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Heat and Drought Tolerance in Wheat: Integration of Physiological and Genetic Platforms for Better Performance Under Stress

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ABSTRACT

Heat and drought stress are currently the leading threat on world's food supply, limiting wheat yield. The extent and severity of stress affected agricultural land is predicted to worsen as a result of inadequate irrigation resources, declining water tables and global warming. Drought/heat tolerance is crucial to stabilize and increase food production since domestication has limited the genetic diversity of crops including wild wheat, leading to cultivated species, adapted to artificial environments, and lost tolerance to stress episodes. Breeding for this trait is complicated as it is controlled by polygenes and their expressions are influenced by various environmental elements and molecular methods such as molecular markers, quantitative trait loci (QTL) mapping strategies, and expression patterns of genes should be applied to produce heat/drought tolerant genotypes. Understanding the mechanism of stress tolerance along with a plethora of genes involved in stress signaling network is important for wheat improvement. Integrating physiology and biotechnological tools with conventional breeding techniques will help to develop wheat varieties with better grain yield under stress during reproductive and grain-filling phases. We briefly consider mechanisms of adaptation and highlight recent research examples through a lens of their applicability to improve the efficiency of wheat under stressful field conditions. Improvement for stress tolerance can be achieved by the introduction of drought and or heat related genes and QTLs to modern wheat cultivars.

Keywords: drought, heat, yield, tolerance, climate change, wheat, stress.

I. Introduction

The average global temperature is reported to be increasing at a rate of 0.18°C every decade (Hansen *et al.* 2012; Annual Climate Summary, 2010).Future climates will also be affected by greater variability in temperature and increased frequency of hot days (Pittock,2003).To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how tolerance to heat can be improved (Halford, 2009). Drought, being also a very important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al.* 2009;Rad *et al.* 2012). As a consequence of severe climatic changes across the globe, threat of the occurrence of more frequent drought spells is predicted. Available water resources for successful crop production have been decreasing in recent years. Furthermore, in view of various climatic change models scientists suggested that in many regions of world, crop losses due to increasing water shortage will further aggravate its impacts.

Wheat (*Triticum aestivum* L.) is very sensitive to high temperature and trends in increasing growing season temperatures have already been reported for the major wheat-producing regions (Alexander *et al.* 2006;Hennessy *et al.* 2008). Though, heat stress affects the metabolic pathways at every stage of life of wheat finally leading to yield reduction, the effect of high temperature is particularly severe during grain filling; these losses may be up to 40% under severe stress (Wollenweber *et al.* 2003, Hays *et al.* 2007). Other effects of high temperatures are decreased grain weight, early senescence, shriveled grains, reduced starch accumulation, altered starch-lipid composition in grains, lower seed germination and loss of vigor (Balla *et al.* 2012). End-of-season or 'terminal' heat stress is also likely to increase for wheat in the near future (Mitra and Bhatia, 2008; Semenov and Halford, 2009).Also available water resources for successful crop production have been decreasing in recent years. As a consequence of severe climatic changes across the globe, threat of the occurrence of more frequent drought spells is predicted. Drought stress can influence plants in terms of membrane integrity, root depth and extension, opening and closing of stomata, cuticle thickness, inhibition of photosynthesis, decrease in chlorophyll content, reduction in transpiration, growth inhibition, hormone composition, protein changes, osmotic adjustment and antioxidant production (Szegletes *et al.* 2000; Lawlor and Cornic 2002; Yordanov *et al.* 2000; Praba *et al.* 2009) to stand with some osmotic changes in their organs. Drought can also cause pollen sterility, grain loss, accumulation of abscisic acid in spikes of drought-susceptible wheat genotypes, and abscisic acid synthesis genes in the anthers (Ji e*t al.* 2010). In relation to current development of cultivars, which are higher yielding even in water-limited environments, one of the major targets is *Triticum* species, being one of the leading human food source, accounting for more than half of total human consumption (Fleury *et al.* 2010;Habash *et al.* 2009).

II. Impacts of heat and drought stress on wheat

A. Photosynthesis and chlorophyll content: Photosynthesis is the most sensitive physiological process to elevated temperature (Wahid *et al.* 2007) and any reduction in photosynthesis affects growth and grain yield of wheat (Al-Khatib and Paulsen, 1990, 1999). Heat stress reduces photosynthesis through disruptions in the structure and function of chloroplasts, and reductions in chlorophyll content. Oxidative stress may induce lipid peroxidation leading to protein degradation, membrane rupture and enzyme inactivation (Sairam *et al.* 2000). Rubisco is more sensitive to increased temperatures than the rest of the enzymes involved in carboxylation. PSII appears to be influenced by temperatures above 45°C but is not severely affected by moderately high temperatures (<40°C) (Allakhverdiev *et al.* 2008). Prasad *et al.* (2008b) reported that the most important reasons for PSII sensitivity to high temperature are heat-induced increase in thylakoid membrane fluidity and electron-transport dependent integrity of PSII. The inhibition of PSII electron transport under heat stress is often indicated by a sharp increase in the basal level of chlorophyll fluorescence that corresponds to photosynthetic inhibition (Ristic *et al.* 2007). Heat-stress induced damage and disruption of the integrity of thylakoid membranes also causes the photophosphorylation to cease (Dias and Lidon, 2009).

Drought has a direct impact on the photosynthetic apparatus, essentially by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO_2 supply, together with an increased accumulation of carbohydrates, peroxidative destruction of lipids and disturbance of water balance. Many studies have shown the decreased photosynthetic activity in wheat under drought stress due to stomatal or non-stomatal mechanisms (Ahmadi, 1998; Del Blanco *et al.* 2000). Stomata are the entrance of water loss and CO_2 absorbability and stomatal closure is one of the first responses to drought stress which result in declined rate of photosynthesis. Stomatal closure deprives the leaves of CO_2 and photosynthetic carbon assimilation is decreased in favor of photorespiration. Down regulation of Rubisco large subunit has been observed in drought stressed susceptible wheat lines (Bota *et al.* 2004, Demirevska *et al.* 2009) showing its involvement in drought tolerance mechanism. The activity of photosynthetic electron chain is finely tuned to the availability of CO_2 in the plant and photosystem II (PS II) often declines in parallel under drought conditions. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al.* 2009). Drought decreased photosynthetic rate and high temperature hastened the decline in photosynthetic rate in wheat. Interactions between the two stresses are pronounced, and consequences of drought on all physiological parameters are more severe at high temperature than low temperature. The synergistic interactions indicate that productivity of wheat is reduced considerably more by the combined stress than by either stress alone, and much of the effect is on photosynthetic processes (Shah and Paulsen 2003).

Mohammadi *et al.* (2009) reported significant negative correlation between chlorophyll content and grain yield under heat and drought stresses and revealed that chlorophyll content can be a significant selection criterion for higher yielding lines under heat and drought stress.

B. Water relations: Leaf relative water contents (LRWC), leaf water potential, stomatal conductance and rate of transpiration are influenced by leaf and canopy temperature. In dry environments, higher temperatures lead to higher vapor pressure deficits, which drive higher evapotranspiration. There is limited information on the dynamics of water and heat balance for wheat during reproductive and grain-filling stages, but an example of the dynamics in seedlings occurred in the study by Machado and Paulsen (2001). During reproductive and grain-filling phases, water is needed for stem and peduncle elongation to raise the ear up through the unfolding leaf to the top of the canopy; cell expansion and growth of all parts of the ear; facets of flowering, such as pollen ripening, rapid extension of stamen filaments and fertilization; grain growth and filling.Water flow for many of these processes involves crossing membranes, possibly facilitated by aquaporins. Elevated temperature tends to increase hydraulic conductivity of membranes and plant tissues due to increased aquaporin activity, membrane fluidity and permeability (Martınez-Ballesta,2009) and, to a greater degree, reduced water viscosity with increasing temperature (Cochard *et al.* 2007). Alternatively, increased permeability of membranes may cause flowers and grains to dehydrate, particularly if gradients driving water flow into flowers or grains are disrupted by heat stress. Environmental conditions that increase the rate of transpiration also result in an increase in the pH of leaf sap, which can promote ABA accumulation and lead to reduction in stomatal conductance. Increased cytokinin concentration in the xylem sap was shown to promote stomatal opening directly as well as decrease the sensitivity of stomata towards ABA (Wilkinson and Davies, 2002). Wheat genes Rht-B1b and Rht-D1b showed strong association with drought tolerance as well as stomatal conductance (Rebetzke *et al.* 2012).

C. Grain number and size: Both grain number and weight is sensitive to elevated temperature. Elevated temperatures reduce the duration between anthesis and physiological maturity which is associated with a reduction in grain weight. Variability in terms of high temperature effects on wheat grain number and size appears to be related to genotypic differences in heat tolerance (Viswanathan and Khanna Chopra, 2001;Tahir and Nakata, 2005). Elevated temperatures can also cause grain shrinkage through ultrastructural changes in the aleurone layer and endosperm cells as observed by Dias *et al.* (2008) when day/night temperatures increased from 25/14°C to 31/20°C. In the absence of heat stress, the aleurone layer of a wheat grain has large cells surrounding a starchy endosperm. Drought related reduction in yield and yield components of plants could be ascribed to stomatal closure in response to low soil water content, which decreased the intake of CO_2 and, as a result, photosynthesis decreased (Cornic, 2000; Flexas *et al.* 2004). Drought and temperature stress applied before grain filling shortened the grain filling period and reduced grain weight and specific weight in wheat (Yang and Zhang, 2006; Ehdaie *et al.* 2006). Drought led to shortened duration of maturation, grain filling duration and reduced grain yield, mean grain weight, grain number and thousand grain weight in wheat when imposed at different phenophases (Barbanas *et al.*2008; Kaur and Behl, 2010). Water deficit during early endosperm development might inhibit kernel growth by decreasing endosperm cell division, decreasing the number of endosperm nuclei and correspondingly endosperm fresh weight, starch accumulation and dry mass at maturity (Ober *et al.* 1991). Kaur *et al.* 2011 reported that drought during endosperm cell division reduces grain sink potential and subsequently mature grain mass, mainly by disrupting cell divisions in peripheral and central endosperm and thus reducing endosperm length and breadth to a considerable extent in wheat. The interaction of high temperature and drought stresses resulted in stronger reduction of pericarp thickness and endosperm size in wheat than either stress alone. Grain filling duration has been used as a parameter to identify heat tolerant wheat genotypes (Yang *et al.* 2002b; Mohammadi *et al.* 2008b). Sadat *et al.* (2013) revealed the utility of SSR marker linked with various heat tolerant traits like grain filling duration, HSI (Heat Susceptibility Index), single kernel weight of main spike, grain filling duration under heat stress in MAS for screening 25 bread wheat genotypes to heat stress. However, limited research has been done to identify genetic markers associated with heat tolerance in different plants. Thus, there is an urgent need to understand genetic factors affecting heat tolerance as well as to identify new diagnostic markers to be deployed in MAS, which will ensure faster yield gains under stress environments.

III. Phenotyping for heat and drought tolerance in wheat with physiological traits:

For screening out transgenic wheat lines with desirable heat/drought tolerance, the physiological traits and processes which can be genetically manipulated to improve wheat adaptation to stress have to be taken into account. The genetic basis of drought tolerance in wheat is still elusive. At present the physiological traits (PTs) linked to heat tolerance appear to be a superlative accessible tool since they exhibit the favorable allele combination for drought tolerance (Table 1). Such alleles interact with the environment and genetic background which includes variation in gene expression and hence are still poorly understood through the QTL approach (Reynolds and Tuberosa, 2008). Hybridization of heat tolerance PTs may not always have a predictable outcome related to net crop yield particularly in varying environmental conditions, but breeding such varieties with complementary PTs could augment the cumulative gene effect (Reynolds and Rebetzke, 2011). Thus the physiological phenotyping along with gene discovery can be valuable to pin down desired alleles and understand their genetic mechanism.

IV. Tolerance mechanisms

The capability of crop plants to survive and produce good grain yield under stress is generally regarded as stress tolerance. Plant responses to heat/drought stress are mediated by an intrinsic capacity to endure basal thermo-tolerance and, after acclimation, the ability to gain thermo-tolerance.

A. Antioxidant defense system: The antioxidant defense system in plants involves both enzymatic and non-enzymatic antioxidant systems. To minimize the affections of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes e.g. superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX). Non-enzymatic antioxidants cooperate to maintain the integrity of the photosynthetic membranes under oxidative stress. The enzymatic components may directly scavenge ROS or may act by producing a non-enzymatic antioxidant. Efficient destruction of O^{2-} and H_2O_2 in plant cells requires the concerted action of antioxidants. O^{2-} can be dismutated into H_2O_2 by SOD in the chloroplast, mitochondrion, cytoplasm and peroxisome. SOD is involved in post-translational modification and reported to play key role in drought tolerance (Budak *et al.* 2013). POD plays a key role in scavenging H_2O_2. CAT is a main enzyme to eliminate H_2O_2 in the mitochondrion and microbody (Shigeoka *et al.* 2002) and thus help in ameliorating the detrimental effects of oxidative stress. It is found in peroxisomes, but considered indispensable for decomposing H_2O_2 during stress. In addition to detoxification via the tripeptide glutathione, GST isoforms may also act as glutathione peroxidases and thus are considered as an integral part of oxidative stress responses. Evidences suggest that drought causes oxidation damage from increased production of ROS with deficit defense system of antioxidant in plants (Seki *et al.* 2002; Chinnusamy *et al.* 2004). The transcript of some of the antioxidant genes such as glutathione reductase (GR) or the ascorbate peroxidase (APX) is higher during the recovery of water deficit period and may play a role in the protection of cellular machinery against photo-oxidation by ROS. Resistant genotypes may cope with drought stress through alternate ROS scavengers e.g. catalase-1, GST and SOD as noticed in some drought resistant wheat genotypes (Budak *et al.* 2013). Balla *et al.* (2009) demonstrated that upon exposure to heat stress, during the reproductive phase, activities of enzymatic antioxidants were substantially increased in heat-tolerant genotypes of wheat and have been correlated with heat the capacity to acquire thermo-tolerance (Sairam *et al.* 2000; Mittler, 2002; Almeselmani *et al.* 2009).

B. Osmolyte accumulation: Osmotic adjustment is a remarkable part of plants' physiology by which they respond to water deficits. In this process, plants decrease their cellular osmotic potential by the accumulation of solutes. These compounds include proline, glutamate, glycine-betaine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose, oligosaccharides and inorganic ions like K^+. These compounds help the cells to maintain their hydrated state and therefore function to provide resistance against drought and cellular dehydration (Ramanjulu and Bartels, 2002; Chaves *et al.* 2003). Osmolytes in low accumulation function in protecting macromolecules either by stabilizing the tertiary structure of protein or by scavenging ROS produced in response to drought (Zhu, 2001). In wheat, P5CS (pyrroline-5-carboxylate synthase) gene on 7A chromosome has been found to be involved in osmotic adjustment and showed positive correlation with drought tolerance (Morgan and Tan, 1996; Sawahel and Hassan, 2002).Abebe *et al.* (2003) reported the tolerance of mannitol accumulating transgenic lines of wheat to water stress and salinity.Trehalose over-expression helps in the maintenance of an elevated capacity for photosynthesis primarily due to increased protection of PS II against photo-oxidation (Garg *et al.* 2002). Proline is one of the amino acids, which appear most commonly in response to stress. Wheat is marked by low level of these compatible solutes and the accumulation and mobilization of proline was observed to enhance tolerance to water stress (Nayyar and Walia, 2003). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2010). Hong-Bo *et al.* 2006 investigated the role of proline as a wheat anti-drought defence protein under drought.

C. Molecular basis of tolerance: Expression of heat shock proteins (HSPs) is the most studied molecular response under heat stress. HSPs save proteins from heat-induced aggregation and thus during the recovery period, facilitates their re-folding (Maestri *et al.* 2002; Rampino *et al.* 2009). Accumulation of Hsps coincides with acquisition of stress tolerance. Hsps are induced by water stress in several plants (Coca *et al.* 1996; Campalans *et al.* 2001). Transgenic *Arabidopsis* plants overexpressing AtHSP17.7 accumulate high levels of AtHSP17.7 protein and show enhanced tolerance to drought and salinity (Sun *et al.* 2001). The concept that low RWC impairs protein structure explains the necessity of molecular chaperones to accumulate under a range of stresses. The abundance of small heat shock proteins (sHsps) in plants and their functional characteristics of binding and stabilizing denatured proteins suggest that sHsps play an important role in plant stress tolerance (reviewed in Wang *et al.* 2004).

Late embryogenesis abundant (LEA) proteins are influenced by drought stress and they help other proteins retrieve after denaturation during water stress (Campbell and Close, 1997). There have been a lot of works during the last two decades to engineer LEA producing genes for promoting crop water stress resistance. Sivamani *et al.* 2000 indicated that barley group 3 LEA gene HVA1 assists to increase wheat growth under drought stress. The over-expression of gene HVA1 in leaves and roots of rice and wheat leads to improved tolerance against osmotic stress as well as improved recovery after drought and salinity stress. Wheat LEA genes, PMA1959 (encoding group one of LEA protein) and PMA80 (encoding LEA protein's second group) improved water deficit resistance in rice (Cheng *et al.* 2002). In wheat, protein contents of groups one, two, and three of LEA have been detected. The Em gene of wheat which encodes LEA protein first group has been vastly researched (Cheng *et al.* 2002; Litts *et al.* 1987). Group three of LEA protein has also been distinguished in seedlings of wheat (Curry *et al.* 1991; Ried and Walker-Simmons, 1993). Dehydrins, also known as group 2 LEA proteins accumulate in response to both dehydration as well as low temperature In durum wheat, protein of groups two (dehydrins) and four of LEA proteins were studied by Ali-Benali *et al.* 2005.Dehydrins help to stabilize macro-molecules against heat-induced damage (Brini *et al.* 2010). Dehydration-responsive element binding (DREB)genes belong to largest family of transcription factors which are induced abiotic stresses. In wheat, Dreb1 genes are located on 3A, 3B and 3D chromosomes. Mapping of Dreb-B1 genes showed that is located between Xmwg818 and Xfbb117 on 3BL chromosome. Dreb-B1 gene is responsible for abiotic stress tolerance in wheat such as it provides tolerance against drought. It provides tolerance to salinity, low temperature and ABA as well (Wei *et al.* 2009). Dreb1/Dreb2 homologous genes have been isolated from many crops viz. wheat, maize, rice and from perennial ryegrass (Lata and Prasad, 2011).

Vacuolar H+translocating pyrophosphatase (V-PPase) is an important enzyme linked to plant development as well as resistance to abiotic stress. Wheat V-PPase genes, TaVP3, TaVP2, and TaVP1 were investigated by Wang *et al.* (2009). Kam *et al.* (2007) also detected the responsible genes in wheat for water stress. They observed that TaRZF70 as a RING-H2 zinc finger gene presented various responses to drought stress which was up-regulated in the leaf and down regulated in the root. TaRZF38 and TaRZF70 were expressed in the wheat root while TaRZF74 and TaRZF59 were expressed in embryo and endosperm at the highest level. Drought stress influences RD gene (responsive to desiccation). Available information on drought responsive genes is still limited as their roles have not been thoroughly determined.

D. Quantitative trait loci (QTL) analysis: QTL analysis and other subsequent study through molecular markers in wheat revealed that chromosome 5B, 4B and 7B carry important genes for drought tolerance (Dashti *et al.* 2007). A QTL on chromosome 5B located between two markers (M51P65 and Psr136) showed positive correlation with drought tolerance. However, QTLs discovered on chromosome 4B and 7B (between M62P64d - Rht and M83P65d - M21P76n markers respectively) showed negative effect on drought tolerance. Reduced height genes (Rht) are responsible for short stature in wheat (Borojevic and Borojevic, 2005). Dwarfing wheat genes Rht- B1b, Rht-D1b and Rht8 have been identified (Gasperini *et al.* 2012) and found positively correlated with drought tolerance. Recently, several QTLs have been identified in wheat for heat tolerance during the reproductive phase. Byrne *et al.* (2002) detected QTLs for heat tolerance under hot and dry conditions on chromosomes 2B and 5B in a spring wheat population. Dhanda and Munjal (2006) reported both dominant and additive types of gene action at the genetic direction of MTS. The QTL for thousand grain weight (TGW) was dissected in to single Mendelian gene and mapped on short arm of chromosome 7D by Röder *et al.* (2008). Nine QTLs across the wheat genome for effective tiller per plant were mapped by Li *et al.* (2010) using a set of 168 doubled haploid (DH) lines, derivatives of a cross between two winter wheat cultivars Huapei 3 and Yumai 57. Similarly, 3 QTLs for stay green have been mapped on chromosome 1A, 3B and 7D by Kumar *et al.* (2010). The other traits like early ground cover, leaf glaucousness (Richards, 1996), leaf rolling (Araus, 1996), biomass, canopy temperature (Reynolds *et al.* 2001), etc. have been mapped independently in various genetic backgrounds. Paliwal *et al.* (2012) used the parameter heat susceptibility index (HSI) for thousand grain weight (HSITGW), canopy tem perature (HSICT) and grain filling duration (HSIGFD) to identify the QTLs for heat tolerance.

V. Improving genetic adaptation of wheat to stress

Development and selection of crop varieties is, most often, aimed at improving yield under existing climatic conditions. With the changing climate, in particular episodes of high temperature during the reproductive phase, ideotypes with physiological, morphological, and molecular traits unique for heat tolerance are required (Semenov and Halford, 2009). Recent advances in molecular biological, functional, and comparative tools open up new opportunities for the molecular improvement of modern wheat. Recently developed techniques enable faster identification and characterization of heat/drought-related gene(s) and generegion(s). Natural variants of modern species harbor a large repertoire of potential stress related genes and hold a tremendous potential for wheat improvement. Introduction of these components of wheat can be performed either with breeding through marker-assisted selection or transgenic methods. Recent increase in sequence availability due to recently developed high-throughput sequencing strategies has provided several high quality genetic markers for breeding. Transgenic strategies with enhanced transformation and selection methods are currently being developed.

A. Marker-assisted selection: MAS is most often performed based on physio-morphological characteristics related to yield under stress conditions. Markers that are utilized in such a context include SSR (simple sequence repeat) markers, Xgwm136, and NW3106, which are linked to genes that effect tillering capacity and coleoptile length, respectively (Gulnaz *et al.* 2011). Other selection markers are linked to Rht (reduced height) genes, which are known to be associated with harvest index.Additionally, transcription factor-derived markers, especiallyDREB proteins hold a great potential as PCR-based selection markers that can be useful in MAS (Wei *et al.* 2009). However, the isolation of transcription factors is a challenge since they belong to large gene families containing members with high sequence similarities. Identification and successful isolation of a single drought-related locus is compelling also in general due to the complex genomic structure of wheat. The polyploid nature of the genome also makes molecular analysis complicated (Barnabas *et al.* 2008) due to repetitions of DNA sequences. Natural genetic variation may be used through direct selection under heat stress during the reproductive phase or through QTL mapping and subsequent marker-assisted selection.

B. Use of Transgenics: An alternative to ongoing breeding programmes is transgenic methods, which enable the transfer of only the desired loci from a source organism to elite wheat cultivars, avoiding possible decrease in yield due to the cotransfer of unwanted adjacent gene segments. Until now, transcription factors have been the most appealing targets for transgenic wheat improvement, due to their role in multiple stress-related pathways. In two different lines of research, overexpression of cotton and A. thaliana DREB was performed in wheat, resulting in transgenic lines with improved drought tolerance (Guo *et al.* 2009; Pellegrineschi *et al.* 2004; Hoisington and Ortiz,2008). In another study, a barley LEA protein, HVA1, was also overexpressed in wheat, and overexpressors were observed to have better drought tolerance (Bahieldin *et al.* 2005). It wheat will be transferred to the fields as a common is not unreasonable to predict in the following decades that GM (genetically modified) commercial crop. However, to pace this process, new transgenics methodologies should be developed since the current methods are laborious and time consuming. In a recent study, drought enhancement of bread wheat was established with the overexpression of barley HVA1, using a novel technique, which combines doubled haploid technology and *Agrobacterium* mediated genetic transformation (Chauhan and Khurana, 2011).

C. Use of Proteomics: New studies are focusing to study wheat tolerance attheproteomic level to target different proteins and understand their role in stress. The differential expression at biochemical and protein level expression could be a simpler approach to understanding and manipulating drought stress in plants (Jiang *et al.* 2012). Consequently comparative proteomics may provide a clearer picture and alternate way to evaluate and characterize drought resistant genes and proteins in wheat varieties. Global changes in the plant gene expression during growth, development and exposure to environmental variations is reflected in respect to changes at the level of various enzymes and or proteins of various metabolic pathways (Hakeem *et al.* 2012). This suggests the importance of analyzing wheat proteome to understand the molecular basis of heat tolerance in some wheat cultivars. In fact, with the availability of sensitive and accurate proteome analysis technique, proteomics has emerged as a powerful tool in discovering genes and pathways involved in abiotic stress response in crop plants (Chen and Harmon 2006; Kosová *et al.* 2011). The key proteins/enzymes and metabolic pathways identified from tolerant wheat lines could be potentially targeted for designing tolerant varieties of wheat.

VI. Conclusion and future perspectives

The wheat crop is grown in diverse agro-ecological conditions ranging from temperate to subtropical climates. Thus, considerable climatic differences in temperature and relative humidity exist in these areas and wheat crop experiences wide seasonal variations. The synergistic interactions between heat and drought indicate that productivity of wheat is reduced considerably more by the combined stress than by either stress alone. Simultaneous drought and heat stresses are more detrimental than either stress alone. The generation of novel plant varieties displaying tolerance to abiotic stress is highly expected to cope with the unfavorable environment challenges. Although molecular markers discovered through QTL, proteomic and gene functional analysis suggest positive correlation with stress tolerance, the complex nature of hexaploid wheat genome makes it difficult to clearly identify the locus of extremely important markers. Traditional breeding, utilization of germplasm resources and transgenic approaches as well as physiological breeding is advocated for significant gain in yield under abiotic stress environments. Despite the current knowledge on the scientific basis of heat/drought tolerance, more information is needed to understand and be able to manipulate such complex quantitative trait. In recent decades, application of high-throughput screening, “omics” strategies on Triticum species with differential drought tolerance copingabilities, has revealed several stressrelated candidate gene(s) or gene block(s). Furthermore, using a variety of bioinformatics, molecular biology, and functional genomics tools, drought-related candidates were characterized, and their roles in drought tolerance were studied. With the recent advances in sequencing technologies, genome sequence of bread wheat is almost complete by the efforts of ITMI (The International Triticeae Mapping Initiative) and IWGSC (International Wheat Genome Sequencing Consortium). Availability of whole wheat genome sequence will contribute to the ongoing studies of exploring the extensive reservoir of alleles in drought/heat tolerant wild germplasm, and this also enables better marker development, genome analysis and large scale profiling experiments. Affordable next-generation sequencing and novel transformation techniques now allow fundamental research to be performed on crops. The future efforts will be to integrate and translate these resources into practical higher yielding field products.

Table 1. List of morpho-physiological traits and adaptation mechanism under heat/drought stress.

Trait	Adaptation mechanism	Reference
Leaf rolling	Avoidance	Araus, 1996
Leaf glaucousness	Avoidance	Richards, 1996; Tsunewaki and Ebana, 1999; Bennett *et al.* 2011
Transpirational cooling (cooler canopy)	Avoidance	Reynolds and Rebetzke, 2011; Pinto *et al.* 2010
Stomatal conductance	Avoidance	Reynolds *et al.* 1994
Early maturation	Avoidance	Tewolde *et al.* 2006
Alteration of membrane lipid composition (Membrane stability)	Avoidance	Shanahan *et al.* 1990; Ciuca and Petcu, 2009
Photosynthetic rate	Tolerance	Rijven, 1986; Al-Khatib and Paulsen, 1990, 1999
Chlorophyll content	Tolerance	Al-Khatib and Paulsen, 1984; Farooq *et al.* 2009
Accumulation of osmo-protectants	Tolerance	Sawahel and Hassan, 2002; Abebe *et al.* 2003; Hong-Bo *et al.* 2006
Antioxidant defense	Tolerance	Almeselmani *et al.*, 2009; Sairam *et al.* 2000
Signaling cascade and transcriptional control	Tolerance	Kaur and Gupta, 2005
Expression of stress proteins	Tolerance	Balla *et al.* 2009; Maestri *et al.* 2002

Fig. 1. Schematic illustration of heat/drought induced signal transduction mechanism and development of stress tolerance in plants. Stress imposes injury on cellular physiology and results in metabolic dysfunction. Stress injury and ROS generated in response to stress also trigger a detoxification signaling by activating genes responsible for damage control and repair mechanism leading to stress tolerance. Partly adopted from Wahid *et al.* 2007

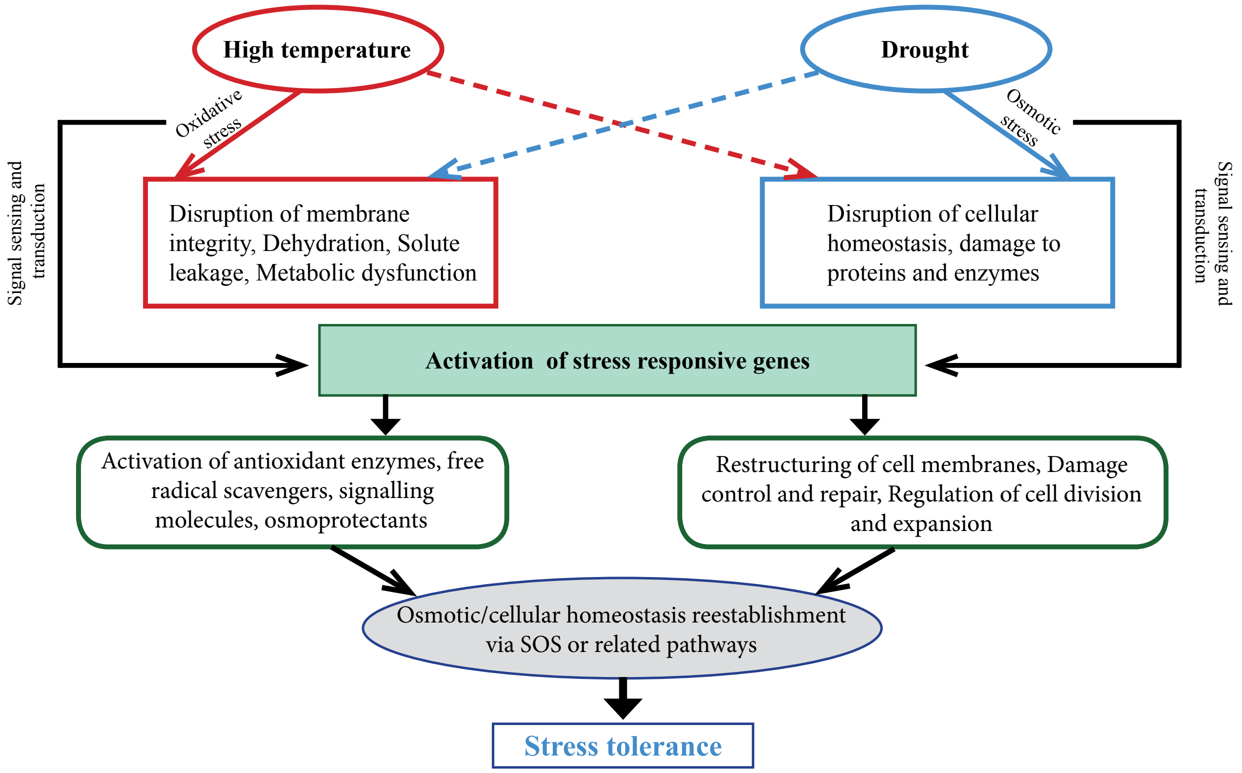
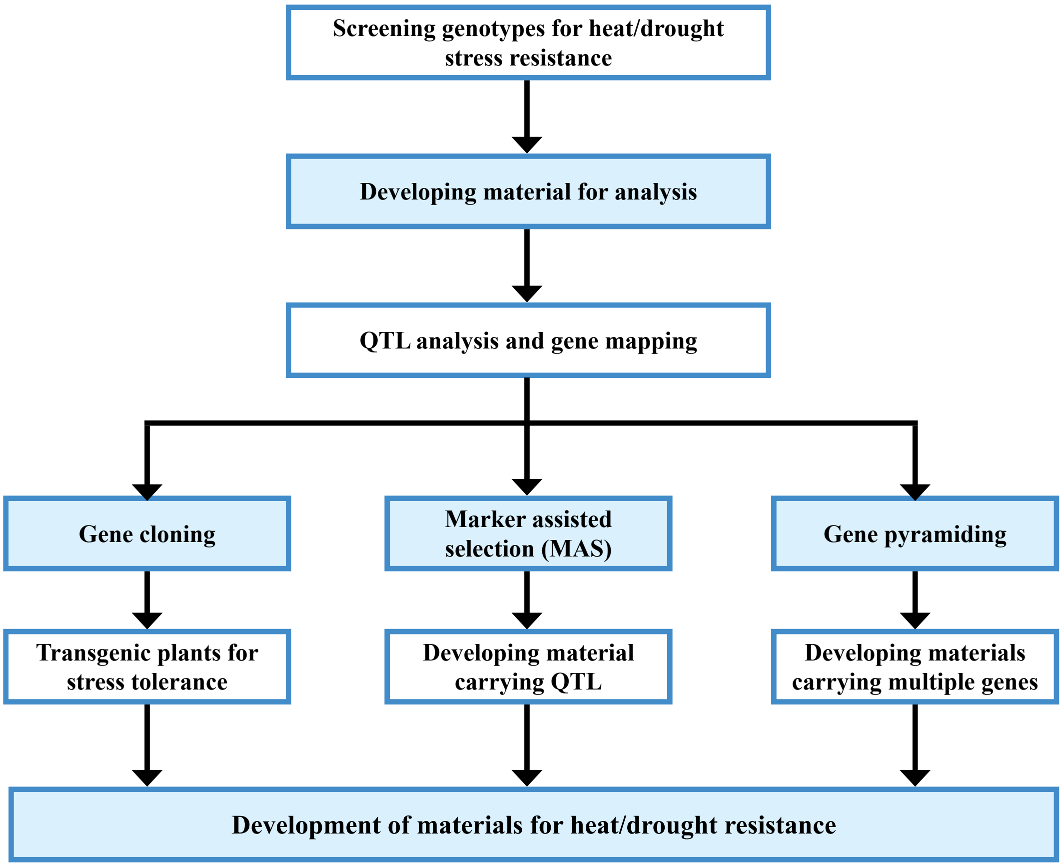


Fig. 2. Developing materials for heat/drought resistance. Genotypes are screened for stress resistance, which are used for the development of genetic materials for QTL analysis and gene mapping. For gene cloning, identified gene or major QTL are analyzed in detail using large populations. Cloned gene is transferred into widely adapted varieties. To develop materials carrying gene or QTL, DNA markers having link to QTL are used for marker-assisted selection. Likewise, marker assisted selection is used for developing materials of gene pyramiding. The gene cloning, marker assisted selection and gene pyramiding are usefull for developing materials for drought resistance. Modified from Budak *et al.* 2013.



