, ketones, malondialdehyde and other related products (Garg & Manchanda, 2009). Malondialdehyde is a three carbon compound produced as a result of peroxidation of polyunsaturated fatty acids and metabolism of arachidonic acid. Some of these products are used to produce coloured reaction to find out the extent of damage in the membranes (Hameed *et al*., 2012). Increase in fluidity of cell themembrane resulted in activation of signal which are lipid-based cascades and increased cyto-skeletal reorganization (Bita and Gerates, 2013).

Light energy may be utilized by the plants in three different ways, for example, in photosynthesis, dissipated as heat energy and may be re-emitted as light in terms of chlorophyll fluorescence. As these processes occur in competition, therefore by measuring one process, i.e. chlorophyll fluorescence the extent of photosynthetic capacity in photosystem II (PSII) may be determined which has become one of the reliable tools for measuring photosynthesis under stress conditions (Czyczyło *et al.,* 2013; Georg *et al*., 2013, Hemantaranjan *et al*., 2014). Therefore, the genotypes PBW 373, Raj 3765, UAS 320, WH 1124, WH 1142, and WH 1164 may be utilised not only for improvement of grain yield, but also for majority of heat tolerance related traits under present study. This indicated complementation of high yielding genes with heat tolerance/resistance genes in these varieties which may provide adaptability over wider area under heat stress conditions. The varieties PBW 373 and Raj 3765 are old varieties released for cultivations under heat stressed areas of India, while the variety WH 1124 and WH 1142 were recently released for cultivation under late sown and drought prone areas of northern India, respectively (Anonymous, 2015). Among other genotypes which performed better for physiological traits and indices of heat tolerance, WH 730 is registered for late heat tolerance, while HD 3059, Sonalika, WH 1021 are widely cultivated under the areas subjected under heat stress in India (Anonymous, 2014). The above genotypes along with UP 2845, NW 2036, etc. which had also better performance for physiological traits and indices of heat tolerance indicating a higher proportion of genes contributing towards heat tolerance than grain yield potential may be used for incorporation of these desirable traits in existing wheat germplasm.

Various research workers revealed that formazan production was comparatively more in heat tolerant genotypes than in heat sensitive (Wahid *et al*., 2007; Varshney *et al*., 2011). This may probably due to because the heat sensitive cultivar has poor tolerance mechanisms to face the initial moderate level of heat stress and consequently the genotype may not develop the adaptive mechanism to face the severe stress conditions. While the tolerant plants could activate the adaptive mechanisms during initial moderate heat stress and become capable to tolerate the severe heat stress. This may be due to increased formazan production during heat stress (Block and Brouwer, 2002). The tetrazolium test is based on the fact that viable cells can reduce tetrazolium salts into soluble formazans under heat stress (Berridge *et al.,* 1967). This assay indicates the ability of a plant tissue to continue the electron transport and the inhibition of tetrazolium tetrazolium triphenyl chloride results into inactivation of dehydrogenase thus producing less formazan production under heat stress (Chen *et al*., 1982). The amount of tetrazolium triphenyl chloride is decreased by the mitochondrial dehydrogenases and the production of formazan is regulated by cytochrome oxidase in the mitochondria (Rich *et al*., 2001). Thus, cell viability test is being widely for quantification of acquired thermal tolerance in plants (Farooq *et al*., 2011), because, inhibition of tetrazolium triphenyl chloride reduction is an indicator of mitochondrial dysfunction or inactivation of respiratory enzymes resulting in to the variable response of cell viability. The genotypes, namely, NIAW 34, Raj 3765 and WH 1021, having high values of formazan production or better mitochondrial viability also better performance for heat stress related traits may be used as the source for improvement of heat tolerance in bread wheat.

To have effective selection for heat tolerance the number of characters as well as the genotypes need to be reduced in order of their merit. Thus, the extent of genetic diversity is a pre-requisite for selection and improvement of heat tolerance (Parker *et al.,* 2002; Sharma *et al.,* 2014). The spatial diversity for heat tolerance may further help to may provide beneficial alleles for improvement of heat tolerance and yield potential (Dodig *et al*., 2010; Sun *et al*., 2013). The heat tolerant includes the genotypes like Sonalika which is an old, widely grown variety under heat stress and released for cultivation under heat stress areas of India since 1966. The other varieties, namely, WH 1124 and HD 3059 are recent release, while DBW 16, WH 1021, NIAW 34, PBW 644, NI 5439, Raj 3765 are old varieties released for cultivation under heat/ drought stress areas of India (Anonymous, 2015). The genotype WH 730 has unique characteristics for heat tolerance in addition to its higher yields under heat stress for which it was registered for heat tolerance with the National Bureau of Plant Genetic Resource, New Delhi. The resistance in these varieties was primarily contributed by the parents, namely, Kauz, Pastor, *Aegilops squrossa*, and some already very popular varieties cultivated under heat/drought stress areas of India, namely, GW 322, Lok 1 etc. The genotypes may be used with high yielding well adapted genotypes to develop a desirable material under heat stress conditions.

Conclusions

The genotypes Raj 3765, NIAW 34, Sonlika, WH 730, PBW 373, WH 1124, WH 1164, WH 1123, MP 3336, UP 2845, WH 1142 etc. were important for low reduction of grain yield under heat stress, while DL 153-2, HI 1571, HD 3059, HP 1744, NW 5054, DBW 16 etc. were important for better performance of physiological traits, namely, CMS, TTC and CTD. In addition, the genotypes Raj 3765, UAS 120, WH 1124 and WH 1164 etc. indicated a desirable combination resistance potential with yield potential which may provide higher grain yield over the wide range of area, while the genotypes DBW 88, HD 2967, HI 1563, HI 1571, NIAW 1951, Raj 4083 and UP 2425 were significantly higher yielder under timely sown conditions. HRI followed by HIS, TTC and CMS were the most promising traits because of their strong associations with other heat tolerance parameters and significance for their mean values for majority of the genotypes under heat stress conditions. The diversity analysis further revealed that the contrasting genotypes in terms of heat tolerance which may be used for improvement of heat tolerance in bread wheat.

Table 1. Pedigrees bread wheat of the genotypes

Sr.	Genotypes	Parentage details	Sr.	Genotypes	Parentage details
1	DBW 16	RAJ 3765/WR 484//HUW 468	29	PBW 343	ND/VG1944//KAL//BB/3/YACO'S'/4/ VEE#5'S'
2	DBW 88	KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	30	PBW 373	ND/VG9144//KAL/BB/3/YACO'S'/4/ VEE#5'S'
3	DBW 90	HUW468/WH730	31	PBW 590	WH594/RAJ3814//WH485
4	DBW 95	K9908/PBW534	32	PBW 644	PBW175/HD2643
5	DL 153-2	TANORI 71/NP 890	33	PBW 660	WG6761/WG6798
6	HD 2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ ATTILA	34	PBW 688	W7561/HD2808
7	HD 2851	CPAN3004/WR426/HW2007	35	RAJ 3765	HD2402/VL639
8	HD 2932	KAUZ/STAR//HD2643	36	RAJ 4083	PBW343/UP2442/WR258/UP2425
9	HD 2967	ALD/CUC//URES/HD2160M/HD2278	37	Sonalika	1154.388/AN/3/YT54/N 10B/LR 64
10	HD 2985	PBW 343/PASTOR	38	UAS 320	UAS257/GW322//DWR195
11	HD 3043	PJN/BOW//OPATA*2/3CROC_1/A. Squarrosa(224) //OPATA	39	UP 2425	HD 2320/UP 2263
12	HD 3059	KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	40	UP 2843	CPAN073/UP2382//OPATA/RAYON// KAUZ
13	HD 3090	SFW/VAISHALI//UP2425	41	UP 2844	HD2844/FRTL/AGRI//NAC
14	HI 1563	MACS2496*2/MC10	42	UP 2845	CPAN4022/UP2382//KAUZ//BOW/NKT
15	HI 1571	Raj 3077/WLT277//HW2045	43	WH 730	CPAN 2092/Improved Lok-1
16	HPW 251	WW24/LEHMIP2-U149	44	WH 1021	GW296/SONAK
17	HUW 667	RAJ1972/HUW468	45	WH 1080	PRL/*2PASTOR
18	K 1114	HP1731/HUW234	46	WH 1105	MILAN/S87230//BABAX
19	MP 3304	GW322/J485	47	WH 1123	NI5663/RAJ3765//K9330
20	MP 3336	HD2402/GW173	48	WH 1124	MUNIA/CHTO//AMSEL
21	MP 3379	Raj 3077/DL788-2	49	WH 1126	WBLL1*2/VIVITSI
22	NW 2036	BOW/CROW/BUC/PVN	50	HP 1744	CIANO/PARULA//CHILERE/GARUDA
23	NIAW 34	CNO 79/PRL "S"	51	WH 1142	OEN/Ae. Sq. (TAUS)/FCT/3/2*WEAVER
24	NIAW 1951	HD2781/NIAW301	52	WH 1164	RL6043/4*NAC//2*PASTOR
25	NW 2036	BOW/CROW/BUC/PVN	53	WH 1179	OASIS/SKAUZ//4*BCN/3/3*PASTOR
26	NW 5054	THELIN//2*ATTILA*2/PASTOR	54	WH 147	E4870/C303//5339/PV18
27	NI 5439	NI8883/MP1055	55	WH 542	JUPATECO/BLUE JAY//URES
28	PBW 175	HD 2160/WG 1025	56	WH 711	ALD'S'HUAC//HD2285/3/HFW 17

Table 2. Mean sum of squares of wheat genotypes for various traits under heat stress conditions

Source of Variation	DF	Grain yield (TS)	Grain yield (LS)	TTC (LS)	CMS (LS)	Days to 50% heading (TS)	Days to 50% heading (LS)	CFL (LS)	CTD (LS)
Replication	4	6.41	3.82	116.63	13.30	0.82	1.43	0.0005	0.43
Year (Y)	1	161.35	541.95*	0.95	826.98*	1.01	137.52	0.0000	25.07
Genotype (G)	55	26.61	7.92	144.43	95.77	49.50	45.88	0.0086	2.50
Interaction (Y×G)	55	0.33	1.48	89.26	104.41	8.62	11.87	0.0001	1.78
Error	220	3.36	1.82	12.97	14.30	2.48	2.60	0.0007	0.15
Total	335								

*, **: Significant at 5% and 1% level of significance, TS: Timely Sown conditions, LS: Late Sown conditions, TTC: Tetrazoliom Tripenyl Chloride test, CMS: Cell Membrane Stability index, CFL: Chlorophyll Fluorescence , CTD: Canopy Temperapture Depression.

Table 3. Mean performance of bread wheat genotypes for various traits under heat stress conditions

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
1	DBW 16	10.25	5.45	59.40*	62.84	86.00*	0.77*	5.90	1.07	0.41
2	DBW 88	17.30**	5.28	54.64	58.92	89.61	0.72	5.80	1.32	-1.45
3	DBW 90	13.30	9.31	52.27	58.85	91.67	0.71	7.50	0.68**	1.79
4	DBW 95	6.25	4.12	36.6	36.18	90.28	0.62	2.33	1.11	-0.97
5	DL 153-2	8.14	3.35	62.94**	66.19*	93.67	0.66	3.23	1.34	-2.46
6	HD 2733	10.42	4.15	48.00	52.16	95.17	0.70	3.67	1.37	-2.93
7	HD 2851	13.02	4.35	45.98	54.51	83.00**	0.68	4.17	1.52	-1.19
8	HD 2932	12.75	12.41**	53.16	59.28	91.17	0.71	6.93**	1.13	-0.77
9	HD 2967	17.18**	4.46	43.32	50.23	84.61**	0.62	3.37	1.33	-0.44
10	HD 2985	9.64	5.45	47.42	51.24	92.00	0.69	6.17	0.99	-0.62
11	HD 3043	12.42	7.55	55.33	59.75	94.67	0.65	6.23*	0.89	-0.24
12	HD 3059	13.22	7.65	65.20**	67.85*	89.67	0.81**	6.47*	0.96	0.60
13	HD 3090	10.05	5.61	40.62	44.45	93.00	0.69	5.33	1.01	-0.85
14	HI 1563	16.22*	4.07	50.94	58.08	84.72**	0.73	2.70	1.53	-1.88
15	HI 1571	18.08**	6.07	50.57	69.19**	89.94	0.62	6.23	1.52	-3.05
16	HPW 251	7.62	5.31	43.36	52.86	86.28*	0.63	5.43	0.69**	1.3

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
17	HUW 667	10.98	4.11	40.41	46.92	87.00	0.70	3.03	1.36	-1.11
18	K 1114	14.42	7.78	42.78	62.59	92.00	0.65	5.43	1.05	-0.27
19	MP 3304	11.35	3.63	43.49	49.09	87.67	0.65	5.37	1.28	-0.88
20	MP 3336	12.73	9.38	52.13	47.99	86.67	0.69	6.47*	0.60**	3.17**
21	MP 3379	11.05	4.45	36.74	45.56	86.00*	0.71	7.60*	1.36	-0.92
22	NW 2036	13.08	8.75	61.31*	65.52*	95.00	0.78**	5.43	0.76*	0.61
23	NAIW 34	12.52	9.38	63.35**	69.75**	90.00	0.77*	6.23	0.57**	2.55**
24	NIAW1951	16.12*	8.25	37.1	51.56	89.39	0.64	6.03	1.11	0.06
25	NW 2036	14.75	8.36	39.65	53.15	90.06	0.73	6.67	0.99	0.59
26	NW 5054	14.90	4.38	53.73	57.1	85.39*	0.63	3.60	1.61	-2.45
27	NI 5439	11.25	5.22	41.58	49.77	86.06*	0.73	6.20	1.22	-0.25
28	PBW 175	12.42	3.25	53.52	56.56	88.00	0.65	3.47	1.68	-3.12
29	PBW 343	11.34	9.5	55.56	64.55	96.00	0.67	6.03	0.57**	0.87
30	PBW 373	15.38	10.51*	51.98	60.81	98.00	0.62	6.93**	0.72**	0.78
31	PBW 590	10.29	5.48	49.81	55.61	85.00**	0.71	4.87	1.07	0.64
32	PBW 644	13.49	7.25	67.73**	66.11	88.33	0.75	5.20	1.06	0.37