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Tomato Varieties with High Indices of Productivity and Resistance to Environmental Factors

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ABSTRACT

In this paper, the results of the complex evaluation of valuable morphobiological and agronomic characteristics of tomato varieties, created at the Institute of Genetics and Plant Physiology of the Academy of Sciences are presented. In order to demonstrate the variability of agronomic characters and to specify the value of analyzed genotypes, their comparative evaluation was carried out by some of biological parameters: yield, production rate, average fruit weight, vegetation period, pericarp thickness, resistance to heat and cold stress. The varieties Jubiliar 60/20, Prestij, Elvira, Mihaela, Milenium and Tomiş have determinate growth and are distinguished by plant height, precocity, yield and production. Also, these varieties differ by the important fruit characters as well as mass (large and medium), shape (round, flat-round and cylindrical), the number of lodge (2-3 and above), pericarp thickness (medium and large) and mesocarp thickness (medium, large and extra large). The evaluated varieties manifest increased productivity and good taste properties. The results identify the genotypes that combine precocity, high productivity and resistance to environmental factors. These can serve as initial material for breeding.

Keywords: tomato, breeding, resistance, cold, draught.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world. By degree of use it occupies the second place in the world, being surpassed only by potatoes. Tomato fruits are notable for their taste, dietetic and medicinal properties, as well for divers use (Ershova, 1978; Avdeev, 1982). As per FAO data tomatoes are grown in the world on an area of 4 million ha. The most tomato producing counties are China (974,000 ha) and India (520,000 ha) while Moldova produced 84,070 tons of tomatoes in 2009 (http://faostat.fao.org/).

At the current stage of vegetable development special attention should be paid to qualitative as well as quantitative traits to improve food security of the country. In agrocenosis the increase of crop yields is not only due to optimized growth conditions, but also due to the use of more productive and resistant genotypes.

It has been shown that the role of genotype for both quantitative and qualitative components of yield is higher, when the pedo-climatic and climatic conditions are unfavorable. Expansion of sown fields and plantations occupied by the varieties and hybrids resistant to abiotic and biotic environment extremes substantially reduces harvest losses and production costs. Positive effects can be obtained by using a sufficient number of specially selected genotypes for specific agro-ecological zones and taking into account the considerable variability in growing conditions of the plants. Now we have a critical need - to develop and implement the regional models of species and hybrids, to develop and introduce some detailed “passports” of homologated and of perspective forms of crops (Kilchevsckiy, 1984, 1987; Juchenco, 1988, 2005; Pivovarov, 1990, 2003).

Contemporary breeding demonstrates the need to create of lines, varieties and hybrids with high environmental resistance. Importance of adaptive breeding in creation of varieties that combine resistance to stress factors with high productivity is recognized by many researchers. (Lapton, 1981; Kilchevsckiy and Hotileva, 1997; Mihnea *et al.* 2002, Iurlova, 2006; Lupashku *et al.* 2008). .Creating of new tomato varieties that will combine the high productivity and ecological resistance has one of the priority directions of contemporary breeding. (Lapton, 1981; Mihnea *et al*., 2002; Lupashku *et al.* 2008)**.**

Development of new varieties and hybrids that are high yielding ,possessing better fruit quality and longer shelf life, resistant to biotic and abiotic stress and are able to provide high harvests under the conditions of positive low temperature (2-3^{0}C) and of low light is worthwhile an indispensable component of solving a global problem of creating more economic technologies in terms of bioenergetics. That is why, today, in order to create new forms of tomato breeders have to consider the whole complex characters especially the productivity, fruit quality, the resistance to abiotic (including climate change) and biotic stresses.

For achieving such objectives, breeding activities were carried out during the period 1995-2011 in the Institute of Genetics, Physiology and Plant Protection to obtain new lines and varieties of tomatoes, which satisfy the requirements of precocity, productivity, resistance to temperature fluctuations and quality. This paper focuses on recently developed tomato varieties in our institute.

Material and methods

The experiments were performed under field condition, on the experimental areas of the Institute of Genetics and Plant Physiology and in the Lab of Applied Genetics. Experimental materials consisted of six tomato varieties obtained through intraspecific and interspecific crosses: Mihaela, Elvira, Jubiliar 60/20, Prestij, Milenium and Tomiş along with two control varieties Soiearis and Peto 95.

Field experiments were conducted in 3 repetitions, in randomized blocks with the distance between rows - 70 cm and between plants - 30 cm. During the vegetative period phonological observations were made. Morphological description was done according to "Guidelines on the testing of vegetable and root crops", Baculina V.A, *et al*. (1982), and "Guidelines for the conduct of tests for distinctness, uniformity and stability" (1992). The scale of resistance to cold temperature was measured following VIR method (Ivackin, 1979).

For assessing the tomato genotypes by sporofit resistance at high temperatures methodological recommendations the VIR were used based on plant growth capacity maintained at high temperatures during 6 hours (Smirnova and Garanko, 1990) . Statistical analysis was performed as described by Dospehov, (1979).

Results and discussions

Morphological description of observations revealed that five varieties of tomato have the determinate type of growth and only the Prestij variety is semi-determinate. The plants are medium branched (Mihaela, Prestij and Tomiş varieties have 5-6 branches and 3-5 branches the rest). By plant height the varieties were placed as follows: Elvira, Jubiliar 60/20, Milenium - 40.0 to 50.0cm, Mihaela, Tomiş - 50,0-60.0cm and the variety Prestige - 65.0 - 70.0 cm.

For all studied varieties the leaf is standard, low and intermediate corrugated, sectate in large, medium and small segments, dark green color of leaf for Millennium and Tomiş varieties, green for Prestij and green-gray to the rest of varieties.

Flowers are regular, yellow colored, inflorescence is simple, 3-5 flowers for Jubiliar 60/20, 5-6 flowers - Elvira, Prestij and Tomiş, and 6-8 flowers - Mihaela and Milenium. The first inflorescence usually appears after the 5-6 node, the following-after 1-2.

The main differences were tested by productivity, fruit quality and fruit main characters. By the fruit form they can be divided into the following groups: 1- circular (Elvira, Mihaela, Milenium and Tomiş), 2- slightly flattened (Prestij), 3- cylindrical (Jubiliar 60/20). Fruits on cultivar Jubilee 60/20 are little edge, at the rest the fruits is smooth.

By fruit mass, the majority of varieties have large fruits (105,0…130,0g), only two varieties (Mihaela and Milenium) have medium fruits (71,7…95,0g). In breeding a high attention is given to thickness of mezocarp. The size of mezocarp determines the destination of fruit (fresh use, juice or paste). According to existing standard, tomato fruits are divided into five groups by the named index: very small (2,0 cm), small (2,1 to 3,0 cm), medium (3, 1 to 4,0 cm), large (4,1 to 5,0 cm), very large (> 5 cm). By this character, the pulp at varieties Jubiliar 60/20, Mihaela, Milenium and Tomiş is medium, and the Prestij and Elvira - is large.

Pericarp thickness represents an important indicator that influence market yields. Data from the specialized literature (Bakulina, 1970; Blashiuk, 1983; Kuzeomensckiy, 2004; Mihnea, 2008) provide evidence for a considerable genotypic variability of the mentioned trait. The studied varieties were assessed in terms of pericarp size and significant differences were ascertained (Table 1). According to the existing standards of tomato morphological traits, pericarp can be: thin (<3 mm), average (3-6 mm), and thick (>6 mm). Based on the comparative analysis of the results, tomato varieties were divided into two groups: with thick pericarp (Jubiliar 60/20, Mihaela, Prestij), with pericarp of medium thickness (Elvira, Milenium and Tomiş). Therefore, varieties created in IGFP, along with other valuable character, also possess carrying capacity of fruits.

Number of seminal lodge represents an internal morphological character of the sinecarpelar fruit based on which the number of overgrown carpels is determined that forms the pistil and type of placenta. Usually, in tomatoes the number of cameras ranges from 2-3 to 5-9. Large fruits have a large number of lodges, the medium-sized have a smaller number. Numbers of seminal lodges differ from one variety to another. Depending on this aspect two types of fruits were found: with 2-3 seminal lodges (Prestige, Jubiliar 60/20, Mihaela, Milenium) and with 3 ... 5 seminal lodges (Elvira, Tomiş).

It is known that no matter in what direction the breeding is carried out, the specialists firstly draw attention to precocity, yield and fruit quality. Vegetation period is an index that determines the biological precocity of tomato, the possibility to cultivate in certain areas. According to the literature it is related to productivity, resistance to cold, chemical composition, resistance to pests and diseases (Ershova, 1978; Avdeev, 1982). Phonological observations made during the vegetation period showed significant differences on the growing season, depending on variety and climatic conditions. Based on the vegetation period, tomatoes are classified in: ultra-early (<105 days), early (106-110 days), medium (111-115 days), late (116- 120 days) and very late (> 120 days).. As a result of the investigations a high diversity of varieties in the basis of vegetation period was found.

The varieties created in our institute can be classified in four groups: very early (Tomis, Milenium), early (Elvira), medium early (Mihaela, Prestij), late (Jubiliar 60/20).

In order to determine the role of the genotype factor, year and interaction of genotype and year, for overall productivity and fruit quota of product - culture the factor analysis was done (Table 2, 3).

The data showed that in the case of tomato cultivation by seedling, conditions of the year had a higher share than the genotype - 76, 23 and 50,29%, respectively, for general productivity and fruit quota. Role of genotype was more important in the case of the second clue (28,66%), than in the case of overall productivity (16,42%). The share sum of genotype (28,66%) and its interaction with the environment (7.98%) reveal their quite high role (36,64%) in the obtaining quality production.

Role of genotype factor is also demonstrated by the differential reaction of varieties to the year conditions and according to the character. For example, in the case of overall productivity, the Mihaela variety presented the most stable indices (56,6-59,7t/ha), and Jubiliar variety – the most variable (42,0-72,3 t/ha) (Fig. 1 A).

Regarding the market fruit quota, it was noticed a reduced variability at Prestij variety (80,8-82,6%) and Solearis variety (79,5-82,0%), but quite high at Elvira variety (78,8-87,1%) (Fig 1 B).

In comparison to the plants cultivated by seedling, to those cultivated from seeds the role of genotype is increased significantly (48,93%) for the overall productivity, and for the market fruit quota has increased a lot the share of genotype vs year interaction factor (44,61%) (Table 3).

The data presented reveal that by this method of cultivation the tomatoes achieve more definitive genetic potential of plants, especially regarding their capacity of interaction with the environment. For example, in the case of overall productivity, variety Milenium presented a smaller variation (46,4-60,1t/ha) (Fig.2 A), and in the case of fruit quota – Tomis variety (89,4-94,5%) (Fig. 2 B).

Peto 95 variety demonstrated very large limits (81,4-91,8%), which makes difficult the character forecasting.

Especially attention was attracted by the varieties: Tomis (Fig. 3) Mihaela (Fig. 4), Jubiliar 60/20 (Fig. 5) which achieved very significant production in 2008 and 2009 years.

Evaluation of tomato resistance to heat and drought (Fig. 6), indicates that all varieties show a high resistance to cold and medium resistance to heat (Elvira, Jubiliar 60/20, Prestij, Tomis). Increased heat resistance indices were registered for the varieties Milenium and Mihaela, resistance that was 73.1% and 64.7% respectively.

The chemical composition of fruits among the studied varieties (Table 4) (in comparative culture competition) shows the quality value of fruit. Thus created varieties differ from control by all biochemical indicators. It should be mentioned that all varieties exceeded the standard by index sugar / acidity, which is an indicator of fruit quality. This shows that created varieties manifested only increased productivity, but high taste properties also.

Conclusions

The varieties created at Institute of Genetics, Physiology and Plant Protection: Jubiliar 60/20, Elvira, Mihaela, Milenium, Tomis are determinate while Prestij is semi-determinate, medium leafy and branched. They differ by plant height, fruit size, precocity, total and market yields. Also, they are distinguished by a complex of fruit characters: fruit mass (large and medium), shape (round, slightly-flatbed and cylindrical), the number of loge (2-3 and more), thickness of pericarp (medium and thick), and thickness of mezocarp (medium, large and very large).

Created varieties show increased productivity and high taste properties, high resistance to cold and heat. They can be recommended for use in breeding programmer to create new varieties.

Table 1. Comparative analysis of tomato varieties after a complex morphological character

Variety	Fruit weight, g	Fruit form	Number of seminal lodge	Pericarp thickness, mm.	Mezocarp thickness, cm
Jubiliar 60/20	105.0±8,17	cylindrical	2.8±0,13	8.6±0,22	3.8±0,62
Prestij	120.0±10,9	slightly flattened	3.0±0,01	7.6±0,37	4.4±1,62
Elvira	130.0±5,93	circular	4.5±0,37	5.4±0,26	4.6±1,59
Mihaela	95.0±3,24	circular	2.6±0,16	8.0±0,21	3.8±0,93
Milenium	71.7±3,50	circular	2.4±0,18	3.0±0,31	3.9±0,84
Tomiş	102.0±3,00	circular	3.7±0,15	5.8±0,27	4.2±0,96
Solearis (martor)	110.0±7,55	slightly flattened	4.3±0,33	5.4±0,22	4.8±1,59

Table 2. Factor analysis of the source of variation of productivity and fruit quota of tomatoes growing by seedling

Source of variation	Degree of freedom	Squares sum of effects	Share in source of variation, %
		General productivity	
Tomato genotype	4	208.4*	16.42
Year	2	967.4*	76.23
Genotype x year interaction	8	79.8*	6.29
Aleatory effects	30	13.5	1.06
		Quota of market fruits	
Tomato genotype	4	38.8*	28.66
Year	2	68.1*	50.29
Genotype x year interaction	8	10.8	7.98
Aleatory effects	30	17.7	13.7

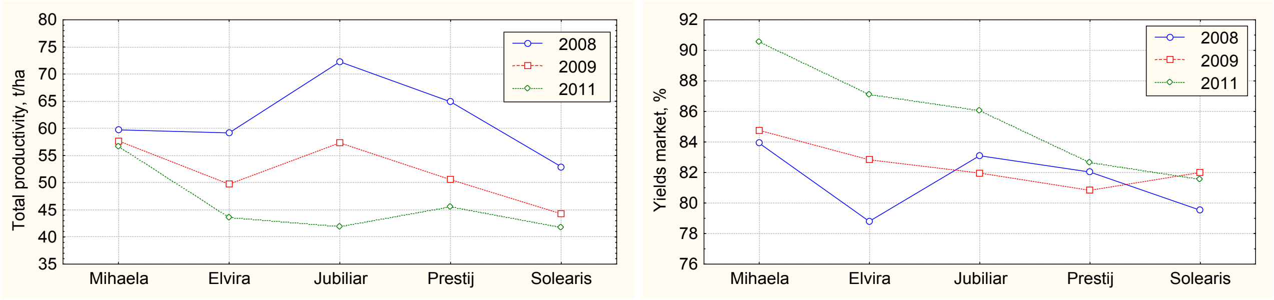
* - p≤0,05

Table 3. Factor analysis of the source of variation of productivity and market fruit quota of tomatoes growing by seeds

Source of variation	**Degree of freedom**	**Squares sum of effects**	**Share in source of variation, %**
		General productivity	
Genotip de tomate	2	389.79*	48.93
An	3	337.55*	42.37
Interacţiune genotip x an	6	63.06*	7.91
Efecte aleatorii	24	6.29	0.9
		Quota of market fruits	
Genotip de tomate	2	30.4*	29.54
An	3	19.3	18.76
Interacţiune genotip x an	6	45.9*	44.61
Efecte aleatorii	24	7.3	7.09

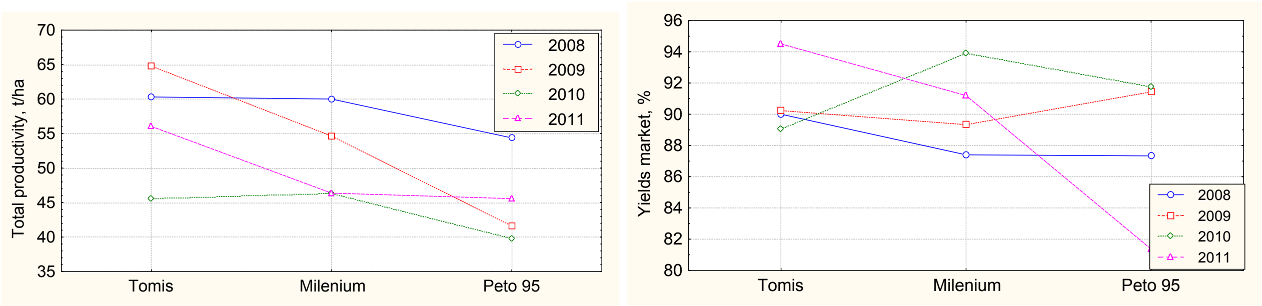
*- p≤0,05

Fig.1. Influence of the year conditions on the overall productivity (A) and market fruit quota (B) of tomatoes cultivated by seedling



A **B**

Fig.2. The influence of year conditions on overall productivity (A) and fruit quota (B) at tomatoes cultivated by seeds



A **B**

Figure 3. Variety early Tomiş

Figure 4. Variety medium early Mihaela

Figure 5. Variety medium late Jubiliar 60/20

Figure 6. Evaluation of tomato varieties for resistance to cold and drought
1. Jubiliar 60/20 2. Prestij 3. Elvira 4. Mihaela 5. Milenium 6. Tomiş 7. Solearis (standard)

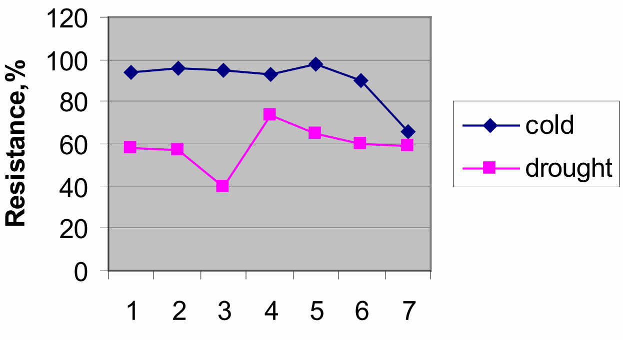


Table 4. Chemical composition of tomato fruits

Variety	**Dry matter %**	**Sugar %**	**Vitamin C, мг/%**	**Acidity %**	**Sugar/acidity indice**
Jubiliar 60/20	5.5	5.4	46.0	0.50	10.8
Prestij	6.2	5.5	52.0	0.78	7.1
Elvira	6.0	5.4	47.6	0.66	8.2
Mihaela	6.0	4.5	35.2	0.58	7.8
Milenium	6.0	4.5	35.2	0.58	7.8
Tomiş	5.2	4.3	27.3	0.50	8.6
Solearis (standard)	5.7	5.0	52.0	0.80	6.6

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The Estimation of Combining Ability and Heterosis Effect for Yield and Yield Components in Tomato (*Solanum lycopersicum* Mill.) at Lowland

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ABSTRACT

A study was conducted in a 6 × 6 full diallel cross set of tomato including reciprocals to estimate the general combining ability, specific combining ability and heterosis for yield per plant (g) and yield components, namely number of fruits per plant, individual fruit weight (g) fruit length (cm), fruit diameter (cm), locule number, and fruit thickness (cm). The experiment was conducted from March to August 2013 at Bogor Agricultural University Experiment Field, Bogor-Indonesia. Randomized Complete Block Design was used with three replications. Data from Fl generation and parents were analyzed using the Griffing Method. Significant differences among genotypes were obtained for all the traits. The variances for general combining ability (GCA) and specific combining ability (SCA) were highly significant indicating the presence of additive as well as non-additive gene effects except the fruit thickness. The tomato genotype IPB 78 is parental with the best general combining ability for yield per plant, individual fruit weight, fruit length, and fruit thickness. The tomato genotype IPB T73 x IPB T3 proved to be the best general combiner for yield and number of fruits per plant. The tomato genotype IPB T3 x IPB T1 proved to exhibit best heterosis for yield per plant and fruit thickness.

Keywords: combining ability, diallel, GCA, heterosis, SCA

Introduction

Tomato is kind of vegetable which has been cultivated worldwide. Tomato contain nutrition fact as vitamin A, C, lycopene, flavonoid and other minerals that are good for human health (Kailaku *et al*. 2007; Bhowmik *et al.* 2012; Akhtar and Hazra, 2013) Therefore, tomato may be functioned as vegetable, table fruit, drinks, raw material for cosmetic and herbs. In Indonesia, tomato becomes important horticulture commodity. Based on data from Directorate General of Horticulture (2011), in year 2011 the production of tomato in Indonesia reached 954,046 ton with the productivity of 14.2 ton/ha, but this production level still could not be able to fulfill the domestic needs, because in the same year, the total import value reached US$ 9,066,578.

The cultivation of tomato in lowland experience many obstacles, such as low productivity. The nature of its fruit set which is induced by low temperature may cause decreased productivity in tropical lowland area (Dane *et al.* 1991; Hanson *et al.* 2002). One effort in order to increase productivity as well as quality of tomato is through application of different plant breeding methods. The improvement of its characters with high economic values often face challenge when selecting parents with high combining ability. Therefore, the effective study for parent selection is highly needed. The observation to the performance of hybrid offsprings can be conducted using diallel crossing method. This progeny test can be related to the Combining Ability which are very useful in determining the parent combination for the best progeny with potentially high productivity and other selected novel characters (Baihaki, 2000; Syukur *et al.* 2012).

Some information can be obtained from diallel analysisi e.g. general combining ability (GCA) and specific combining ability (SCA) from crossing parental lines. GCA is the performance of line as combination of solely crossing with other lines, whereas SCA is the performance of a hybrid line resulted from the cross with other line (Singh and Chaudary, 1979). Combining ability is a measurement of plant genotype ability in crossing to produce superior plants. Combining ability which is obtained from a cross between two parental lines can provide information regarding cross combinations for better heredity (Sujiprihati *et al.* 2008). The analysis of diallel crossing is needed to predict the additive and dominant effects from a certain population that can be used further to predict the genetic variability and heritability (Baihaki, 2000). This analysis is often used for many kind of plant, such as tomato (Rai *et al.* 2005; Hannan *et al.* 2007a; Hannan *et al.* 2007b; Sekhar *et al.* 2010; Farzane *et al.* 2012; Saleem *et al.* 2013; Saputra *et al.* 2014), chilli (Sujiprihati *et al.* 2007), eggplant (Nalini *et al.* 2011) and corn (Iriany *et al.* 2011).

Beside combining ability, value of heterosis can also be used as one important consideration for selecting paretn genotype and novel hybrid. The information of heterosis value on certain selected genotypes can be very useful for development hybrid novel variety (Amanullah *et al.* 2011). The objective of this research was to obtain the information of GCA, SCA and heterosis value on tested tomato.

Material and methods

The research was conducted from March to August 2013, located at Plant Breeding Laboratory, Faculty of Agriculture, Bogor Agricultural University (IPB) and at Research Field Leuwikopo, IPB (250 m above sea level). The type of soil is latosol. Plant material used were consisted of 6 breeding lines, namely IPBTl, IPBT3, IPBT13, IPBT64, IPB T73 and IPB T78. The hybrid from fully diallel cross used, were 15 F_1 and 15 F_1R (R for Reciprocal) .

The research was carried out using randomized complete block design with three replications. Each experimental unit consisted of 20 plants with an area of 1m x 5m bench covered by black silver plastic mulch. The size of planting rows was 50cm x 50cm. The seedlings were tranplanted to the field after emergence of 4-5 true leaves (around 4 weeks old). Fertilizing was done every week, with solution 10 g Nitrogen: Phosphor: Pothasium (16:16: 16), 250 ml each. Pesticide was sprayed every two weeks with fungicide mancozeb 80% or propineb 2 g l^{-1}, insecticide profenovos with dose 2 ml l^{-1}. The pinching of lateral shoots was done for having optimal growth of the plants. Weeding was also done manually. Harvesting was done when tomatoes have been 75% rippened, every five days, totally eight times.

The observation was conducted on 10 sample plants from each units. The characters observed were namely yield/ plant (g per plant), fruit amount, fruit weight (g), fruit length (cm), fruit diameter (mm), and amount of locule per fruit (cm). The observation of weight, length, and diameter of fruit were measured from the same fruit two days after harvesting.

The data were analysed using Method I of Grifing (Singh and Chaudhary, 1979). The Ratio of Genetic influence (RG) was calculated based on Baker formula (1978). The estimation of heterosis value of hybrid was analysed based on the mean of both parents (mid parent heterosis) and heterobeltiosis value was analysed based on the mean of the better parent (Fehr, 1987). Data analysis was done by Microsoft Excel.

Results and discussion

The analysis of variance showed the existence of significant variation among genotypes for yield (yield per plant) and yield component (number of fruits, individual fruit weight, fruit length, fruit width, number of locule and fruit thickness). Table 1 shows mean squares from the analysis of variance for all the characters. Presence of significant differences among genotypes for all the characters, allowed combining ability analysis (Singh and Chaudhary, 1979).

The analysis of variance for combining ability showed the existence of significant variation due to both GCA and SCA for all the character, indicating that both GCA and SCA effects played important roles in controlling those traits except fruit thickness character for SCA. Highly significant variation due to both of GCA and SCA indicated the importance of additive as well as non-additive gene action in inheritance of all characters except fruit thickness. Hannan *et al.* (2007a) and Hannan *et al.* (2007b) evaluated GCA and SCA on a 10 × 10 diallel set of tomato excluding reciprocals. The result showed highly significant variation for both GCA and SCA for yield per plant, number of fruits per plant, fruit weight per plant. Gaikwad *et al.* (2009) reported highly significant GCA and SCA variances which indicated the importance of both additive and non-additive gene action in the expression of all the characters observed. However, the ratio of components of genetic variance revealed the predominant role of non-additive gene actions in controlling total yield, marketable yield, number of fruits *per* plant, average fruit weight, pericarp thickness, and number of locules. Gul (2011) indicated highly significant GCA and SCA for characters fruit length, fruit width, and fruit weight on 8×8 half diallel cross. Similarly, Farzane (2012) demonstrated highly significant variation due to GCA as well as SCA indicated the role of additive as well as non-additive types of gene action in inheritance of yield and yield component (individual fruit weight and number of locule) characters by DGU and DGK analyzed on full diallel cross 10 × 10. Saputra *et al.* (2014) also showed similar result, that GCA significantly changed the individual fruit weight, fruit size, number of fruit per plant and fruit weight *per* plant. SCA gave significant influence on the character of individual fruit weight, fruit size and fruit weight *per* plant.

The influence of reciprocals occurred in all observed characters. It indicated the influence of female parents or maternal effects. It caused the hybrid performance unequal with their reciprocal. Farzane (2012) showed the influence of reciprocals in yield and yield component (individual fruit weight, number of fruit *per* plant and number of locule).

Based on genetic ratio analysis for character yield *per* plant, number of fruit, individual fruit weight, fruit length, fruit width, number of locule, and fruit thickness are 1.63, 1.62, 1.36, 1.60, 1.10, 1.76 and 1.68, consecutively (Table 2). It indicated the importance of additive more important than non-additive types of gene action in inheritance of all characters. The result was different from Gaikwad *et al.* (2009) since the population was different. Different population will cause different gene action as well. According to Syukur *et al.* (2012), the characters which are controlled by additive genes will be easier to be selected particulary for improving the inbred line varieties.

The positive general combining ability (GCA) effect for yield *per* plant was recorded in IPBT3, IPBT13, and IPBT78, number of fruit per plant (IPBT3, and IPBT78), individual fruit weight (IPBT1, IPBT13, and IPBT78), fruit length (IPBT1, IPBT13, IPBT64 and IPBT78), fruit width (IPBT1, IPBT13, IPBT73, and IPBT78), number of locule (IPBT1 and IPBT IPBT73). The negative GCA for character fruit thickness was record in IPBT1 and IPBT73. The highest GCA effects for yield per plant, individual fruit weight, fruit length, and fruit thickness were record in IPBT78 genotype. The highest GCA effect for number of fruit was record in IPBT1 (1.35) and for locule number in IPBT3 (-0.62). The GCA effect on locule number directed towards negative since the few locule number is preferred. The negative combining ability effect indicated the genotypes or cross combiner contributed to decreasing performance in certain characters while the positive combining effect indicated the genotypes or cross combiner contributed to increasing performance in certain characters. GCA and SCA positive effects are used during genotype selection with high yield. In contrasting, GCA and SCA negative effect are used during genotype selection towards pathogen resistance (Yustiana, 2013).

Table 4 indicated the highest SCA estimated for yield *per* plant in combiner IPBT73 × IPBT13 (482.39) followed by IPBT1 × IPBT73, IPBT3 × IPBT64, IPBT13 × IPBT73 and IPBT64 × IPBT78. The highest SCA estimated for number of fruit was record in IPBT73 × IPBT3 (48.37) and the effect was high in IPBT13 × IPBT3, IPBT13 × IPBT73. The highest SCA estimated for individual fruit weight was record in IPBT64 × IPBT78 (10.35) with the high combiner IPBT78 × IPBT13. Highest estimated SCA for fruit length was recorded in IPBT1 × IPBT78 (5.85) and such effects were higher in IPBT1 × IPBT64 and IPBT73 × IPBT78. The highest estimated SCA for fruit width was record in IPBT78 × IPBT13 (4.25) followed other combiners IPBT1 × IPBT13 and IPBT64 × IPBT13. The highest SCA estimate for number of locule was record in IPBT73 × IPBT3 (-1.88). The highest SCA estimate for fruit thickness was record in IPBT73 × IPBT78 (0.47)

The estimated GCA and heterosis effect was influence by dominant gene action types. Therefore, GCA and heterosis effect are positively associated (Yustiana, 2013). Mid-parent heterosis (MPH) and best-parent heterosis was highest for yield *per* plant (58.8%; 42.2%) and fruit thickness (20.8%; 8.1%) in IPBT3 × IPBT1, number of fruit (82.5%; 50.4%) in IPBT73 × IPBT13, individual fruit weight (37.2%; 31.2%) and fruit width (15.4%; 9.4%) in IPBT78 x IPBT13, fruit length (9.4%; 5.9%) in IPBT1 × IPBT13, and MPH for number of locule (72.8%) in IPBT73 × IPBT64 and it BPH (37.7%) in IPBT78 × IPBT3.

Heterosis value ≥ 20% on yield component of self-pollinating plants as rice gives opportunities to hybrid varieties breeding programs. Based on MPH and BPH value, the results of this research showed that there is a potential to develop hybrids with more yield per plant, number of fruit, individual fruit weight, and number of locule. Hanan *et al.* (2007b) reported similar results that heterosis occur for yield per plant (19.3-34.9%), number of fruit (10.0-20.0%), fruit weight (9.6-48.7%), fruit length (14.8-32.7%) and maximum heterosis for fruit width 10.6%. Ahmad *et al.* (2011) indicated that BPH for yield per plant reach 32.09%. Farzane (2012) reported high MPH for number of fruit (25.03%) and yield per plant (36.82%).

Conclusions

Both additive and dominant gene action types play an important role in controlling yield and yield component in tomato at lowland, but additive gene action was more prominent to controlling yield *per* plant, number of fruit, individual fruit weight, fruit length, fruit width, number of locule and fruit thickness. The influence of reciprocals occurred in all the observed characters. Tomato genotype IPBT78 proved to be the best general combiner for yield and yield components. The best cross combinations were IPBT73 × IPBT13 for yield per plant and number of fruit per plant.

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Table 1. Analysis of variance for yield and yield component in tomato

Source	Df	Mean squares						
		Yield plant^{-1}	Number of fruits	Individual fruit weight	Fruit length	Fruit width	Number of locules	Fruit thickness
Replication	2	541318.65^{ns}	300.45^{ns}	541318.65^{ns}	11.36^{ns}	17.12^{ns}	0.53^{ns}	1.27^{ns}
Genotype	35	401656.43**	2349.11**	401656.43**	141.69**	52.93**	8.41**	0.81**
Error	70	123385.76	238.57	123385.76	11.56	14.28	0.52	0.29

* Significant at P=0.05, ** Significant at P=0.01, ^{ns} non significant

Table 2. Mean squares from a combining ability analysis, additive variance, dominant variance and genetic ratio for yield and yield components in a diallel cross of Tomato.

Source	Df	Yield plant^{-1}	Number of fruits	Individual fruit weight	Fruit length	Fruit width	Number of locules	Fruit thickness
GCA	5	333012.27**	1492.08**	197.90**	138.31**	18.70**	10.73**	0.83**
SCA	15	75424.91**	345.61**	92.55**	34.07**	15.45**	1.48**	0.16^{ns}
Reciprocal	15	123385.76**	984.11**	132.03**	30.03**	19.50**	1.49**	0.30*
Error	70	41128.59	79.52	25.65	3.85	4.76	0.17	0.10
V add		43115.62	192.51	17.92	17.54	0.60	1.55	0.11
V dom		19913.99	154.50	38.84	17.54	6.20	0.76	0.04
RG		1.63	1.62	1.36	1.60	1.10	1.76	1.68
CV (%)		29.76	24.64	22.59	9.12	9.16	17.65	12.76

* Significant at P=0.05, ** Significant at P=0.01, ^{ns} Non Significant

Table 3. General combining ability (GCA) effects of parents for yield and yield component

Genotype	Character						
	Yield plant^{-1}	Number of fruits	Individual fruit weight	Fruit length	Fruit width	Number of locules	Fruit thickness
IPB T1	-116.15	-11.96	4.64	2.46	1.35	0.41	-0.08
IPB T3	58.09	13.82	-4.68	-0.63	-2.32	-0.62	0.10
IPB T13	49.08	-0.94	1.62	0.87	0.75	-0.08	0.18
IPB T64	-102.42	-10.17	-0.21	2.49	-1.04	-0.45	0.17
IPB T73	-176.71	-2.93	-1.19	-2.34	0.89	1.29	-0.25
IPB T78	142.52	1.58	5.17	4.61	0.57	-0.37	0.27

Table 4. Specific combining ability (SCA) effects of parents for yield and yield component

Genotype	Character						
	Yield plant-1	Number of fruits	Individual fruit weight	Fruit length	Fruit width	Number of locules	Fruit thickness
IPBT1 x IPBT3	-250.03	19.71	-15.07	-5.79	-6.09	-0.99	-0.24
IPBT1 x IPBT13	40.04	-9.23	7.32	1.98	3.76	0.14	0.24
IPBT1 x IPBT64	57.30	-0.48	6.33	4.72	2.11	-0.67	0.41
IPBT1 x IPBT73	297.77	8.12	0.69	-4.47	2.13	1.06	-0.13
IPBT1 x IPBT78	-15.88	-5.04	3.41	5.85	-0.44	-0.60	0.23
IPBT3 x IPBT1	-186.21	-25.57	1.83	-0.09	0.83	0.57	-0.52
IPBT3 x IPBT13	-96.11	-10.73	6.82	2.06	3.06	0.78	-0.12
IPBT3 x IPBT64	157.93	-2.61	-0.99	1.26	-1.39	0.07	0.00
IPBT3 x IPBT73	-167.47	-4.23	4.85	1.41	1.42	-0.49	0.02
IPBT3 x IPBT78	132.92	2.42	0.39	0.66	0.64	0.29	-0.14
IPBT13 x IPBT1	-182.75	-9.35	3.42	2.44	1.13	0.53	-0.26
IPBT13 x IPBT3	101.76	22.05	-9.17	-3.01	-3.57	-0.55	-0.32
IPBT13 x IPBT64	-171.50	-4.78	-2.24	-0.19	-1.21	-0.28	0.27
IPBT13 x IPBT73	183.53	27.13	-4.32	-0.20	-2.62	-0.64	-0.08
IPBT13 x IPBT78	-14.76	3.53	-2.83	-2.81	-0.23	0.50	-0.20
IPBT64 x IPBT1	63.38	0.48	1.58	-2.28	2.35	0.89	-0.14
IPBT64 x IPBT3	-27.05	14.26	-7.52	-2.99	-2.89	-0.57	-0.17
IPBT64 x IPBT13	-91.94	-8.87	7.97	2.34	3.45	0.62	0.25
IPBT64 x IPBT73	18.59	5.44	-5.45	-3.98	-0.69	0.28	-0.21
IPBT64 x IPBT78	153.59	-0.29	10.35	3.09	3.37	0.49	-0.02
IPBT73 x IPBT1	18.66	-7.72	7.56	3.65	1.26	-0.89	0.19
IPBT73 x IPBT3	482.39	48.37	-10.53	-1.42	-4.29	-1.88	0.12
IPBT73 x IPBT13	-61.26	-29.72	1.34	0.26	-0.38	0.37	-0.12
IPBT73 x IPBT64	-495.26	-27.67	-7.35	-0.06	-4.78	-1.38	-0.11
IPBT73 x IPBT78	-410.99	-24.59	-1.59	4.33	-2.83	-1.85	0.47
IPBT78 x IPBT1	-118.82	-8.39	2.73	-3.87	2.12	1.00	-0.52
IPBT78 x IPBT3	40.09	18.58	-11.97	-5.85	-3.17	-0.20	-0.13
IPBT78 x IPBT13	-277.20	-36.39	10.26	3.87	4.25	0.38	0.06
IPBT78 x IPBT64	-198.93	-5.95	-9.29	0.45	-4.47	-0.76	-0.11
IPBT78 x IPBT73	-488.79	-6.93	-14.22	-10.53	-3.20	0.77	-0.75

Table 5. Mid-parent heterosis (MPH) and best-parent heterosis (BPH)

Genotype	Yield plant^{-1}		Number of fruits		Individual fruit weight		Fruit length		Fruit width		Number of locules		Fruit thickness	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
IPBT1 x IPBT3	-28.6	-36.0	7.9	-32.9	-23.4	-43.0	-10.7	-19.1	-7.5	-17.3	-17.4	-43.0	-14.0	-23.0
IPBT1 x IPBT13	-2.5	-24.5	-36.0	-56.7	14.3	13.2	9.4	5.9	8.1	5.0	-1.7	-20.7	1.9	-10.9
IPBT1 x IPBT64	24.2	12.6	16.2	-14.1	10.6	6.4	-0.4	-9.4	10.8	10.5	1.0	-28.5	9.5	-5.8
IPBT1 x IPBT73	19.0	3.4	8.2	-25.6	23.0	6.6	2.0	-15.0	8.0	5.7	-9.3	-21.6	7.7	4.6
IPBT1 x IPBT78	1.5	-17.9	-18.4	-41.1	15.3	11.2	-2.0	-14.5	7.7	5.0	4.0	-27.9	-6.5	-20.4
IPBT3 x IPBT1	58.8	42.2	37.8	-14.3	1.1	-24.7	-4.5	-13.4	3.1	-7.9	-25.5	-48.6	20.8	8.1
IPBT3 x IPBT13	12.8	-4.5	38.3	16.5	-12.0	-34.1	-5.9	-12.0	-2.4	-14.9	7.9	-12.7	-14.1	-16.3
IPBT3 x IPBT64	26.8	25.2	6.9	-19.4	-24.6	-45.2	-11.4	-26.2	-8.6	-18.1	3.8	-0.7	-7.4	-11.5
IPBT3 x IPBT73	20.9	16.8	60.2	32.0	-8.0	-23.3	3.9	-5.5	-4.8	-16.5	-46.5	-65.9	0.6	-12.2
IPBT3 x IPBT78	31.0	16.6	32.9	2.9	-22.1	-43.3	-19.5	-35.3	-0.3	-8.8	27.0	26.1	-12.1	-16.9
IPBT13 x IPBT1	5.7	-18.1	32.7	-10.1	6.1	5.1	0.6	-2.6	3.9	0.9	-10.7	-27.9	11.0	-2.9
IPBT13 x IPBT3	30.5	10.5	16.4	-1.9	-14.5	-35.9	-6.7	-12.8	-2.9	-15.4	-9.0	-26.4	-6.5	-9.0
IPBT13 x IPBT64	-14.2	-28.1	12.5	-2.0	-8.9	-13.1	-8.3	-19.0	-1.1	-4.2	14.9	-3.6	5.0	3.1
IPBT13 x IPBT73	-2.0	-14.5	24.3	21.0	-23.5	-33.2	-4.9	-18.6	-12.9	-13.5	-13.5	-37.4	-9.8	-23.1
IPBT13 x IPBT78	-11.1	-16.0	-2.4	-12.1	3.3	-1.3	-10.9	-24.4	6.3	0.8	31.8	7.3	-11.2	-13.9
IPBT64 x IPBT1	47.7	33.9	55.7	15.1	-11.3	-14.6	-5.4	-13.9	-4.7	-4.9	-14.8	-39.7	10.9	-4.5
IPBT64 x IPBT3	23.0	21.4	19.1	-10.2	-25.0	-45.5	-14.3	-28.6	-4.2	-14.1	-3.2	-7.4	-2.9	-7.2
IPBT64 x IPBT13	-30.8	-42.0	-2.8	-15.3	-30.3	-33.5	-15.8	-25.6	-11.0	-13.8	7.3	-10.0	-12.2	-13.8
IPBT64 x IPBT73	-60.3	-62.1	-49.0	-54.5	-42.6	-51.9	-19.9	-38.0	-15.6	-17.6	-19.5	-47.6	-11.8	-25.9
IPBT64 x IPBT78	6.6	-6.2	-15.0	-18.1	-7.0	-7.2	-8.4	-12.5	-2.3	-4.5	15.8	11.5	-9.8	-10.9
IPBT73 x IPBT1	-65.6	-70.1	-9.3	-37.6	18.4	2.6	0.9	-15.9	5.0	2.8	-7.3	-19.9	7.6	4.5
IPBT73 x IPBT3	30.3	25.8	82.5	50.4	-2.4	-18.6	1.2	-8.0	2.1	-10.5	-15.9	-46.3	-1.1	-13.7
IPBT73 x IPBT13	8.5	-5.4	21.7	18.4	-1.6	-14.0	-5.4	-19.0	3.1	2.4	19.1	-13.8	-4.2	-18.3
IPBT73 x IPBT64	-25.1	-28.5	-19.7	-28.4	-7.7	-22.6	-19.9	-38.0	5.4	2.9	72.8	12.6	-23.2	-35.5
IPBT73 x IPBT78	-83.0	-84.4	1.7	-6.2	-40.3	-49.9	-24.8	-43.7	-14.0	-17.8	-20.5	-49.1	-13.6	-28.2
IPBT78 x IPBT1	36.7	10.6	8.5	-21.7	9.5	5.6	-0.9	-13.5	4.2	1.6	-20.2	-44.7	3.4	-11.9
IPBT78 x IPBT3	53.1	36.2	81.5	40.5	-22.0	-43.2	-23.8	-38.8	0.0	-8.6	38.7	37.7	-11.3	-16.2
IPBT78 x IPBT13	16.6	10.1	15.6	4.0	37.2	31.2	1.0	-14.2	15.4	9.4	44.1	17.3	-3.8	-6.8
IPBT78 x IPBT64	1.5	-10.7	1.6	-2.0	8.0	7.8	0.2	-4.4	3.7	1.4	10.1	6.0	2.6	1.3
IPBT78 x IPBT73	35.8	24.7	-2.4	-9.9	-5.9	-20.9	-14.4	-35.9	-2.3	-6.7	-22.5	-50.4	0.4	-16.5

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Creating Varieties of The Perennial Cereal Grasses by The Polycross Method in Northern Kazakhstan

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ABSTRACT

In Kazakhstan fodder crops in arable land are mainly perennial grasses, for example wheatgrass, Psathyrostachys juncea, smooth brome and different types of blue grass.

Depending on the conditions of the region's sharply continental climate, development work is carried out to create new varieties of wheatgrass and smooth brome of a hay type with high drought resistance, winter hardiness, a high forage seeds yield and high quality as feed and resistance to disease. In the Barayev Centre the traditional and modern emerging techniques are used in developing grasses such as creating complex hybrid populations, including synthetic populations by the use of the polycross method. In the polycross nursery the nine best examples of clones of broad-eared wheatgrass and eight best clones of smooth brome were selected. The obtained polycross hybrids were evaluated according to the following criteria: combining ability in terms of green mass yield, dry matter and seeds, crude protein content, drought resistance, hardiness, plant height and herbage vigour. After the analysis of combining ability the synthetic populations were formed: 5 components were included in a synthetic wheatgrass (K-1027, K-418, K-1029, K-1024, K-1043), and 4 components in a smooth brome (K-681, K-621, K-712, K-641). Synthetic populations of broad-eared wheat grass variety Shortandinsky shirokokolosy and a smooth brome variety named Ishimsky Yubileiny were transferred to the Kazakhstan state variety testing center in 2005-2007. Recently, these varieties have been released in the regions of northern Kazakhstan.

Keywords: wheatgrass, smooth brome, polycross method, clone, combining ability, synthetic population..

Introduction

In Kazakhstan, most fodder crops in arable land are perennial grasses. With a proper selection of species and varieties for different areas and with the necessary care they provide cheap, varied and nutritious feed. They are dominated by wheatgrass, *Psathyrostachys juncea*, smooth brome and different types of blue grass. The variety of crops and quality seeds contribute significantly to increase productivity and quality of feed products. The climate in the north of Kazakhstan is extremely continental, very arid and dry with cold, moderately snowy winters, very often with little snow. Annual precipitation is 250-350 mm, which characterizes the area as arid.

Analysis of methods of creating perennial grass varieties included in the State register of the Republic of Kazakhstan shows that 60% of the varieties have been created using mass selection, 20% by individual selection, 2% by biotypical selections and 1% by hybridization. Under the conditions of Northern Kazakhstan, when creating better and more productive, adaptable, cold, drought, disease and pest resistant varieties of smooth brome and wheatgrass, the most perspective method may be using of heterosis effect.

The complex polyploidal nature and predominantly cross pollination of smooth brome and wheatgrass presupposes wide use in their selection of the heterotic effects, which can be maintained for a relatively long time in so-called panmictic populations. On the other hand, panmictic populations cannot drastically change their attributes and properties in space and time under steady environmental conditions and biocenotic ratios during the reproduction of the variety.

For anemophilous cross-pollinated species, heterosis can be achieved by creating synthetic populations (synthetics) as developing these species with preliminary emasculation is quite time-consuming and not cost-effective. In addition, individual specimens in created complex hybrid populations can freely interbreed with each other and during the reproduction of such varieties populations a balanced system is formed, which can maintain the heterosis effect for a relatively long time. One of the essential steps in the heterosis synthetic population formation is to determine the combining ability (CA) of their components. The polycross test is assigned an important role in determining the CA. This method is less labour intensive than top crosses and paired crosses, and provides a good amount of high-grade seeds.

If earlier a crop was considered synthetic, having been developed by crossing, mixing, planting in rows of two or more varieties, or cloning of inbred lines with subsequent re-pollination (Henson and Carnahan 1959), in the last decade the creation of synthetic varieties presupposes definition of combining ability (Kedrov-Zihman 1974). The main condition in organising polycross nurseries is that each sample should have an equal opportunity to be pollinated by the pollen of all test samples of the analysed group. There are various plans (schemes) of polycross nursery layouts (Frandsen and Frandsen 1948; Shaepman 1952; Walther 1959; Knowles 1955; Osipova 2006). In the end, their effectiveness is verified by creating cultivars. Success in the selection of synthetic varieties would be significantly promoted by not only an established approach to quality selection of components, but also by the determination of an optimal number for each specific case with consideration being given to the specific nature of each culture. Today the question of the minimum and maximum number of components to create synthetics is controversial. Special experiments conducted with forage grasses to identify the optimal number of components for the formation of synthetics on the basis of CA showed that combining 10 to 20 of the best clones can achieve higher yields of forage than the union of less than 10 and more than 20 clones (Frandsen and Frandsen 1948). Knowles (1955) conducted experiments with five synthetic varieties composed of 4, 5, 6, 7 and 8 clones that had CA higher than average, which was estimated after free pollination. Synthetic varieties consisting of 5 and 6 clones gave first generation fodder yields above 112% compared with the standard. The remaining synthetic varieties didn’t have any significant differences with the standard. As for the seed crop was concerned, all five varieties yielded substantial gain compared to the standard grade.

According to Osipova (2006), the optimal number of constituents to create the synthetic materials may be 9, but it does not exclude other options. Unfortunately, in the Republic of Kazakhstan this successful method is not widely used in selection of grasses, apparently because of the great complexity of its implementation. In the Barayev Centre selection work of the perennial grasses was carried out using traditional and modern methods of selection such as creating complex hybrids, including synthetic populations using the polycross method. This work is aimed at the creation of new varieties of hay grass (wheatgrass, brome) with high drought resistance, winter hardiness, high yield in terms of forage and seeds, high quality feed and disease resistance. The aim of our research is to use the polycross method and to create varieties of wheatgrass and smooth brome adapted to the conditions of Northern Kazakhstan.

Materials and methods

Studies were carried out in the Barayev Research and Production Center for Grain Farming (Akmola region, Kazakhstan). The starting material used in the polycross nurseries was pre-selected wild populations of broad-eared wheatgrass and smooth brome, which have a number of economically important characteristics and properties.

Polycross nurseries were laid out according to method of Kedrov-Zihman (1974). The polycross progeny’s test was performed in a control nursery laid out in the standard method. The combining ability (general - GCA and specific - SCA) was determined by the methods that were developed for cross pollinates at the Russian National Institute of Plants (Kobylyansky *et al.*,1977). According to this method GCA was estimated for the yield of hybrids in percentage of average yield of all hybrids in the field experiment; SCA was determined as percentage of average yield of standard. In the initial stages surveys and observations of the selection process were conducted in accordance with the guidelines for the selection of perennial grasses in the Russian National Institute of Feed (Smurigyn, Novoselova and Konstantinova 1985). The experimental material was processed according to Dospehov (1985) guidelines, using the «SNEDECOR» software package.

In the collection nurseries the broad-eared wheatgrass and smooth brome were planted with wide-row method. Amassive selection of individual plants was conducted according to the following criteria: plant vigor, height, leaf color, bush clump shape, bushiness, foliage, ears (panicles), drought resistance, hardiness and resilience to diseases and pests. Seeds from the best plants of selected wild populations were combined and propagated on the isolated areas where negative mass selection was conducted as well.

In our studies, the polycross nurseries of wheatgrass and smooth brome were laid out in a square shape. Clones from isolated propagation nurseries were used as the starting material. The clones of the 9 best samples of broad-eared wheatgrass and 8 best of smooth brome were included in the polycross nursery (Table 1).

Clones or plants of broad-eared wheatgrass were planted in late April-May, in rows in four blocks (replicates) of 60x60 cm, with one plant or clone *per* nest. As smooth brome forms a creeping rhizome from a bushy node, which extends in width as the plant grows and forms underground shoots; its seedlings were planted in blocks of 80x80 cm, which did not allow the bushes to coalesce within the first three years of life.

Seeds from polycross nurseries were gathered separately from each bush (excluding shelter belts), and then the seeds of each species were mixed from all replicates for polycross progeny testing for CA (combining ability) in accordance with the main economically important properties.

Results and discussion

This research was focused on the selection of plant forms with a significant level of CA based on the characteristics most widely used in fodder and, above all, yields of green mass, dry matter and seeds. However, the selection of components for the formation of synthetics which would combine the heterotic effects showing the most economically important traits is not always possible due to its discrete manifestation, including its constituent yield elements (Osipova 2006).

The estimated general combining ability (GCA) of the plants under study facilitated the identification of forms that could be graded as medium (level 3), high (level 4) and very high GCA (level 5) according to the main economically important traits. The formation of synthetics was performed considering not only the GCA, but specific combining ability (SCA) as well. Because the number of components with a discharge of 3 to 5 was too small, which could lead to the loss of valuable genotypes (Table 2), samples were selected which were distinguished by a complex of economically important properties such as: green mass, dry matter and seeds yield, crude protein content, drought resistance, hardiness, plant height, grass, etc., for example, even if the sample was not of a high yield but was the most drought-resistant or tall, it was included in the synthetics (syn) group (Table 3).

In terms of green mass yield, dry matter and seeds of broad-eared wheatgrass hybrids with a high level of GCA, one hybrid of the 9 studied, stood out (level 5) and three hybrids were marked as medium (level 3); to a green mass yield and dry matter with a very high level of SCA there were 5 polycross hybrids and the excess was 12.4-86.6% and 13.5-91.9%, respectively. In terms of seed yield with very high GCA and SCA there was only one polycross hybrid, the remaining samples were low or very low in character (rank 1-3). These 5 polycross hybrids excelled in other economically important traits as well: plant height, drought tolerance, winter hardiness, crude protein content. The difference in plant heights was from 3 to 5 cm between standard and hybrid plants, the difference in the crude protein content was 1.3-2.1% respectively.

From 8 studied smooth brome polycross hybrids there were only 2 hybrids which reached medium GCA level (level 3) for the green mass yield, the others had a low level of GCA and SCA as well. Only one polycross hybrid had a high GCA level (4) for dry matter yield and a very high SCA level (5), the second hybrid had the medium GCA and SCA levels (3); others had a low level ranking of 1-2. For seed yield in polycross hybrids smooth brome had two very high GCA and SCA rankings, their excess in GCA and SCA was on 28.6-66.6%. The rest showed low CA levels with regard to basic economically important traits (crude protein content, plant height, drought tolerance and winter hardiness) four polycross hybrids showed a good result.

An increase in hybrid on the standard in plant height was from 4 to 7 cm, the content of crude protein - 0.6-0.9%. These hybrids were more drought-resistant and winter-hardy.

So after the analysis of CA the synthetic populations were formed: for wheatgrass 5 components were included in syn (K-1027, K-418, K-1029, K-1024, K-1043); for smooth brome - 4 components (K-681, K-621, K-712, K-641).

The results of such differentiated selection of synthetics components have proved effective in creating synthetic varieties: the yield of synthetic populations of perennial grasses formed using polycross method, it was above standards in competitive strain testing (Table 4).

Synthetic populations of broad-eared wheatgrass called Shortandinsky shirokokolosy and smooth brome Ishimsky yubileiny were transferred to the Kazakhstan State Variety Testing Center in 2005-2007.

Currently, the variety of wheatgrass Shortandinsky shirokokolosy was released for the North-Kazakhstan region in 2011.

According to the results of the State variety trials in the North-Kazakhstan region the seed yield of the new variety of wheatgrass was higher by 27% than yield of previously recognized standard variety - Batyr. Green mass yield of wheatgrass Shortandinsky shirokokolosy exceeded the standard by 19%; dry weight by 20%.

The variety of smooth brome Ishimsky yubileiny was released for the North Kazakhstan and Kostanay region in 2011.

According to the results of the State variety trials in the North-Kazakhstan region the green mass yield of smooth brome Ishimsky yubileiny on average for two years (2010-2011) was 12.2 t/ha, dry matter -3.49 t/ha, seeds yield - 0, 47 t/ ha, exceeding the standard by 13%, 19.1%, 17.5% , relatively. The variety differed from the standard by height.

Thus, the use of the polycross method with the estimation of the CA allows creating varieties of smooth brome-grass and wheat grass for the environment of Northern Kazakhstan.

The number of constituents in the formation of synthetic materials is determined by the individual characteristics of the base material for the studied species and it can vary from 4 to 5, but does not exclude other options.

Table 1. Selected samples of smooth brome and wheatgrass in a nursery of the polycross

Species	Number of selected samples	Name and origin of the parental population
Broad-eared wheatgrass	9	K-1027 Karaganda region (Kazakhstan), K-418 Kostanay region (Kazakhstan), K-104, IK-1061 Akmola region (Kazakhstan), K-1083 Novosibirsk region (Russia), K-1024 Orenburg region (Russia), K-1000 Altay region (Russia), K-1029 Povolzhie (Russia); K-1021 Armeniya
Smooth brome	8	K-641Altay region (Russia), K-673 Chelyabinsk region (Russia), K-621 Kemerov region (Russia), K-681, K-683, K-679 Ekaterenburg region (Russia), K-712 Krasnoyarsk region (Russia), K-647 Bashkortostan (Russia)

Table 2. The combining ability in polycross progenies selected from wild specimens of smooth brome and wheat grass included in the synthetics (syn)

Variety	**Dry matter %**		**Vitamin C, мг/%**		**Sugar/acidity indice**	
	GCA	**SCA**	**GCA**	**SCA**	**GCA**	**SCA**
			Broad-eared wheatgrass			
K-381	$\frac{112.4}{2}$	$\frac{163.0}{5}$	$\frac{117.6}{3}$	$\frac{161.3}{5}$	$\frac{117.2}{2}$	$\frac{124.5}{3}$
K-382	$\frac{116.1}{3}$	$\frac{168.3}{5}$	$\frac{115.3}{3}$	$\frac{158.1}{5}$	$\frac{98.6}{1}$	$\frac{104.8}{1}$
K-409	$\frac{116.7}{3}$	$\frac{169.2}{5}$	$\frac{113.5}{3}$	$\frac{155.6}{5}$	$\frac{80.0}{1}$	$\frac{85.1}{1}$
K-418	$\frac{119.5}{3}$	$\frac{173.3}{5}$	$\frac{118.8}{3}$	$\frac{162.9}{5}$	$\frac{97.7}{1}$	$\frac{103.8}{1}$
K-427	$\frac{128.7}{5}$	$\frac{186.6}{5}$	$\frac{140.0}{5}$	$\frac{191.9}{5}$	$\frac{140.7}{5}$	$\frac{149.5}{5}$
LSD05	5.3	5.4	6.3	6.2	12.1	12.5
			Smooth brome			
K-681	$\frac{109.4}{2}$	$\frac{111.0}{2}$	$\frac{97.7}{1}$	$\frac{99.0}{1}$	$\frac{142.9}{5}$	$\frac{166.6}{5}$
K-621	$\frac{113.1}{3}$	$\frac{115.0}{3}$	$\frac{128.5}{4}$	$\frac{129.9}{5}$	$\frac{128.6}{5}$	$\frac{150.0}{5}$
K-712	$\frac{105.1}{3}$	$\frac{107.0}{2}$	$\frac{109.0}{2}$	$\frac{110.0}{2}$	$\frac{85.7}{1}$	$\frac{100.0}{1}$
K-641	$\frac{99.0}{1}$	$\frac{101.0}{1}$	$\frac{112.9}{3}$	$\frac{114.1}{3}$	$\frac{85.7}{1}$	$\frac{100.0}{1}$
LSD_{05}	3.3	3.3	2.7	2.8	2.3	2.2

Note. Numerator - GCA (% to the average of all hybrids in the experiment) and SCA (% of the average yield in the standard experiment); the denominator - the level of GCA and SCA.

Table 3. The ratio of components in the formation of synthetic populations of wheatgrass and smooth brome.

Species	Total components syn_1	The number of components included in the syn_1						
		Extracted by the CA			Other economically valuable features			
		productivity			High crude protein content	Drought-resistance	Hardiness	Height
		Green weight	Dry matter	Seeds				
Wheatgrass (syn_1)	5	$\frac{5}{100}$	$\frac{5}{100}$	$\frac{2}{40}$	$\frac{5}{100}$	$\frac{5}{100}$	$\frac{5}{100}$	$\frac{5}{100}$
Smooth brome (syn_1)	4	$\frac{4}{100}$	$\frac{3}{75}$	$\frac{4}{100}$	$\frac{4}{100}$	$\frac{4}{100}$	$\frac{4}{100}$	$\frac{3}{75}$

Note. The numerator is the number of the separated components syn1, denominator indicates the percentage of ratio.

Table 4. Yield of synthetic populations of broad-eared wheatgrass and smooth brome formed using the polycross method of different schemes, competitive strain testing (average for 6 years).

Crop	Productivity, t/ha					
	Green mass		Dry matter		-	
	x	% tost	x	% tost	x	% tost
Broad-eared wheatgrass						
syn_1	9,1*	115,2	4,91*	116,1	0,27*	122,7
St, Karabalukskiy 202	7,9	100,0	4,23	100,0	0,22	100,0
Smooth brome						
syn_1	17,4*	123,4	6,71*	124,7	0,40*	133,3
St, Limanniy	14,1	100,0	5,38	100,0	0,30	100,0

Note. *- significant at the 5% level of significance

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Research Article

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Analysis of Progeny For Productivity Traits and Summer Dormancy of *Dactylis glomerata* L.

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ABSTRACT

Desertification constitutes a significant risk for the persistence of native population. For forage plants growing in areas subject to prolonged and severe summer drought, the most important agronomic characteristic is not the ability to produce during drought but the ability to survive, recover in autumn, and grow actively during the rainy seasons. Dormancy is an adaptive response defined as growth suppression in summer despite irrigation, is an effective adaptation to drought observed in cocksfoot. Nevertheless, summer dormancy in cocksfoot is associated with low vegetative productivity. The objective of this study was to select hybrids with good level of production and range of dormancy in a progeny generated between a summer dormant and a summer active genotype. The preliminary results showed that some hybrids had important level of senescence similar to dormant parent with good spring biomass production, despite an expected negative correlation ($r=-0.119$) between the level of senescence under summer irrigation and biomass production under favourable conditions in spring.

Keywords: cocksfoot, hybrids, senescence, biomass production, summer dormancy.

Introduction

Climate change is affecting most regions of the world in recent decades, including Mediterranean region. These changes, concerning rainfall distribution and its scarcity, global heating and increased drought, are considered as a major obstacle to agricultural production, (IPCC, 2007). They affect as well the sustainability of rainfed agriculture and allow rangeland degradation. However, in these areas, the persistence of perennial herbaceous plants is mainly determined by plant survival over successive summer droughts (Lelievre and Volaire, 2009).

Dactylis glomerata L. (Cocksfoot) is an important perennial grass, having high fodder quality and increased drought resistance due to its ability to use water in the summer and grow up under relatively high temperatures. Mediterranean cocksfoot populations have developed valuable adaptive traits such as summer dormancy, defined as an absence of growth in summer despite irrigation. It is "an endogenously controlled and coupled series of processes comprising the cessation of leaf growth and senescence of herbage expressed under non-limiting water conditions in summer" (Norton *et al*., 2008).

This trait is associated to reduced water consumption (Lolicato, 2000) and to an increased survival (Oram, 1990). This could be of great interest to develop perennial grasses in Mediterranean region. Nevertheless, summer dormancy in cocksfoot is associated with low vegetative productivity (Shaimi *et al.,* 2009).

Our aim is to find hybrids with good level of production and range of dormancy in a progeny generated between a summer dormant and a summer active genotype.

Materials and methods

180 hybrids from the cross between a dormant cocksfoot (*Dactylis glomerata* ssp. Hispanica) and productive (*Dactylis glomerata* ssp. *glomerata*) and their parents were transplanted in the field, in the experimental INRA station of Guich (Latitude 34 ° 03'N, Longitude 06 ° 46'W) Rabat, Morocco. Planting was done in February 2011, spaced one meter *per* plant, in three repetitions, over a sandy soil of 1100 m^2. A fertilizer was applied at planting and consisted of 14, 28 and 14 kg/ha of nitrogen, phosphate and potash, respectively. Extra nitrogen (40 kg/ha) was applied after each cut. Soil was covered with a plastic mulch to prevent weeds, only holes of plants were uncovered. In 2012, forage yield was harvested manually on 31st of January, 2nd of April, 15th of May and 11th of September. Green material from each plant was oven dried at 70 ° C for 72 h and dry weight was recorded. The percentage of plant senescence was scored on 16th of July, according to scale (0= all tissues green, 100= no visible green tissues) and used to assess summer dormancy. Spring biomass was calculated by adding the biomass harvested after the second and the third cuts. Summer biomass refers to September harvest following full summer irrigation. Plant height was measured at flowering time for all the plants. The analyses were performed with the Statview (SAS institute Inc., Cary, NC, USA).

Results and discussion

Results show a large variability in the progeny for spring and summer biomass, height and plant senescence. In spring, correlations were established between functional traits and variables associated with aerial biomass productivity.

Spring production differed significantly ($P<0.0001$) among genotypes. A Mediterranean cultivar of cocksfoot 'Medly' was more productive than Kasbah in spring, 22% of hybrids were more productive to both parents. In summer some hybrids produced more than Medly, while Kasbah produced little biomass.

Plant height differed among hybrids and parents ($P<.0001$). Height of 63% of hybrids exceeded the parents (Figure 1). Spring biomass was positively correlated with plant height ($r=0.593$),this correlation is presented in (Figure 2). The same result was, reported by Mefti *et al.,* (2008) on perennial grasses.