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
33	PBW 660	15.31	5.28	35.40	40.58	88.06	0.70	5.90	1.49	-2.29
34	PBW 688	10.79	5.12	59.58*	57.02	83.33**	0.69	5.07	1.20	0.43
35	Raj 3765	16.03*	11.46**	64.45**	69.72**	93.67	0.75	6.90**	0.65**	2.39**
36	RAJ 4083	18.27**	7.67	35.81	52.59	88.72	0.74	4.07	1.32	-1.27
37	Sonalika	12.62	8.47	58.58	66.91*	90.00	0.70	5.77	0.75**	1.59*
38	UAS 320	14.40	11.02*	43.45	55.55	99.33	0.65	6.53*	0.72**	0.24
39	UP 2425	18.91**	9.35	51.35	61.05	85.61*	0.71	5.13	1.15	0.81
40	UP 2844	13.35	6.21	47.98	52.87	98.00	0.63	6.43**	1.22	-2.68
41	UP 2845	10.48	8.45	42.23	50.09	97.67	0.65	5.33	0.44**	0.82
42	WH 1021	5.86	4.58	60.30*	65.00	83.67**	0.75	7.23**	0.50**	1.85**
43	WH 1080	9.62	6.4	46.15	40.41	99.33	0.65	5.47	0.76**	-1.24
44	WH 1105	11.38	6.88	51.41	46.25	96.00	0.76	6.80**	0.90	-0.77
45	WH 1123	13.57	11.2	59.61*	58.45	92.00	0.77*	6.40	0.56**	2.55**
46	WH 1124	11.91	10.28*	64.82**	63.85	95.83	0.77*	6.93**	0.31**	2.45**
47	WH 1126	10.05	6.2	58.04	59.37	90.50	0.79**	5.30	0.87	0.28
48	HP 1744	5.73	4.88	61.67*	64.72	88.67	0.75	5.73	0.95	-0.26

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
49	WH 1142	15.28	10.83*	49.79	47.25	93.00	0.80**	6.37	0.66**	2.21**
50	WH 1164	15.12	12.75**	56.98	63.52	91.00	0.79**	6.53*	0.55**	3.38**
51	WH 1179	15.35	9.48	58.67	68.59**	90.06	0.68	5.27	0.87	1.46**
52	WH 147	13.98	5.58	39.92	53.19	82.33**	0.65	6.20	1.37	-0.22
53	WH 542	7.18	8.34	57.04	62.07	90.33	0.75	3.67	1.22	-1.36
54	WH 711	8.48	4.45	42.68	46.51	94.67	0.64	6.17	1.08	-1.72
55	WH 730	8.29	6.62	55.57	70.91**	85.33**	0.75	5.50	0.46**	2.53**
56	UP 2843	12.72	9.35	61.59*	67.49*	97.00	0.69	6.10	0.60**	0.93
Mean		**12.44**	**7.06**	**51.14**	**56.95**	**90.36**	**0.70**	**5.55**	**1.00**	**0.00**
SE(m)		1.497	1.10	2.94	3.09	1.32	0.02	0.32	-	-
SE(d)		2.117	1.56	4.16	4.37	1.86	0.03	0.45	0.09	0.45

*, **: Significant at 5% and 1% level of significance

Table 4. Correlation coefficients of bread wheat genotypes for among various traits under heat stress conditions

Character	TTC	CMS	Days to 50% heading	CFL	CTD	HSI	HRI
Grain yield	0.314*	0.365**	0.446**	0.330*	0.539**	0-.661**	0.668**
Tetrazoliom tripenyl chloride test (TTC)	1.000	0.797**	0.101	0.504**	.159	-0.391**	0.394**
Cell membrane (CMS) stability index		1.000	-.007	0.342*	0.182	-0.298*	0.361**
Days to 50% heading			1.000	-.095	0.274	-0.438**	0.000
Chlorophyll Fluorescence (CFL)				1.000	0.255	-0.342*	0.468**
Canopy temperature depression (CTD)					1.000	-0.549**	0.498**
Heat sensitivity index (HSI)						1.000	-0.847**

*, **: Significant at 5% and 1% level of significance; HRI: Heat Respone Index

Table 5. Principal components of various traits in bread wheat for heat tolerance

Character	PC 1	PC 2	PC 3	PC 4	PC 5
Heat sensitivity index	-0.907	-0.023	-0.223	0.023	-0.069
Heat response index	0.877	0.022	-0.258	0.056	0.032
Cell membrane stability index	-0.058	0.983	-0.097	-0.091	0.048
Tetrazoliom tripenyl chloride test	0.124	0.851	0.132	0.191	-0.048
Days to 50% heading	0.00	-0.013	0.963	-0.042	0.035
Chlorophyll Fluorescence	-0.02	0.002	-0.034	0.989	0.023
Canopy temperapture depression	0.003	0.000	-0.001	0.012	1.003
Grain yield	0.15	0.067	0.197	0.109	0.066
Eigen value	3.78	1.564	0.993	0.587	0.545
Variance	47.251	19.555	12.412	7.334	6.818
Cumulative variance	47.251	66.806	79.218	86.552	93.37

Figure 1. Scree Plot principal components for various traits in bread wheat

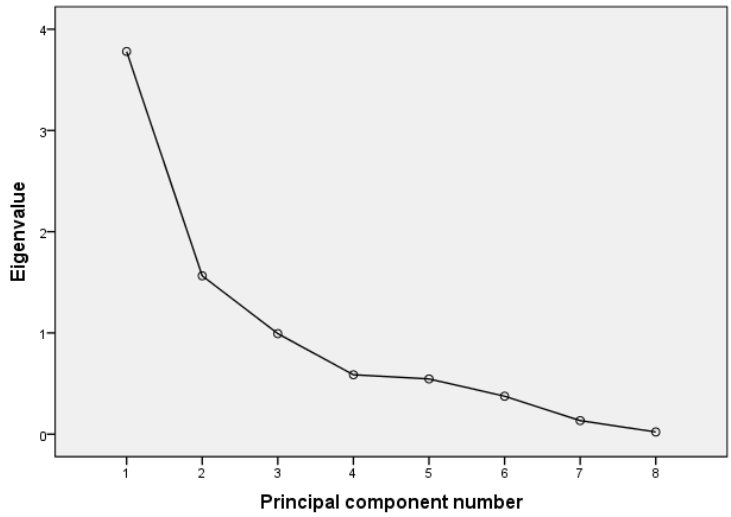


Figure 3. Scatter diagram of bread wheat genotypes under heat stress conditions

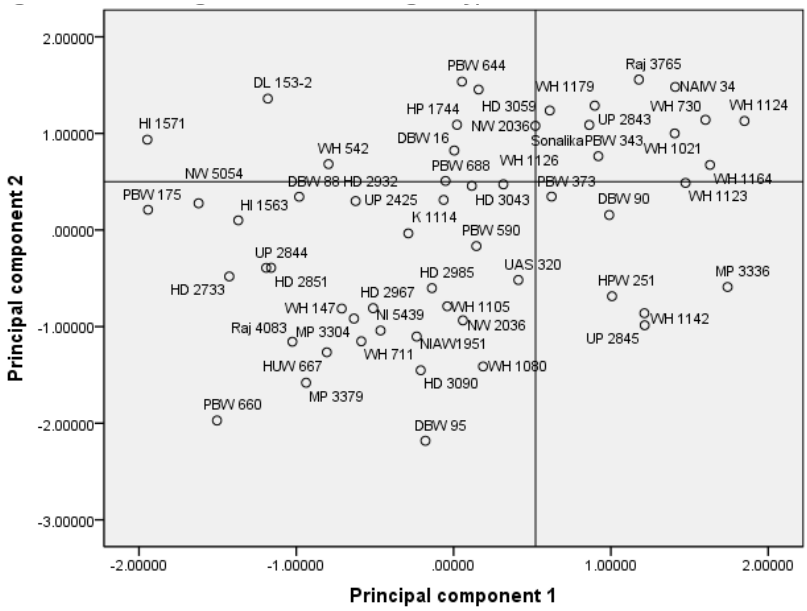
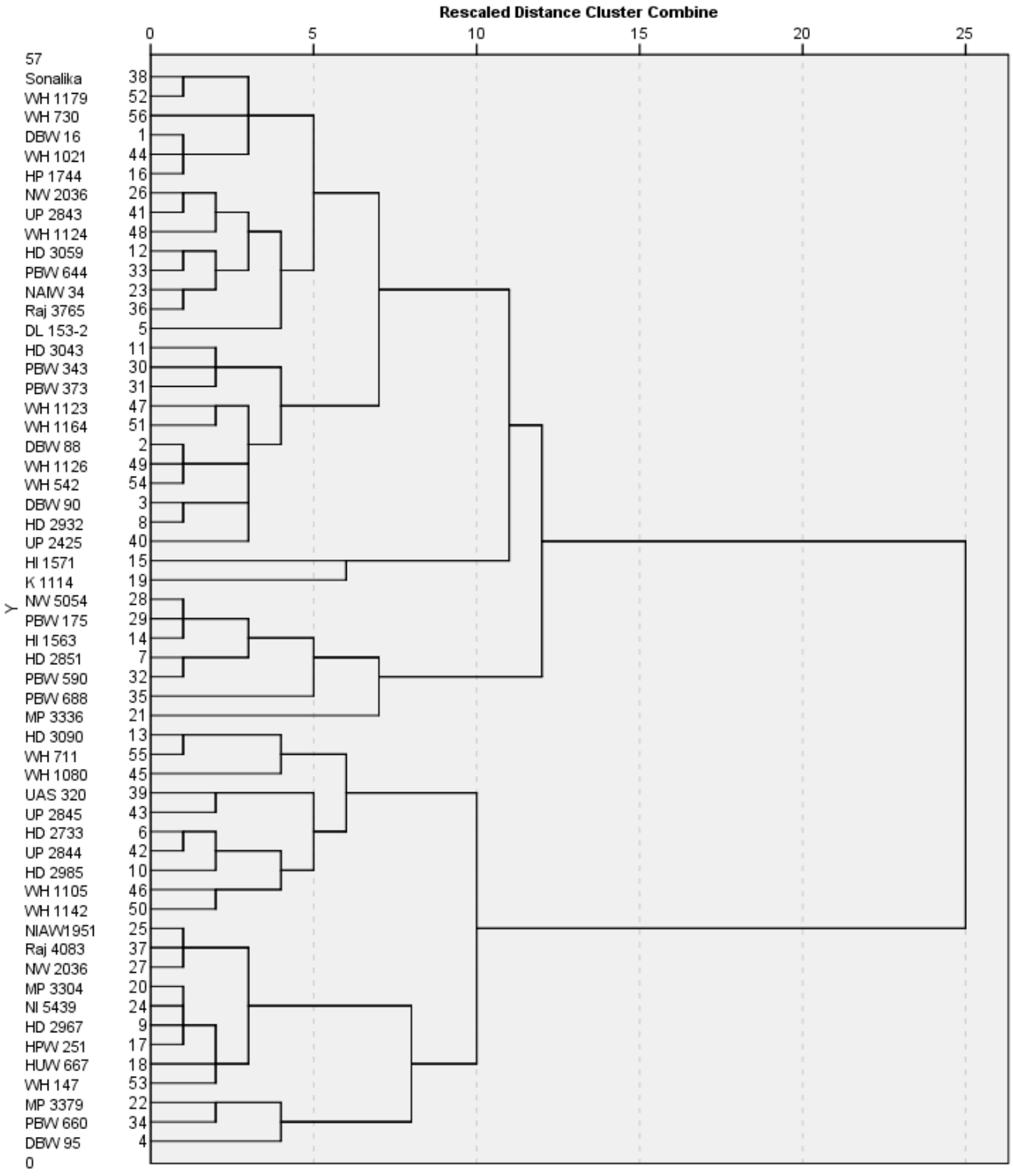


Figure 4. Weekly maximum and minimum temperature during the growth season

Figure 2. Clustering of bread wheat genotypes under heat stress conditions



References

Allakhverdiev SI, Kreslavski, VD, Klimov VV (2008). Heat stress: An overview of molecular responses in photosynthesis. *Photosyn. Re*s. 98: 541-550

Anonymous (2014). Progress Report of All India Coordinated Wheat & Barley Improvement Project 2013-14, Director's Report. Ed. Indu Sharma, Indian Institute of Wheat and barley Research, Karnal, India, p. 120.

Anonymous (2015). Progress Report of All India Coordinated Wheat & Barley Improvement Project 2014-15, Director's Report. Ed. Indu Sharma, Indian Institute of Wheat and barley Research, Karnal, India, p. 116.

Asseng S, Fosterw I, and Turner NC (2011). The impact of temperature variability on wheat yields. *Global Change Biol.* (online). doi:10.1111/j.1365-2486.2010.02262.x

Asthir Bavita (2015). Protective mechanisms of heat tolerance in crop Plants. *J. Plant Interactions* 10(1): 202-210.

Berridge MV, Tan AS, McCoy KD and Wang R. (1967). The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. *Plant Physiol*. 42: 1423-1426.

Bidinger FR, Mahalakshmi V and Rao GDP (1987). Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. I. Factors affecting yields under stress. *Aust. J. Agric. Res*. *38*:37-48.

Bita CE and Gerats T (2013). Plant to lerance to high temperature in a changing environment: scientific fundamental sand production of heat stress-tolerant crops. *Plant Sci.* 4(273):1-18**.**

Blum A, Klueva N and Nguyen HT (2001). Wheat cellular thermotolerance is related to yield under stress. *Euphytica*. 117, 117-123.

Chen HH, Shen ZY and Li PH (1982). Adaptability of crop plants to high temperature stress. *Crop Sci*. 22: 719–725.