Dormancy was approached by senescence score, since Norton *et al.* (2006) suggested that leaf senescence could be used to identify summer dormancy. Plant senescence levels ranged from 5 to 95%. Some hybrids expressed high levels of senescence exceeding parents. Under summer irrigation, spring biomass was inversely correlated with senescence of aerial tissues ($r=-0.119$). However this correlation is weak and many hybrids produced sufficient spring biomass and also had enhanced senescence scores (Figure 3).

Our results show that it is possible to combine the two traits, summer senescence and spring productivity in some hybrids from the progeny. The significant variability among the studied cocksfoot progeny could be exploited to create cultivars with a high level of summer dormancy and a good vegetative production.

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Figure 1. Distribution of cocksfoot population generated from summer dormant and summer-active cultivars for plant height (cm)

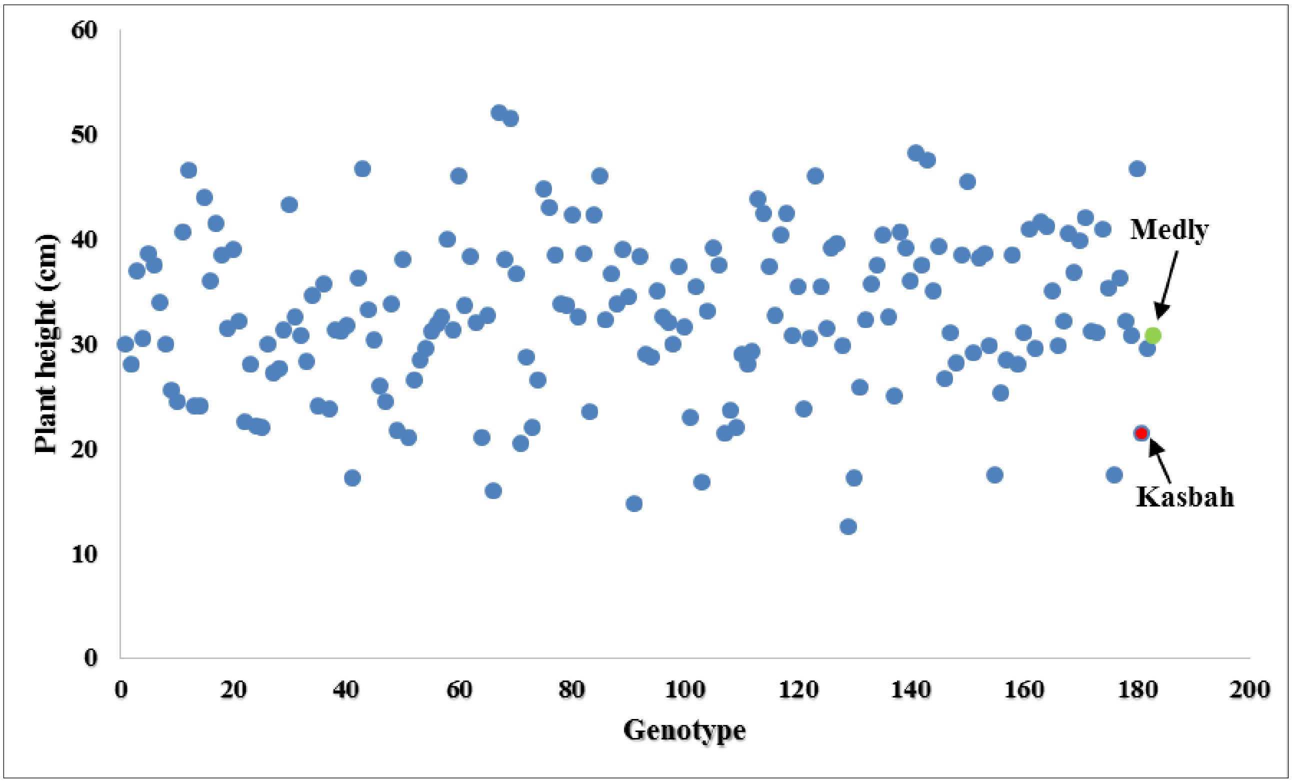


Figure 2. Relationships between spring biomass (g/plant) and plant height (cm) under summer irrigation of cocksfoot population generated from summer dormant and summer-active cultivars

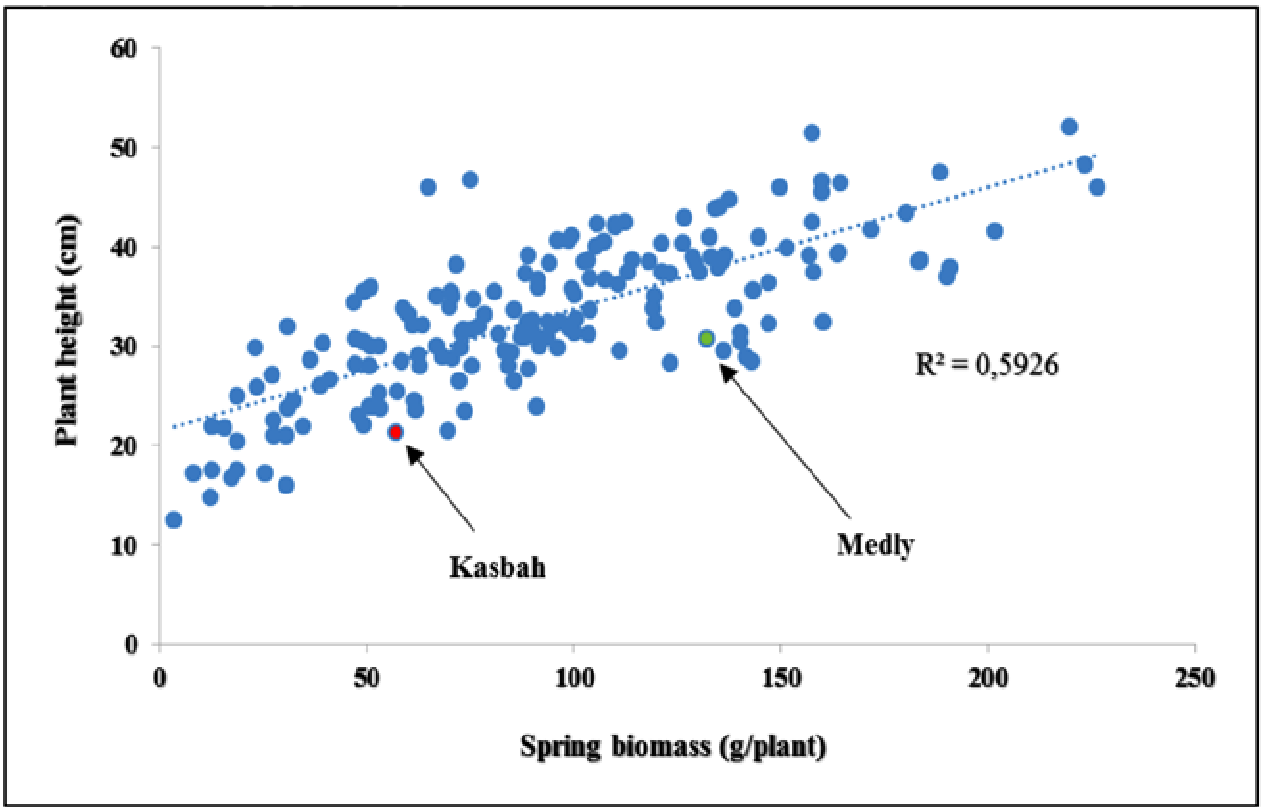
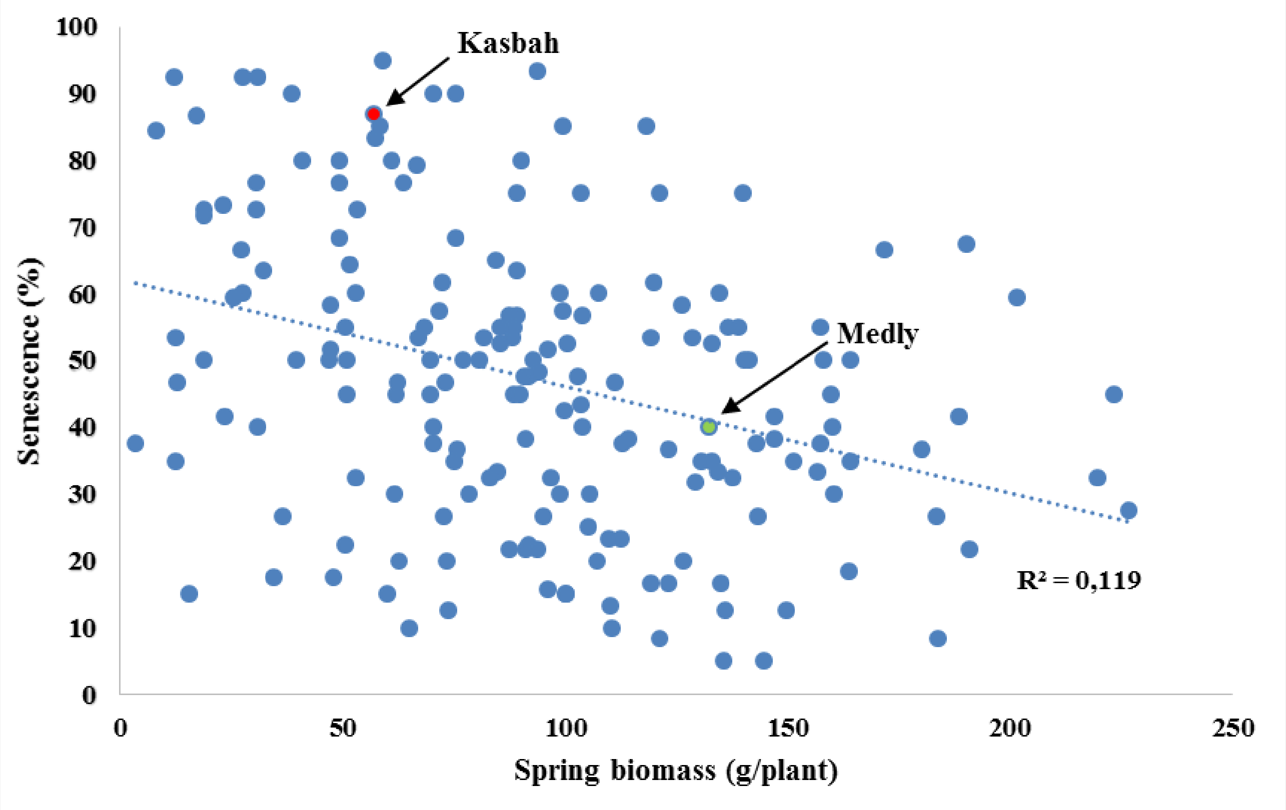


Figure 3. Relationships between spring biomass (g/plant) and senescence (%) under summer irrigation of cocksfoot population generated from summer dormant and summer-active cultivars



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Research Article

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Heterotic Effects for Lint Yield in Double Cross Hybrids on Cotton**

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ABSTRACT

Double-crosses, compared to the single-crosses, have wider genetic diversity hence possess ecologically wider spans and are more adaptable to environmental conditions as mixture of genotypes have better chances of success to cope up with varied environmental conditions. This study was carried out in order to determine the heterotic effects of investigated traits in the population comprising F1 generation from 45 double crosses developed through double cross breeding method, in Diyarbakır ecological conditions in 2010. The trials were conducted using complete block design (RCBD) with three replications. In the study lint yield was determined. Eight hybrid cotton combinations had positive and high values for heterosis and heterobeltiosis in terms of lint yield (kg ha-1) . These were identified as promising for future studies that need to be taken into consideration in these hybrid combinations.

Keywords: cotton, double cross, lint yield, heterosis, heterobeltiosis.

Introduction

Hybrid vigor or heterosis is the converse of the deterioration that accompanies inbreeding. Turner (1953), Marani (1968), and Khan *et al.* (1981) reported varying degree of heterosis which was attributed to cotton fiber yield.

A double cross hybrid results from the cross between two single crosses that are themselves the result of crosses between two selected inbred lines. For successful double cross hybrid development, heterotic effects have to be maximized and the best results are expected when four unrelated or diverse inbred lines are used (Stoskopf, *et al.,* 1993).

Heterosis is the superiority of F_1 over the mean of the parents or over the better parent or over the standard check with respect to agriculturally useful traits. To maximize heterosis, there is a need for utilizing breeding programs aimed at constantly creating variability and increasing genetic diversity between populations that can further be exploited through selection for combining ability between such diverse populations (Kumar, 2008).

In this research, five *Gossypium hirsutum* L. and one *Gossypium barbadense* L., a total of six genotypes of the types , were used to develop 45 F_1 populations following double cross breeding method of hybridization in order to improve the populations for various traits, heterotic effects (heterosis, heterobeltiosis) and to identify best hybrids for future work in breeding elite cotton genotypes.

Material and methods

The research was carried out at the GAP International Agricultural Research and Training Center Research Areas in 2010. This study was carried out in order to determine the heterotic effects of investigated traits in the F_1 generation populations created through 45 double crosses, using the double cross breeding method. The trials were conducted using complete block design (RCBD) with three replications. Each plot consisted of two rows of 12 m length and harvesting was done from the inner 10 m of the rows. The distance between rows and plants was 70cm and 15 cm, respectively. Sowing was done with combine cotton drilling machine on 15th May 2010; all plots received 120 kg ha^{-1} N and 60 kg ha^{-1} P_2O_5. Half of the N and all P_2O_5 were applied at sowing time and the remaining N was given at the square stage in the form of ammonium nitrate.

Fantom (*G. hirsutum* L.), Paum 15 (*G. hirsutum* L.), Stoneville 468 (*G. hirsutum* L.), Giza 75 (*G. barbadense* L.), Delcerro (*G. hirsutum* L.), and Nazilli-84 S (*G. hirsutum* L.) varieties were used as genetic material.

Six parents were crossed to create 15 F_1 hybrids according to hybridization technique suggested by Poehlman (1959) and Griffing (1956). Forty-five double cross progenies were obtained from 15 single cross F_1 hybrids generation following the half diallel method of Singh and Chaudhary (1985). Statistical analysis was made according to Snedecor and Cochran, (1967). Heterosis of all F_1 hybrids was computed according to Fehr (1987) as follows:

$$Ht(\%) = \frac{\bar{F}_1 - \dfrac{\bar{P}_1 + \bar{P}_2}{2}}{\dfrac{\bar{P}_1 + \bar{P}_2}{2}} \text{ x } 100 \qquad Hb(\%) = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \text{ x } 100$$

Where: *Ht:* heterosis; P_1: parent 1; *Hb:* heterobeltiosis; P_2: parent 2; F_1: first generation; *BP:* better parent

The observation was recorded for average lint yield (kg. ha^{-1}) on five randomly selected plants per replicate from each population. The data of all the genotypes were pooled and heterosis (Ht) and heterobeltiosis (Hb) was calculated for average lint yield (Hallauer and Miranda, 1982; Chaing and Smith1967; Fonseca and Patterson, 1968).

Results and discussion

The heterosis and heterobeltiosis for average lint yields are given in Table 1. Heterosis of average lint yield ranged from 24.60% (1x2) x (4x5) {(Paum15 x STV468) x (Fantom x Delcerro)} to -9.79% (3x4) x (5x6) {(Nazilli 84S x Fantom) x (Delcerro x Giza75)}. The double cross combinations (1x2) x (3x4), (1x2) x(3x5), (1x2)x(3x6), (1x2) x (4x5), (1x2) x (4x6), (1x2) x (5x6), (1x3) x (2x5), (1x3) x (2x6), (1x3) x (4x5), (1x3) x (4x6), (1x3) x (5x6), (1x4) x (2x3), (1x4) x (2x5), (1x5) x (2x3), (1x5) x (3x4), (1x5) x (3x6), (1x5) x (4x6), (2x3) x (4x5), (2x3) x (4x6), (2x3) x (5x6) exhibited significant positive heterosis among all the combinations (Figure 1). Turner (1953), Marani (1968), and Khan *et al.* (1981) reported similar results and found varying degree of heterosis which was attributed to cotton fiber yield.

Heterobeltiosis of average lint yield ranged from 11.69% (1x2)x(4x5) {(Paum15xSTV468) x (Fantom x Delcerro)} to -18.81% (1x3) x (5 x 6) {(Paum 15 x Nazilli 84S) x (Delcerro x Giza75)}. The double cross combinations (1x2)x(3x4), (1x2)x(3x5), (1x2) x(3x6), (1x2)x(4x5) exhibited significant positive heterobeltiosis among all the combinations (Figure 1) Stoskopf, *et al.*, (1993) reported similar results and suggested that heterotic effects have to be maximized and the best results are expected when four unrelated or diverse inbred lines are used.

It can be concluded that lint yield is main components for productivity. Therefore, selection for lint yield might results in the improvement of production and the promising double crosses like (1x2)x(3x4), (1x2)x(3x5), (1x2)x(3x6), (1x2)x(4x5) (Ht>10 and Hb>5%) may be further tested on large plots over different locations and seasons before recommending them for commercial utilization.

Table 1. Heterosis (%) and heterobeltiosis (%) of double cross combinations for average lint yield

Double Cross Combinations	Lint Yield (kg. ha^{-1})	Ht (%)	Hb (%)	Double Cross Combinations	Lint Yield (kg. ha^{-1})	Ht (%)	Hb (%)
(1x2)x(3x4)	1999.5	16,58	9,18	(1x5)x(4x6)	1471.1	5,44	-0,28
(1x2)x(3x5)	2027.6	18,82	10,72	(1x6)x(2x3)	1690.4	2,27	-9,37
(1x2)x(3x6)	1891.7	15,69	3,29	(1x6)x(2x4)	1564.2	-4,81	-15,25
(1x2)x(4x5)	2045.4	24,60	11,69	(1x6)x(2x5)	1550.0	-3,8	-13,01
(1x2)x(4x6)	1756.6	11,65	-4,09	(1x6)x(3x4)	1389.3	-8,58	-13,10
(1x2)x(5x6)	1680.3	7,67	-8,25	(1x6)x(3x5)	1394.5	-7,72	-11,82
(1x3)x(2x4)	1791.4	0,70	-2,94	(1x6)x(4x5)	1413.8	-2,25	-2,62
(1x3)x(2x5)	1740.8	-0,36	-2,29	(2x3)x(4x5)	1762.6	6,28	-5,50
(1x3)x(2x6)	1664.7	-3,80	-4,78	(2x3)x(4x6)	1660.5	4,42	-10,97
(1x3)x(4x5)	1575.8	-0,40	-7,98	(2x3)x(5x6)	1638.1	3,84	-12,17
(1x3)x(4x6)	1507.5	-0,42	-11,97	(2x4)x(3x5)	1717.8	0,24	-6,93
(1x3)x(5x6)	1390.4	-7,38	-18,81	(2x4)x(3x6)	1602.0	-2,45	-13,20
(1x4)x(2x3)	1859.5	9,73	-0,30	(2x4)x(5x6)	1517.7	-3,20	-17,77
(1x4)x(2x5)	1711.2	3,52	-3,96	(2x5)x(3x4)	1673.5	-0,99	-6,07
(1x4)x(2x6)	1537.3	-6,05	-12,07	(2x5)x(3x6)	1578.3	-1,98	-11,41
(1x4)x(3x5)	1514.7	-2,46	-4,23	(2x5)x(4x6)	1497.8	-3,27	-15,93
(1x4)x(3x6)	1352.2	-8,72	-11,28	(2x6)x(3x4)	1676.3	0,17	-4,12
(1x4)x(5x6)	1341.8	-4,63	-11,96	(2x6)x(3x5)	1648.8	-0,97	-5,69
(1x5)x(2x3)	1777.0	6,39	-4,72	(2x6)x(4x5)	1500.6	-6,22	-14,17
(1x5)x(2x4)	1650.3	-0,61	-10,59	(3x4)x(5x6)	1302.9	-9,79	-18,50
(1x5)x(2x6)	1617.0	0,32	-7,51	(3x5)x(4x6)	1458.5	0,70	-7,78
(1x5)x(3x4)	1600.1	4,10	0,08	(3x6)x(4x5)	1365.5	-5,52	-5,95
(1x5)x(3x6)	151,71	4,12	2,83				

(**1:** Paum 15; **2:** STV 468; **3:** Nazilli 84S; **4:** Fantom; **5:** Delcerro; **6:** Giza 75)

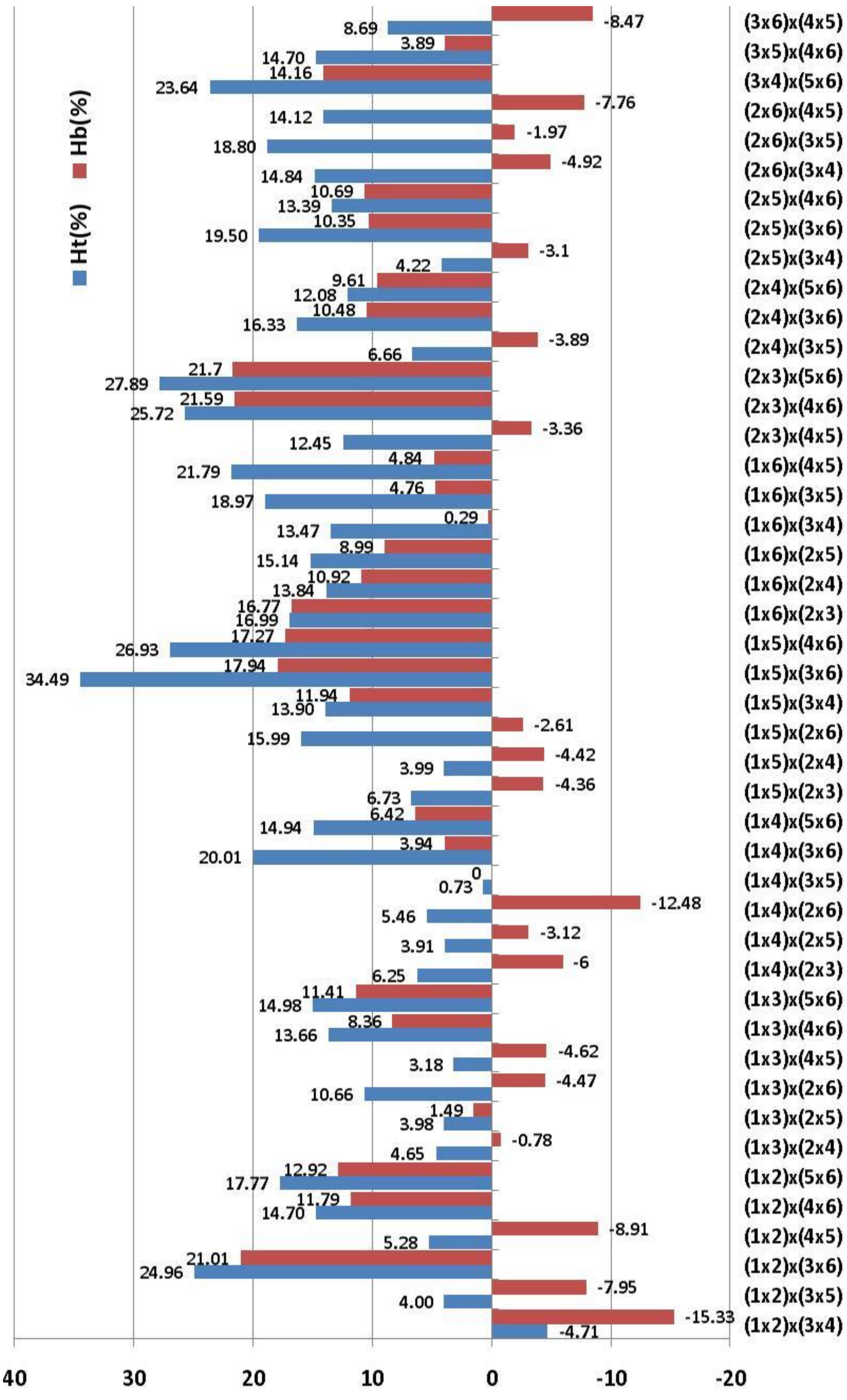

Figure 1. Heterosis (%) and heterobeltiosis (%) of double cross combinations in terms of lint yield

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Determination of Yield Performances of Oleic Type Sunflower (*Helianthus annuus* L.) Hybrids Resistant to Broomrape and Downy Mildew

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the most important edible oil crop in Turkey. Downy mildew and broomrape are the most important limiting factors for yield production in sunflower areas both in Turkey and also Eastern Europe and Black Sea countries. High or mid oleic type sunflower have recently started to gain importance year by year in the world because of that high oleic acid sunflower oil is more appropriate for frying as it is more beneficial to health. Therefore, higher oleic varieties will be demanded frequently in the future both in Turkey and also in the world. The study is involved the oleic type sunflower hybrids developed in Hybrid Sunflower Breeding Project conducted by Trakya Agricultural Research Institute, Edirne, Turkey. The measured values were evaluated in oleic type hybrids evaluated in regional yield trials in dry conditions at Edirne and Luleburgaz in the project during 2011 and2012. Oleic type hybrids resistant to broomrape and downy mildew were tested in yield trials. Some experimental hybrids exhibited higher performance than controls in some locations both for seed yield and seed weight and promising candidate hybrids were selected to promote in registration trials. Based on yield trials in the study, some oleic type candidate hybrids exhibited higher performance in terms of grain yield than other varieties. These hybrids were also resistant to broomrape and downy mildew and their oleic acid contents were measured as over 80% which were characterized as high oleic varieties while some of them were categorized mid oleic acid type as having 60-75% oleic acid content.

Keywords: sunflower, oleic acid, hybrid, yield, broomrape, downy mildew, resistance

Introduction

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) and broomrape (*Orobanche cumana* Wallr) are the most important limiting factors for yield production in sunflower areas both in Turkey, Spain, and also Eastern Europe and Black Sea countries which have more than 60% of world sunflower production (Kaya *et al*. 2012).

Downy mildew occurs depending on the climatic conditions during the sunflower growing season and mildew increases especially in rainy season when sunflower seeds stay longer than ten days under the soil due to low temperature during the sowing time. The mildew infection type is calling as primary (systemic) or secondary infection and the primary infection causes substantial yield reductions up to 100%, whereas secondary infection has non-significant importance on the production of sunflower (Spring *et al*. 1991; Fernandez-Martinez *et al*. 2009; Kulkarni *et al*. 2009; Jocic *et al*. 2010; Vear 2010; Kaya *et al*. 2012).

The metalaxyl seed treatment is the most effective way for chemical control of the primary infection of downy mildew at early stages of development of sunflower. However, the chemical control lost the effectiveness due to appeared high virulent new races in recent years. Therefore; genetic control seems to be the best solution for downy mildew nowadays. Downy mildew has more than determined twenty races in sunflower and resistance is controlled by several single dominant genes called *Pl*-genes providing vertical resistance. Genetic resistance to new races of downy mildew has been determined in wild sunflowers and has been transferred into cultivated sunflower genotypes (Tan *et al*. 1992; Shindrova *et al*. 1994; Kulkarni *et al.* 2009; Jocic *et al.* 2010; Vear 2010; Kaya *et al*. 2012).

Broomrape which is angiosperm parasite reduces up to 100% of sunflower seed yield. Like downy mildew, it results into appearance of new more virulent races of the parasite overcoming the existing sources of resistance (Vranceanu *et al*. 1980). New races other than 5 known races are observed recently that infested sunflower production areas largely in many countries such as Turkey, Spain, Bulgaria, Romania and Russia (Shindrova *et al*. 1998; Kaya *et al*. 2009; Evci *et al*. 2011b; Fernandez-Martinez *et al*. 2013). There has been no chemical control for broomrape until the last ten years so the genetic control was only solution. However, Clearfield system which combines Imidazilonone (IMI) herbicides and IMI-tolerant hybrids designing two technologies working together is also effective chemical way to control both weeds and broomrape in sunflower. On the other hand, new resistant genes were also developed by sunflower breeders against those new races and these resistant hybrids are used widely in the market. Both IMI and genetic resistance is effective solution for broomrape control in sunflower currently and IMI types mostly preferred in intensive weed problem areas (Kaya *et al*. 2009 and 2012; Fernandez-Martinez *et al*. 2013).

Classical sunflower oil fatty acid composition is saturated acids 11% (stearic, palmitic), oleic 20% and linoleic acid 69% and it has a large utilization for cooking or margarine (Baydar and Erbas, 2005). However, high (over 80%) or mid oleic type (60-70%) sunflower oil have recently started to gain importance year by year in the world because of that high oleic acid sunflower oil is more appropriate for frying and is more beneficial for health. Oleic type sunflower production reached over 50% in Spain and France, almost 100% in US, but it was just started recently in Turkey and some Eastern European countries too (Kaya *et al*. 2008; 2010; 2012).

High oleic content is firstly discovered in Russia by Soldatov utilizing from chemical mutations in Pervenent population (Demurin and Borisenko 2011). Oleic acid content is determined by Ol genes and it is determined by genetic factors which is also highly influenced by environmental factors (mainly night temperatures during grain filling period) (Pacureanu-Joita *et al*. 1999 and 2005; Baydar and Erbas, 2005; Izquierdo *et al*. 2006; Evci *et al*. 2009; Fernandez-Martinez *et al*. 2009; Demurin and Borisenko 2011). Today, mostly Pervenent mutation sources are using widely in sunflower breeding programs. Due to this recent trend, higher oleic varieties will be demanded frequently in the future both in Turkey and also in the world (Kaya *et al*. 2012).

Use of genetically resistant hybrids is definitely the most effective, economically feasible and environmentally friendly solution for controlling both broomrape and downy mildew in sunflower (Vear 2010). Therefore, most of the sunflower breeding program is designed as primary goal to develop resistant inbred lines and hybrids to both broomrape and downy mildew together as well as having high yielding and quality performances (Pacureanu-Joita *et al*. 1999; Fernandez-Martinez *et al*. 2009; Kaya *et al*. 2012).

National Sunflower Research Project being conducted by Trakya Agricultural Research Institute (TARI) in Edirne is primary public breeding program in Turkey. Many sunflower hybrids and inbred lines have been developed until today in National project. The yield trials in this project have been set up with the objective of developing sunflower genotypes genetically resistant to dominant races of downy mildew and new races of broomrape in both oleic and linoleic types in Trakya Region which covers almost 60% of sunflower production in Turkey. The study covered 2011-12 cycles of that National project with joint project of Trakya Birlik and the aim of study was to determine yield and quality performance of high oleic type sunflower hybrids resistant to broomrape and downy mildew in different locations.

Materials and methods

The experiments involving candidate sunflower hybrids and lines developed by National Sunflower Research Project conducting by TARI were conducted in 2011 and 2012 in the present study. Two regional yield trials in 2011 in Lüleburgaz and Edirne locations and one in Edirne in 2012 were also conducted in the study. Total 22 candidate hybrids existed in 2009 and 13 candidates in 2010 with four controls. Commercial hybrids such as linoleic types Tunca belonging to Limagrain and Bosfora belonging to Syngenta Seed Company and as high oleic types LG 5400 belonging to Limagrain Seed, P64H34 belonging to Pioneer Seed Co, Oliva from May Seed Co and Oleko belonging to Syngenta Seed Company were used as controls in the yield trials.

The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,5 m long with the 70x35 cm plant spacing. The middle 2 rows were harvested and the border rows were discarded, and plot size was 4.16 m² at harvest. Trials were planted mostly in mid April and they were harvested manually in mid September in each year. Oleic acid content (%), seed yield (kg ha^{-1}), 1000 seed weight (g), flowering and physiological maturity (days), plant height (cm), head diameter (cm), oil content (%) were measured. Oil contents of the hybrids were determined using Nuclear Magnetic Resonance (NMR) analysis at TARI institute lab whereas fatty acid contents including oleic acid were measured by Gas Chromatography (GC) in Trakya Birlik lab using 122-2361 DB-223 type colon. Briefly, samples from sunflower genetic material were ground, at least 1 g ground materials was treated with N-Heptan solution followed by 0.5 Ml, Methanol KOH solution. Finally, 100 microliters clarified solution was analyzed in GC after waiting for at least one hour. The data were analyzed statistically in JUMP program.

Broomrape tolerance of hybrids was determined under artificial and natural conditions. To use in artificial tests in growth chamber during winter, seeds were harvested from the broomrape plants collected from different infested fields of Trakya Region. For artificial screening, broomrape seeds in the pots (1-2 g/pots) were mixed with soil (sand, turf and normal soil). Broomrape shoots were counted on the root system of the sunflower plants of each pot after 45 days from planting (Kaya *et al*. 2009). In the natural infested fields in the Malkara, Tekirdag, *Orobanche* test plots were 5 m length, 14-15 plants existed in each row with 35 cm x 1 m plant density in two replications. Susceptible Sanbro hybrid was used as one control after every 40 rows. On the basis of observations, broomrape resistant, tolerant and susceptible hybrids were determined.

The resistance to downy mildew of sunflower hybrids was determined in artificial downy mildew tests in this study. In the tolerance tests, firstly sunflower seeds were germinated in the dark room until seedlings root elongated up to 0,5-1 cm. Then germinated seeds were soaked with spore solution in petri dishes to infest to downy mildew diseases. These germinated seeds after waiting at +16 °C planted into plastic glasses or pots filling with sterilized sand + perlite in growth chamber. Temperature +24 °C was maintained as 12 hours day and 12 hours dark in the chamber under controlled conditions then plants were grown in that conditions (Spring *et al*. 1991; Kulkarni *et al*. 2009; Evci *et al*. 2011a)

When first true leaves reached to 2-3 mm, the pots or glasses were covered with plastic bags strictly without allowing any air to appear 100% humidity. These plants have been staying at +16-17 °C temperature until 24-48 hrs then while susceptible ones were infested with disease and white spores appeared over cotyledons, resistant ones were immune (Viranyi 1985; Spring *et al*. 1991; Evci *et al*. 2011a).

Results

In the study, almost all candidate hybrids exhibited broomrape and downy mildew resistance in the both artificial tests in the lab. There was no broomrape and downy mildew in the hybrids in the field trial observations too. Some experimental hybrids exhibited higher performance than controls in some locations both for seed yield and seed weight and promising candidate hybrids were selected to send registration trials. Based on yield trials in 2011, 10 TR 048, 11 TR 068, 11 TR 076 and 11 TR 072 oleic type candidate hybrids exhibited higher performance in terms of grain and oil yield than other varieties in both locations. Among these high oleic varieties, 11 TR 072, and 11 TR 066 varieties were high oleic acid type, 11 TR 068, 10 TR 048 and 11 TR 076 varieties were mid oleic acid type in 2011 (Table 1-2).

Only 12 TR 013 was observed as promising candidate hybrid resistant to broomrape and downy mildew in seed and oil yield among candidate hybrids existed in 2012 regional yield trial (Table 3). Its oil content was higher than controls (46,4%) and its oleic acid content was 74.72% so that hybrid was categorized as mid oleic type.

Discussion

After evaluating yield performance and other yield traits of candidate hybrids, 9982-R, 9987-R, 9979-R and 99791-R sunflower restorer lines of these promising sunflower hybrids having higher seed and oil yield performance as well as resistance to broomrape and downy mildew resistant , these were also sent to registration trials too. These male lines also depicted broomrape and downy resistance as well as high and mid oleic content in the tests. As a result, National Sunflower project reached the target for developing both higher seed and oil yielding inbred lines and also being broomrape and downy mildew resistance which are the biggest problems in Turkish sunflower production areas.

Table 1. List of morpho-physiological traits and adaptation mechanism under heat/drought stress.

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	Oleic A %	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
11 TR 077	227	4	101,3	116	1	51,1	58,03	40,9	63	98	152	16
P64H34 (C)	239	2	106,7	105	2	44,1	85,45	44,0	62	102	133	16
LG 5400 (C)	242	1	108,0	104	3	42,8	86,31	46,8	63	99	138	15
11 TR 072	229	3	102,2	103	4	44,8	84,42	40,9	63	101	127	13
11 TR 068	224	7	100,2	103	5	45,7	72,53	40,8	62	99	138	14
10 TR 048	225	5	100,5	102	6	45,2	58,70	42,2	62	102	133	15
11 TR 076	224	8	99,8	101	7	45,3	68,16	41,6	61	100	138	14
11 TR 066	224	6	99,9	98	8	43,6	83,21	41,7	62	102	135	15
OLEKO (C)	216	9	96,4	92	9	42,7	89,15	40,6	60	98	134	17
11 TR 070	196	16	87,4	92	10	47,1	57,83	38,5	62	101	129	15
11 TR 075	199	13	89,0	91	11	45,5	83,44	41,6	62	99	133	15
11 TR 064	210	10	93,7	90	12	42,9	73,01	44,2	63	99	136	14
OLİVA (C)	199	11	88,9	90	13	45,0	86,73	37,9	62	102	118	15
11 TR 065	199	12	89,0	90	14	45,3	72,92	38,5	62	103	133	14
11 TR 069	182	21	81,3	90	15	49,6	57,44	40,4	65	-	128	15
11 TR 071	198	14	88,4	88	16	44,5	86,78	40,5	63	101	120	14
11 TR 063	196	15	87,6	86	17	43,7	85,37	37,8	65	102	133	14
11 TR 074	185	20	82,7	86	18	46,2	77,70	35,8	64	101	140	13
11 TR 062	194	17	86,5	85	19	43,8	81,99	40,9	64	103	143	15
11 TR 073	185	19	82,6	83	20	45,1	78,87	38,4	64	103	136	13
11 TR 067	190	18	84,8	82	21	43,4	75,16	40,5	62	103	132	15

Rk: Rank; Oil Y: Oil Yield; Oil C: Oil Content; Oleic A: Oleic Acid; TSW: Total Seed Weight; Flowr: Flowering Time; PM: Physiological Maturity; PH: Plant Height; HD: Head Diameter

CV (%) =8,6 LSD=253,2 kg ha^{-1} for seed yield,
CV (%) =8,6 LSD=114,3 kg ha^{-1} for oil yield,

Table 2. Sunflower hybrids in Yield Trial-4 at Lüleburgaz in 2011

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
11 TR 076	247	1	127,8	111	1	44,8	39,8	59	99	143	18
11 TR 077	226	2	116,7	100	2	44,4	48,4	61	97	155	21
OLEKO (C)	219	3	113,5	100	3	45,4	48,8	62	97	148	17
10 TR 048	214	4	110,8	96	4	44,7	45,0	60	100	155	18
11 TR 072	207	5	107,1	95	5	45,8	51,7	61	101	149	21
11 TR 065	198	6	102,4	89	9	44,8	44,5	61	102	156	17
11 TR 071	197	7	101,9	87	10	44,1	53,8	63	100	151	22
11 TR 068	194	8	100,2	95	6	49,2	42,8	61	98	159	21
11 TR 066	193	9	99,6	83	14	43,1	42,9	62	102	154	21
OLİVA (C)	191	10	99,0	93	7	48,7	49,0	62	101	162	20
LG 5400 (C)	189	11	97,7	93	8	49,0	49,1	62	99	130	17

Continuing table 2

Hybrids	**S Yield (kg ha^{-1})**	**Rk**	**Rate to Std(%)**	**Oil Y (kg ha^{-1})**	**Rk**	**Oil C (%)**	**TSW (g)**	**Flower (Day)**	**PM (day)**	**PH (cm)**	**HD (cm)**
11 TR 067	187	12	96,9	86	11	46,1	52,7	62	102	163	19
11 TR 074	186	13	96,3	86	12	46,4	36,3	62	99	154	15
11 TR 075	183	14	94,8	83	15	45,5	43,6	61	98	150	18
11 TR 073	181	15	93,7	80	17	44,0	51,8	62	101	160	17
11 TR 062	179	16	92,7	86	13	48,0	42,7	61	101	146	19
P64H34 (C)	174	17	90,1	83	16	47,4	42,2	64	100	123	15
11 TR 063	164	18	84,7	73	18	44,8	40,2	61	102	142	18
11 TR 070	149	19	77,3	73	19	48,6	53,1	63	100	146	19
11 TR 064	145	20	74,9	66	20	45,6	49,1	61	98	156	17

CV (%) =8,6 LSD=253,2 kg ha^{-1} for seed yield,
CV (%) =8,6 LSD=114,3 kg ha^{-1} for oil yield,

Table 3. Sunflower hybrids in Yield Trial-1 at Edirne in 2012

Hybrids	**S Yield (kg ha^{-1})**	**Rk**	**Rate to Std(%)**	**Oil Y (kg ha^{-1})**	**Rk**	**Oil C (%)**	**Oleic A %**	**TSW (g)**	**Flower (Day)**	**PM (day)**	**PH (cm)**	**HD (cm)**
BOSFORA(C)	253	1	107	110	1	43,6	37,01	44,88	64	100	167	17
P 64 H 34 (C)	243	2	103	105	3	43,3	86,90	38,44	63	105	148	16
12-TR-013	235	3	99	109	2	46,4	74,72	36,55	66	100	146	21
12-TR-001	229	4	97	87	15	38,0	61,66	34,04	65	105	138	14
TUNCA (C)	227	5	96	101	4	44,4	42,22	35,10	67	102	151	14
12-TR-003	226	6	96	101	5	44,8	83,66	27,65	67	102	154	14
12-TR-008	225	7	95	99	6	43,8	59,96	35,72	65	105	165	17
LG 5400 (C)	223	8	94	97	8	43,3	85,36	38,64	65	100	147	14
12-TR-012	221	9	94	97	9	44,0	83,36	42,46	66	106	161	17
12-TR-009	216	10	91	97	10	44,7	86,79	31,45	66	107	156	15
12-TR-012	212	12	90	90	14	42,5	80,88	34,12	67	106	174	14
12-TR-005	209	13	89	93	13	44,5	-	32,24	64	106	161	17
12-TR-007	206	14	87	86	16	41,9	60,48	32,24	64	105	159	16
12-TR-004	203	15	86	97	11	47,8	78,90	28,48	67	105	154	16
12-TR-002	203	16	86	86	17	42,5	82,94	34,68	66	102	152	15
12-TR-006	202	17	86	96	12	47,3	82,41	40,85	65	108	152	18
12-TR-014	170	18	72	75	18	44,0	54,36	34,25	66	99	145	15

CV (%) =9,30 LSD=287,3 kg ha^{-1} for seed yield,
CV (%) =9,31 LSD=126,7 kg ha^{-1} for oil yield,

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Interrelationship Between 1000 Seed Weight with Other Quantitative Traits in Confectionary Sunflower

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ABSTRACT

Breeding for increase in 1000 seed weight, results in increased seed yield. Therefore it is considered an important criterion in the development of confectionary sunflower hybrids. In this paper, we studied mutual relationships among several quantitative traits on one hand, and between 1000 seed weight on the other. Path coefficient analysis was used to separate direct and indirect effects of studied traits on 1000 seed weight, and to identify traits that could be used as selection criteria in sunflower breeding. The research was conducted during three vegetation seasons on 22 NS high-protein two-line confectionary sunflower hybrids, produced within the breeding program at IFVCNS, Novi Sad, Serbia. Significant and highly significant correlations were found among the largest number of examined traits. The analysis of simple correlation coefficients showed a highly significant positive correlation between 1000 seed weight and length of seed (0.717^{**}), seed hull ratio (0.609^{**}) and significant positive correlation with thickness of seed (0.549^{*}). A significant negative interdependence was determined between 1000 seed weight and seed protein content (-0.538^{*}). Path coefficient analysis for 1000 seed weight at phenotypic level showed that the length of seed and thickness of seed had a highly significant direct positive effect on 1000 seed weight ($DE=0.849^{**}$; $DE=0.748^{**}$). Width of seed had a strong negative direct effect on 1000 seed weight ($DE=-387^{*}$). A weak direct negative effect of kernel protein content and seed hull ratio was established, whereas seed protein content had a weak direct positive effect on 1000 seed weight. This indicates that length and thickness of seed have high influence on 1000 seed weight.

Keywords: confectionary sunflower, correlations, 1000 seed weight, path coefficient analysis, quantitative traits

Introduction

Although sunflower is mainly grown for the production of vegetable oils in the world, it is one of the most preferred confectionary seeds in Turkey, Eastern Europe, US, Canada, and in some Asian countries, such as China, Pakistan, Iran, Middle East countries, etc. (Pekcan *et al.* 2015). Confectionary sunflower produces large seeds with low oil contents, which are used in baking and as a snack food (Lu and Hoeft 2009). Although the favored seed color of confectionary hybrid in Turkey is white with grey stripes, consumers from Balkan countries such as Serbia, Bulgaria, Moldova and Romania prefer black seeds (Sincik and Goksoy 2014). The major breeding objectives for all sunflower types should be high yields and quality of oil, proteins and other products for food and non-food industries, approach to management of resistance genes, and stability of sunflower resistance to certain pathogens (Škorić *et al.* 2012). Besides their seed oil content, seeds of confectionary and oil type sunflower are distinguished by their hullability, shell color, seed weight and morphology, and kernel-to-pericarp weight ratio (Hladni *et al.* 2011). Seed of high protein sunflower is usually black with white stripes, or colorful and significantly bigger than the seed of oil type sunflower, with thicker hull loosely connected to the kernel. The shell is easily separated from the kernel and allows the whole seed to be dehulled (Gonzalez-Perez and Vereijken 2007). The selection for seed size i.e. for increased mass of 1000 seeds may be an important criterion in sunflower yield increase (Jocić *et al*. 2000). The best confectionary types should have the oil content lower than 30%, and husk content up to 50% (Kaya *et al*. 2009). However, large kernel size is the characteristic of confectionary sunflower most preferred by customers. Thus , in addition to seed yield, 1000 seed weight and seed size are major interests in confectionary sunflower breeding (Pekcan *et al*. 2015).

Confectionary sunflower hybrid breeding is directed towards the increase of protein content and quality (>25%), mass of 1000 seeds (>100 g), hectoliter mass, oil stability with decrease of its content in the seed (<40%), increase of kernel ratio and decrease of shell ratio, uniformity in seed size and color, dehulling, as well as tolerance to dominant diseases in the cultivation region (Hladni *et al*. 2009).

Mass of 1000 seeds belongs to the major yield components, breeding for increase of the mass of 1000 seeds leads to seed yield increase, so this trait is used as selection criteria when creating sunflower hybrids (Miller and Fick 1997; Kaya *et al*. 2003; Goksoy and Turan 2007; Hladni *et al*. 2008; Yasin and Singh 2010; Kholghi *et al*. 2011). Seed protein content and mass of 1000 seeds demonstrated a strong positive correlation with protein yield, which means that breeding for these components is simultaneously breeding for protein yield (Hladni *et al*. 2011). Plant breeders commonly prefer yield components that indirectly increase yield (Kaya *et al*. 2007).

The aim of this paper was to determine the interdependence between 1000 seed weight and seed protein content, kernel protein content, hull ratio, length of seed, width of seed and thickness of seed.

Materials and methods

The research was conducted during three vegetation seasons on 22 confectionary sunflower hybrids, produced within the breeding program at the Institute of Field and Vegetable Crops. Novi Sad, Serbia. Twenty-two high protein two-line confectionary hybrids: NS-H-1, NS-H-2, NS-H-3, NS-H-4, NS-H-5, NS-H-6, NS-H-7, NS-H-8, NS-H-9, NS-H-10, NS-H-11, NS-H-12, NS-H-13, NS-H-14, NS-H-15, NS-H-16, NS-H-17, NS-H-18, NS-H-19, NS-H-20, NS-H-21, NS-H-22, created by crossing cytoplasmic male sterile female line and male line with a fertility restorer genotype, were examined during three vegetation seasons (2008, 2009, 2010), at three locations: Rimski Šančevi, Erdevik in the Vojvodina region, and Kula in central Serbia. The following traits were examined: 1000 seed weight, seed protein content, kernel protein content, hull ratio, length of seed, width of seed and thickness of seed. The plot where the experiment was conducted was 28 m^2 in size and 70cm x 28cm plant density was used. Seeds were planted by hand in 4 rows in April, and all plants from the two middle rows were harvested in September except for the first plants on each plot. The experiment was done as a randomized complete block design with 3 replications. Mass of 1000 seeds (g) was measured on a random sample of absolutely clean and air-dried seed. Seed protein content and kernel protein content was determined by a conventional micro Kjeldahl method. Hull ratio was determined by dehulling the seeds and their separation into kernel and hull. Length of seed, width of seed and thickness of seed (mm) was measured using a vernier caliper.

Mutual relationships of the examined characteristics, and their direct and indirect effects on seed yield, were analyzed using the path coefficient analysis (Wright 1921; Dewey and Lu 1959; Ivanović and Rosić 1985). Statistical analysis was performed using R (2014).

Results and discussion

In the development of new high-protein hybrids for confectionary use, it is important to find the traits that are easily determined and show their interdependence with 1000 seed weight, based on which those traits could be defined as selection criteria.

The analysis of simple correlation coefficients showed a significant negative correlation between 1000 seed weight and seed protein content (-0.538^*). This result is in contradiction to the research performed by Radić *et al*. (2013) who determined weak positive correlations between 1000 seed weight and seed protein content, and findings of Joksimović *et al*. (1999), Dagustu (2002) and Drumeva *et al*. (2011), who determined a significant positive correlation between 1000 seed weight and seed protein content.

A highly significant positive correlation was found between 1000 seed weight and the length of seed as well as hull ratio. A significant positive correlation was found between thickness of seed and width of seed. A nonsignificant positive correlation was determined between width of seed and kernel protein content and 1000 seed weight (Table 1). This result is in agreement with the findings of Kaya *et al*.

(2008) who stated a very strong positive correlation between hull ratio and 1000 seed weight, and Li *et al*. (2010) who found a strong positive correlation between hull ratio and 1000 seed weight. However, our results are in disagreement with Ergen and Saglam 2005; Kaya *et al*. 2009, who found a strong negative correlation between hull ratio and 1000 seed weight.

A highly significant negative correlation was observed between seed protein content and both seed hull ratio and length of seed, whereas it exhibited a significant negative correlation with seed thickness, and a nonsignificant negative correlation with kernel protein content and width of seed. These results are in agreement to the findings of Ergen and Saglam (2005) who determined a strong negative correlation between seed protein content and hull ratio.

The analysis of the simple correlation coefficient showed a significant positive correlation between kernel protein content and hull ratio, and a nonsignificant positive correlation with length of seed, width of seed and thickness of seed (Table 1). Hull ratio demonstrated a highly significant positive interdependence with length of seed and width of seed (0.734^{**};0.671^{**}), and a significant positive interdependence with thickness of seed. The analysis of simple correlation coefficient showed a significant negative correlation between length of seed and thickness of seed, as well as a nonsignificant positive interdependence with thickness of seed. Width of seed had a highly significant positive correlation with thickness of seed (Table 1).

Since the values of simple correlation coefficients did not provide clear connections between the examined characteristics on one hand and 1000 seed weight on the other, their correlations were further analyzed by using path coefficient analysis to determine the involvement of correlation coefficients in a direct and indirect effect on a specific trait (Table 2). Path coefficient analysis for 1000 seed weight at the phenotypic level showed that the length of seed and thickness of seed had a very strong direct positive effect on 1000 seed weight ($DE=0.849^{**}$; $DE=0.748^{**}$), which is in accordance with the simple correlation coefficient. Width of seed had a strong negative direct effect on 1000 seed weight ($DE=-387^{*}$), which is discordance with the simple correlation coefficient as the simple correlation coefficient is significant positive. The effect of the simple correlation coefficient was masked with the indirect effect of the width of seed through length of seed and thickness of seed. Seed protein content demonstrated a weak positive direct effect (DE=0.113) on 1000 seed weight, while the simple correlation coefficient is very strong and in the opposite direction. The direct effect of seed protein content was masked by its negative indirect effect through the length of seed (ID=-0.515) and thickness of seed (ID=-0.280), and by the positive indirect effect through the width of seed (IE=0.113).

Kernel protein content had a weak negative direct effect on 1000 seed weight (DE=-0.052), while the simple correlation coefficient is weak and of the positive direction. The existence of a weak positive simple correlation coefficient between 1000 seed weight and kernel protein content is the result of the indirect positive effect of kernel protein content through length of seed (IE=0.274) and thickness of seed (IE=-0.188). Joksimović *et al*. (2004) found that protein content had a very strong negative direct effect on 1000-seed weight (-0.840), which was in agreement with the very strong negative correlation based on simple correlation coefficients.

Hull ratio had a weak negative direct effect (DE=-0.019) on 1000 seed weight, while the simple correlation coefficient is very strong and of the positive direction. This correlation was masked with the positive indirect effect of hull ratio through length of seed (IE=0.623) and thickness of seed (IE=0.369), as well as the negative indirect effect of hull ratio through width of seed (IE=-0.260).

The differences in the presented results can be explained by the fact that different plant material was used by the authors in their research. In sunflower breeding for productivity, it is important to find the traits which are easy to evaluate, demonstrate their causal connection with 1000 seed weight, and therefore can be used as selection criteria. Higher 1000 seed weight is an ultimate objective of confectionary sunflower researchers. The focus should be placed on traits with a very strong positive direct effect on 1000 seed weight. Presence or absence of correlations can contribute to the right choice of examined traits, so as to enhance the efficiency of some selection criteria.

Conclusion

The main direction in breeding low oil content confectionary sunflower is directed towards the increased mass of 1000 seeds, higher protein content and quality, with the decrease in shell ratio. Within the development of new high-protein hybrids for confectionary use, it is important to find the traits that can be easily determined, and at the same time show their interdependence with 1000 seed weight. The applied path coefficient analysis gave a somewhat different picture than the one given by the correlation analysis. Path coefficient analysis has partitioned the direct and indirect effects of the quantitative traits on 1000 seed weight of sunflower. It allowed us to detect those components which exhibit the highest effect on 1000 seed weight expression. The data obtained in this investigation, as well as various literature data, indicate that the characteristic such as length and thickness of seed (0.849**; 0.748**) are the main 1000 seed weight components which should be used as selection criterion in sunflower breeding. Width of seed had a strong negative direct effect on 1000 seed weight (DE=-0. 387*). On the basis of the research in this paper it appeared that the length and thickness of seed were the most important traits for 1000 seed weight, and can be used for the improvement of seed yield and evaluation of sunflower breeding materials.

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Table 1. Simple correlation coefficients of quantitative traits and 1000 seed weight.

Trait		**KPC**	**SHR**	**LS**	**WS**	**TS**	**TSW**
		X2	X3	X4	X5	X6	y
SPC	X1	-0.355	-0.687**	-0.607**	-0.293	-0.374*	-0.538*
KPC	X2		0.521*	0.323	0.197	0.252	0.285
HR	X3			0.734**	0.671**	0.494*	0.609**
LS	X4				0.424*	0.175	0.717**
WS	X5					0.730**	0.463*
TS	X6						0.549*

** *F test significance at level P<0.01* * *F test significance at level P<0.05* ns- *not significantly different*

X1	seed protein content (SPC)	X5	width of seed (WS)
X2	kernel protein content (KPC)	X6	thickness of seed (TS)
X3	hull ratio (HR)	Y	1000 seed weight (TSW)
X4	length of seed (LS)		

Table 2. Analysis of direct and indirect effects of the six traits on 1000 seed weight

Components	**DE (P)**	**IE (Pxr)**	**CC (r)**
Seed protein content (SPC)	0.113		
Indirect effect KPC		0.018	
Indirect effect HR		0.013	
Indirect effect LS		-0.515	
Indirect effect WS		-0.113	
Indirect effect TS		-0.280	
Total			-0.538
Kernel protein content (KPC)	-0.052		

Continuing table 2

Components	DE (P)	IE (Pxr)	CC (r)
Indirect effect SPC		-0.040	
Indirect effect HR		-0.010	
Indirect effect LS		0.274	
Indirect effect WS		-0.076	
Indirect effect TS		0.188	
Total			0.285
Hull ratio (HR)	-0.019		
Indirect effect SPC		-0.077	
Indirect effect KPC		-0.027	
Indirect effect LS		0.623	
Indirect effect WS		-0.260	
Indirect effect TS		0.369	
Total			0.609
Length of seed (LS)	0.849**		
Indirect effect SPC		-0.068	
Indirect effect KPC		-0.017	
Indirect effect HR		-0.014	
Indirect effect WS		-0.164	
Indirect effect TS		0.130	
Total			0.717
Width seed (WS)	-0.387*		
Indirect effect SPC		-0.033	
Indirect effect KPC		-0.010	
Indirect effect HR		-0.013	
Indirect effect LS		0.360	
Indirect effect TS		0.546	
Total			0.462
Thickness of seed (TS)	0.748**		
Indirect effect SPC		-0.042	
Indirect effect KPC		-0.013	
Indirect effect HR		-0.009	
Indirect effect LS		0.148	
Indirect effect WS		-0.283	
Total			0.549
Coefficient of R^2 determination	0.753		

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Influence of Environments on The Amount and Stability of Grain Yield in The Modern Winter Wheat Cultivars II. Evaluation of Each Variety

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ABSTRACT

Cultivars grown at the farmers' fields were selected and tested for three consecutive years at eight locations in Bulgaria, which were representative for the entire territory of the country and had contrasting soil and climatic conditions for crop growing. Significant variations of grain yield were found among the investigated cultivars regardless of their specific response to the year conditions and the location. The interaction genotype x environments was significant and high, and was of non-linear type. The changeable environmental conditions caused different reactions of the cultivars, which allowed dividing them into groups according to the plasticity and stability they demonstrated. The variation in this experiment determined through Principal Component Analysis (PCA reached level four, which is comparatively rare for this trait. On the whole, PC1 had low value (49%), while PC2 was high (16%). There were several cultivars with very high PC2 values, exceeding several times the values of their respective PC1. The percent of variation caused by the environment was significant for grain yield under the conditions of Bulgaria. The investigated cultivars differed not only by grain yield but also by their plasticity and stability under changeable environments, the percent of the genotype effect being about 12 % for the entire experiment. It was found that each cultivar can give high grain yield at high ecological stability regardless of its genetic potential for quality. Best balance between grain yield and stability was found in cultivars Aglika, Demetra, Iveta (first quality group), Galateya, Slaveya (second quality group) and Todora, Kristal and Pryaspa (third quality group).

Keywords: wheat, grain yield, cultivars, genotype x environment, stability.

Abbreviations: **GY** = grain yield, **b_i** = Regression coefficient **(A)**, **σ^2** = Deviation from regression **(A)**, **Residual** = Residual variance **(A)**, **GY-b_i** = General adaptability index **(B)**, **GY-σ^2** = "General stability" index **(B)**, **HV** = Variance of heterogeneity **(C)**, **IN. Corr** = Variance of incomplete correlation **(C)**, **GE** = Genotype x environment interaction **(C)**, **W^2** = Ecovalence (percent of genotype from total variation) **(D)**, **SV** =Variance of stability **(E) (F)**, **Ysi**= Size and stability of the trait **(F)**

Introduction

The study on the interaction of the genotype with the environmental conditions when considering grain yield *(GxE)* is very important for winter wheat due to its genetic and physiological specificity as a crop of the micro climate. Cultivars developed under certain conditions perform best under these conditions and it is difficult for them to compete with cultivars developed in different regions (Tayyar, 2010; Muhe and Assefa, 2011). This makes very important the investigations on the factors which cause changes in the direction and value of the genotype x environment interaction in the breeding of this crop (Tadesse *et al.,* 2010; Rachovska *et al.,* 2011). The breeding efforts are directed toward developing accessions with higher productivity than that of the cultivars already used in practice, which is very difficult against the background of the level already achieved (Tsenov *et al.,* 2009; Aminzadeh, 2010). Therefore the necessity arises to systematically improve the wheat plant by enhancing its tolerance to stress (Boyadjieva *et al.,* 2009; Mohammadi *et al.,* 2010; Arain *et al.,* 2011, Bennett *et al.,* 2012); this has created serious prerequisites for high and stable grain yield over years. Increasing the adaptability of the new cultivars is a main goal of many breeding programs both in spring (Ferney *et al.,* 2010), and in winter wheat (Paunescu and Boghic, 2008; Sharma *et al.,* 2010). Reasons for this are the investigations revealing possibility to combine high stability with high grain yield (Tsenov *et al.,* 2008). In their study Botwright *et al.,* (2011) report very high interaction of the cultivar with the environment, a prerequisite for high adaptability at level of the yield 8 t/ha. Therefore it can be assumed that there are actual possibilities of linear type of interaction of the genotype with the environment that would lead to desirable combination of high yield levels with stability (Aminzadeh, 2010; Tsenov *et al.,* 2011a).

Stability is the ability of the cultivars to express their genetic potential under a wide range of conditions so that the grain yield from the stable genotype is always high even at significantly high genotype x environment interaction (Tsenov *et al.,* 2011b). In the investigations of Purchase (1997), Annicchiarico (2002) there is the definite statement that the analysis on the genotype x environment interaction is important at all levels of the breeding process – from determining of the biotype for a certain region (Dolatabad *et al.,* 2010) and evaluation on the combining ability of the parental components for crossing (Yan and Hunt 2002) to the proper distribution of the most suitable cultivar (Tayyar, 2010).

As already mentioned above, the interaction of the cultivar with the environment is complex and depends on unpredictable conditions and on the behavior of the group and each variety in it. Grain yield from wheat is always strongly influenced by the growing conditions, and the specific expression of each genotype against the background of the behavior of a group of varieties is too complex for specific analysis (Ferney *et al.,* 2006). The more the factors of the environment (year and location), the more complex and multi-layered the interaction is and is therefore impossible to analyze by a single evaluation approach. In this relation Lin *et al.,* (1986) and Becker and Léon, (1988) have developed concepts for proper analysis and interpretation of the results from this type of researches, which are still valid. These concepts, on their part, require the application of several directly opposite statistical parameters which help to make proper interpretation of the genotype x environment interaction and to evaluate the plasticity and stability of the used varieties (Pacheco *et al.,* 2005, Chapman, 2008).

The aim of this investigation was to determine the specific reaction of each genotype involved in the trial under the typical conditions for grain production in Bulgaria by using different and mutually complementary criteria (parameters, indices) for evaluation fro their adaptability and grain yield stability.

Material and methods

The grain yield from 24 Bulgarian wheat cultivars was investigated at 8 locations during 2007-2009. Data were used from post-registration testing of the national Executive Agency of Variety Testing,

Field Inspection and Seed Controlat in 8 locations in Bulgaria (Table 1) out of the total 12 locations investigated and therefore their numbering is incomplete. The methods for conducting the field trial have already been presented in detail in our previous communication (Tsenov and Atanasova, 2013). The reasons for excluding four locations and one season (2010) from the database are explained in it.

The behavior of each investigated cultivar was followed through its grain yield under variable environments (location and season). The ordering of the initial data and their analysis was done with XLSTAT 2009.

The genotype x environment interaction was determined by using three statistical programs specifically suitable for the purpose of this investigation: GEST (Ukai *et al*., 1996), STABLE (Kang and Magari 1995) and GGE biplot (Yan and Kang, 2003). Different aspects of the genotype x environment interaction were analyzed by calculating several of the most common parameters and indices for evaluation and analysis on this interaction grouped and designated as follows:

(**A**) -coefficient of regression [bi], deviation of each cultivar from the regression [σ^2] and residual variation [Residual] according to (Finlay and Wilkinson, 1963),

(**B**) -index of general adaptability (GY-b_i) according to (Vulchinkov and Vulchinkova, 2007) and index of “general stability” (GY-σ^2), suggested **in this investigation** as an additional element of evaluation

(C) -variance of stability [σ^2i], heterogeneity variance

[HV], variance of incomplete correlation [IN. Corr], interaction of the genotype with the environment (GE) according to Muir *et al*., (1992)

(D) -ecovalence [W^{2}i] according to Wricke (1962)

(E) -variance of stability [SV] according to Shukla (1972)

(F) -parameter of yield stability [Ysi] according to the approach of Kang and Magari (1995).

In Tables 4 and 5 a part of the parameters of the groups (**A, B, C, D, E**) are presented as percent from the average level of the respective parameter. This was done with the aim to more precisely compare the data of each cultivar because the absolute values were very close and their direct comparing was very difficult. For informative purpose the mean values of each parameter are represented as absolute value in the last row of each table.

The data from the Principal Component Analysis and the graphic analysis (Jmp 10) are at the basis of the detailed comparison of the ecological plasticity and stability according to the investigated trait of each involved cultivar. For better substantiation of the existing variations between the cultivars, the obtained values of the parameters and indices were analyzed with the help of several additional statistical programs (Statistica 7, Statgraphics XV). They were used to calculate the parameters of the Principal Component Analysis (PCA), of the descriptive statistics, of the correlation values and the variance analysis. Rank correlations (Kendall –Tau) were calculated with the help of the software StatPlus 2009 Professional.

Results and discussion

Figure 1 presents grain yield from the 24 cultivars in a reduced scheme of 8 locations and three years of investigation, as mentioned in the first communication (Tsenov and Atanasova, 2013). The high variation of the character depending on each investigated factor, including the variation caused by the genotype, is evident. Significant differences between the cultivars were observed in all three years; in 2007, when there was a long drought, the differences were highest (Tsenov *et al.,* 2014); with regard to locations, the differences were also clearly outlined (Figure 2). The applied statistical analysis clearly delineated the differences in the data on grain yield depending on the location where the trial was conducted, as well as the low values of the PCA to the second level (66%). According to the data five groups of locations can be differentiated: *(1)*-1(Selanovtsi), *(2)*-2(Pordim), *(3)*-3(Brushlen), 10(Gorski izvor) and 11(Ognyanovo), 6(DAI), *(4)*-8(Burgas), *(5)*-9(Radnevo).

The variation caused by the differences in the conditions over years and locations is the reason for their significant interaction with the cultivar (Table 1); this, on its part, is a sufficient prerequisite for objective evaluation of the behavior of the individual genotype as a level, adaptability and stability of grain yield.

Even after elimination of some of the levels of the individual factors, the interaction of grain yield with the environment was complex, and its variation reached level four of the principal component analysis (Table 3). This was entirely in accordance with the established high effect of heterogeneity indicated in Table 2. The values of the separate components gradually decreased from PCA1 to PCA4, but they were significant and could not be ignored. They showed non-linear type of the genotype's interaction with the environment which made very difficult the evaluation of the individual cultivar with regard to its behavior under the conditions of the environment. It is known that the levels of the first two components are important and provide some information on the stability of the genotype. The evaluation of the variation of each cultivar is represented in Figure 3 through the PCA 1 values.