Crul T, Toth N, Piotto S, Literati-Nagy P, Tory K, Haldimann P, Kalmar B, Reensmith L, Torok Z, Balogh G, Gombos I, Campana F, Concilio S, Gallyas F, Nagy G, Berente Z, Gungor B, Peter M, Glatz A, Hunya A, Literati-Nagy Z, Vigh LJ, Hoogstra-Berends F, Heeres A, Kuipers I, Loen L, Seerden J, Zhang D, Meijering RAM, Henning RH, Brundel BJJM, Kampinga HH, Koranyi L, Szilvassy Z, Mandl J, Sumegi B, Febbraio MA, Horvath I, Hooper PL and Vigh L (2013). Hydroximic acid derivatives: pleiotropic hsp co-inducers restoring homeostasis and robustness. *Curr.Pharm. Des*. 19: 309-346.

Czyczyło-Mysza IMT, Marcin´ska I, Skrzypek E, Karbarz M, Dziurka M, Hura T, Dziurka K, and Quarrie SA (2013). Quantitative trait loci for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. *Mol Breeding* 32:189-210.

De Block M and De Brouwer D (2002). A simple and robust in vitro assay to quantify the vigour of oilseed rape lines and hybrids. *Plant Physiol. Biochem*.40(10) : 845-852.

Dhanda SS and Munjal R (2012). Heat tolerance in relation to acquired thermotolerance for membrane lipids in bread wheat. *Field Crops Re*s 135:30-37.

Dhanda SS and Munjal R (2006). Inheritance of cellular thermotolerance in bread wheat. *Plant Breeding.* 125: 557-567.

Dodig D, Zoric M, Knezjevic D, King SR, Sjurlan-Momirovic G (2010). Assessing drought tolerance and regional patterns of genetic diversity among spring and winter bread wheat using simple sequence repeats and phenotypic data. *Crop Pasture Sci*.61: 812-824.

Eisen MB, Spellman PT, Brown PO, Botstein D (1998). Cluster analysis and display of genome-wide expression ilatteriis. *Proc. Natl. Acad. Sci.*, *USA* 95, 14863–14868.

Essemine J, Govindachary S, Ammar S, Bouzid S, Carpentier R (2011). Abolition of photosystem I cyclic electron flow in *Arabidopsis thaliana* following thermal-stress. *Plant Physiol. Biochem.* 49 235-243.

Everitt BS (1993). Cluster Analysis. Wiley, New York, NY.

Everitt BS and Dunn G (1992). Applied Multivariate Data Analysis. Oxford University Press, New York, NY.

Farooq Muhammad, Bramley Helen, Palta Jairo A and Siddique K HM (2011). Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Crit. Rev. Plant Sci.* 30(6): 491-507.

Fischer RA and Maurer R (1978). Drought resistance in spring wheat cultivars.I. Grain yield response. *Aust. J. Agric. Res*. 29:897-907.

Fisher KS, Johanson EC and Edmeades GO (1982). Breeding and selection for rought resistance in tropical maize. *In: Drought Resistance in Crops with Emphasis on Rice*, 377-399. IRRI, Manila, Philippines.

Fitter AH and Hay RKM (2002). Environmental Physiology of Plants, 3rd ed.; Academic Press:London, UK.

Garg N and Manchanda G (2009). ROS generation in plants: boon or bane? *Plant Biosyst*. 143:81-96.

Georg L, Georg N and Mauricio H (2013). Physiological response of sugar beet (*Beta vulgaris*) genotypes to a temporary water deficit, as evaluated with a multiparameter fluorescence sensor. *Acta Physiol Plant.* 35:1763-1774.

Gigon A, Matos AR, Laffray D, Yasmine ZF and Pham Thi AT (2004). Effect of drought stress on lipid metabolism in the leaves of arabidopsis thaliana (*Ecotype Columbia*). *Ann. Bot.* 94: 345-351.

Gupta NK, Agarwal S, Agarwal VP, Nathawat NS, Gupta S and Singh G (2013). Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. *Acta Physiol Plant* 35:1837-1842.

Hameed A, Goher M and Iqbal N (2012). Heat stress-induced cell death, changes in antioxidants, lipid peroxidation and protease activity in wheat leaves. *J. Plant Growth Regul*.31:283-291.

Harb A, Krishnan A, Ambavaram MMR and Pereira A (2010). Molecular and physiological analysis of drought stress in arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol. 154: 1254-1271.

Hasanuzzaman M, Nahar K, Alam MdM, Roychowdhury R and Fujita M (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.* 14:9643-9684.

Hays DB, Mason RE and Do JH (2007). Wheat production in stressed environments. In: Buck, H.T., Nisi, J.E. and Salomon, N. (eds) Springer, Dordrecht.

Hemantaranjan A, Nishant Bhanu A, Singh MN, Yadav DK, Patel PK (2014). Heat stress responses and thermotolerance. *Adv Plant Agric Res*. 1:1-10.

Ibrahim AMH and Quick JS (2001). Heritability of heat tolerance in winter and spring wheat. Crop Science. 41: 1401-1405.

Jha UC, Bohra A and Singh NP (2014). Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. *Plant Breeding* 133(6): 679-701.

Kornyeyev D, Holaday S and Logan B (2003). Predicting the extent of photosystem II photoinactivation using chlorophyll a fluorescence parameters measured during illumination. *Plant Cell Physiol*. 44:1064-1070.

Kültz D (2005). Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol.* 67: 225-257.

Mirza H, Kamrun N, MdMA, Rajib R and Masayuki F (2013). Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. *Int J Mol Sci*. 14(5): 9643-9684

Magdalena K, Izabela B and Anna K (2015). Fractionation of Buckwheat Seed Phenolics and Analysis of Their Antioxidant Activity. *Pol. J. Food Nutr. Sci*.65 (4):243-249

Morales D, Rodriguez P, Dellamico J, Nicolas E, Torrecillas A and Sanchez-Blanco MJ (2003). High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biol. Plant*. 47, 203-208.

Munjal R and Dhanda SS (2016). Assessment of drought resistance in indian wheat cultivars for morpho-physiological traits. *Ekin J. Crop Breeding Genetics* 2(1):74-81.

Penuelas J and Filella I (2001). Responses to a warming world. *Science* 294: 793-794.

Parker GD, Fox PN, Langridge P, Chlamers K,Whan B, Ganter PF (2002). Genetic diversity within Australian wheat breeding programs based on molecular and pedigree data. Euphytica 124: 293-306

Pastore A, Martin SR, Politou A, Kondapalli KC, Stemmler T (2007). Unbiased cold denaturation: low- and high-temperature unfolding of yeast frataxin under physiological conditions. *J. Am. Chem. Soc.* 129:5374-5375.

Rich PR, Mischis LA, Purton S and Wiskich JT (2001). The sites of interaction of triphenyltetrazolium chloride with mitochondrial respiratory chains. *FEMS Microbiol. Let*t. 202(2): 181-187.

Rodríguez M, Canales E and Borrás-Hidalgo O (2005). Molecular aspects of abiotic stress in plants. *Biotechnol. Appl*. 22: 1-10.

Sareen S, Kundu S, Malik R, Dhillon OP and Singh SS (2015). Exploring indigenous wheat (*Triticum aestivum*) germplasm accessions for terminal heat tolerance. *The Indian Journal of Agricultural Sciences*. 85(2)**:**.37-42.

Schöffl F, Prandl R and Reindl A (1999). Molecular responses to heat stress. In: Shinozaki, K., Yamaguchi-Shinozaki, K. (Eds.), Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. R.G. Landes Co., Austin, Texas, pp. 81-98.

Sharma P, Sareen S, Saini M, Verma A, Tyagi BS and Sharma I (2014). Assessing genetic variation for heat tolerance in synthetic wheat lines using phenotypic data and molecular markers. *Australian journal of crop sciences.* 8(4): 515-522.

Singh P and Dwivedi P (2015). Morpho-physiological responses of wheat (*Triticum aestivum* L.) genotypes under late sown condition. *Vegetos* 28 (1) : 16-25.

Snedecor GW and Cochran (1981). Statistical Methods. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

Sullivan CY (1972). Mechanisms of heat and drought resistance in grain sorghum and methods of measurements. In: N.G.O. Rao and L.R. House (Eds "Sorghum in seventies"), Oxford and IBH, ND, pp 112-120.

Sun J, Luo H, Fu J and Huang G (2013). Classification of genetic variation for drought tolerance in Tall fescue using physiological traits and molecular markers. *Crop Sci.* 53: 647-654.

Towill LE and Mazur P (1974). Studies on the reduction of 2,3,5-triphenyltetrazolium chloride as a viability assay for plant tissue culture. Can J Bot 53: 1097-1102.

Varshney RK, Bansal KC, Aggarwal PK, Datta SK and Craufurd PQ (2011). Agricultural biotechnology for crop improvement in a variable climate: hope or hype? *Trends Plant Sci*.16:363–371.

Vigh L, Horváth I, Maresca B and Harwood JL (2007) Can the stress protein response be controlled by 'membrane-lipid therapy'? *Trends Biochem. Sci.* 32: 357-363.

Vigh L, Maresca B and Harwood JL (1998). Does the membrane's physical state control the expression of heat shock and other genes? *Trends Biochem. Sci.* 23: 369-374.

Wahid A, Gelani S, Ashraf MM and Foolad R (2007). Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199-223.

Wang JZ, Cui LJ, Wang Y and Li JL (2009). Growth, lipid peroxidation and photosynthesis in two tall fescue cultivars differing in heat tolerance. Biol. Plant. 53:247-242.

Xue Q, Weiss A and Baenziger P (2004). Predicting phenological development in winter wheat. *Climate Research. 25*(3), 243-252.

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Genotypic Variability in Triticale (*Triticale hexaploide* Lart.) for Response of Azotobacter Inoculation in Semi Arid Conditions

Akhand Pratap SINGH* **Vivek Kumar SRIVASTAVA**** **Rishi Kumar BEHL***** **O.P. BISHNOI******

* Director
** Head, Dept. of Biotechnology , Raja Balwant Singh Engineering Technical Campus, Bichpuri (Agra), India
*** Professor (Retd)
**** Assistant Scientist (Wheat) CCS Haryana Agriculture University, Hisar, Haryana, India

Corresponding author e-mail: vivekrbscollege@yahoo.com

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ABSTRACT

Triticale hexaploide Lart. commonly known as Triticale is a manmade cereal obtained from cross between wheat (*Triticum*) and rye (*Secale*). It is used for food and feed purposes in view of its high nutritive value. In the present study, we focused on enhancement in the crop production of Triticale (*Triticale hexaploide* Lart.) under semi arid conditions at Agra, India. A pot experiment was conducted to evaluate the effect of *Azotobacter* and chemical fertilizers on seven variety of triticale *viz.* R2TL3001, R1TL3005, R1TL3006, R1TL2942, R2TL3002, R1TL3003, R1TL3004 and a local variety of wheat which was treated as check. The results of R1TL2942, R1TL3003 and R1TL3006 with *Azotobacter* were observed as best yielding variety of Triticale with 56-59 grains and with 55-58 grains per spike respectively. The findings show that R1TL2942, R1TL3003 and R1TL3006 are the potential variety of Triticale that could grow under Indian climatic conditions.

Keywords: Triticale, wheat, Azotobacter.

Introduction

Triticale (*Triticale hexaploide* Lart.) is a hybrid cereal of wheat (*Triticum*) and rye (*Secale*) which was developed by using conventional plant breeding followed by embryo culture. Its origins could be traced back to the Scotland (Ammar. *et al*., 2004). The research work on triticale was started in 1950's in Canada and the first Triticale variety was release in 1972 by University of Manitoba. Triticale was developed to integrate the grain qualities of wheat with less input requirements along with the hardiness of rye. However, it has had limited take-up in the India till date while triticale has made a significant impact in Europe, notably in Poland, Hungary, Germany where over 4 million hectares are grown with good performance. It becomes an accepted crop worldwide, competitive with local grains. The triticale varieties are equal or higher yielding in comparison of other crops for grain, forage and biomass production, for feed and food along with industrial applications. It has superior adaptation under stress conditions like wise drought, acidic soils, excess moisture and situations of high fertility where other crops yield less and are poorly adapted. The grain of triticale is much suitable as feed for ruminants and monogastrics, especially for silage and swine feed. Triticale develop a new levels of sustainable flexibility for crop planning, especially for enabling year-long forage supplies using grazing or conserved forage. Triticale could also play a special role in integrated cropping systems which provide the crop diversity in the rotation, a break in pest, disease and weed cycles, and seasonal flexibility. At the same time, high yields of triticale silage or grain will returned to the livestock operation that generated the manure *(*Chapman *et al.,* 2005*).* This is well known that micro-organisms play an important role in number of chemical transformations in

soils and influenced the availability of major nutrients like nitrogen, phosphorus, potassium and sulphur to the plants. Thus, the application of chemical fertilizer may be reduced by 20-50% with the use of bio-fertilizers (Balyan, 1998). Azospirillum, Azotobacter, Blue green algae (BGA), VAM and Phosphate Solubilizing Bacteria (PSB) can be used as bio-fertilizers to increase the crop production (Singh, *et al*., 2006). Therefore the present was planned to evaluate the response of Triticale genotypes to inoculation with Azotobacter.

Materials and Methods

Investigation was carried out during Rabi season of 2016-17 in the research plots of the Department of Biotechnology, Raja Balwant Singh Engineering Technical Campus, Bichpuri, Agra. A pot experiment was conducted to evaluate the effect of Azotobacter and chemical fertilizer on seven variety of triticale namely R2TL3001, R1TL3005, R1TL3006, R1TL2942, R2TL3002, R1TL3003, R1TL3004 and a local variety of wheat kept as check. All the experiments performed in this study were replicated three times in a randomized block design. There are four plant replicates in all of fourty eight pots. The soil of the experimental field was sandy loan in texture with a pH 7.84 the soil was low in available nitrogen (174.40 kg ha^{-1}), medium in available phosphorus (25.80 kg $P_2 O_5$ ha^{-1}) and rich in available potash (220.70 kg K_2O ha^{-1}). The Triticale and wheat varieties were sown during Rabi season on January 04, 2017. The culture solution was prepared in the concentration of 100 g/l of Azotobacter, in 10% jaggory solution and mixed with the fine and dry soil before sowing. The recommended dose of NPK in the ratio of 120:60:60 kg/ha was applied as per treatment in the form of urea, single super phosphate and potash. One-third amount of urea and full dose of single super phosphate and potash were used and mixed thoroughly. Remaining two third of urea was top-dressed in two split doses. The cultural operations viz. irrigation, weed control, earthing up, insect pest control etc. were done in each of the pots uniformly throughout the course of investigation whenever necessary. The parameters for the experimental setup includes the date of germination, initiation of first flag leaf, initiation of spike, plant height, number of spikes, number of spikelets/spike, number of grains per spike along with the test weight.