Variations in the conditions resulting from one of the two factors (year or location) provoked different response of each cultivar according to the mean level of reaction of 4.1%. Lowest was the variation of the standard cultivars (7)-Pobeda and (13)-Sadovo 1, and of cultivars (12)-Sadovo 772 and (18)-Neven. All other cultivars demonstrated variation above the mean value of the group, meaning that their response to the effects of the environmental factors is of linear type. This is expressed in higher grain yield under favorable conditions and *vice versa*. The values of the second component were radically opposite from the point of view of the cultivars. The mentioned cultivars (7), (12), (13), as well as (15)-Aneta and (20)-Yantur had strongly expressed non-linear variation under changeable environments (Figure 4). Exceptionally low were the values of PCA2 in cultivars (4)-Desislava, (5)-Iveta, (8)-Vyara, (10)-Enola and (11)-Miryana. In general this information shows how each cultivar principally changes the trait under variable conditions from favorable to unfavorable for wheat.

For more detailed and specific evaluation of the cultivar's interaction with the environment, it was analyzed by using the most common statistical approaches (Tables 4 and 5). The values of the cultivars for most of the parameters were very similar and therefore the relative values (%) of each genotype were presented, according to the mean value of each parameter. When the values are above 1.06, the percent of the cultivar is high, and when it is below 0.94, the percent is low. According to the “dynamic” or “agronomic” concept, stable is considered a genotype which follows the dynamics of the environmental conditions by changing its character. According to (Becker and Leon, 1988) with this approach more stable is the cultivar which has regression coefficient (bi) about (1) and the lowest possible deviation from the regression straight line (σ^2). According to the data in columns 5 and 6 such were cultivars Demetra, Iveta, Enola, Miryana, Slaveya and Neven. On the other hand cultivars Albena, Pobeda, Sadovo 1, Sadovo 772, Kristal, Svilena and Todora were highly variable by yield in comparison to the rest of the cultivars. Furthermore, the latter cultivars had high values of the parameter in column 7 which is additional evidence for their high variation.

With index (GY-bi) – column 8 and (GY-σ2) – column 9, the situation was the opposite, the higher values revealed higher degree of compromise combination of grain yield with stability. The index (GY-σ^2) is introduced here as an addition to the information provided by the index of general stability (GY-bi), described in detail by (Vulchinkov and Vulchinkova, 2007). The reason for this is that the extraction of the value of the regression coefficient (bi) from the mean value of the trait is not always completely informative from the point of view of the cultivar’s deviation from the regression straight line of the group. In our opinion this deviation [σ^2] is also important and at close values of (bi) about 1 (in 12 out of the 24 cultivars) it more correctly reflected the difference in the variation of the individual cultivar, provided that the difference in its variation here was from 33 to 188%. This allowed positioning its values in the group of indices (**B**).

Table 5 presents data on the degree of variation of each cultivar expressed through the different statistical approaches designated in the material and methods section as statistical groups C, D and E. The genotypes were positioned in descending order according to the values of ecovalence [W^2] (Wricke 1962) in column 6. The lower the values of each parameter for a given cultivar, the lower is its variation as a percent against the background of the total variation under the conditions of this experiment. The values of the separate parameters of the groups were almost overlapping although different statistical approaches were used for their calculation, a fact mentioned many times in similar investigations (Tsenov *et al.* 2006). This means that each of the parameters from a given group of approaches can be equally used for evaluation of the genotype. According to these data a half of the cultivars demonstrated low interaction with the conditions of the factors because their percent in the total variation was low. These were cultivars Demetra, Petya and Iveta and the standards Enola and Pryaspa. Highest was the interaction with the environment of the cultivars which are standards: Aglika, Pobeda, Sadovo 1 and the cultivar Sadovo 772. The low values of the ecovalence (W^2) and the variance of stability (SV) in such cultivars as Desislava and Yantar were related to very high values of the parameters of group (C). This fact indicates that these cultivars demonstrate a very complex interaction with the environment and their response cannot be foreseen from the point of view of environmental variations. On the whole it is very high but due to the high values of the three parameters it is not adequate at all to the response of the group of cultivars. Similar are the data on cultivar Viara. The data on cultivars Aneta, Kristal, Svilena and Todora showed very high values of the parameters in groups **(D)** and **(E).** This is an indication for the strong variation of these varieties at low level of interaction with the conditions (low values of [GE])**,** which implies non-linear interaction. Such an assumption is valid for all cultivars which show disagreement of the values of the parameters from group **(C)** with the parameters from groups **(D)** and **(E)**.

Analyzing the data from the different Tables through the well known approaches appropriate for this purpose, we encountered the fact that the data on the respective cultivars disagreed, sometimes considerably, which made the formulation of the correct conclusions on their behavior difficult.

This was the reason for calculating the correlations between the values of the trait and the values of the parameters for evaluation of the genotype’s stability and plasticity on the whole (Table 6). Grain yield was in positive correlation only with the regression coefficient ($r=0.780^{**}$). The correlations were negative with the other parameters for evaluation, but not significantly high. It should be so in principle because these parameters investigate and demonstrate the variation and interaction of the trait with the environment and do not relate directly to its level. Similar by value and direction (negative) were the correlations of (bi) and all other parameters for evaluation presented in column 3. The correlations between all other parameters were significantly high and positive (columns 4, 5, 6, 7, 8, 9). Therefore each of these parameters can be used for correct evaluation of the stability and plasticity as a main parameter or in a group with each of the other parameters.

It was a considerable inconvenience in the process of writing the discussion section that the values of the individual statistical parameter for each cultivar showed disagreement by value and direction of expression. The stability and adaptability of the cultivar is highly important and therefore, it was the aim of this investigation. This was the reason for applying one of the integral methods (Kang and Magari 1995*),* the approach of which allows making a compromise evaluation of the level of grain yield and its stability under the conditions of the environment through the values in column 5 of Table 5. Cultivars Todora-(24), Aneta-(15), Neven-(18) and Pryaspa-(22) possessed the best combination between yield and stability, although they showed high variation of grain yield (c.f. Table 3). Cultivars Kristal-(21), Aglika-(1) and Iveta-(5) had excellent combination between high and stable yield, as well as low variance of the investigated factors of the environment. Most unstable were the standards Pobeda-(7), Sadovo 1-(12), Yantur-(20) and cultivar Milena-(6). The data clearly illustrate that when making specific analysis it is possible to identify cultivars with high general adaptability. Although the objectivity and correctness of the method used for evaluation has been demonstrated many times (Plamenov *et al.* 2009; Rachovska *et al.* 2011; Dimova *et al.* 2012) we decided to compare it to a similar and improved statistical method developed by Yan and Kang (2003).

In the recent years this method (Vulchinkov and Vulchinkova, (2007) has been used in many studies for evaluation of the interaction of the genotype with the environment although its objectivity has been criticized with regard to the spatial position of the cultivars (Vulchinkov and Vulchinkova, (2007) its application gives good evaluation on the behavior of specific cultivars or lines and on the suitability of the separate locations for concrete analysis on the productivity or quality of the respective crops (Yan and Rajcan 2002; Ferney *et al.* 2010; Yan and Holland, 2010). According to the investigation of Rubio *et al.* (2004) this method can be successfully used to group the genotypes by phenology and by their ecological origin. Comparing this method to the most widely used traditional approach for analysis of the genotype x environment interaction (Eberhart and Russell 1966) it has been found that it has a number of advantages in determining stable maize hybrids with high grain yield (Alwala *et al.* 2010). Figure 5 shows the spatial distribution of the investigated cultivars through principal component analysis. The cultivars positioned to the right of the blue line (grain yield) and above the red line (stability) possess good combination between stability and size of grain yield. The small red circle on the red line indicates the position of the most suitable yield-plus-stability combination. These were cultivars Iveta-(5), Aneta-(15), Neven-(18), and the two standards Aglika-(1) and Pryaspa-(22). The position of cultivar Todora-(24) showed high yield but lower stability, which was also valid for cultivar Viara-(8). The standard cultivars Pobeda-(7), Enola-(10), Sadovo 1-(12) and Yantar-(20) demonstrated significantly lower and simultaneously unstable grain yield in comparison to the other standards and investigated cultivars. Additional information on which cultivar gave highest grain yield is presented in Figure 6. High grain yield from cultivars Aneta-(15), Neven-(18) and Pryaspa-(22) was obtained at six out of the eight locations, with the exception of DAI and Radnevo. At the same time cultivar Todora showed maximum grain yield at these two locations.

The ranking of the investigated cultivars by the two discussed methods coincided to a large extent, meaning that their ranking in Table 7 can be considered correct. The correlation between the ranking by parameter [Y_S(i)] and grain yield was very strong and positive (Table 8). The presence of negative correlations with all parameters of plasticity and stability (Table 5, column 2) is an indication that during the ranking the effects of the interaction with the environment have been taken into account and that the ranking by yield is different. The correlation of grain yield with the index of general adaptability [GY-bi] was very strong (r=0.956), as well as its correlation with the index [Y_S(i)] (r=0.844). High and positive were the correlations of the index [GY-σ^2] with grain yield (r=0.681), with the index of general adaptability (r=0.672) and the parameter of yield stability [Y_S(i)] (r=0.579).

It follows that by using the values of this new index, ranking with the aim to make evaluation is also possible and entirely correct. The application of each of the two indices separately (Figures 7 and 8) leads to different ranking of the cultivars. This difference was additionally investigated (Table 9) and it was found that the strongest correlation with grain yield showed index [Y_S(i)] (r=0.708**), which was an evidence that it gave considerably lower reading of the effect of variation.

On the other hand, the lack of significant correlation of King's parameter [Y_S] with the two indices implies that their values probably take into account to a greater extent the effect of variation (GxE). Additional evidence for this assumption is provided by the established high values of the correlations of grain yield with the two indices, which, however, had lower values. The relation of parameter [Y_S] with the new index [GY-σ^2] must be strong, because the correlation they showed according to grain yield was similar. When the investigated cultivars demonstrate higher variation as deviation from the regression curve (σ^2) than the variation of their regression coefficients, as in our case, then the suggested index of "general" stability can be correctly used for ranking of the cultivars by grain yield. Its use changes to a certain degree the ranking of the cultivars, but it is not significant against the background of ranking by the other indices, which makes it applicable. The main reason for using the index of "general" stability [GY-σ^2] is the application of the «dynamic» concept of stability when the trait changes as formulated by (Becker and Leon, 1988), according to which the deviation from the regression curve should be as low as possible for the stability of the cultivar to be highest.

The evaluation of the behavior of a given genotype under specific and changeable conditions of the environment provides valuable information on several aspects: how the cultivar responds to changeable conditions, how plastic and adaptable it is under a wide set of environments (locations and seasons) and what is the area of its eventual distribution. This knowledge is important for breeding as well, to apply proper approaches of purposeful selection for specific locations (regions) with similar growing conditions. It is known that cultivars with high adaptability have linear genotype x environment interaction. The cultivars with very high stability usually are not highly productive and therefore it is necessary to use special methods and approaches for combining of high productivity with high stability (Kaya and Taner, 2003; Fan *et al.* 2007). According to the commonly accepted definition, a "stable" cultivar performs comparatively well under unfavorable conditions and not so well under favorable conditions. The breeder's "ideal" cultivar possesses high productivity, shows regression coefficient (bi) approximate to 1 (plasticity) and the lowest deviation of factual data from the regression curve (σ^2) (stability). From this point of view the use of the suggested new index «general stability-[GY-σ^2] is logical and acceptable. The results from a part of the cultivars confirmed the generally accepted thesis of high yield and low stability. Almost all cultivars with the exception of the standard Pryaspa-(22), which are highly productive, demonstrated high variation, i.e. low stability. There are several cultivars with high grain yield also relatively stable under the investigated conditions of the environment; these cultivars most thoroughly met the criterion of the "ideal standard". These were cultivars Iveta-(5), Demetra-(3) and Karat-(17). It can be concluded that the combination of high yield and stability can be achieved in cultivars regardless of their genetic potential for grain quality.

The discussed approaches for evaluation of each particular cultivar according to the data are applicable and complementary. The evaluation on the plasticity and stability of the cultivar is not an easy task, provided that cross interaction of the genotype with the environment has been established (Table 3). Furthermore, the Principal Component Analysis of the data revealed high effect of random factors, which was about 25% from the total variation of grain yield. In this situation the established correlations between the parameters and regularities of the applied approaches are especially valuable because of their statistical significance. The great number of investigated locations and their specific interaction with the year conditions had such high effect on the grain yield that significant differences between the cultivars on the whole were very difficult to determine.

Conclusions

Under the conditions of Bulgaria the interaction of the cultivar with the environmental conditions by grain yield was complex and non-linear, although the percent of the genotype was only about 12 from the total variation of the experiment.

Any cultivar can have high grain yield and high ecological plasticity regardless of its quality potential.

Best balance of grain yield with its stability was found in cultivars Aglika, Demetra, Iveta (quality group A); Galateya, Slaveya (quality group B), Aneta and Karat (quality group C), and Todora, Kristal and Pryaspa (quality group D).

In the investigated group of cultivars there were cases of compromise combination of grain yield with stability at the highest possible levels. In this respect cultivars Aglika, Demetra, Iveta (quality group A); Galateya, Slaveya (quality group B), Aneta and Karat (quality group C), and Todora, Kristal and Pryaspa (quality group D) most completely meet the criterion of the "ideal" cultivar.

Cultivars Sadovo 1-(13) and Pobeda-(7) accepted and used as standards in Bulgaria had the lowest productivity and were most affected by the growing conditions.

Table 1. Geographic position and soil types of the growing locations

No	Location	Coordinates		Altitude (m)	Soil type
1	Selanovtsi, District Vratsa	N43°40’	E24°01’	168	Carbonate chernozem
2	Pordim, District Pleven	N43°23’	E24°51’	183	Less Haplustoll
3	Brushlen, District Ruse	N43°59’	E26°22’	31	Haplustoll
6	DZI, District Dobrich	N43°43’	E28°10’	250	Haplustoll
8	Burgas, District Burgas	N42°32’	E27°27’	25	Haplustoll Vertisols
9	Radnevo, District Stara Zagora	N42°18’	E25°58’	135	Haplustoll Vertisols
10	Gorski izvor, District Haskovo	N42°01’	E25°25’	178	Haplustoll Vertisols
11	Ognyanovo, District Pazardzhik	N42°09’	E24°22’	206	Alluvial meadow

Figure 1. Graphic representation of grain yield as a result from the direct effect of the factor year

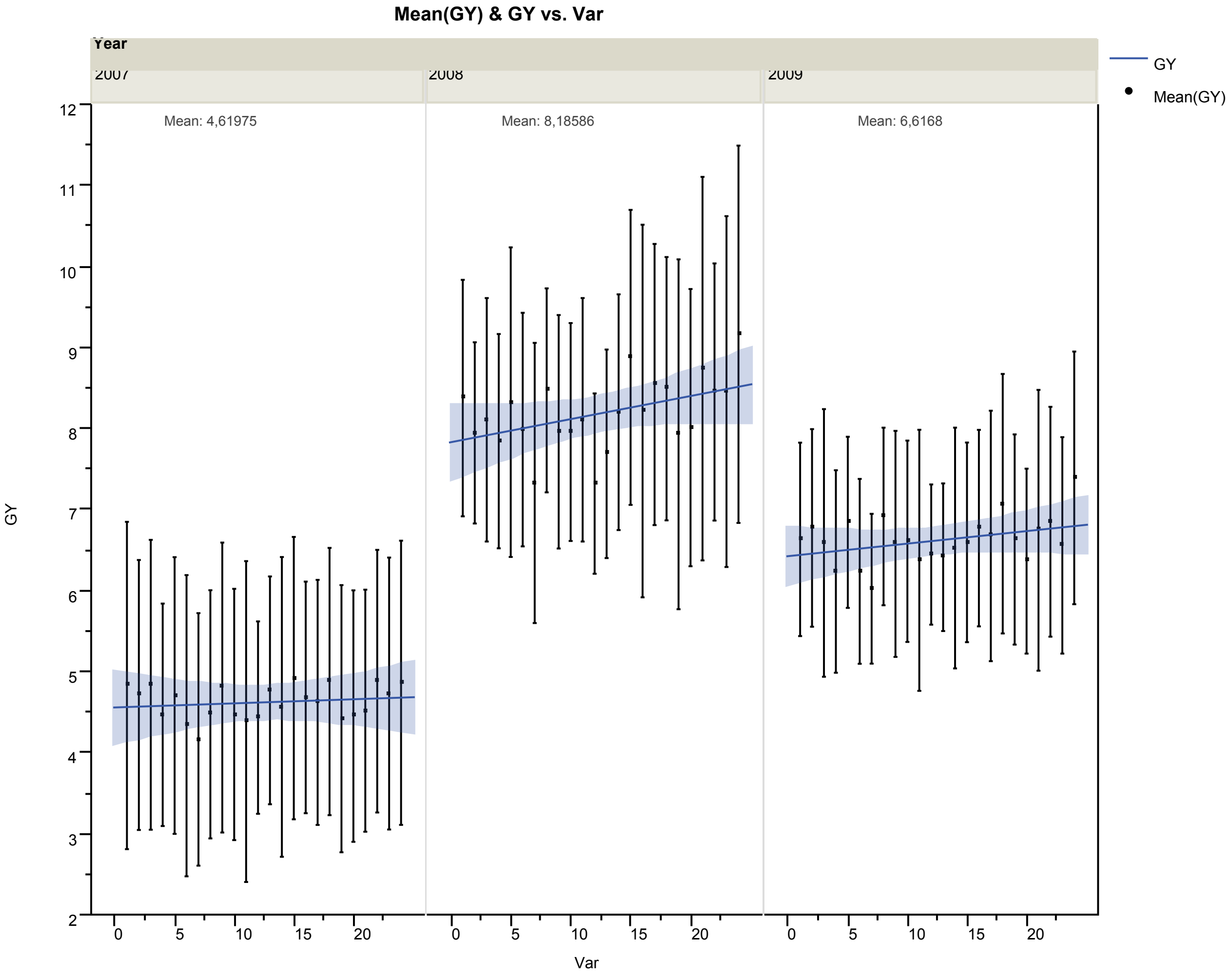


Figure 2. GGE analysis and visualization of grain yield variation according to the location

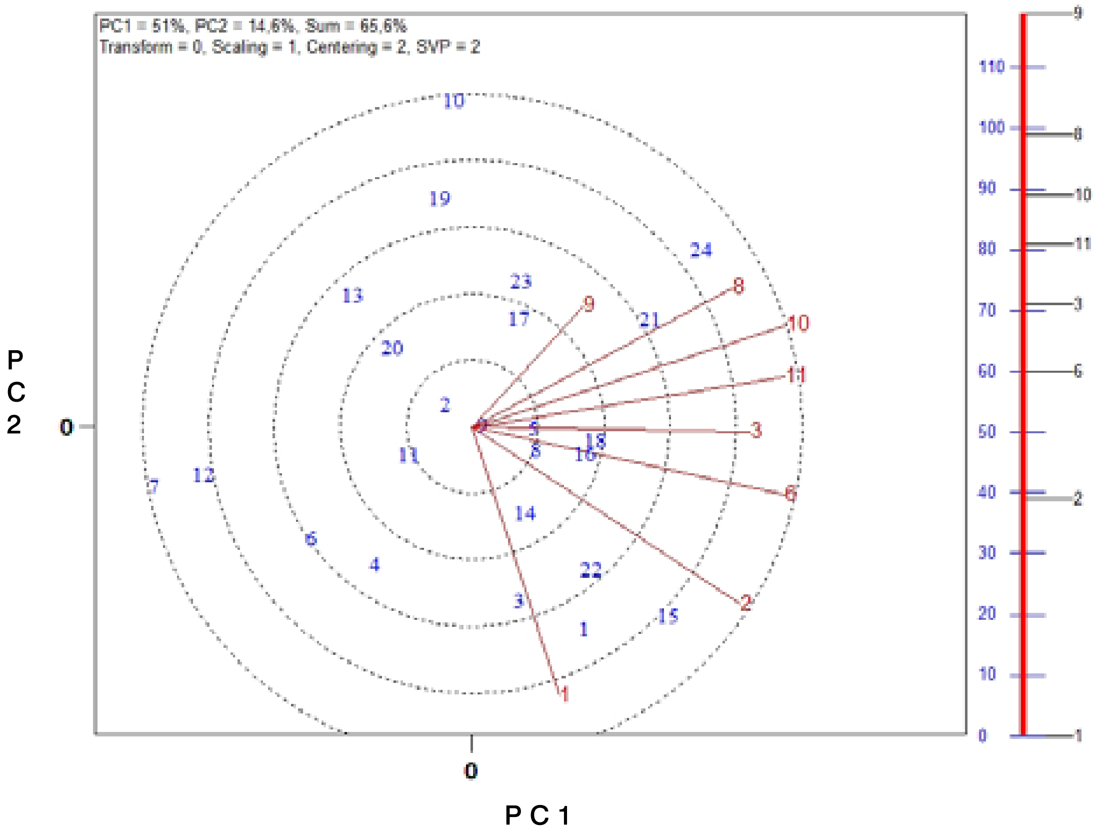


Table 2. ANOVA of the genotype x environment interaction during the three-year period of investigation

Source	**d.f.**	**F**	***p-value***
Genotypes	23	7.93	0.00000
Environments	7	100.23	0.00000
Interaction	192	3.43	0.00120
Heterogeneity	23	1.73	0.00000
Residual	322	0.41	0.00370
Pooled Error	576		

Table 3. Principal Component Analysis (PCA) of grain yield

Components	**F1**	**F2**	**F3**	**F4**
Eigenvalue	1.202	1.020	0.276	0.156
Variability (%)	47.000	16.900	7.157	4.350
Cumulative %	48.400	65.300	72.460	76.800

Figure 3. Principal Component Analysis (F1) of the genotype's contribution (%)

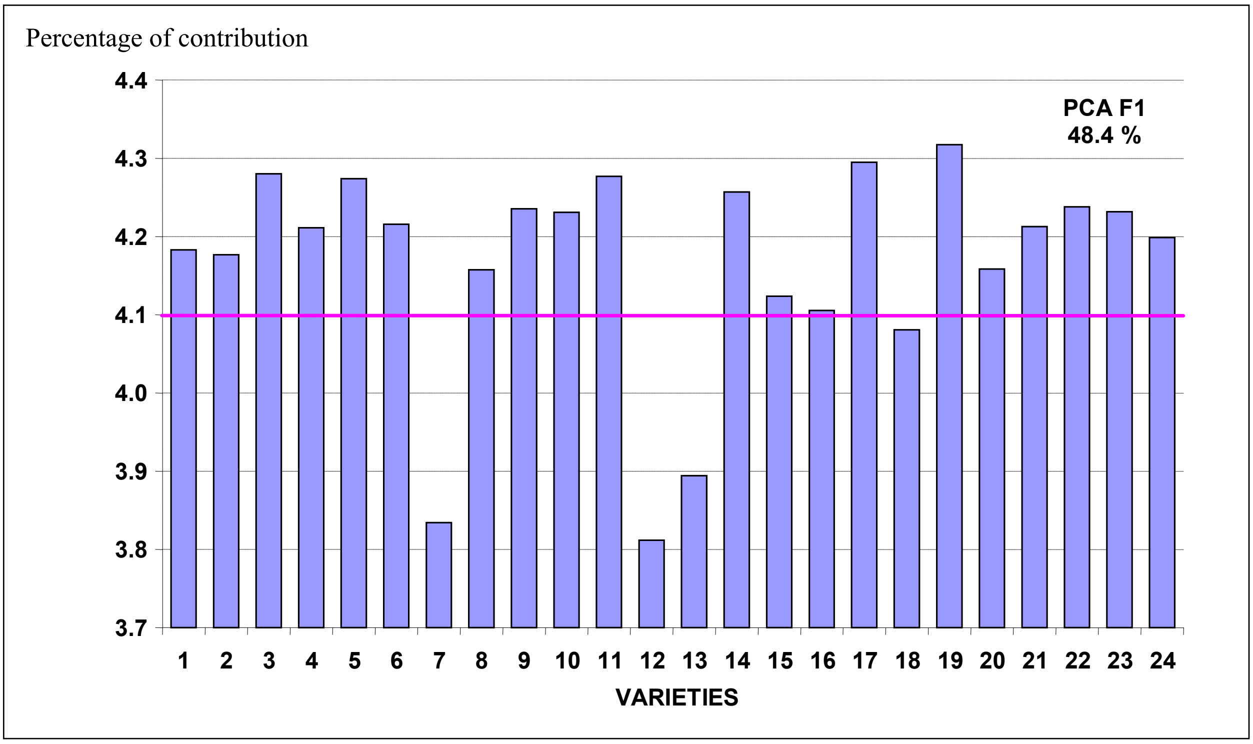


Figure 4. Principal Component Analysis (F2) of the genotype's contribution (%):

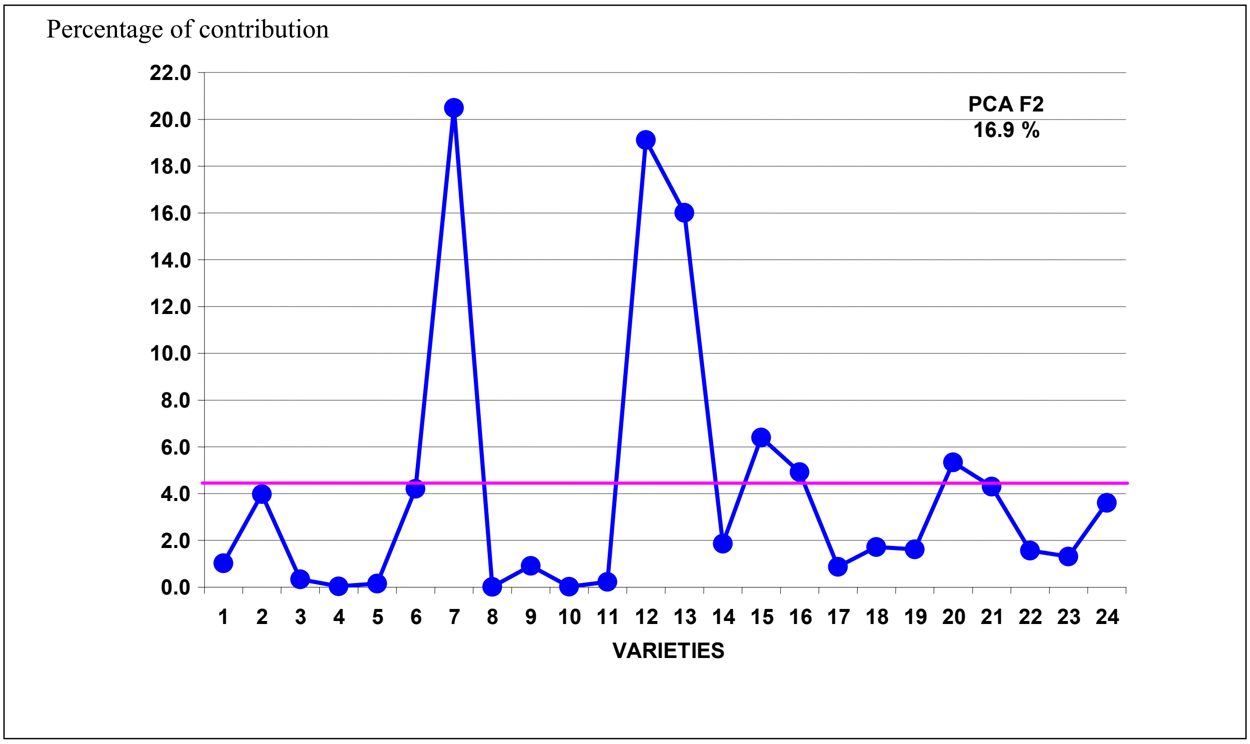


Table 4. Evaluation on the genotype x environment interaction according to the respective mean value of groups **A** and **B**

No	**Variety**	**Group of quality**	**GY, t/ha**	**A** *(Finlay and Wilkinson, 1963)%*			**B** *(Vulchinkov and Vulchinkova (2007)*	
				bi %	**$\sigma 2$ %**	**Residual %**	**GY-bi**	**GY-$\sigma 2$**
1	2	3	4	5	6	7	8	9
1	**Aglika***	A	6.53	106	108	131	100	86
2	Albena	A	6.46	93	131	90	101	105
3	Demetra	A	6,67	102	60	76	103	117
4	Desislava	A	6.23	92	105	83	97	103
5	Iveta	A	6.72	103	50	78	104	117
6	Milena	A	6.19	93	82	119	96	84
7	**Pobeda***	A	5.72	84	100	122	89	71
8	Viara	B	6.65	97	121	106	104	101
9	Galateya	B	6.49	96	101	92	101	105
10	**Enola****	B	6.47	99	57	86	100	107
11	Miryana	B	6.30	101	86	80	97	107
12	**Sadovo1****	B	5.90	76	186	109	94	82
13	Sadovo 772	B	6.25	82	182	121	99	84
14	Slaveya	B	6.51	99	60	88	101	107
15	Aneta	C	6.86	107	46	109	106	105
16	Geya1	C	6.65	105	65	111	103	99
17	Karat	C	6.50	108	78	82	99	110
18	Neven	C	6.61	102	33	114	102	96
19	Petya	C	6.35	102	88	76	98	110
20	**Yantar*****	C	6.25	100	86	97	96	96
21	Kristal	D	6.69	119	188	105	101	103
22	**Pryaspa*****	D	6.68	105	65	90	103	110
23	Svilena	D	6.40	112	168	125	97	86
24	Todora	D	7.05	119	154	111	107	108
	Mean (abs. value)		**6.46**	**1.00**	**0.42**	**2.20**	**4.10**	**4.26**

***Check varieties**:* **- for A group of quality,* ** *- for B group of quality,* *** *- for C group of quality*

Table 5. Evaluation on the interaction genotype x environment according to the respective mean value of statistical groups **C, D** and **E**

No	**Variety**		**C** *(Muir et al. (1992)*		**D** *(Wricke 1962)*	**E** *(Shukla 1972)*
		HV (%)	**IN. Corr (%)**	**GE (%)**	**W^2 (%)**	**SV (%)**
1	2	3	4	5	6	7
3	Demetra	89	89	89	*72*	*71*
19	Petya	86	86	86	*72*	*71*
5	Iveta	50	50	50	*75*	*73*
11	Miryana	100	100	100	*76*	*75*
10	**Enola****	51	51	51	*82*	*81*
17	Karat	66	66	66	*82*	*81*
4	Desislava	142	142	142	*83*	*83*
14	Slaveya	55	55	55	*84*	*83*
22	**Pryaspa****	62	62	62	*87*	*87*
9	Galateya	54	54	54	*88*	*88*
2	Albena	50	50	50	*89*	*89*
20	**Yantar*****	309	309	309	*92*	*92*
8	Viara	170	170	170	102	102
15	Aneta	52	52	52	106	106
16	Geya1	79	79	79	107	107
18	Neven	66	66	66	109	110
6	Milena	71	71	71	116	116
21	Kristal	54	54	54	118	119
23	Svilena	50	50	50	124	125
24	Todora	50	50	50	124	125
1	**Aglika***	239	239	239	126	127
7	**Pobeda***	60	60	60	127	128
13	Sadovo 772	141	141	141	129	130
12	**Sadovo1****	256	256	256	130	131
	Mean (abs. value)	**4.17**	**4.16**	**8.33**	**4.91**	5.61

Table 6. Pearson’s correlation values between the statistical parameters of stability

Variables	**GY**	**bi**	**σ2**	**Residual**	**HV**	**IN. Corr.**	**GE**	**W²**
1	2	3	4	5	6	7	8	9
bi	0.780**							
σ2	-0.236	-0.120						
Residual	-0.152	-0.058	0.412*					
HV	-0.157	-0.073	0.818***	0.433*				
IN. Corr.	-0.222	-0.127	0.434*	0.993***	0.462*			
GE	-0.229	-0.125	0.628**	0.934***	0.716**	0.950***		
W²	-0.208	-0.110	0.612**	0.947***	0.696**	0.956***	0.998***	
SV	-0.207	-0.110	0.611**	0.947***	0.695**	0.956***	0.997***	0.999***

Figure 5. Rank of cultivars based on their mean value and stability of locations

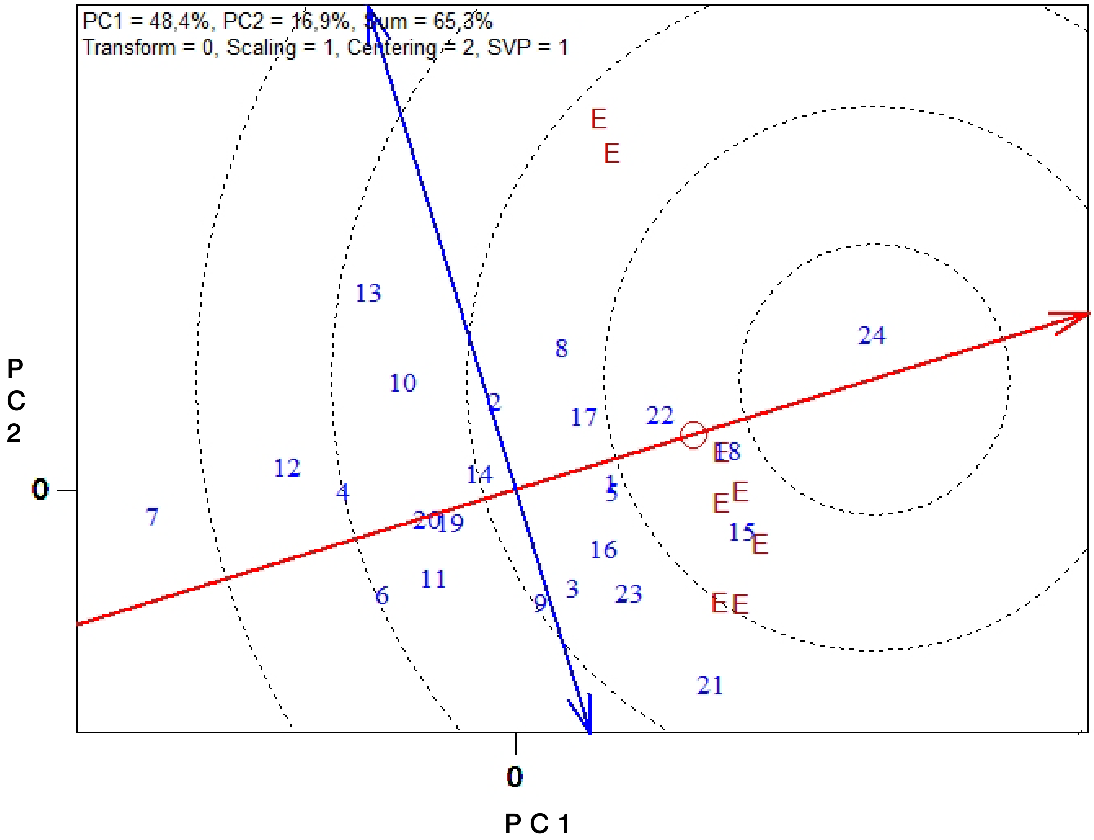


Table 7. Rank of cultivars by grain yield and its stability through the method of F (Kang, 1993)

Number	Variety	GY	GY Rank	Adjustment to R.	F (Y_s)
1	2	3	4	5	6
24	Todora	6.87	24	2	26+
15	Aneta	6.72	23	2	25+
18	Neven	6.64	22	1	23+
22	**Pryaspa***	6.60	21	1	22+
21	Kristal	6.57	20	1	21+
5	Iveta	6.53	19	1	20+
1	**Aglika***	6.53	18	1	19+
16	Geya 1	6.51	17	1	18+
8	Viara	6.49	16	1	17+
3	Demetra	6.43	15	1	16+
17	Karat	6.41	14	1	15+
23	Svilena	6.38	13	1	14+
14	Slaveya	6.36	12	1	13+
2	Albena	6.33	11	-1	10
9	Galateya	6.25	10	-1	9
11	Miryana	6.23	9	-1	8
10	**Enola***	6.23	8	-1	7
19	Petya	6.19	7	-1	6
13	Sadovo 772	6.17	6	-1	5
4	Desislava	6.10	5	-1	4
20	Yantar*	6.10	4	-1	3
6	Milena	6.02	3	-1	2
12	**Sadovo 1***	5.90	2	-2	0
7	**Pobeda***	5.67	1	-2	-1
	Overall mean	6.34			12.6
	LSD (p=0.05)	0.34			

Figure 6. Which cultivar performs best at which location?