Results and Discussion

All of the studied triticale varieties germinated between 13-15 January 2017. The dates for the initiation of first flag leaf ranged between 16-25 February 2017 while the initiation of spikes started from 28 February 2017 to 10 March 2017. In some cases (R1TL3002 and R1TL3004) the initiation of spikes was observed upto 20 March, 2017. The maximum number of spikelet of 55±0.66 per plant was investigated in R1TL2942 treated with Azotobacter followed by R1TL3006 and R1TL3003 with the number of spikelet of 53±2.08 and 51±4.25 respectively. The per plant yield (g/plant) of R1TL2942, R1TL3006 and R1TL3003 treated with Azotobacter was observed as 52±0.63, 51±2.50 and 49±4.58 respectively. These findings are encouraging as the yield of control crop was observed as 25±0.69. The data on various plant parameters are given in Figure 1. Other varieties of triticale were not very much promising with respect to plant yield. The results of the experiments in which the triticale seeds were grown under chemical fertilizers are summarized in Figure 2. With the chemical fertilizer treatment all the seven varieties of triticale were observed as plant yield between 19±2.08 to 23±0.62 which is less or nearby the check. With the above discussion it is clear that the *Azotobacter* inoculums may be used as a biofertilizer for the triticale and it may reduce the use of chemical fertilizers and save the environment. The Azotobacter may be used as a component of nutrient supply system for the sustainable agriculture. The finding were also supported by the findings of Kader *et al.,* 2002 in which they studied the effect of *Azotobacter* inoculums on wheat and found that it reduces the use of urea N by 20 percent.

Figure 1. Effect of Azotobacter on investigated varieties of triticale

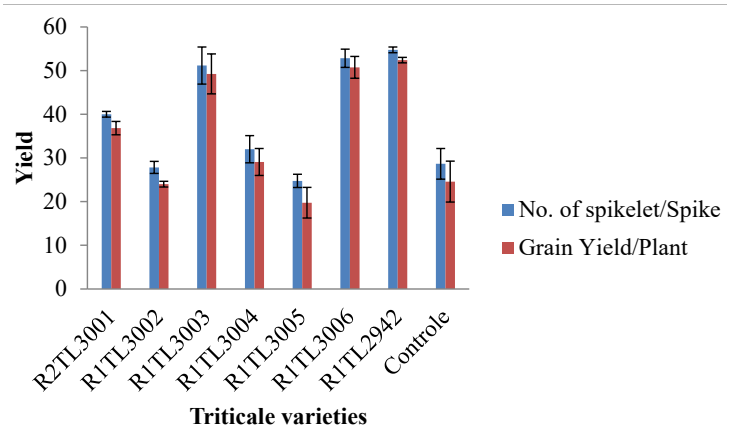
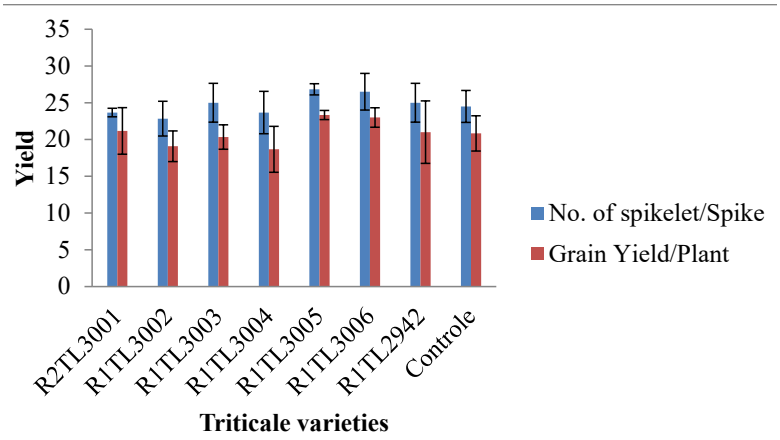


Figure 2. Effect of chemical fertilizer on investigated varieties of triticale



References

Ammar K, Mergoum M, Rajaram S (2004). The history and evolution of triticale; Triticale improvement and production; 2004; 1-9

Balyan JS (1998). Studies on maize stover management and N, P application in maize-wheat crop sequence; 1st Int. Agron., Congress held at New Delhi, Nov.; 23-27.

Kader MA, Mian MH, Hoque MS (2002). Effects of Azotobacter inoculants on the yield and nitrogen uptake by wheat; Journal of Biological Science 2(4); 259-261

Singh SR (2006). Response of bio-fertilizers and pesticides on yield and quality of cabbage, radish and brinjal in vegetable-based rotation system; Applied Biological Research; 8:112; 33-36

Additional Suggested Readings

Chapman B, Salmon D, Dyson C, Blackley K (2005). Triticale Production and Utilization Manual; Alberta Agriculture, Food & Rural Development (AAFRD)

http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd10535

Research Article

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Anther Culture in Red Cabbage (*Brassica oleraceae* L. var. *capitata* subvar. *rubra*): Embryogenesis and Plantlet Initiation

Aslıhan CILINGIR[1] Senay Murat DOGRU[2] Ertan Sait KURTAR[3*] Ahmet BALKAYA[1]

[1] Agriculture Faculty, Horticulture Dep., Ondokuz Mayis University, Samsun, Turkey
[2] Black Sea Agricultural Research Institute, Samsun, Turkey
[3] Vocational High School of Bafra, Ondokuz Mayis University, Samsun, Turkey

* Corresponding author e-mail: ertansaitkurtar@hotmail.com

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ABSTRACT

Cabbages, cauliflower and broccoli breeding activities are very low level compared with the other vegetable species. This study was conducted to determine the efficacy of the anther culture technique on the in vitro embryogenesis and according plantlet initiation and to the author's knowledge, this is the first reports in red cabbage species. The anthers at uninucleate microspore stage (about 3-5 mm long) were collected at florescence times and then cultured on a solid MS and B5 medium with the addition of 2,4-D, NAA, Glutamine, Serine, Silver nitrate, IAA, BAP and different sucrose concentration to induce callogenesis, embryogenesis, and plantlet initiation. The culture medium, sucrose concentration, and genotype were found to be highly effective on androgenesis. Among the genotype, Zencibas cv. produced the best result with a combination of M medium+30 g/l sucrose for embryogenesis and plantlet initiation, while, Integro F_1 and Cabellero F_1 did not generate an androgenic response. This results can assist cabbage breeders to develop new hybrid cultivars in breeding programs.

Keywords: Red cabbage, anther culture, embryogenesis, plantlet initiation.

Introduction

Brassica vegetables are an important and highly diverse group of crops grown world-wide that belong mainly to the species *Brassica oleracea* and *Brassica campestris* (Monteiro and Lunn, 1998; Balkaya *et al*., 2005). Red cabbage is a member of the genus *Brassica* within the economically important *Brassicaceae* family. Cabbages breeding studies have been increased the last years according to the other *Brassica* vegetable species (Balkaya *et al*. 2016). Thus, it is well known that all *Brassica* spp. are open pollinated and improve new F_1 cultivars with desirable agronomic traits need to many times and also intensive efforts. Moreover, red cabbage is a biennial crop for seed production, and selfing depression, incompatibility and male sterility are seen in some genotypes, frequently (Dogru and Balkaya, 2015). Thus, doubled haploid (DH) technology is a unique solution and a new tool to the recovery of genetically uniform parental lines for the release of hybrid varieties in a short time (Kurtar and Balkaya, 2010; Dogru *et al*., 2016). These valuable DH lines could be obtained from anther-microspore culture, ovule-ovary culture and pollen irradiation in many species.

Androgenesis is an effective and widely used technique for the generating homozygote haploid lines of numerous species. Various techniques for microspore/anther culture have been developed for *Brassica* species (Cardoza and Stewart, 2004). The first successful production of haploid plants via androgenesis was reported in broccoli by Keller and Armstrong (1983), in Brussels sprouts by Ockendon (1984) and in white head cabbage by Kameya and Hinata (1970).

To our recent knowledge, there are no reports about androgenesis in red cabbage species via anther culture. Thus, this study was conducted to determine the frequency of anther culture for our future breeding efforts, firstly. So, the effects of medium, genotype and sucrose concentration were primarily investigated.

Materials and methods

Materials, preparation, and culture

In this study, the three red cabbage cultivars named open pollinated local variety, commercial Zencibas, Integro F_1 and Cabellero F_1 were used for anther culture process. The seeds of donor lines were sown in plastic flats (cell volume 80 cm^3 and 45 cells per flat) containing peat-moss. Cabbage seedlings were grown in a controlled glasshouse and 10 seedlings from each genotypes were planted in 7 lt plastic pots. Plant nutrition components were applied and plants were protected with fungicides and insecticides regularly throughout the cultivation.

Carmine staining technique was used for determining the uni-nucleate microspore stage. Flower buds (3-4 mm in length and 1-2 mm in width) were collected in the middle to late uni-nucleate microspore stages (Figure 1a , Figure 1b). The buds were transferred to the laboratory immediately and rinsed under running cold tap water for 15 min in teapots. Subsequently, buds were immersed in 70% ethanol for 1 min then 10% commercial bleach solution for 15 min. Buds were rinsed three times with sterile distilled water for 5 min each time and placed on sterilized filter paper to desiccate excessive surface water. MS and B5 medium supplemented by various PGR's were used for callogenesis, embryogenesis and plantlet initiation (Table 1). The pH of the media was adjusted to 5.8 and solidified with 7 g/l agar-agar for all culture processes.

Callus induction, callus maturation, embryogenesis and plantlet initiation

1.0 mg/l 2,4-D was used with the addition of 100 g/l sucrose for both MS and B5 medium for callus induction. Anthers without filaments were excised from buds and cultured in jars containing 25 ml of induction medium. Jars were sealed with stretch film and incubated in a growth chamber at 26±1°C and illuminated with white fluorescent 32 W lamps (3000 lux) under 16/8 h (day/night) photoperiod. Five anthers for each jar and ten jars for each replicate were cultured for each medium and genotype.

When calli enlarged (Figure 1c, Figure 1d) they were cut into pieces (about 5 x 5 mm) and sub-cultured onto maturation and embryogenesis media supplemented by a combination of 30 and 100 g/l sucrose + 1.0 mg/l NAA + 800 mg/l Glutamine + 10 mg/l Serine, 2.0 mg/l Silver nitrate and 1.0 mg/l BAP at same conditions.

Five weeks old maturated calli were transferred fresh differentiation media (Figure 1e) and were incubated at same conditions. The differentiation media were refreshed 2 times at 2 weeks interval to maintain callus formation and stimulate plantlet initiation.

Data collection and statistical analysis

A factorial experiment based on completely randomized design with five replications was used. Five jars for each replicate were cultured for each medium, genotypes and sucrose concentration. The data were analyzed by SPSS and mean values were separated based on Duncan's multiple range test.

Results

At the end of this study, various types of callus form (whitish, greenish, yellowish) were obtained from the anthers in callus induction procedure. The anthers differentiated and turned callus form within 5 or 6 weeks of culture (Figure 1e). It is obvious that strong genotypic differences were determined for callogenesis, embryogenesis and plantlet initiation processes (Table 2). Interestingly, among the cultivars, Zencibas cv. had an only positive reaction and the others (Integro F_1 and Cabellero F_1 cv.) were found to be highly recalcitrant and they did not generate any response.

Besides, the medium was the other important factor in all processes and the highest responses were found statistically signıficant from MS medium. In respect to callogenesis, MS medium was twofold higher than B5 and the percentage of callus induction was 52% in MS and 26% in the B5 medium for Zencibas cv. However, sucrose concentration had a determinative effect on callus maturation, embryogenesis, and plantlet initiation. The mean percentage of callus maturation changed from 20.0% (by 100 g/l sucrose in B5) to 56.0% (by 30 g/l sucrose in MS) and MS medium + 30 g/l sucrose produced the favorable maturation results.

Some fragment of maturated calli turned mazerine blue within 3 or 4 weeks of embryogenesis process. Then, the primary embryos were seen in some callus within 5 or 6 weeks (Figure 1f-1k), subsequently, mini plantlets were seen on some callus after 3 or 4 weeks (Figure 1l and Figure 1m). Likewise, medium and sucrose concentration were found to be effective on the frequency of embryogenesis and plantlet initiation and the highest responses were observed by the combination of MS + 30 g/l sucrose. The mean

percentage of embryogenesis were ranged from 0% (by 100 g/l sucrose in B5) and 30% (by 30 g/l sucrose in MS) when it was determined by 7.5% (by 100 g/l sucrose in MS) and 12.5% (by 30 g/l sucrose in B5). On the other hand, the frequency of the mean number of plantlets per callus was 0.58 and 0.68 with the addition of 30 g/l sucrose in MS and B5 medium, respectively. Although MS + 100 g/l sucrose gave the promising outcome, the frequency was found to be the lower than average.

Discussion

To our knowledge, this is the first report on anther culture in red cabbage. According to our preliminary results, the success of androgenesis was found to be the highly depend on genotypes, medium composition, and sucrose concentration, statistically. In view of androgenic response, the genotypic reaction was found to be a key factor in our research for all process, particularly. Hence, only open pollinated local cultivar Zencibas released androgenic response, besides, the other hybrid cultivars were found to be unresponsive. This is in line with findings of many researchers, and the differences of the genotypic reaction were observed from androgenesis in winter rape (Smykalova *et al*., 2006), in rapeseed (Custers, 2003; Weber *et al*., 2005) and in oilseed rape (Gu *et al*., 2004). It is clearly reflected that genotype is the main problem for androgenesis in *Brassica* (Zhang *et al*., 2008; Lee *et. al*., 2014).

Medium composition and sucrose concentration were effective on callus induction and embryogenesis. The MS medium combined with low sucrose concentration produced the maximum percentages of embryonic callus, besides, B5 medium and higher sucrose concentration had a negative effect on both callogenesis and embryogenesis. Medium effect on embryogenesis has also been reported for microspore embryogenesis in *B. oleracea* (Dias, 1999) and *B. campestris* (Wakui *et al*., 1994), in agreement with our findings. MS medium with BAP + 2.4-D + low sucrose (20 and 30 g/l) produced the highest embryonic response in broccoli. The anthers were lost their viability in higher sucrose concentrations (50 and 60 g/l) and embryogenesis was interrupted, comparing to B5 medium (Mousa *et al*., 2014). Similarly, the best embryogenic response was obtained from on MS medium with 20 g/l sucrose + 1 mg/l BA+0.001 mg/l NAA (Krzyzanowska *et al*., 2006). On the other hand, MS medium with 1 mg/l BA produced the maximum percentages of embryos in cabbage (Gorecka and Krzyzanowska, 2007), and BAP was the most efficient PGR for enhancing shoot multiplication and elongation in broccoli (Ravanfar *et al*., 2009)

Conclusion

Anther culture is highly recommended as an alternative method to microspore culture in embryogenesis and plantlet initiation for red cabbage in dihaploidization process. However, this technique should be improved either the production high quantity in vitro regenerants or used for a broad spectrum for future breeding efforts. Moreover, the success of this technique is highly depending on genotype, and it is the main obstruction of dihaploidization process. Thus, our further investigations will be realized on fecund lines, effective PGR combinations, and concentrations, improve the physiological condition of donors and also microspore culture for alternatively.

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Table 1. The composition of MS and B5 media

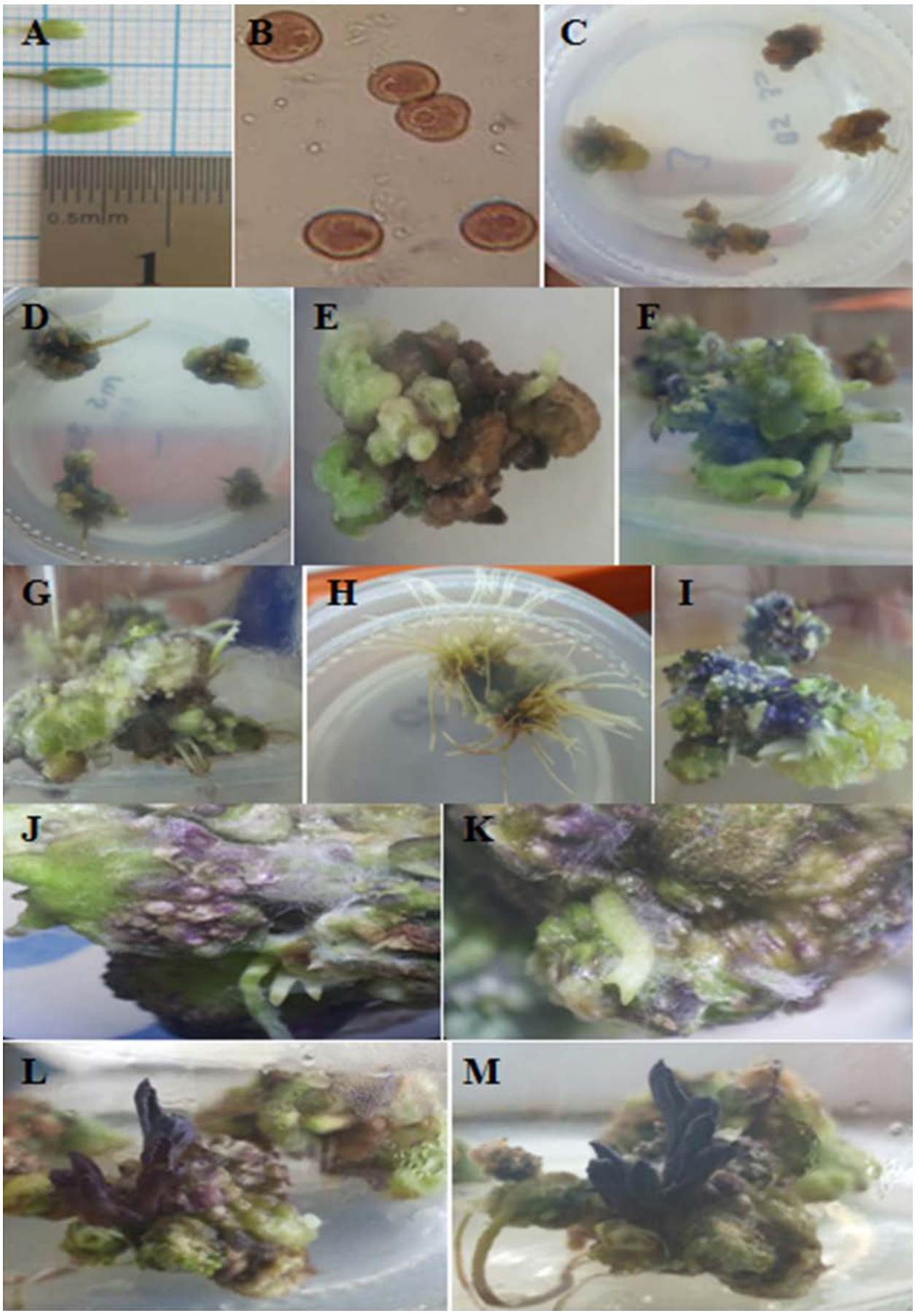
	Media	Sucrose (g/l)	2,4-D	NAA	Glutamine	Serine	$AgNO_3$	BAP
					(mg/l)			
Callus induction	B5							
	MS	100	1	1	-	-	-	-
Callus maturation, embryogenesis, plantlets initiation	B5	100	-	1	800	10	2	1
	MS	30						

Table 2. The effects of media on callus induction (CI %), callus maturation (CM %), embryo induction (EI %) and plantlet initiation (PI plant/per callus)

Genotype	Media	CI	CM		EI		PI	
			30	100	30	100	30	100
Zencibas	MS	52.0 ^{A}	56.0 ^{A}	40.0 ^{B}	30.0 ^{A}	7.5 ^{BC}	0.58 ^{A}	0.13 ^{BC}
	B5	26.0 ^{B}	40.0 ^{B}	20.0 ^{C}	12.5 ^{B}	0.0 ^{D}	0.68 ^{A}	0.0 ^{D}
	Avr.	*39.0*	*48.0* ^{A}	*30.0* ^{B}	*21.3* ^{A}	*3.8* ^{B}	*0.63*	*0.07*

Different letters indicate a significant difference (P<0.05).

Figure 1. (A) Female flowers (B) Microspores at uni-nucleate stage (C and D) Callus induction and enlargement (E) Callus maturation (F-K) Embryogenesis (L and M) Plantlets initiation



References

Balkaya A, Yanmaz R, Apaydın A (2005). Morphological characterisation of white head cabbage (*Brassica oleracea* var. *capitata* subvar. *alba*) genotypes in Turkey. New Zealand Journal of Crop and Horticultural Science. 33:333-341.

Balkaya A, Sarıbaş Ş, Özgen T (2016). The last status of winter vegetable species in Turkey. Turkish Seed Union Magazine, 5(20):8-12.

Cardoza V, Stewart CN (2004). Brassica Biotechnology: Progress In Cellular and Molecular Biology. In Vitro Cellular & Developmental Biology-Plant 40:542-551.

Custers JBM (2003). Microspore culture in rapeseed (*Brassica napus* L.). In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds.): Double Haploid Production in Crop Plants. Kluwer, Dordrecht 185-193.

Dias JS (1999). Effect of activated charcoal on *Brassica oleracea* microspore culture embryogenesis. Euphytica 108: 65-69.

Dogru SM, Balkaya A (2015). The Different Applications for Shortening Seed Production and Their Effect Mechanism in Cabbage. Alatarım Dergisi 14 (2): 29-37.

Dogru SM, Cilingir A, Kurtar ES, Balkaya A (2016). Usage of Haploid and Doubled-Haploid Techniques in the Cultivar Breeding of Cabbage Group Vegetables. Turkish Seed Union Magazine 5(20): 30-33.

Gorecka K, Krzyzanowska D (2007). The use of anther culture for obtaining head cabbage homozygous lines. In: Cell culture and somatic cell genetics of plants, Vasil, I.K. (Eds.) Akademia Press, New York, pp: 302-310.

Gu HH, Hagberg P, Zhou WJ (2004). Cold pretreatment enhances microspore embryogenesis in oilseed rape (*Brassica napus* L.). Plant Growth Regulation. 42: 137-143.

Kameya T, Hinata K (1970). Induction of haploid plants from pollen grains of Brassica. Japanase Journal of Breeding. 20: 82-87.

Keller WA, Armstrong KC (1983). Production of haploid via anther culture in *Brassica oleracea* var. *italica*. Euphytica 32: I5l-I 59.

Krzyżanowska D, Krystyna G, Waldema K, Urszul K (2006). The Effect Of Genotype And Medium On Plant Regeneration From Androgenic Embryos. Journal of Fruit and Ornamental Plant Research Vol. 14 (1) 121:127.

Kurtar ES, Balkaya A (2010). Production of in vitro haploid plants from in situ induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) via irradiated pollen. Plant Cell Tissue and Organ Culture. 102: 267-277.

Lee MH, Lim CJ, Lee IH, Song JH (2014). High-purity Seed Production of Doubled Haploid Chinese Cabbage [*Brassica rapa* L. ssp. *pekinensis* (Lour.)] Through

Microspore Culture. Plant Breeding and Biotechnology. 2014 (June) 2(2):167-175.

Mousa Magdi AA, Ashraf GH, Hassan SA, Mohammed FM (2014). Improved androgenesis of broccoli (*Brassica oleracea* var. *italica*) anthers using sucrose and growth regulators. Asian Journal of Crop Science. 6(2): 133.141.

Monteiro A, Lunn T 1998. Trends and perspectives of vegetable brassica breeding. World Conference on Horticultural Research, 17-20 June 1998, Rome, Italy.

Ockendon DJ (1984). Anther culture in Brussels sprouts (*Brassica oleracea* var. *gemmifera*). I. Embryo yields and plant regeneration. Annals of Applied Biology. 105: 285-291.

Ravanfar SA, Aziz MA, Kadir MA, Rashid AA, Sirchi MHT (2009). Plant regeneration of *Brassica oleracea* subsp. *italica* (Broccoli) CV Green Marvel as affected by plant growth regulators. African Journal of Biotechnology. Vol. 8 (11), pp. 2523-2528.

Smykalova I, Vetrovcova M, Klima M, Machackova I, Griga M (2006). Efficiency of Microspore Culture for Doubled Haploid Production in the Breeding Project "Czech Winter Rape". Czech Journal of Genetics and Plant Breeding. 42(2): 58-71.

Weber S, Unker W, Friedt W (2005). Improved doubled haploid production protocol for *Brassica napus* using microspore colchicine treatment in vitro and ploidy determination by flowcytometry. Plant Breeding. 124: 511-513.

Wakui K, Takahata Y, Kaizuma N (1994). Effect of abscisic acid and high osmoticum concentration on the induction of desiccation tolerance in microspore-derived embryos of Chinese cabbage (*Brassica campestris* L.). Breed Science. 44: 29-34.

Zhang W, Fu Q, Dai XG, Bao MZ (2008). The culture of isolated microspores of ornamental kale (*Brassica oleracea* var. *acephala*) and the importance of genotype to embryo regeneration. Scientia Horticulturae. 11: 769-772.

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Change in Traits' Association with Intermating

Agdew BEKELE[1*] Sada R. VERMA[2] Rishi K. Behl[2]

[1] Southern Agricultural Research Institute (SARI), Hawasa Ethiopia
[2] CCS Haryana Agricultural University, India

[*] Corresponding author e-mail: agdew_bek@yahoo.com

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ABSTRACT

The present investigation was conducted to examine the extent of phenotypic and genotypic correlation in bi-parental mating and selfing for yield and related components. Sixty plants from every cross of barley (IBON-W-61 x DWR91, BH935 x BH902 and BH902/DWRUB64) were randomly selected and crossed as well as selfed to generate 30 BIPs and 30 selfed progenies in 2012-13. In 2013-14 those slefed and intermated progenies were grown in a compact family block design with three replications. Data were recorded on days to heading, days to maturity, plant height, spike length, grain/spike, effective tiller number per plant, biomass yield per plant, grain yield per plant, harvest index and 1000-grain weight. The genotypic and phenotypic correlation coefficients were calculated between pair of characters. From the study it was concluded that intermating has improved mean performance and variance of characters by breaking linkages between genes and resulted in breakage of coupling phase and repulsion phase linkages that led to decreased and increased correlations, respectively.

Keywords: Intermating, selfing, biparental, coupling phase, repulsion phase.

Introduction

Archaeological evidence has suggested that barley is an oldest crop cultivated during ancient times at about 10,000 to 12,000 years ago. It is most likely originated in the Fertile Crescent area of the Near East (Anonymous, 2012), since the wild progenitor of barley, *Hordeum spontaneum*, is still found in this area. Barley grain is used as animal feed, human food and malt. The use of barley which brings the largest added value, however, is for the production of malt, which has contributed to the crop's expansion to the world. Developed countries use barley predominantly for animal feed and malt production. In India, the major barley production has been for cattle feed and food, however, recently there is a considerable demand for malt barley due to an increase in consumption of beer and malt based products in India and other countries (Verma *et al*., 2008; Verma *et al*., 2011).