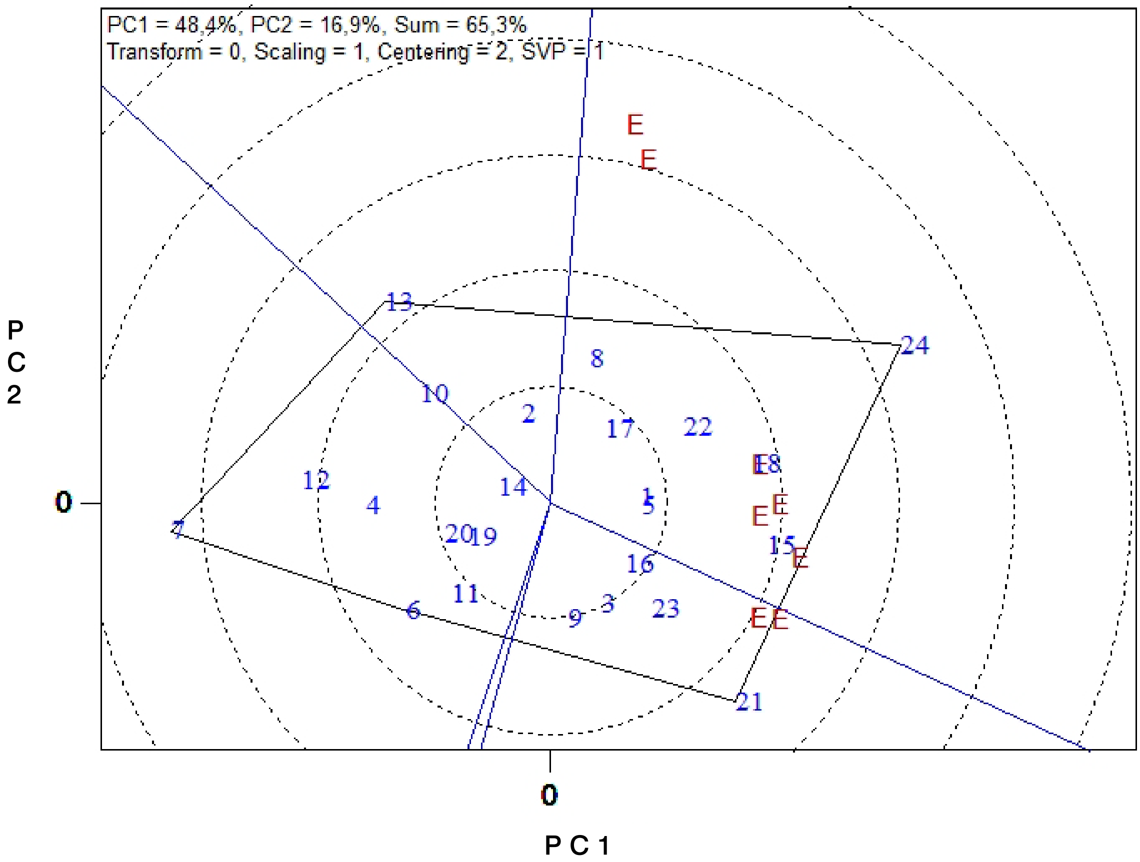


Figure 7. Index of the general adaptability of the cultivar (GY-bi), according to Vulchinkov and Vulchinkova, (2007)

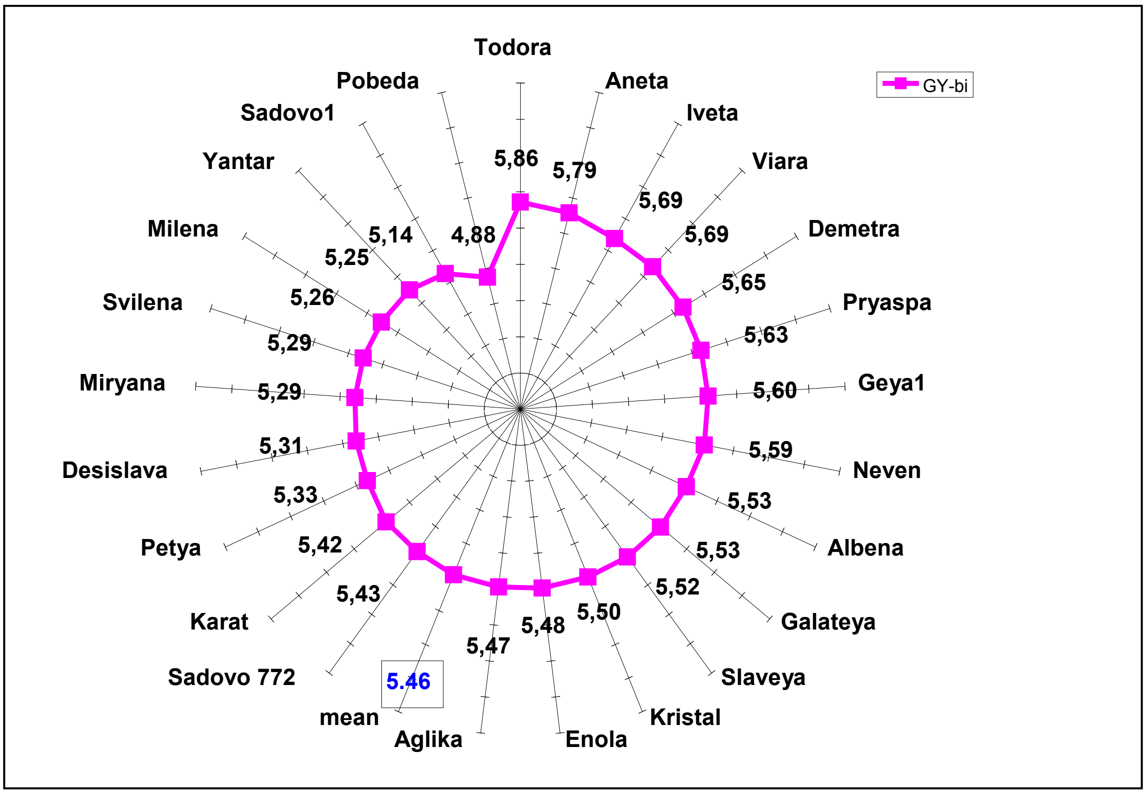


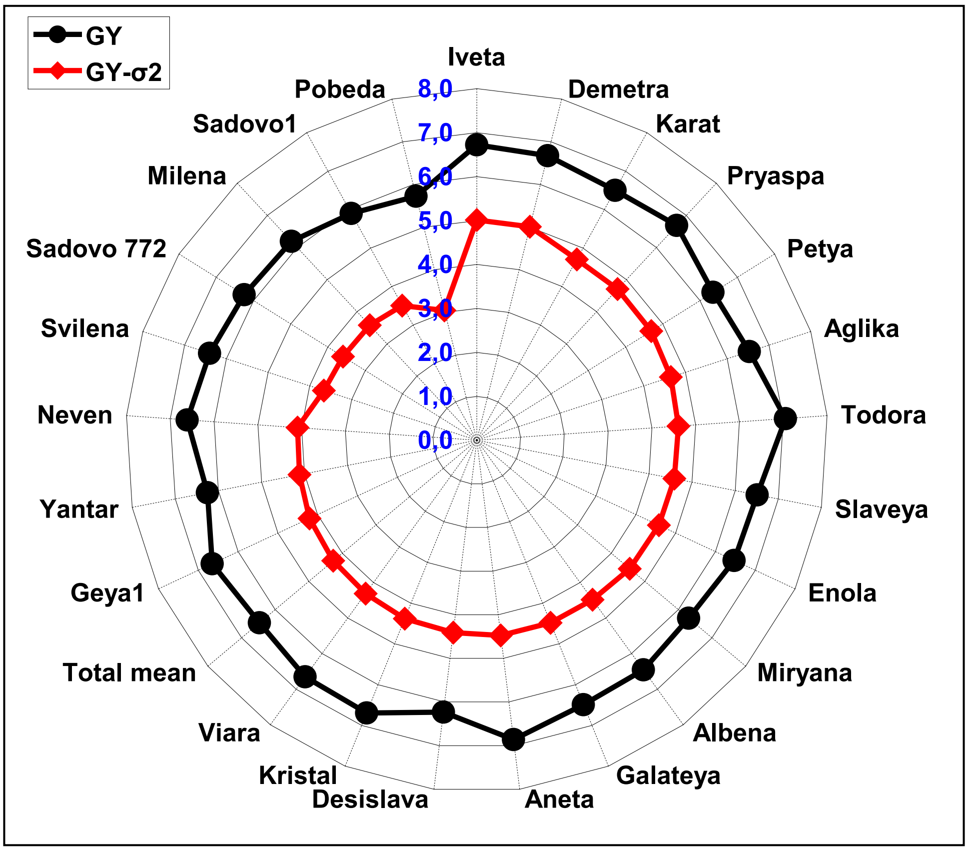
Table 8. Pearson's correlation matrix at the most important parameters of resistance and adaptability of grain yield

Variables	GY			GY-bi			GY-σ^2		
	r	p-value	R^2	r	p-value	R^2	r	p-value	R^2
GY-bi	0.956***	0.0000	0.915***						
GY-σ^2	0.681**	0.0001	0.763**	0.672**	0.0000	0.651**			
Y_S	0.914***	0.0000	0.835***	0.844**	0.0000	0.713**	0.579**	0.0080	0.629**

Table 9. Kendall -Tau rank correlations of the stability indices with adaptability

Variables	Y_S	p-value	GY	p-value
GY	0.708**	0.0000		
GY-b	0.376	0.0173	0.467**	0.0011
GY-σ^2	0.129	0.4273	0.684**	0.0082

Figure 8. Index of general stability [GY- σ^2] of each investigated cultivar



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Assessment of Drought Resistance in Indian Wheat Cultivars for Morpho-Physiological Traits

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ABSTRACT

A set of diverse 28 wheat genotypes was evaluated for drought stress related traits, under irrigated and drought stress conditions for two years. The genotypes differed significantly for all the traits under drought stress environment, while under irrigated conditions nonsignificant differences were observed for triphenyltetrazolium chloride test, cell membrane stability, relative water content and osmotic potential. The genotypes NW 1014 and WH 1127 appeared to be drought tolerant, while C 306, HW 2004, Lok 1, NIAW 34, PBW 175, WH 1098, WH 1126, WH 1142, WH 1181 and WH 1182 indicated a combination of drought tolerance, avoidance and escape mechanisms, the genotypes HD 2858, PBW 343, WH 283 and WH 711 had tendency of escape, but susceptible and the remaining genotypes were susceptible. Correlation coefficient analysis indicated that the genotypes having better mitochondrial survival ability, membrane stability and water relation parameters under drought stress also had higher values for grain yield, drought susceptibility index (DSI) and drought response index (DRI). DRI appeared to be the most important among all the traits, because the genotypes having high DRI values also had high grain yield under drought stress conditions and high values for drought related traits.

Keywords: bread wheat, water relation parameters, cell membrane stability, drought susceptibility index, drought response index

Introduction

Drought stress is one of the major limiting factors of wheat productivity worldwide (Moayedi et al. 2011). Several drought tolerance screening methods have been developed, but their efficiency for incorporation of drought tolerance is restricted due to low heritability and a high magnitude of genotype x environmental interactions. Thus, progress for infusing drought tolerance may be achieved by using physiological characters in complement with conventional breeding for grain yield under drought stress. Cell membrane stability (CMS) measured by conductivity test and mitochondrial cell viability as measured by the reduction of tetrazoliumtriphenyl chloride test (TTC) received a considerable attention for measuring drought tolerance in wheat genotypes. Membrane disruption may result in crowding of cellular components which may be due to decrease in cellular volume resulting in protein denaturation and viscosity by increasing the permeability in plasma membranes (Kocheva et al. 2014).

Among water relation parameters relative water content was regarded as one of the reliable criteria for assessment of plant water status in mid 1980s (Arjenaki et al. 2012). Also, relative water content is related to cell volume and can reliably indicate the relation between the water absorbed by plant and the water consumed through transpiration. The use of RWC was considered for determination of water status in plant leaves to their fully turgid condition in various crops (Ibrahim et al. 2014). In addition to the maintenance of water content, solute potential (accumulation of various compatible solutes) is an important component of drought tolerance. These include amino acids (e.g., proline), sugars (e.g., fructon, sucrose), inorganic ions (e.g., potassium), organic ions (e.g., malate), ammonium compounds (e.g., glycine betaine) and polyols (e.g., manitol). These solutes help in protection of structure of membranes, proteins, oxidative damage and higher structural stabilization under drought stress and also help to maintain various metabolic and physiological functions. These may also contribute to drought avoidance with increased root growth and soil water extraction under drought stress (Boussadia et al. 2013).

Chlorophyll fluorescence also indicates drought resistance of the genotypes through carbon reduction cycle. Through the use of ATP and NADPH, metabolism of carbon influences the proton gradient, electron acceptor of PSII, and finally fluorescence yield under drought stress. Chlorophyll quenching analysis is a non-invasive and reliable method to determine the function of PS II (Batra et al. 2014). This study was planned to determine the role of traits viz., triphenyltetrazolium chloride, cell membrane stability, relative water content and osmotic potential, DSI and DRI under drought stress and their complementation with grain yield for improvement of drought tolerance.

Materials and methods

Twenty eight genotypes of wheat (*Triticum aestivum* L.) differing in their mean performance under drought stress (Table 3) were grown under normal and drought stress environments during the years 2009-2010 and 2011-2012 under field conditions at the CCS Haryana Agricultural University, Hisar, Haryana, India. The experiments were conducted in a randomized complete block design with three replications for both environments (irrigated and drought) with a plot size of two rows of 3m length and a spacing of 23 × 10cm. Both experiments were sown during the first week of November in each year. In Irrigated experiment five irrigations were provided including pre-sowing irrigation, while under drought stress conditions only pre sowing irrigation was provided in each year. Data for days to 50% heading, grain yield per plant, relative water content, osmotic potential, chlorophyll fluorescence, triphenyltetrazolium chloride and cell membrane stability were recorded at anthesis. The average of five competitive plants selected randomly from each genotype per replication.

Relative water content: The relative water content (RWC) was calculated according to Turner (1981) and evaluated from the equation: RWC (%) = [{(FW – DW)/(TW – DW)}] ×100, where FW is the fresh weight of the leaves, TW is the weight at full turgor and DW is the dry weight of leaves.

Cell membrane stability: CMS was measured by the method given by Blum and Ebercon (1981) for wheat. The leaf membrane stability (CMS) was determined from the following equation: CMS (%) = 1− (T_1/T_2) × 100, where T_1 is the initial conductance and T_2 is final conductance value.

Chlorophyll fluorescence: Chlorophyll fluorescence measurements (F_v/F_m) were taken about 4 cm from the base of abaxial surface of flag leaves by using a portable handy Plant Efficiency Analyser, PEA (Hansatech, UK) at 15 days after anthesis. The florescence signals were detected as F_v/F_m. The data were analyzed using software biolyser 4.0 programme (R. Maldonado Rodriguez, Bioenergetics laboratory at the University of Geneva, Switzerland).

TTC reduction assay: Cell viability was assayed by the conversion of 2, 3, 5 triphenyltetrazolium chloride (TTC) into red formazan by dehydrogenase activity of viable cells. The level of mitochondrial viability was determined by measuring the percentage reduction of TTC to formazan using the following formula: TTC (%) = ODh / ODc × 100, Where ODh and ODc represent the optical density measured spectrophotometrically at 485 nm for second and first set respectively.

Leaf osmotic potential: Leaf osmotic potential measurements were made for samples in drought stress by the method of Blum (1988). Turgid leaf samples were frozen in liquid nitrogen samples were thawed and cell sap was pressed from leaves, which was subsequently analysed for osmolarity (C) (mmol.kg^{-1}) using a model 5520 Vapor Pressure Osmometer. Osmolarity of cell sap was converted from mmol/kg to osmotic potential (MPa) using the formula MPa= – C x 2.58 x 10^{-3}.

Drought susceptibility index (DSI): The drought susceptibility index of individual genotype was calculated by the method suggested by Fischer and Maurer (1978) with the following formula: DSI = (1−Ysi/Ypi)/D, D= (1−Ys/Yp): where, Ys and Ysi are the mean and individual grain yields of genotypes, respectively, under a drought stress environment; Yp and Ypi are the mean and individual grain yields of genotypes, respectively, under a normal irrigated environment.

Drought response index (DRI): Drought tolerance for an individual genotype was computed using the formula given by Bidinger et al .(1987) as DRI =(Ya −Yest)/SES, where Yest and Ya are the yields estimated by regression and actual yields under stress for the cultivars, respectively, and SES is the standard error of the multiple regression.

Total precipitation during the season: The weekly data of precipitation during the season were obtained from the observatory, Department of Meterological Science, CCSHAU, Hisar, India. The data indicated that there was no precipitation after sowing in the month of November and December in both years. During the months of January to February there were approx. 19 mm rains in 2009-10 and 14.5 mm in 2011-12, and there were negligible rains up to second week of April and drought stress was operative. These periods coincide late jointing to boot stage of plant growth under both irrigated and rain fed environments. Drought stress experiments faced drought stress during the month of Feb onward in both years as the rains were not adequate to irrigate experiments. In addition, due to negligible rains during the months of March and April in both years severe drought stress occurred particularly during anthesis and dough stages of plant growth. The data for soil moisture content were collected gravimetrically on the depth of 0-15 cm, 15-30 cm and 30-45 cm depth at anthesis and maturity stages of plant growth throught the length of the experiment in each replication during both years. Soils of Hisar are clay loam and majority of root growth takes place between 0-15 cm depth of soil. The mean values of soil moisture content over the replications and over the years are presented in Table 1.

Results

Variability and mean performance

Significant differences among the genotypes over the environments and over the years and environments for majority of the traits revealed that the genotypes behaved differently in different environments/years (Table 2). Therefore in order to get more consistent results such types of experiments require repetition over the years and /or environments. Non-significant differences among the genotypes for triphenyltetrazolium chloride, cell membrane stability, relative water content and osmotic potential under irrigated conditions may probably due to poor expression of drought adaptive mechanisms in absence of drought stress. The data of these traits generated under irrigated conditions were excluded from further analysis. The mean performance of genotypes for grain yield under irrigated conditions was significantly higher (9.77±1.18 g) than that under drought stress (5.27±0.73 g) conditions indicating considerable impact of drought stress (Table 3). The genotypes HD 2009 (11.23*), HW 2004 (11.55*), WH 1098 (12.79*) WH 1142 (11.45*), WH 1181 (11.85*) and WH 1182 (11.45*) had significantly higher grain yield than their mean value (9.77±1.18) under irrigated conditions. These genotypes were also significantly higher yielder than their overall mean under drought stress conditions indicating that these genotypes may prove better under both environments. Majority of these genotypes also performed significantly better for drought related traits, namely, triphenyltetrazolium chloride test, cell membrane stability, relative water content and osmotic potential, drought susceptibility index and drought response index.

In addition, the genotypes C 306 (6.43*), NIAW 34 (6.45*) and PBW 175 (8.50*) had significant higher grain yield over the mean (5.27±0.73) only under drought stress conditions indicating their superiority only under drought stress. Mean days to heading was significantly early under drought stress (84.38±2.40 days) than under irrigated condition (101.25±2.41 days). The genotypes HD 2858, Lok 1, PBW 175, PBW 343 and WH 711 earlier heading under both environments, while the genotypes NIAW 34 (77.35*), HW 2004 (79.35*), WH 1098 (78.50*) and WH 1182 (77.25*) escaped drought by accelerating their life cycle only under drought stress conditions. This indicated a role of developmental plasticity for days to heading in these genotypes for adaptation under drought stress conditions. But the genotypes C 306, WH 1181 and WH 1127 were very less influenced by drought stress for days to heading. Therefore, it may be assumed that early heading might have a little role in significantly higher grain yields of these genotypes under drought stress. The drought indicator traits, namely, DSI and DRI were significant for the genotypes, namely, C 306, WH 1142, HW 2004, PBW 175, WH 1181, WH 1098 and NIAW 34. This revealed that DSI and DRI were equally effective in predicting grain yields of these genotypes under drought stress, while for WH 1127 only DRI was effective (DRI= 0.75*).

Correlations: Genotypes having higher grain yield under irrigated conditions also had higher grain yield under drought stress conditions ($r = 0.51^{**}$). (Table 4) indicating a complementation of high yield potential with drought resistance potential, but nonsignificant correlations of drought related traits with grain yield under irrigated conditions may be due to lack of drought hardening in absence of drought stress. Under drought stress conditions significant associations of grain yield with TTC ($r = 0.43^{*}$), CMS ($r = 0.45^{*}$), RWC ($r = 0.48^{**}$), OP ($r = 0.41^{*}$), days to heading ($r = -0.41^{*}$), DSI ($r = -0.69^{**}$) and DRI ($r = 0.82^{**}$) indicated that high grain yield was contributed by drought tolerance related traits. DRI appeared to be the most important trait as the genotypes with high score of DRI also had high values for grain yield ($r = 0.82^{**}$), TTC ($r = 0.56^{**}$), CMS ($r = 0.41^{*}$), RWC ($r = 0.65^{**}$), OP ($r = 0.38^{*}$) and low score of DSI ($r = -0.90^{**}$).

Significant correlations of TTC with CMS ($r= 0.38^{*}$), RWC ($r= 0.85^{**}$), OP ($r= 0.63^{**}$), and DRI ($r=0.56^{**}$) indicated that the genotype which had high percentage of cell viability under drought stress also had high values of membrane stability maintaining high solute potential and were better in terms of resistance parameters under drought stress. There was significant association of DSI and DRI ($r = - 0.90^{**}$). DSI is an effect of all the traits contributing towards grain yield under drought stress (Fischer and Maurer, 1978), DRI can be made free from the influence of the characters relating to escape, avoidance and high yield potential through multiple regression technique (Arraudeau, 1989) as in case of present set of material.

Disscussion

Variability and mean performance

Drought stress causes disruption of water, ion and organic solute movement across the plant membranes, which affects the process of photosynthesis and transpiration and decrease the capacity of plasma-lemma to retain the solute which may be due to increased porosity and loosening of plasma membranes. Another reason for membrane disruption may be due to drought-induced denaturation of enzymes related membranes, which are responsible for maintaining chemical gradients in the cell under heat stress. (Antelmo et al. 2010). The genotypes PBW 175, WH 1181, PBW 644, UP 2425, NW 1014, Raj 3765 and WH 1098 performed were better not only for membrane stability, but also for other drought stress related traits including osmotic potential which may due to for accumulation of solutes in cells namely, sugars, sugar alcohols, amino acids, glycebetaines and protein etc. Majority of the solutes in bread wheat genotypes were K^{+} in early stages of drought stress and molecules including glycinebetaine, glucose and proline in later stages of plant growth which were involved helping maintenance of turgor and relative water conduct in leaves under drought stress (Arjenaki et al. 2012).

Resilience to phenological development also helps in adaptation to drought stress through accelerated heading and maturity. The genotypes WH 1182, WH 1142, HW 2004, NIAW 34, and WH 1098 indicated escape mechanism by completing their heading significantly early under drought stress as compared to irrigated conditions, while higher grain yields of the genotypes WH 1181, WH 1126 and WH 1127 were comparatively more influenced by drought avoidance/tolerance mechanisms as indicated by higher values of DSI and DRI for these genotypes with stable days to heading. Involvement of these genotypes in crossing programme with that of high yielding types may provide desirable segregants under drought stress conditions.

Correlations

The genotypes with increased values of mitochondrial viability, cell membrane stability and water relation parameters, namely, RWC and OP also had high grain yield and favorable values of DRI and DSI indicating the contribution of these traits towards grain yield under drought stress conditions. This indicated that selection for CMS and or TTC under drought stress may give desirable results (Dhanda and Munjal, 2012). Grain yield is controlled by polygenes, complex inheritance, low heritability and is influenced by complex environmental interactions. Therefore, under drought stress conditions high grain yield potential should be complemented with a specific drought related trait which may buffer against severe reduction in the grain yield. Water status performs several functions in plants under drought stress. It regulates several biological reactions and maintains fliud medium which controls macromolucular structure required in operation of drought adaptive mechanisms. Recently intracellular $Ca^{2}+$ has been found to be involved in operation plant responses to drought stress and also participate in signal transduction process of plants which play an important role in accumulation of compatible solute under drought stress. (Nurit et al. 2012). Significant correlation of DSI and DRI and with grain yield and water relation traits suggested the combination of drought escape/avoidance traits with drought tolerance operating for high grain yield under drought stress.

Conclusions

A significant impact of drought stress was observed as the grain yield was reduced about 50% under drought stress conditions. All the traits under study showed significant variation particularly under drought stress conditions. Drought Response Index appeared to be the most important among all the traits studied, because the genotypes having high values for drought response index also had high grain yield under drought stress conditions. The genotypes NW 1014, and WH 1127 appeared to have tendency of drought tolerant, while C 306, HW 2004, Lok 1, NIAW 34, PBW 175, WH 1098, WH 1126, WH 1142, WH 1181 and WH 1182 indicated a combination of drought tolerance, avoidance and escape mechanisms, HD 2858, PBW 343, WH 283 and WH 711 tendency of escape, but susceptible and the remaining genotypes were susceptible. Accordingly these genotypes may be used in breeding programme as per requirement of the area of cultivation.

Table 1. Mean values for soil moisture content (%) at heading and maturity stages plant growth during the years 2009-10 and 2011-12

Soil sampling distance (m)	**Depth of soil (cm) at anthesis stage**			**Depth of soil (cm) at Maturity stage**		
	0-15	15-30	30-45	0-15	15-30	30-45
0-3	15.4	19.6	22.6	13.5	18.3	21.2
6-9	14.2	20.3	23.5	12.3	17.3	20.7
9-12	13.6	18.1	21.1	11.6	16.4	19.6
Mean	14.4	19.3	22.4	12.5	17.3	20.5

Table 2. Mean sum of squares of wheat genotypes over two years (2009-2010 and 2011-2012) and environments

Source of variation	**Degree of freedom**	**Character**		**Source of variation**	**Degree of freedom**	**Character**				
		Grain yield	**Days to heading**			**TTC**	**CMS**	**CHFL**	**RWC**	**OP**
Rep/year/env	8	3.17	0.22	Rep/year	4	166.60**	13.82	0.001	5.6	1.8**
Env (E)	1	2,579.50**	14009.10**	-	-		-	-	-	-
Year (Y)	1	723.81**	57.50**	Year (Y)	1	62.20**	826.87**	0.01**	11.3	0.9*
E xY	1	80.01**	81.03**	-	-	-	-	-	-	-
Genotype (G)	27	46.00**	62.9**	Genotype (G)	27	311.07**	195.10**	0.020**	121.5**	1.5**
GxE	27	29.11**	132.01**	-	-			-	-	-
GxY	27	1.299	15.11**	G x Y	27	205.05**	212.69**	0.001	78.5**	0.7*
GxExY	27	1.142	26.61**	-	-		-	-	-	
Error	216	5.201	5.069	Error	108	26.02	28.50	0.002	11.4	0.3
Total	335	-	-	Total	167	-	-	-	-	-

*, ** : Significant at 5% and 1% level of significance, respectively; Degree of freedom (DF), Grain yield (GY), Days to heading (DH), TriphenylTetrazolium Chloride (TTC) Cell membrane Stability(CMS), Chlorophyll Fluorescence (Fv/Fm), Relative Water Content (RWC) and Osmotic Potential (OP)

Table 3. Mean performance of wheat genotypes over two years (2009-2010 and 2011-2012) for various characters under irrigated (Irr) and drought stress (Dr) conditions

Genotype	Grain yield		TTC	CMS	Chfl	RWC	OP	Days to heading		DSI	DRI
	Irr	Dr	Dr	Dr	Dr	Dr	Dr	Irr	Dr		
C 306	8.12	6.43*	55.16*	55.46	0.75*	87.90*	-1.14*	97.02*	80.27	0.43*	1.36*
DBW 16	9.58	5.45	54.23*	49.58	0.70	87.90*	-2.88	100.94	81.69	0.88	0.13
DBW 17	16.23*	5.85	48.15	55.12	0.75*	75.80	-2.85	103.88	86.87	1.31	-1.01
HD 2009	11.23*	6.15*	45.18	62.85*	0.65	73.40	-3.00	96.04*	76.01	0.92	0.00
HD 2687	6.30	3.20	42.21	58.39*	0.67	70.20	-2.87	104.86	95.50	1.01	-0.35
HD 2858	9.56	5.46	46.35	52.63	0.73*	74.50	-2.45	95.06*	75.25*	0.88	-0.29
HW 2004	11.55*	6.90*	43.28	56.23	0.74*	87.50*	-1.19	98.98	79.35*	0.82*	0.81*
Lok 1	7.55	4.56	46.25	53.59	0.63	73.20	-2.75	93.10*	75.01*	0.81*	-0.64
NIAW 34	9.85	6.45*	48.15	57.64*	0.72	73.50	-2.24	98.00	77.35*	0.71*	0.76*
NW 1014	8.75	4.78	53.26*	55.16	0.73*	85.70*	-1.29	115.64	98.15	0.93	0.71*
PBW 175	9.85	8.50*	55.62*	67.29*	0.78*	89.50*	-0.56*	93.10*	77.01*	0.28*	2.74*
PBW 343	8.60	3.15	48.12	45.26	0.67	65.50	-2.32	95.45*	74.65*	1.29	-2.34
PBW 373	5.65	3.14	43.15	40.56	0.66	70.40	-2.89	112.70	94.95	0.91	-0.31
PBW 644	14.12*	5.85	52.63*	59.48*	0.68	84.20*	-2.03	106.16	92.90	1.20	-0.03
PBW550	8.45	3.48	43.26	54.65	0.61	70.10	-2.46	105.84	95.40	1.20	-0.68
Raj 3765	6.84	3.11	58.26*	59.36*	0.69	88.26*	-0.45*	98.00	83.35	1.11	-1.37
UP 2425	5.96	3.75	53.26*	62.18*	0.70	86.40*	-1.63	112.70	96.05	0.76	0.37
UP 2565	11.53*	3.05	48.56	55.21	0.61	75.60	-1.15*	107.80	88.85	1.50	-2.35
WH 1081	6.50	3.70	45.58	51.38	0.73*	81.50*	-1.52	87.22*	87.10	0.88	-0.45
WH 1098	12.79*	7.98*	59.26*	69.15*	0.73*	91.60*	-0.41*	102.90	78.50*	0.77*	1.51*
WH 1126	9.12	5.45	55.16*	51.69	0.65	88.60*	-2.39	102.90	86.98	0.82*	0.61*
WH 1127	10.25	6.13*	45.62	49.52	0.74*	71.80	-1.26*	102.90	85.05	0.82	0.75*
WH 1142	11.45*	6.98*	51.45*	47.15	0.77*	83.60*	-1.3*	101.04	81.35*	0.80*	1.07*
WH 1181	11.85*	9.30*	59.57*	61.53*	0.77*	91.60*	-0.26*	98.98	85.16	0.44*	3.50*
WH 1182	12.16*	7.26*	45.55	61.25*	0.66	73.50	-1.1*	99.30	77.25*	0.82*	0.90*
WH 283	9.23	4.26	43.18	61.85*	0.98*	69.20	-2.75	96.04*	75.35*	1.10	-1.37
WH 542	7.30	3.25	51.25*	55.48	0.66	85.40*	-2.25	100.94	93.70	1.13	-0.65
WH 711	15.23*	3.85	38.15	50.29	0.74*	65.30	-2.15	97.02*	76.90*	1.53	-3.37
Mean	**9.77**	**5.27**	**48.64**	**55.04**	**0.70**	**76.20**	**-1.84**	**101.25**	**84.38**	**0.98**	**0.00**
CD (5%)	**1.18**	**0.73**	**2.47**	**2.26**	**0.02**	**3.20**	**0.31**	**2.41**	**2.40**	**0.12**	**0.53**