A six-row and two-row feature of barley is an important morphological architecture (Palmer *et al.,* 2009). Through selection and domestication process, the six-rowed barley was evolved from the two-row wild type (Pourkhiranandish and Komatsuda, 2007) as a result of spontaneous mutations. Following this mutation, six-row was favored more than the two-row during selection because of the three fold number of grains per spike.

Two-rowed barleys usually have a more number of tillers per plant and larger, heavier grain than six-rowed varieties. On the other hand, six-rowed types usually have more grains per inflorescence. Thus the compensatory effects of yield components lead to similar levels of yield potential (Hayes *et al*., 2003). In six-rowed barley all the florets are fertile and produce grains, while in the two-rowed plants the outer florets of each group are sterile. Two-row character is dominant over the six-row character in F_1 (Khan, 1985) and in the F_2 population this character is segregated into two row, six-row and intermediate types. Genetically, there are at least five independent

loci controlling the six rowed spike phenotype in barley (Pourkhiranandish and Komatsuda, 2007). Six-rowed spike *1* (*vrs1*), a recessive gene located on chromosome 2HL, is found in six-rowed, where as cultivated two-rowed barleys have dominant alleles for *Vrs1*.

The gain in barley yield has come largely due to manipulating genes responsible for lodging and disease resistance which made barley plant responsive to agronomic practices. Thus, the realized increase in barley productivity is to be maintained. Further efforts to increase yield have become relatively difficult because of the fact that the ongoing breeding methods such as pedigree method are limited due to several drawbacks like limited parent participation, low genetic variability, reduced recombination, and rapid fixation of genes following selfing. The improvement following such method may further be restricted due to association between genes for desirable and undesirable characters and there is no chance to regain desirable genes that may have lost in the selected plants. In view of these observations, a fresh look on generating new genetic variability for yield and its component traits has become essential.

Most of the agronomic traits are quantitative in nature and the expression of the desired combinations is hidden because of tight linkages among the interacting gene blocks. Different mating designs have been suggested to exploit the hidden variability by breaking the linkages in the breeding materials of self pollinated crops. One of these designs is the biparental mating in the early segregating generations, mostly in F_2, that forces recombination and breaking down of undesirable linkages among traits (Comstock and Robinson, 1952) than the selfing series. The biparental mating has been reported to effect fostered recombination in rice (Manickavelu *et.al*., 2006; Mahalingam *et al*., 2011a; Mahalingam *et al*., 2011b), in wheat (Yunus and Paroda, 1982; Verma, 1989) and in six row-barley (Prakash and Verma, 2006). However, the information is scanty in case of barley. Therefore, the present investigation was designed to investigate the relative efficiency of inter-mating and selfing in breaking the undesirable linkage blocks between the yield component traits to affect association among those component traits.

Materials and Methods

The experimental materials used for the first crop season of this study during 2012-13 comprised of F_2 generation of three inter-varietal crosses of barley, namely, cross I (IBON-W-61/DWR91- two-row / two-row), cross II (BH935/BH902 - two-row / six-row) and cross III (BH902/DWRUB64 - six-row / six row). From each cross 1,000 F_2 plants were space planted in 2012-13 *rabi* season at the research station of the department of Genetics and Plant breeding, CCS Haryana Agricultural University, India. Out of F_2 populations 60 plants were selected from each cross for paired crossing to generate biparental populations (BIPs) and to get selfed generations from 30 female parent plants. Crossing was conducted following the normal hand emasculation procedure and more than five tillers of the maternal plants were allowed to self pollinate for production of F_3 selfed seed. Then, seeds of 30 BIPs and the respective selfs were harvested separately to grow the next experiment.

In the *rabi* season of 2013-14, 30 families in two populations (BIPs and their respective F_3s) in each cross along with their parents and checks were grown in a compact family block design with three replications. In each replication, two compact blocks were set for each population and each compact block was further partitioned to 33 plots for families, parents and checks. In each cross, families were randomized with in replication and progenies with parents and checks were randomized with in family block following the standard procedure for the design (Nageswara, 2007). Each plot consisted of a single row with 3 meters length and the spacing between rows and plants were 30 cm and 15 cm, respectively.

Data was recorded on days to heading, days to maturity, plant height, spike length, grains per spike, effective tiller number per plant, biomass yield per plant, grain yield per plant, harvest index and 1000 grain weight. Days to heading was recorded when ears of 50% the plants in the plot were fully emerged from the flag leaf sheath, while days to maturity was recorded when the plants of plot had fully turned to yellow on plot bases. The rest traits were measured from five competitive selected plants from the middle of the row after maturity of the crop.

The data recorded on quantitative characters of yield and its components were subjected to statistical analysis. For the mean data collected, separate analysis of variances between families for each population in every cross was carried out using the standard ANOVA procedure for the randomized complete block design (Nageswara, 2007). Following ANOVA, the nature and extent of association between yield and its component traits was examined by computing phenotypic and genotypic correlation coefficients for each population in every cross using the Plant Breeding Tools software version 1.1 (PBTools, 2013) as suggested by Miller *et al*. (1958) and Kwon and Torrie (1964) as:

$$r_{pxy} = \frac{\text{cov } pxy}{\sqrt{(\sigma^2 px)(\sigma^2 py)}}$$

$$r_{gxy} = \frac{\text{cov } gxy}{\sqrt{(\sigma^2 gx)(\sigma^2 gy)}}$$

where r_{pxy} is phenotypic correlation coefficient and r_{gxy} is genotypic correlation coefficient between characters x and y; cov_{pxy} and cov_{gxy} are phenotypic covariance and genotypic covariance between characters x and y, respectively; σ^2_p and σ^2_g are phenotypic and genotypic variance, respectively, for the respective character.

Results and Discussion

Analysis of variance

The analysis of variance results for mean family of ten characters studied in biparental and selfed populations of three crosses revealed significant variation among families as source of variation for most the assessed traits in BIP and F_3s of three crosses (Table 1). Hence, all the families in both populations of all three crosses differed significantly from each other with respect to all the assessed characters. Previous research findings indicated that biparental mating generated adequate variability in crops like rice (Amudha *et al*., 2006; Mahalingam *et al*., 2011a; Mahalingam *et al*., 2011b), wheat (Yunus and Paroda, 1983; Fredrickson and Kronsrad, 1985; Verma, 1989; Nematualla and Jha, 1993;), barley (Prakash and Verma, 2006), sesame (Vinayan and Govindarasu, 2010) and okra (Raju *et al*., 2010; Guddadamath *et al*., 2011).

Correlation of characters

Correlation of traits signifies that when one trait is selected, the other associated trait is also changed. The short-term response to selection depends not only on the heritabilities of the selected traits but also on the genetic and phenotypic covariances among traits (Falconer, 1989). Genetic correlations between traits, which arise due to pleiotropy or linkage relations among genes controlling the traits, are considered factors affect the direction of short term phenotypic evolution (Norry *et al*., 2000). The correlation between traits can be negative or positive in which a negative (for genes to increase one trait and decrease the other one) correlation arises from repulsion linkage of genes controlling the two traits while the positive (genes increase both traits) association occurs due to coupling phase of linkage (Sharma, 2008). In general the genotypic correlation varies from one population to another and also over time.

In the present investigation, the phenotypic and genotypic correlation coefficient analysis was carried out for combinations of all measured traits in three crosses for two populations (Tables 2, 8 & 9), and the result revealed that for most of the character associations, the genotypic correlation coefficients were higher, in magnitude, than the corresponding phenotypic correlation coefficients for both populations in three crosses. This indicated that the association between characters was, in general, inherited or controlled genetically. However, there are certain cases that the phenotypic correlation coefficients were closer to, or greater than (like biomass with effective tiller number and grain yield per plant in all crosses) the corresponding genotypic coefficients suggesting that environment had effect on those correlations. Most of the previous research findings also confirm higher magnitude of genotypic correlation coefficient compared to the corresponding phenotypic one (Yunus and Paroda, 1982; Waitt and Levine, 1998; Al-Tabbal and Al-Fraihat, 2012).

The considerable shift of correlations in the biparental populations compared to selfed progenies was observed in this investigation. When comparing the phenotypic and genotypic correlation coefficients among characters between BIP and F_3 selfs, the situation varied depending on traits associated and crosses. As many as 20, 16, and 14 new associations (either from non-significant to significant or vice versa) appeared in BIPs compared to selfed progenies in cross I, cross II, and cross III, respectively.

In BIPs of cross I, grain yield/ plant showed a significant positive association with spike length and grains per spike in F_3 were broken and changed to non-significant correlation in biparental progenies. The grain yield/plant's positively significant correlation coefficient with traits such as effective tiller /plant, harvest index and 1000-grain weight showed increment; however, its association reduced to positively significant with biomass yield/plant. In cross II, the association of grain yield/plant with plant height, spike length and days to heading was reduced to non-significant in intermated population from a significant positive association in selfed progenies; but its association with effective tiller per plant and harvest index was significantly and positively improved in BIPs form non-significant correlation in F_3 while its association with biomass yield/plant, 1000-grain weight and days to maturity improved

in magnitude towards positive and significant in BIPs form significant positive association in F_3. The association of grains/spike with grain yield/plant was changed towards significant negative association in biparental from significant positive correlation in selfed progeny. The association of grain yield/plant with other traits in cross III revealed significantly and positively improved association with effective tiller number/plant, slightly reduced with significant positive magnitude when associated with biomass yield/plant and harvest index, and the correlation was significantly reduced to non-significant correlation with 1000-grain weight in BIPs compared to F_3. Yunus and Paroda (1982) reported the improvement of association of grain yield with days to heading, days to maturity and plant height towards a positively significant correlation coefficient in BIPs in one cross of bread wheat. Similarly, Nematualla and Jha (1993) noticed in wheat that the significant association of grain yield/plant with plant height, number of tillers per plant, spike length, number of spikelets/spike, grains per spike and 1000-grain weight was considerably reduced in magnitude in BIPs compared to F_3. Verma (1989) also demonstrated considerable improvement of correlation coefficient in magnitude in BIPs for association of grain yield/plant with tillers per plant, 1000-grain weight and biomass yield/plant in one cross of bread wheat, while the correlation coefficient considerably reduced with 1000-grain weight in the other cross.

Grains per spike established significant positive association with plant height, spike length and harvest index in F_3 reduced in magnitude in BIPs, while its significant positive correlation with effective tiller number per plant, 1000-grain weight and days to heading in F_3 was broken and became non-significant in BIPs in cross I. In cross II, grains/spike had significant positive association with plant height and biomass yield in selfed was broken to non-significant in BIPs. However, previous research report showed significant positive association between grains/ear with plant height and days to maturity in wheat (Yunus and Paroda, 1982). The magnitude of significant negative association of grains/spike with 1000-grain weight in F_3 was increased in BIPs which was in line with findings of Verma (1989) and Yunus and Paroda (1982) in bread wheat. The non-significant association of harvest index and days to heading with grains/spike in selfed progenies was changed to significant correlation in BIPs to negative (harvest index) and positive (days to maturity) directions. In cross III, the significant positive correlation of grains/spike with plant height in F_3 was broken to non-significant in BIPs but its association with spike length considerably increased. However, the significant correlation of days to heading (negative) and days to maturity (positive) with grains/spike appeared in BIPs from non-significant correlation in F_3.

The significant positive association of plant height with spike length in F_3 was broken in cross I and reduced in magnitude in crosses II & III in BIPs. Similarly, in cross I, effective tiller number per plant showed increased significant positive associations with harvest index and 1000-grain weight in BIPs from significant correlation in F_3 while the magnitude reduced when it was associated with biomass yield/plant. In crosses I & III, the association of biomass yield/plant and 1000-grain weight with effective tiller numbers/plant was changed to significant positive association in biparental from non-significant correlation in selfed progenies. Association of biomass yield per plant with 1000-grain weight in F_3 was broken in cross I, reduced in magnitude in cross III, and significantly improved in cross II. Thousand grain weight established a significant positive association with harvest index in BIPs in cross I and cross III. The significant decrease or increase as well as magnitude change in correlations between different yield contributing characters have also been reported in different crops like sesame (Martinez and Cordoba, 2004; Vinayan and Govindarasu, 2010), in cotton (Meredith and Bridge, 1971; Tyagi, 1987), in pearl millet (Singh and Murty, 1973), in safflower (Naike *et al*., 2009) and in okra (Guddadamath *et al*., 2012).

From the above elaboration, it is evident that reshuffling of genes responsible for correlations (Yunus and Paroda, 1982) at genotypic level amongst some of the characters in three crosses resulted in new recombination, probably due to changes from coupling to repulsion phase linkage or vice versa. An increase in a genetic correlation coefficient can be obtained if linkages were in a predominant repulsion phase (Meredith and Bridge, 1971; Tyagi, 1987). Miller and Rawlings (1967) suggested that breakage of coupling phase linkages tended to decrease the correlation, whereas that of repulsion phase linkages increased their magnitude (ignoring the sign). Form those suggestions, the results of the present investigation indicate to have involved both repulsion and coupling phase linkage as both decreases and increases the correlation. For some of the associations between characters, more changes will likely to occur in these populations following successive intermating (Nematualla and Jha, 1993). In rare cases, the correlation has been changed from significant positive in F_3 to significant negative in BIPs in cross I for traits between effective tiller

number per plant & plant height and spike length & biomass; however in cross III, the association between biomass yield/plat and days to heading changed from significant negative in F_3 to significant positive in BIPs which indicates the breakage of repulsion linkage phase in both biparental mating and selfing approaches following recombination.

In conclusion, the new correlations appeared in biparental compared to selfed progenies changed from either significant to non-significant or non-significant to significant either in desirable or undesirable association. And it was evident that reshuffling of genes responsible for correlations which might have involved both repulsion and coupling phase linkage that decreases and increases the correlation, respectively. This warranted that selection may be resorted to initiate subsequent cycles of intermating which may lead to further improvement.

Table 1. Mean square due to families for BIPs and F_3s across three crosses

Agronomic Trait	Mean squares due to families					
	Cross I		Cross II		Cross III	
	BIP	F_3	BIP	F_3	BIP	F_3
Days to heading	9.2**	33.5**	37.4**	31.0**	7.64	5.4
Days to maturity	2.96**	12.3**	7.5**	21.1**	3.1**	2.71
Plant height (cm)	53.3*	165.1**	94.1**	93.85**	32.95*	64.9**
Spike length (cm)	0.5*	1.6**	0.71**	1.23**	0.64	0.95**
Grains/spike	5.3**	9.75**	53.8**	35.8**	37.1**	43.7**
Effective tiller number	5.8*	5.52**	8.24*	5.35**	6.01**	2.36**
Biomass yield/plant (g)	242.7*	295.6**	408.4*	206.6**	385.5**	178.1**
Harvest index (%)	26.1**	29.1**	35.3	27.6**	26.9**	17.9**
1000 grain weight (g)	15.1**	39.1**	82.9**	84.1**	125.2**	79.9**
Grain yield/plant (g)	45.2**	53.0**	67.7**	29.3*	66.8**	41.1**

* and **, value is significant at 5 and 1% level, respectively

Table 2. The genotypic (above diagonal) and phenotypic (below diagonal) correlations of traits in F_3 and BIP families of cross I

	PH	SL	GPS	ETN	BM	GY	HI	TGW	DH	DM	Population
PH		0.605**	0.722**	0.475**	0.247	0.329	0.307	0.758**	0.374*	-0.076	F_3
		0.235	0.713**	-0.625**	-0.647**	-0.110	0.313	0.015	0.004	-0.085	BIP
SL	0.555**		0.603**	0.404*	0.371*	0.610**	0.687**	0.423*	0.224	0.130	F_3
	0.155		0.587**	0.013	-0.644**	-0.236	0.246	0.230	0.587**	-0.026	BIP
GPS	0.652**	0.612**		0.529**	0.263	0.555**	0.737**	0.425*	0.539**	0.248	F_3
	0.502**	0.554**		-0.002	-0.208	0.212	0.472**	0.255	0.236	0.153	BIP
ETN	0.345	0.315	0.410*		0.779**	0.770**	0.413*	0.700**	-0.173	-0.188	F_3
	-0.374*	0.048	0.023		0.657**	0.778**	0.568**	0.809**	0.053	0.063	BIP
BM	0.242	0.318	0.239	0.729**		0.864**	0.282	0.756**	-0.364*	-0.045	F_3
	-0.337	-0.197	-0.032	0.675**		0.774**	0.182	0.232	0.011	0.402*	BIP
GY	0.282	0.500**	0.470**	0.760**	0.870**		0.723**	0.793**	-0.161	-0.040	F_3
	-0.133	-0.060	0.184	0.745**	0.785**		0.751**	0.835**	0.121	0.030	BIP
HI	0.194	0.504**	0.567**	0.373*	0.166	0.626**		0.439*	0.206	-0.004	F_3
	0.128	0.104	0.275	0.395*	0.063	0.661**		1.000**	0.121	-0.403*	BIP
TGW	0.502**	0.270	0.270	0.462*	0.446*	0.543**	0.375*		-0.313	-0.655**	F_3
	0.105	0.247	0.324	0.458*	0.225	0.596**	0.664**		0.346	0.055	BIP
DH	0.271	0.239	0.444*	-0.082	-0.255	-0.136	0.133	-0.224		0.731**	F_3
	0.028	0.392*	0.181	-0.010	0.035	0.080	0.050	0.206		0.519**	BIP
DM	-0.085	0.144	0.200	-0.121	-0.020	-0.042	-0.043	-0.38*	0.540**		F_3
	-0.016	0.079	0.138	-0.042	0.197	0.016	-0.246	0.064	0.361*		BIP

* and ** , the correlation coefficient is significant at 5 and 1%, respectively; DH, days to heading; DM, days to maturity; PH, plant height; SL, spike length; GPS, grains per spike; ETN, Effective tiller number; BM, biomass yield/plant; HI, Harvest index; TGW, 1000-grain weight; GY, grain yield/plant.

Table 3. The genotypic (above diagonal) and phenotypic (below diagonal) correlations of traits in F_3 and BIP families of cross II

	PH	SL	GPS	ETN	BM	GY	HI	TGW	DH	DM	Population
PH		0.566**	0.545**	0.325	0.791**	0.658**	-0.367*	0.130	0.018	-0.060	F_3
		0.437*	0.284	0.026	0.390*	-0.073	-0.807**	0.163	0.234	0.155	BIP
SL	0.486**		0.239	-0.152	0.566**	0.430*	-0.311	0.355	0.155	0.175	F_3
	0.328		0.062	0.213	0.212	0.074	-0.21	0.11	0.298	0.151	BIP
GPS	0.389*	0.236		-0.085	0.371*	0.445*	0.015	-0.444*	-0.183	-0.059	F_3
	0.193	0.058		-0.191	-0.140	-0.430*	-0.738**	-0.758**	0.388*	0.234	BIP
ETN	0.173	-0.068	-0.056		0.195	0.227	-0.036	-0.076	-0.083	-0.098	F_3
	0.071	0.205	-0.145		0.998**	0.959**	0.014	0.886**	0.196	-0.073	BIP
BM	0.506**	0.467**	0.309	0.404*		0.744**	-0.573**	0.211	0.333	0.352	F_3
	0.324	0.201	-0.066	0.596**		0.970**	0.050	0.716**	0.523**	0.626**	BIP
GY	0.359	0.339	0.295	0.458*	0.824**		0.116	0.438*	0.394*	0.453*	F_3
	0.016	0.103	-0.271	0.515**	0.868**		0.369*	0.615**	0.224	0.549**	BIP
HI	-0.282	-0.250	-0.026	0.046	-0.363	0.223		0.197	-0.025	0.013	F_3
	-0.580**	-0.202	-0.435*	-0.148	-0.265	0.239		-0.298	-0.308	0.149	BIP
TGW	0.136	0.322	-0.323	-0.002	0.238	0.336	0.123		0.2583	0.154	F_3
	0.103	0.105	-0.560**	0.547**	0.558*	0.513**	-0.105		0.008	0.115	BIP
DH	0.072	0.162	-0.198	-0.041	0.254	0.273	0.005	0.176		0.835**	F_3
	0.195	0.209	0.264	0.111	0.276	0.127	-0.278	-0.004		0.965**	BIP
DM	0.014	0.191	-0.044	-0.057	0.300	0.330	0.028	0.127	0.719**		F_3
	0.204	0.206	0.199	0.028	0.341	0.292	-0.101	0.076	0.769**		BIP

* and ** , the correlation coefficient is significant at 5 and 1%, respectively; DH, days to heading; DM, days to maturity; PH, plant height; SL, spike length; GPS, grains per spike; ETN, Effective tiller number; BM, biomass yield/plant; HI, Harvest index; TGW, 1000-grain weight; GY, grain yield/plant.

Table 4. The genotypic (above diagonal) and phenotypic (below diagonal) correlations of traits in F_3 and BIP families of cross III

	PH	SL	GPS	ETN	BM	GY	HI	TGW	DH	DM	Population
PH		0.907**	0.531**	-0.329	0.132	-0.029	-0.281	-0.156	0.064	0.268	F_3
		0.431**	0.333	-0.008	-0.229	-0.131	0.125	-0.091	-0.947**	0.592**	BIP
SL	0.602**		0.823**	-0.085	0.031	-0.121	-0.281	-0.363*	-0.166	0.323	F_3
	0.307		0.963**	-0.083	0.144	0.021	-0.208	0.013	-0.336	0.292	BIP
GPS	0.397*	0.759**		0.066	0.180	0.041	-0.179	-0.186	-0.283	0.006	F_3
	0.262	0.869**		-0.008	0.078	-0.079	-0.273	-0.093	-0.396*	0.423*	BIP
ETN	-0.156	-0.065	0.025		0.053	0.074	0.072	0.084	-0.106	-0.100	F_3
	-0.049	0.041	0.017		0.720**	0.716**	0.192	0.593**	0.724**	0.049	BIP
BM	0.137	0.056	0.102	0.299		0.866**	0.206	0.800**	-0.980**	-0.330	F_3
	-0.168	0.155	0.062	0.746**		0.833**	-0.076	0.559 **	0.442*	0.067	BIP
GY	0.033	-0.051	0.011	0.281	0.894**		0.664**	0.811**	-0.767**	-0.268	F_3
	-0.083	0.077	-0.044	0.748**	0.857**		0.486**	0.876**	0.319	0.026	BIP
HI	-0.155	-0.201	-0.148	0.096	0.208	0.621**		0.355	-0.026	0.004	F_3
	0.130	-0.121	-0.191	0.205	-0.038	0.478**		0.667**	-0.166	-0.036	BIP
TGW	-0.078	-0.264	-0.173	0.167	0.670**	0.712**	0.369*		-0.263	-0.233	F_3
	-0.034	0.012	-0.096	0.519**	0.517 **	0.789 **	0.633**		0.463**	-0.059	BIP
DH	0.125	-0.080	-0.092	-0.043	-0.412*	-0.370*	-0.098	-0.158		0.264	F_3
	0.049	0.125	0.067	0.230	0.180	0.166	0.004	0.201		-0.550**	BIP
DM	0.208	0.270	0.012	-0.082	-0.250	-0.212	0.002	-0.205	0.150		F_3
	0.384	0.267	0.362*	0.055	0.073	0.040	-0.033	-0.045	0.052		BIP

* and ** , the correlation coefficient is significant at 5 and 1% , respectively; DH, days to heading; DM, days to maturity; PH, plant height; SL, spike length; GPS, grains per spike; ETN, Effective tiller number; BM, biomass yield/plant; HI, Harvest index; TGW, 1000-grain weight; GY, grain yield/plant.

Reference

Al-Tabbal JA and Al-Fraihat AH (2012). Genetic variation, heritability, phenotypic and genotypic correlation studies for yield and yield components in promising barley genotypes. *J. Agri. Sci*. 4 (3): 193-210.

Amudha K, Arumugachamy S and Thiyagarajan K, (2006). Studies on selfed and biparental progenies involving upland rice genotypes. *Crop Res.* 31(1): 157-159.

Anonymous (2012). Barley. Consultative Group for International Agricultural Research Centres (CGIAR). *http://www.cgiar.org/our-research/crop-factsheets/barley/*. Accessed on 20/11/2013.

Comstock RE and Robinson HF (1952). Estimation of average dominance of genes, pp. 494-515. In: *Heterosis*, Gowen, J.W. (Ed), Iowa State College Press-Ames.

Falconer DS (1989). Introduction to quantitative genetics, 3rd edition John Wiley, New York.

Fredrickson LJ and Kronsrad WE (1985). A comparison of intermating and selfing following selection for heading date in two diverse winter wheat crosses. *Crop Sci*. 25: 555-560.

Guddadamath S, Mohankumar HD and Salimath PM (2011). Genetic analysis of segregating populations for yield in okra [*Abelmoschus esculentus* (L) Moench]. *Karnataka J. Agric. Sci.* 24 (2): 114-117.

Guddadamath SG, Mohankumar HD and Salimath PM (2012). Effect of biparental mating on association pattern among quantitative characters in okra [*Abelmoschus esculentus* (L.) Moench]. *Int. J. of Hort.* 2(5): 21-24.

Hayes PM, Castro A, Marquez-Cedillo L, Corey A, Henson C, Jones BL, Kling J, Mather D, Matus I, Rossi C and Sato K (2003). Genetic diversity for quantitatively inherited agronomic and malting quality traits. *Developments in Plant Gent. Breed*. 7: 201-226.

Khan M (1985). Inheritance of awns and rows in barley. *Pakistan J. Agric. Res*. 6(2): 93-96.

Kwon SH and Torrie JH (1964). Heritability and interrelationships among characters of two soybean populations. *Crop Sci*. 4: 196-198.

Mahalingam A, Robin S and Rabindran R (2011a). Genetic studies on enhanced disease reaction of biparental progenies (BIPs) to blast disease of rice (*Oryza sativa* L.). *J. Rice Res*. 4(1 & 2): 15-19.

Mahalingam A, Robin S, Mohanasundaram K and Pushpam R (2011b). Studies on genetic architecture of biparental progenies for important yield attributes in rice (*Oryza sativa* L.). *J. Plant Breed. Crop Sci*. 3(12): 296-301.

Manickavelu A, Gnanamalar RP, Nadarajan N and Ganesh SK (2006). Genetic variability studies on different genetic populations of rice under drought condition. *J. Plant Sci*. 1(4): 332 -339.

Martínez JF and Córdoba (2004). Correlation studies in F_2, F_3 and BIPS Populations of sesame (*Sesamum indicum* L.). Sesame and Safflower Newsletter 19, FAO, Italy, Rome.

Meredith WR and Bridge RR (1971). Breakup of linkage blocks in cotton *Gossypium hirsutum* L. *Crop Sci.* 11:695-698.

Miller PA and Rawlings JO (1967). Breaking up of initial linkage blocks through intermating in cotton population. *Crop Sci*. 7: 637-640.

Miller PA, Williams JC, Robinson HF and Comstock RE (1958). Estimates of genetic and environmental variance and covariance and their implication in selection. *Agron. J.* 50:126-131.

Nageswara RG (2007). Statistics for Agricultural sciences. B.S. Publications, Hyderabad, India.

Naik VR, Bentur MG and Parameshwarappa KG (2009). Impact of biparental mating on genetic variability and path analysis in safflower. *Karnataka J. Agric. Sci.* 22(1): 44-46.

Nematualla and Jha PB (1993). Effect of biparental mating in wheat. *Crop Improve*. 20(2): 173-178.

Norry FM, Vilardi JC and Hasson E (2000). Negative genetic correlation between traits of the *Drosophila* head, and interspecific divergence in head shape. *Heredity* 85: 177–183.

Palmer SA, Moore JD, Clapham AJ, Rose P and Allaby RG (2009). Archaeogenetic evidence of ancient Nubian barley evolution from six to two-row indicates local adaptation. *PLoS ONE* 4(7): e6301. doi:10.1371/journal.pone.0006301.

PBTools (2013). Plant Breeding Tools, version 1.1. International Rice Research Institute, Los Banos, Laguna, Philippines.

Pourkhiranandish M and Komatsuda T (2007). The importance of barley genetics and domestication in global perspective. *Annals of Bot.* 100: 999-1008.

Prakash V and Verma RPS (2006). Comparison of variability generated through biparental mating and selfing in six-rowed barley (*Hordeum vulgare* L.). *Crop Improve*. 33(1): 49-52.

Raju C, Satish RG, Rajesab Shanthakumar G and Nayak A (2010). Genetic variability created through biparental mating in bhendi [Abelmoschus esculentus (L.) Moench]. *Gregor Mendel Found*. J. 1: 33-35.

Sharma JR (2008). Statistical and biometrical techniques in plant breeding. New age international publishers, New Delhi, India.

Singh BB and Murty BR (1973). A comparative analysis of biparental mating and selfing in pearl millet (*Pennisetum typhoïdes*). *Theor. Appl. Genet*. 43(1): 18-22.

Tyagi AP (1987). Correlation studies on yield and fibre traits in upland cotton (*Gossypium hirsutum* L.). Theor Appl Genet. ;74 (2) **:**280-283.

Verma RPS, Kharub AS, Sarkar B and Dinesh K (2011). Barley: A crop for changing climate in India. *Progressive Agri.*, 11: 63-73.

Verma RPS, Kharub AS and Mishra B (2008). Dual purpose barley for green fodder and grain in dry areas of India. Poster presented in *10th Int. Barley Genetics Symposium*, Alexandria during 5-10 April, 2008.

Verma SR (1989). Studies on methods of generating variability and genetic analysis of yield and its component traits in bread wheat (*Triticum aestivum* L.em. Thell). *PhD Thesis*, Haryana Agricultural University, Hisar.

Vinayan MT and Govindarasu R (2010). Relative efficiency of biparental mating, single capsule descent, selected bulk and random bulk selections in sesame (*Sesamum indicum* L.). *Electronic J. of Plant Breed*. 1(4): 666-674.

Waitt DE and Levin DA (1998). Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. *Heredity* 80: 310-319.

Yunus M and Paroda RS (1982). Impact of biparental mating on correlation coefficients in bread wheat. *Theor. Appl. Genet*. 62: 337-343.

Yunus M and Paroda RS (1983). Extent of genetic variability created through biparental mating in wheat. *Indian J.Plant Gen. Breed*. 43 (1): 76-81.

Released Varieties

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Registration of "Saban" Bread Wheat variety

Saban is winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2014.The pedigree of Saban involved cross Trakia/3/MvC410-90/GK Kalaka//MvC410-90/Ftm-II with TE6060-1T-1T-1T-0T. Crossing was made in 2004 and yield test began in 2009-2010 growing year.

The spike of the Saban cultivar is moderately long, white, smooth, with awn and compact. It resembles cultivar Gelibolu. The flag leaf is twisted, dark-green, and with medium glaucousity. Grain is oval, hard and red colour. Saban is a medium-tall cultivar, similar to Flamura 85, Gelibolu and Tekirdağ. Plant height is between 70 and 95 cm depending on the growing conditions. It is medium early and as it has good adaptation ability, it has been grown throughout Trakya-Marmara region and some other parts of Turkey. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Saban is tolerant to powdery mildew (*Erysiphe graminis* f. sp. *tritici*) and susceptible to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia recondita*).

Yield potential is high however; high yield can be obtained if environmental conditions are favorable and good agronomic practices are applied. The highest grain yield obtained was 10561 kg ha^{-1} in Tekirdağ location in 2003-2014 growing years. Mean yield of the variety testing experiment was 8318 kg ha^{-1} in Trakya growing conditions. Suggested planting rate is between 450-500 seeds/m^2.

Grain quality is good. The mean values of some bread making qualities of the variety testing experiment (2001 and 2013) are; test weight 77.8 kg, thousand kernel weight 40.1 g, protein content 13.8%, absorpsion 38.9% and sedimentation (Zel) 38.1 ml, alveograph energy value (W) 140.5. The highest quality values in 2010-2011 growing seasons were; test weight 79.1 kg, protein content 16.7%, gluten value 46.0%, gluten index 91.5% and sedimentation (Zel) 65 ml.

Pre-Basic and Basic seeds of the Saban cultivar have been produced by Trakya Agricultural Research Institute (TARI). Certified seed of the Saban are produced by both private companies and state farms.

Figure 1. Spike and grain of the Saban cultivar

İrfan OZTURK
Trakya Agricultural Research Institute (TARI), D-100 Highway 22100 Edirne, Turkey.
Corresponding author e-mail: irfan.ozturk@tarim.gov.tr

Suggested Readings

Anonymous (2014). Tescil Denemeleri Raporu, Ankara

Öztürk İ and Korkut KZ (2011). Characterization of Drought Resistance and Its Relations with Quality in Bread Wheat (*Triticum aestivum* L.) Genotypes. Namık Kemal Uni. 2011. (Ph. D. Thesis)

Öztürk İ, Avcı R, Kahraman T, Girgin CV and Tuna B (2013). Trakya Agricultural Research Institute Annually Reports, Edirne, 2014. Trakya Agr. Res. Inst., Edirne, Turkey

Öztürk İ, Korkut KZ (2015). Effect of the Drought Application on Different Level of Plant Development Stage on Quality characters in Bread Wheat *(Triticum aestivum* L.) Genotypes. 11. Tarla Bitkileri Kongresi, 7-10 Eylül 2015. Çanakkale (Basımda)

Öztürk İ (2015). Trakya Bölgesi İçin Geliştirilen Yeni Çeşitler. Harman Time Dergi. Agustos 2015, Yıl: 3, Sayı: :30 Sayfa:52-54. ISSN:2147-6004

Registration of "Kopru" Bread Wheat variety

Köprü is winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2015. Köprü emanated from cross Pehl//Rpb8-68/Chrc/3/506/88-113 and selection history is TE 5793-1T-1T-1T-1T-4T-0T. Cross was made in 2001-2002 and yield testing began in 2010-2011 growing year.

The spike of the Köprü cultivar is moderately long, white colour, smooth, with awn and compact. The tip of the spike is so compact. Appearance of the spike looks like Tekirdağ but tip of the spike is different. The flag leaf is twisted, dark-green, and with gloucoisity. The grain is oval, hard, red colour and very large. Köprü is medium-tall cultivar with 95 cm plant height. It has resistance to winterkilling, tolerant to medium drought condition and is medium early.It has high productive tillering capacity. It is suitable for growing on fertile and less fertile soils. Köprü is susceptible to powdery mildew (*Erysiphe graminis* f. sp. *tritici*). It has tolerance to yellow rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia recondita*) and it carries Lr9 gene.

Köprü has high yield potential. Average yield of the 2011 and 2012 growing year in Trakya region was 7153 kg ha^{-1}. The highest yield with 8984 kg ha^{-1} was obtained in 2011-2012 growing season in Tekirdağ location. Suggested planting rate is 450-500 seeds/m^2.

Köprü has good bread making quality characteristics. Some of the quality mean valueof the testing experiment years (2012 and 2014) are; thousand kernel weight 36.2 g, test weight 74.7 kg, grain protein content 12.7%, absorpsion55.7% and sedimentation 38.1 ml, alveograph energy value (W) 134.7 and flour yield 60.8%. Before releasing testing experiment thousand kernel weight 46.3 g, test weight 80.7 kg, grain protein content 12.7%, gluten value 40.2 %, gluten index 66.1% and sedimentation 39.5ml. In the same period (2010-2011) the highest values were; grain protein content 15.1%, gluten value 41.7%, gluten index 81.6%, sedimentation (Zel) 52 ml, absorpsion 66.5%, alveograph energy value (W) 164.

Pre-Basic, Basic seeds and Certified seeds of the Köprü cultivar have been produced by Trakya Agricultural Research Institute (TARI).

Figure 1. Spike, grain and in the field of the Köprü cultiva

İrfan OZTURK
Trakya Agricultural Research Institute (TARI), D-100 Highway 22100 Edirne, Turkey.
Corresponding author e-mail: irfan.ozturk@tarim.gov.tr

Suggested Readings

Anonymous (2015). Tescil Denemeleri Raporu, Ankara

Öztürk İ and Korkut KZ (2011). Characterization of Drought Resistance and its Relations with Quality in Bread Wheat (*Triticum aestivum* L.) Genotypes. Namık Kemal Uni. 2011. (Ph. D. Thesis)

Öztürk İ, Kahraman T, Avcı R, Girgin C and Tuna B (2014). Trakya Agricultural Research Institute Annually Reports, Edirne, 2014. Trakya Agr. Res. Inst., Edirne, Turkey,

Öztürk İ, Korkut KZ (2015). Effect of the Drought Application on Different Level of Plant Development Stage on Quality characters in Bread Wheat *(Triticum aestivum* L.) Genotypes. 11. Tarla Bitkileri Kongresi, 7-10 Eylül 2015. Çanakkale (Basımda)

Öztürk İ (2015). Trakya Bölgesi İçin Geliştirilen Yeni Çeşitler. Harman Time Dergi. Agustos 2015, Yıl: 3, Sayı: :30 Sayfa:52-54. ISSN:2147-6004

Registration of "Yuksel" Bread Wheat

Yüksel is winter bread wheat (*Triticum aestivum* L.) variety selected from IWWIP program and selection was made by Trakya Agricultural Research Institute (TARI) and registered in 2016. Yüksel resulted from cross OK81306/STAR'S' and selection history is CMSW92WM00167S-17WM-05WM-015WM-010WM-3WM-0WM-5T-0T. Selection was made in 2009-2010 and yield testing began in 2013-2014 growing year.

The spike of the Yüksel cultivar is moderately long, white colour, smooth, with awn and compact. Appearance of the spike looks like Flamur 85 but tip of the spike is different. The flag leaf is twisted, dark-green, and with slightly gloucoisity. The grain is oval, hard, red colour and large. Yüksel is medium-tall cultivar with plant height variying from 78 cm to 100 cm. It has resistance to winter killing, tolerant to medium drought condition and is medium early. It has high productive tillering capacity. It is suitable for growing on fertile and less fertile soils. Yüksel is resistance to leaf rust (*Puccinia recondita*) and it carries Lr9 gene, yellow rust (*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*) resistance. It has tolerance to *Septoria tritici* leaf disease.

Yüksel has high yield potential. Average yield of the 2013 and 2015 growing year in Trakya region was 8077 kg ha^{-1}. The highest yield with 9310 kg ha^{-1} was obtained in 2013-2014 growing season in Edirne location. Suggested planting rate is 500-550 seeds/m^2.

Yüksel has good bread making quality characteristics. Some of the quality value of the testing experiment years (2013 and 2015) are; thousand kernel weight 35.2-42.8 g, test weight 73.1-78.8 kg, grain protein content 12.3-14.3%, absorpsion 56.5-60.9% and sedimentation 41-51 ml, alveograph energy value (W) 111-199, flour yield 67.1-72.5%. Before releasing testing experiment thousand kernel weight 40.6 g, test weight 80.8 kg, grain protein content 13.8%, gluten value 36.6%, gluten index 96.7% and sedimentation 56 ml.

Pre-Basic, Basic seeds and Certified seeds of the Yüksel cultivar have been produced by Trakya Agricultural Research Institute (TARI).

Figure 1.Spike and grain of the Yüksel cultivar

İrfan OZTURK
Trakya Agricultural Research Institute (TARI), D-100 Highway 22100 Edirne, Turkey.
Corresponding author e-mail: irfan.ozturk@tarim.gov.tr

Suggested Readings

Anonymous (2016). Tohumluk Tescil ve Sertifikasyon Merkezi Müdürlüğü Raporu, Ankara

Öztürk İ, Korkut KZ (2016). Stability Parameters and Effect of the Drought Application on Different Growth Stage in Some Quality Characters of Bread Wheat (*Triticum aestivum* L.) Genotypes. ICBC 2016. 15. International Cereal and Bread Congress İstanbul.

Öztürk İ, Kahraman T, Avcı R, Girgin VÇ, Aşkın OO, Tuba B, Tülek A (2016). Effect of Temperature during Shooting and Grain Filling Period on Yield and Some Quality Parameters of the Bread Wheat (*Triticum aestivum* L.) Cultivars. ICBC 2016. 15. International Cereal and Bread Congress İstanbul.

Öztürk İ, Kahraman T, Avcı R, Girgin VÇ, Tülek A, Akın K, Aybeke M, Tuna B, Kurt C (2016). Effect of the Environmental Condition on Yield and Some Agronomic Characters in Bread Wheat (*Triticum aestivum* L.) Genotypes Under Trakya Region. Current Problems And Horizons For The Agricultural Education, Science And Business 12-13 May 2016. Trakia Universityy, Stara Zagora Bulgaria.

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Journal article:

Toker C (1998). Adaptation of kabuli chickpeas (Cicerarietinum L.) to the low and high lands in the West Mediterranean region of Turkey. Turk J Field Crop 3:10-15.

Toker C and Canci H (2003). Selection of chickpea (Cicerarietinum L.) genotypes for resistance to ascochyta blight [Ascochytarabiei (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (Cicerarietinum L.) sown in autumn.Euphytica 151: 201-205.

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Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (CicerarietinumL.). Euphyticadoi:10.1007/s001090000086

Book:

Toker C (2014). YemeklikBaklagiller. BISAB, Ankara.

Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM(2007) Abiotic stresses. In: Chickpea Breeding and Management,YadavSS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:

FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 15 May 2013.

Dissertation (Thesis):

Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (Cicerarietinum L.).Dissertation, Akdeniz University, Antalya.

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Abbreviations

Abbreviations should be defined at first mention and used consistently.

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Plant Breeders Union of Turkey
www.bisab.org.tr

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Adakale Street, No: 22/12 Kızılay, 6420 Cankaya/Ankara - TURKEY
Phone: +90 312 433 30 65-66 Fax: +90 312 433 30 06
Email: bisab@bisab.org.tr