*, ** : Significant at 5% and 1% level of significance, respectively; Degree of freedom (DF), Grain yield (GY), Days to heading (DH), TriphenylTetrazolium Chloride (TTC) Cell Membrane Stability (CMS), Chlorophyll Fluorescence (Fv/Fm), Relative Water Content (RWC) and Osmotic Potential (OP), Irr: Irrigated, Dr: Drought stress

Table 4. Correlation coefficients of among characters in bread wheat under irrigated (Irr) and drought stress (Dr) conditions

Character	Env	Grain yield		TTC	CMS	Chfl	RWC	OP	Days to heading		DSI	DRI
		Irr	Dr	Dr	Dr	Dr	Dr	Dr	Irr	Dr	DSI	DRI
Grain yield	Irr	1.00	0.51^{**}	-0.02	0.20	0.18	-0.02	0.14	-0.05	-0.31	0.25	-0.01
	Dr	0.51	1.00	0.43^{*}	0.45^{*}	0.33	0.48^{**}	0.41^{*}	-0.22	-0.41^{*}	-0.69^{**}	0.82^{**}
TTC	Dr	-0.02	0.43	1.00	0.38^{*}	0.04	0.85^{**}	0.63^{**}	0.10	0.07	-0.47^{*}	0.56^{**}
CMS	Dr	0.20	0.45	0.38	1.00	0.23	0.40^{*}	0.44^{*}	-0.09	-0.13	-0.32	0.41^{*}
Chfl	Dr	0.18	0.33	0.04	0.23	1.00	0.09	0.12	-0.26	-0.34	-0.24	0.19
RWC	Dr	-0.02	0.48	0.85	0.40	0.09	1.00	0.56^{**}	0.08	0.16	-0.55^{**}	0.65^{**}
OP	Dr	0.14	0.41	0.63	0.44	0.12	0.56	1.00	-0.21	-0.13	-0.31	0.38^{*}
Days to heading	Irr	-0.05	-0.22	0.10	-0.09	-0.26	0.08	-0.21	1.00	0.76^{**}	0.20	0.01
	Dr	-0.31	-0.41	0.07	-0.13	-0.34	0.16	-0.13	0.76	1.00	0.19	0.00
Drought susceptibility index		0.25	-0.69	-0.47	-0.32	-0.24	-0.55	-0.31	0.20	0.19	1.00	-0.90^{**}
Drought response index		-0.01	0.82	0.56	0.41	0.19	0.65	0.38	0.01	0.00	-0.90	1.00

*, ** : Significant at 5% and 1% level of significance, respectively; Degree of freedom (DF), Grain yield (GY), Days to heading (DH), TriphenylTetrazolium Chloride (TTC) Cell Membrane Stability (CMS), Chlorophyll Fluorescence (Fv/Fm), Relative Water Content (RWC) and Osmotic Potential (OP), Irr: Irrigated, Dr: Drought stress

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The Effect of Supplementary Irrigation on Leaf Area, Specific Leaf Weight, Grain Yield and Water Use Efficiency in Durum Wheat (*Triticum durum* Desf.) Cultivars

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ABSTRACT

The main aim of the present study is to understand the impact of irrigation on Leaf area, Specific Leaf Weight, Grain yield and Water use efficiency. Ten durum wheat (*Triticum durum* Desf.) genotypes of diverse origin were evaluated under two conditions (Irrigated and non irrigated conditions). After flowering, ten flag leaf of each plot were cut for measuring the length, width and mass of leaf. Leaf area (LA) while Specific Leaf Weight (SLW) was measured mathematically . Water use efficiency of yield was calculated using the following equation: WUE_{yield} = Grain Yield / evapo-transpired water. In this study the difference between Leaf area under stressed and non stressed condition equal 17.24%. In addition, water stress reduced the specific leaf weight (41.86%). Irrigation condition affects positively the grain yield (12.42%) and negatively water use efficiency (12.11%). WUE_{yield} of wheat under Mediterranean conditions was the highest with a deficit irrigation consisting of two-thirds of the water required at full irrigation (i.e. WUE_{yield} at full irrigation was lower).

Keywords: durum wheat, leaf area, specific leaf weight, WUE, irrigation.

Introduction

Durum wheat (*Triticum durum* Desf.) is widely grown in stressful environments. Under stressed conditions, the maintenance of high leaf net CO_2-exchange rates and higher water use efficiency was associated with higher wheat yields (Austin, 1987). Leaf area (LA) plays an important role in plant growth analysis. Leaf area and leaf weight measurements are required to calculate several growth indices, which are leaf area index (LAI), net assimilation rate (NAR), specific leaf area (SLA), specific leaf weight (SLW), and leaf area duration (LAD) (Gardner *et al*. 1985). The leaves, being the site of photosynthetic activity, appear to have an obvious relation to the plant's grain yield ability (Sharma *et al*. 2003). Flag leaf makes a major contribution towards the grain weight (41-43%) and is the major photosynthetic site during the grain filling period (Ibrahim and Elenein, 1977). Flag leaf area is an indicator of potential grain yield in wheat and since the flag leaf plays a predominant role, its size is likely to be important (Monyo and Whittington, 1973). Photosynthesis is the primary source of dry matter production and grain yield in crops. The improvements of leaf photosynthesis have occurred with the advance of high-yielding cultivars breeding (Jiang *et al*. 2002). The flag leaf is considered to be a primary source of assimilates for grain filling and grain yield due to its short distance to the pike and the fact that it stays green for longer than the rest of the leaves. Positive correlations have been found between flag leaf size and yield (Briggs and Aytenfisu, 1980). Recently, the leaf area index or other indices of vegetation have been used in agricultural models for biomass estimation and yield prediction (Major *et al.* 1986). There are various methodological approaches to measure plant leaf area. Direct measurement of leaf area is usually time consuming and labor intensive and this action usually causes canopy damage. But leaf area can be estimated non-destructively by using mathematical formulae, which only require simple measurements of the leaf lamina. Potdar and Pawar (1991) evaluated non-destructive leaf area estimation in banana (*Musa acuminata* Colla.) and showed a strong relationship between leaf area and various combinations of leaf length (L) and leaf width (W). Pekson (2007) also showed that there was a high correlation between leaf area and a combination of lamina length (L) and lamina width (W) in *Vicia faba* L. Serdar and Demirsoy (2006) developed a mathematical equation to estimate leaf area in chestnut (*Castanea sp*.) by measuring leaf length and leaf width and calculated different combination of them. Their result showed that there was a strong relationship between estimated leaf area and actual leaf area (R^2=0.99). Cho *et al.* (2007) found that estimation of individual LA, leaf fresh weight (LFW) and LDW in hydroponically grown cucumbers (*Cucumis sativus L*.) can be done with high accuracy using leaf length, leaf width and leaf chlorophyll value (R^2=0.98, R^2=0.96, R^2=0.96 respectively). McKee (1964), Pearce *et al.* (1975), and Dwyer and Stewart (1986), reported a general equation to estimate individual leaf area of maize (*Zea mays L*.):

$$\text{Leaf area} = L \times W \times A$$

Where LA, L, W and A are leaf area, leaf length, leaf maximum width and A constant (A=0.75), respectively. Other researchers obtained A values between 0.72 and 0.79, for example 0.72; (Keating and Wafula, 1992), 0.73; (Stewart and Dwyer, 1999), and 0.79; (Birch *et al.* 1998). Specific leaf area (SLA), that is, the light-capturing surface built by the plant per unit investment of dry mass, is an indirect measure of the return on investments in a productive organ (Niklas *et al.* 2007). If light capture was the sole governing factor of leaf function, SLA would tend to be infinite to maximize return on dry mass investment. However, maximum SLA is constrained by a minimum of dry mass needed to construct support, protection or transport tissues, such as veins or epidermis, which are generally dense. SLA is further particularly sensitive to changes in the external environment and in the internal functioning of the plant, as extensively documented both by experimental and observational studies (Gunn *et al.* 1999; Niinemets, 2001; Poorter and Nagel, 2000). However, until recently the dependence of SLA on leaf size had not been comprehensively assessed (Milla and Reich, 2007). SLA and leaf size (measured as A (cm^2) are functionally linked by definition (SLA=A/M (cm^2/mg) where M is leaf mass (g). Thus, to quantify how a given change in leaf size affects SLA we examined the scaling relationship of M to A. Landsberg (1990) used the inverse of SLA, namely specific leaf weight (SLW in mg/cm^2), as an indicator of leaf toughness in her studies of insect herbivory and eucalypt dieback. Water is the main abiotic factor limiting plant production in several regions of the world, with crop growth and economic yield being severely affected by water availability (Araus *et al.* 2002).

The water use (WU; i.e. the water consumed) and water use efficiency (WUE; in general terms, the efficiency of this consumed water to assimilate carbon, produce biomass or grain yield) are crucial parameters where water is scarce, as in semi-arid regions with Mediterranean climate (e.g. Mediterranean basin in south Europe, North Africa and West Asia as well as Western Australia and parts of South Africa and Chile). Agronomists and crop physiologists, however, define WUE rather from an integrative approach, i.e. the accumulated dry matter divided by the water used by the crop in the same period (Abbate *et al.* 2004). In a broad sense, assimilated dry matter can be considered as the total biomass (commonly, aboveground parts) or, alternatively, as the accumulated dry matter partitioned the economical product (for cereals, the grains). Thus, it may be defined as WUE for the biomass ($WUE_{biomass}$) and the grain yield (WUE_{yield}) (Hatfield *et al.* 2001). The aim of this study is to evaluate the effect of stressed and non stressed conditions on Leaf area (LA) and Specific Leaf Weight (SLW) and its relationships with grain yield and water use efficiency in ten durum wheat Cultivars.

Material and methods

The experiments (under rain-fed and irrigation conditions) were conducted in the experimental field of ITGC (Technical Institute of Field Crops) of Sétif (5°20’E, 36°8’N, 958m above sea level), Algeria; during the 2010-2011 cropping season. A set of 10 genotypes (Table 1) of durum wheat (*Triticum durum* Desf.) were planted on November 30, 2010, genotypes were grown in randomized block design with four replicates. The seeds were sown using an experimental drill in 1.2mx2.5m plots consisting of

6 rows with a 20 cm row space and the seeding rates for both experiments were about 300 seeds per m^2. The plots were fertilized with SULFAZOT (26% N, 35% S, 120 Kg/ha) at tillage stage. Weeds were removed chemically by TOPIC (0.75L/ha) and GRANSTAR (15g/ha). All plots of the irrigation experiment were irrigated by using a Sprinklers system and the volume of water input for each plot was controlled. Two irrigation regimes were applied. The first irrigation was performed at the time of Elongation (20/04/2011) (30 zadoks cods). The second irrigation was applied on (08/05/2011) after heading (50 zadoks cods). After flowering, ten flag leaf of each plot were cut for measuring the length, width and mass of leaf. Leaf area (LA) and Specific Leaf Weight (SLW) were measured mathematically. LA=L × W × A (Spagnoletti Zeuli and Qualset, 1990); where LA, L, W, and A are leaf area, leaf length, leaf maximum width and A constant (A=0.607) respectively. SLW and leaf area (LA) are functionally linked by definition (SLW=M/LA (mg/cm^2)) when M is leaf mass (g) (Radford, 1967). Water use efficiency was calculated using the following equation:

$$WUE_{yield} = \text{Grain Yield / evapo-transpired water}$$

(Tambussi *et al.* 2007)

Grain yield was determined from sub-samples taken from harvested grains of each plot.

Evapo-transpired water is estimated by using software AquaCrop Version 3.1. The input necessary to estimate the evapo-transpired water by AquaCrop software were:

– Daily rainfall of growing season;

– Daily Reference evapo-transpiration (ET_0) estimated by using ET_0 software (2000) and according to Penman Montheil equation modified and recommended by FAO (1998);

– The different layers and types soil of experimental field;

– Morpho-physiological characteristics of crop (Genotypes) and growing cycle of each genotype.

Results and discussion

As shown in Table 2, analysis of variance revealed that Leaf area, Specific Leaf Weight, WUE_{yield} and grain yield were highly significant (P<0.001) under irrigation regime treatment. In addition, the genotypic effect was highly significant (P<0.001) for Leaf area, Specific Leaf Weight and grain yield under both conditions, WUE_{yield} was highly significant (P<0.001) under irrigated condition and significant (P<0.01) under non irrigated condition. Moreover, interaction effect of irrigation regime × genotype was highly significant for Leaf area and Specific Leaf Weight.

3.1. *Leaf area (LA)*

The results of the present study indicated that the two different conditions of growth (stress and non stress conditions) had different considerable effects on leaf area. Under stressed condition, leaf area ranged from 11.46 cm^2 for Polonucum to 19.37 cm^2 for Oued Zenati with an average of 14.96 cm^2 over all genotypes, but under irrigated condition (non stressed) leaf area varied between 13.83 cm^2 for Altar to 30.66 cm^2 for Oued Zenati with an average of 18.09 cm^2 over all genotypes. In this study, the difference between Leaf area under stressed and non stressed condition amounted to 17.24% (Figure 1). The maximum leaf area *per* culm was observed just before heading when the flag leaf had fully emerged (Puckridge, 1971). The water stress significantly reduced leaf area due to the reduced cell division. Water stress may reduce turgor pressure and hence cell expansion, resulting in approximately the same dry mass being contained within a smaller leaf area, thus raising density (Hsiao, 1973; Rascio *et al.* 1990).

3.2. *Specific Leaf Weight (SLW)*

A survey of literature revealed that morpho-physiological traits such as flag leaf area (Fischer and Wood, 1979), specific leaf weight, leaf dry matter (Aggarwal and Sinha, 1984; Misra, 1995) had been widely used as selection parameters contributing towards drought tolerance for various crop plants in addition to grain yield. With regard to genotype effects and under stressed condition, Polonucum had high value of SLW 0.0349 mg cm^{-2}, but Mexicali had low value 0.0162 mg cm^{-2}. Under non stressed condition, specific leaf weight ranged from 0.062 mg cm^{-2} for Bousselem to 0.062 mg cm^{-2} for Dukem with an average of 0.043 mg cm^{-2} over all genotypes. Figure 2 shows that water stress reduced the specific leaf weight (41.86%). Munamava and Riddoch (2001) reported that specific leaf weight (SLW) and specific leaf area (SLA) decreased with stress, especially when water stress was applied at booting stage.

3.3. *Grain Yield (GY)*

The results of the present study indicated that the two different conditions of growth (stress and non stress condition) had different considerable effects on grain yield. Under stressed condition, grain yield ranged from 52.20 Qx ha^{-1} for genotype

Oued Zenati to 64.63 Qx ha^{-1} for genotype Waha with an average of 58.50 Qx ha^{-1} over all genotypes, but under well watered condition, grain yield ranged from 57.45 Qx ha^{-1} for genotype Oued Zenati to 75.55 Qx ha^{-1} for genotype Sooty with a mean of 66.8 over all genotypes. Drought resistance is usually quantified by grain yield under drought. Wheat grain yield under drought, however, depends on yield potential as well as the phenology of the genotype (Acevedo, 1991) . In this study, the difference between grain yield under stressed and non stressed condition equal 12.42% (Figure 3). Although stress typically depresses grain yield (Hsiao, 1973), it can elevate the value of other components of the economic yield, such as quality of grain protein (Guttieri *et al*. 2000). Moreover, Donaldson (1996) and Nazeri (2005) have reported that water deficit after anthesis stage decreased grain filling period, kernel weight and crop production. According to Blum (1988), identification of high potential varieties under optimum moisture and water deficit conditions (slow stressing) has been a principal breeding approach for durum and bread wheat genotypes.

3.4. Water use efficiency (WUE_{yield})

The results of the present study show that there is a highly significant difference between stressed and non stressed conditions and genotypes. Under stressed condition, WUE_{yield} ranged from 9.21 kg mm^{-1} ha^{-1} for Oued Zenati to 12.44 kg mm^{-1} ha^{-1} for Sooty. In irrigated condition WUE_{yield} varied between 9.25 kg mm^{-1} ha^{-1} for Oued Zenati and 14.29 kg mm^{-1} ha^{-1} for Waha; the difference in WUE_{yield} between irrigated and non irrigated conditions accounted 12.11% (Table 2). Oweis *et al*. (2000) reported that WUE_{yield} of bread wheat under Mediterranean conditions was the highest with a deficit irrigation consisting of two-thirds of the water required at full irrigation (i.e. WUE_{yield} at full irrigation was lower). In fact, increase in WUE_{yield} under water limitation are reported in several studies and climatic conditions (Abbate *et al*. 2004). However, there are other reports in wheat where no increase in WUE (neither WUE_{yield} nor $WUE_{biomass}$) was found under water-deficit treatments (Xue *et al*. 2003).

Conclusion

This study confirmed that the supplementary irrigation affect significantly Leaf area, Specific Leaf Weight, Grain yield and Water use efficiency. The difference between Leaf area under stressed and non stressed condition was 17.24%, this suggest that the water stress significantly reduced leaf area due to the reduced cell division. In addition, water stress reduced the specific leaf weight by 41.86%. Many studies reported that specific leaf weight (SLW) and specific leaf area (SLA) decreased with stress, especially when water stress was applied at booting stage. The difference between grain yield under stressed and non stressed condition was 12.42 %. Water deficit after anthesis stage decreased grain filling period, kernel weight and crop production. The difference in WUE_{yield} between irrigated and non irrigated conditions was 12.11%. Many studies reported that WUE_{yield} of bread wheat under Mediterranean conditions was the highest with a deficit irrigation consisting of two-thirds of the water required at full irrigation (i.e. WUE_{yield} at full irrigation was lower).

Table 1. Name and origin of the ten genotypes used in the study

Cultivar	Name	Origin	Cultivar	Name	Origin
1	Bousselem	Algeria	**6**	Altar	CIMMYT
2	Hoggar	Algeria	**7**	Dukem	CIMMYT
3	Oued Zenati	Algeria	**8**	Kucuk	CIMMYT
4	Polonicum	Algeria	**9**	Mexicali	CIMMYT
5	Waha	Algeria	**10**	Sooty	CIMMYT

Table 2. Response of Leaf area (LA), Specific leaf weight (SLW), water use efficiency of grain yield (WUE_{GY}) and grain yield (GY) of ten durum wheat genotypes tested.

	LA		**SLW**		**WUE_{GY}**		**GY**	
Genotype	**Irr**	**Non-Irr**	**Irr**	**Non-Irr**	**Irr**	**Non-Irr**	**Irr**	**Non-Irr**
Oued Zenati	30,66 a	19,37 a	0,0457 bc	0,0251 bc	9,25 d	9,21 e	57,46 d	52,20 d
Altar	13,83 d	14,75 bcd	0,0395 bcd	0,0262 b	12,37 bc	11,38 abc	69,14 abc	55,94 bcd
Sooty	16,43 cd	13,12 de	0,0486 b	0,0250 bc	13,96 ab	12,44 a	75,55 d	63,15 abc
Polonucum	24,41 b	11,46 e	0,0426 bcd	0,0349 a	10,01 d	9,65 de	60,18 cd	56,48 abcd
Waha	17,11 c	12,02 de	0,0454 bc	0,0333 a	14,29 a	10,86 abcd	65,94 abcd	64,63 a
Dukem	14,66 cd	17,15 ab	0,0620 a	0,0205 bcd	14,14 a	11,97 ab	72,70 ab	63,94 ab
Mexicali	15,77 cd	17,29 ab	0,0454 bc	0,0162 d	13,18 abc	10,45 abce	63,44 bcd	59,64 abcd
Kucuk	15,67 cd	16,35 abc	0,0343 cd	0,0196 cd	11,93 c	12,11 a	73,53 a	53,96 d
Hoggar	14,93 cd	13,36 cde	0,0381 bcd	0,0226 cd	13,28 abc	10,27 cde	62,36 cd	60,05 abcd
Bousselem	16,48 cd	14,75 bcd	0,0304 d	0,0215 bcd	12,16 c	11,16 abcd	67,75 abc	55,01 cd
Mean	18,099	14,967	0,043	0,025	10,95	12,46	66,80	58,50
Min	13,83	11,46	0,0304	0,0162	9,25	9,21	57,45	52,20
Max	30,66	19,37	0,062	0,0349	14,29	12,44	75,55	64,63
CV %	30,20	17,04	20,10	23,89	9,75	13,7	9,00	7,55
LSD 0,05	2,68	3,18	0,0122	0,0060	1,67	1,6	9,77	8,15
Genotype effect	***	***	***	***	***	**	***	***
Irrigation effect	***		***		***		***	
Interaction effect	***		***		*		ns	
% Differences	17,24 ↑		41,86 ↑		13,78 ↓		12,42 ↑	

Means followed by the same latter are not significantly different, CV: coefficient of variation, ns: no significant, * Significant difference at $P < 0.05$, ** significant difference at $P < 0.01$, *** significant difference at $P < 0.001$

Figure 1. The effect of irrigation on leaf area in all genotypes tested.

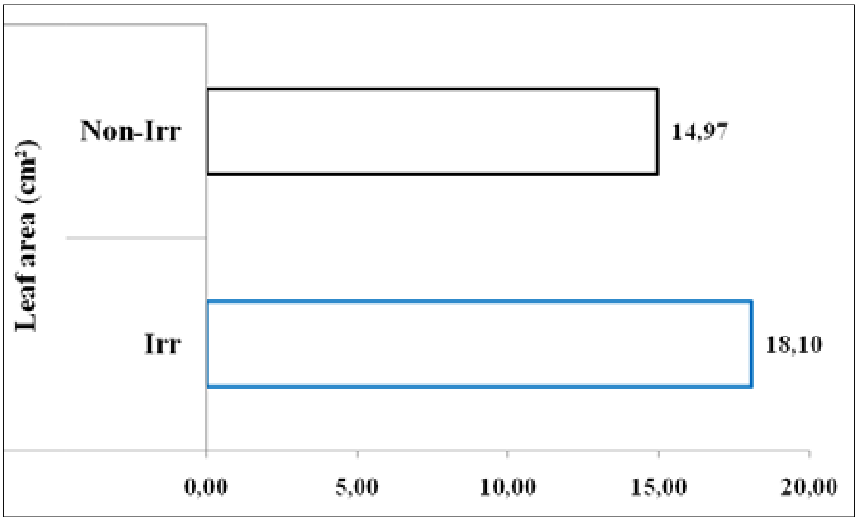


Figure 2. The effect of irrigation on Specific leaf weight in all genotypes tested.

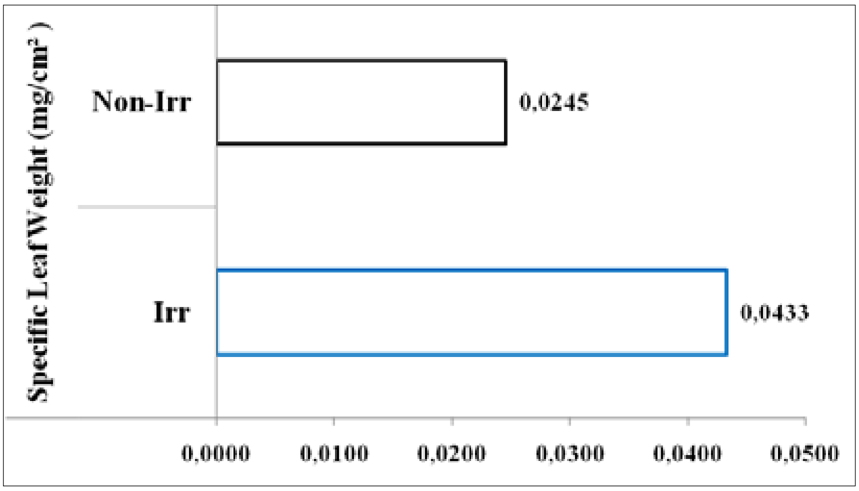
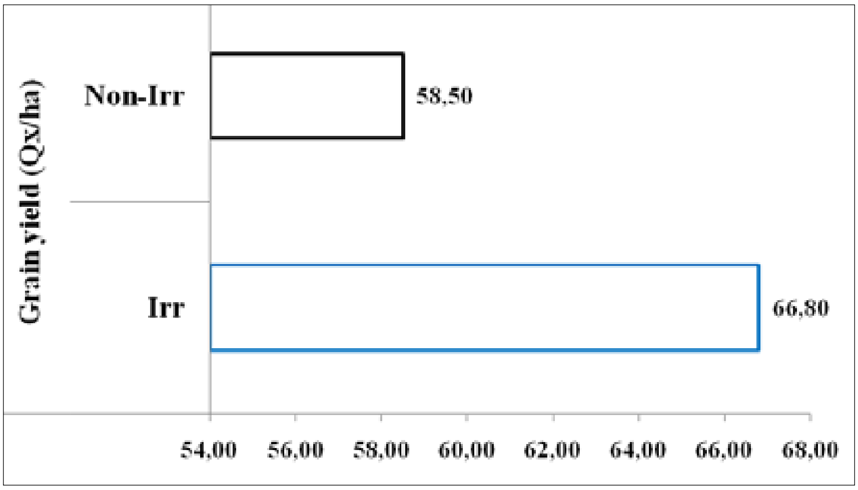


Figure 3. The effect of irrigation on Grain yield in all genotypes tested.



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GGE Biplot Analysis of Multi-Environment Yield Trials in Barley (*Hordeum vulgare* L.) Cultivars

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ABSTRACT

Identification of the genetic stability and adaptation of released varieties are very important for breeding programs. Genotype x Environment Interaction (GEI) is extensively observed by breeders as differential ranking of variety yields among environments or years. Therefore, four spring barley varieties, registered in different years, were evaluated at eight environments in different years. The experiments were performed according to a complete randomized block design with four replications. Stability and genotypic superiority for yield was determined using ANOVA and GGE biplot analysis. Genotype x environment interaction was found to be highly significant ($P < 0.01$) for grain yield. The GGE biplot indicated that three mega-environment were occurred in terms of varieties. Kendal and Altikat, took place in same mega-environment, while Samyeli in the second, Sahin 91 in third. On the other hand, Kendal and Altikat showed general adaptability (E1, E2, E5, E7 and E8), while Samyeli and Sahin 91 exhibited specific adaptation to E4 and E3 respectively. Considering both techniques, Samyeli and Sahin 91 came forward with low yielding, while Kendal and Altikat with high yielding and stability. Results indicated that GGE biplot is illuminant methods to discover stability and adaptation pattern of varieties in practical recommendations.

Keywords: spring barley, genotype x environment interaction, GGE biplot, grain yield, stability.

Introduction

Barley is an excellent feed grain, fall and winter pasture, and forage crop in South-eastern Anatolia Region of Turkey. Barley has a wide range of adaptation, growing best on fertile, well-drained soils. Spring or winter and two- or six- row varieties are available. Spring barleys are grown in majority of region, while winter types only north of region. Basically this region is divided into three sub-regions. The first sub-region includes the Syrian border having low rainfall and drought conditions. Therefore, barley is one of a few plants that are grown without irrigation in this sub-region. The second sub-region have good conditions for barley and consisting of four province broadcast (Adıyaman, Diyarbakır, Batman, Siirt). The third sub-region includes north of South-eastern Anatolia Region. Many factors of barley wheat are affected depending on agro-ecologicalical conditions of these sub-regions (Mizrak 1986).

The cultivars which are used in South-eastern Anatolia Region are different depending on sub-regions, as three main sub-regions have different conditions to cultivate barley cultivars. So it is very important to identify cultivars for specific sub-region. For that matter multi-environment trials (MET) are conducted to evaluate stability performance of genotypes under different environmental conditions *via* biplot analysis (Farshadfar *et al.* 2012; Yan 2000). Any genotype cultivated in varying environments show significant fluctuations in yield and yield components performance. These problems are affected by the different agro-ecological conditions and are referred to as genotype-by-environment (GE) interaction (Allard and Bradshow 1964). Furthermore, GE interaction decreases the genetic advance in plant breeding programs through minimizing the relation between phenotypic and genotypic values (Comstock and Moll 1963). Therefore, GE interaction must be either exploited by selecting superior genotype for each specific target environment or avoided by selecting widely adapted and stable genotype across wide range of environments (Ceccarelli 1989).

The breeding program of GAP International Agricultural Research and Training Center released five varieties between 1993-2013 years. The varieties, which released (2008-2013) in South-eastern Anatolia Region have different genotype features with superior grain yield, quality and other desirable characteristics over a wide range of different environmental conditions. Genotype by environment interaction (GxE) makes it difficult to recommend the best performing and most stable genotypes. Plant breeding programs should take GEI into consideration as well as an estimate of its magnitude, relative to the magnitude of G and E effects, which affects yield and yield components. The objective of this investigation was to use GGE Biplot to evaluate genetic improvement of varieties and detects in performance and stability of new varieties in eight diverse environments in South-eastern Anatolia Region with higher precision by removing the noise caused by E or genotypes.

Material and method

Plant genetic materials

The experimental material comprising of three new and one old barley varieties which were evaluated in eight rain-fed environments in different growing season (Table 1). The experiment was conducted in a randomized block design with four replications. The seeding rate was used 450 seeds m^{-2}. Plot size was 7.2 m^{-2} (1.2 × 6 m) consisting of 6 rows spaced 20 cm apart. Sowing was done by Wintersteiger drill. The fertilization rates for all plots were used 60 kg N ha^{-1} and 60 kg P ha^{-1} with sowing time and 60 kg N ha^{-1} was applied to plots at the early stem elongation. Harvest was done using Hege 140 harvester up on 6 m^{2}.

Statistical analysis

The grain yield data were subjected to combined analysis of variance (ANOVA) to determine the effects of environment (E), genotype (G), and their interactions. The data were graphically analyzed for interpreting GE interaction using the GGE biplot software (Yan 2001). GGE biplot methodology, which is composed of two concepts, the biplot concept (Gabriel 1971) and the GGE concept (Yan *et al.* 2000), was used to visually analyze the wheat-barley disomic addition lines MET data. This methodology uses a biplot to show the factors (G and GE) that are important in genotype evaluation and that are also the sources of variation in GE interaction analysis of MET data (Yan 2001). The graphs generated based on (1) relationships between testing environments based on the angles between the vectors of the environments. (2) Ranking of cultivars on the basis of yield and stability, (3) ranking of test environment relative to the highest yielding cultivar, (4) comparison of cultivars to an ideal cultivar, (5) ranking of cultivars relative to the test environment with highest yielding performance and (6) "which-won-where" pattern to identify the best genotypes in each environment for four genotypes of South-eastern Anatolia Region.

Results and discussion

Analysis of variance showed that the impacts of Environments (E), Genotypes (G) and Genotype × Environment Interaction (GEI) are highly significant. The percentage of the total sums of squares accounted for by G, E, and GE interactions were used as an indicator of variation attributed to grain yield. The biplot analysis of variance of grain yield of the four cultivars tested in eight environments showed that 82.89% of the total sum of squares was attributable to environmental effects, only 4.39% to genotypic effects and 12.7% to GEI effects (Table 3). Because environment accounted for 82.89% of the total variation for grain yield/ha, the effect of environmental sites was expected to be high. Majority of grain yield variation, explained by environments, showed that the environments were diverse and a major part of variation in grain yield can be resulted from environmental changes. But notional addition of GE constituent variance was very high as compared to the G component of variance showing that genetic improvement of this study is low. Yan and Kang (2003) reported high magnitude of E constituent to the extent of 80% in wheat and 59% in soybean. Also, Brar *et al.* (2010), Mohammadi and Amri (2011), reported more than 78% estimates for E components in *Taramira* and wheat through the environment and years. The heritability of genotype estimates were 7.67 to 18.53%, for seed yield (Letta *et al.* 2008; Brar *et al.* 2010). On the other hand, some researchers reported heritability of environment estimates between 40.5 to 84.8% for grain yield (Dash and Pandey 2009; Singh *et al.* 2009).

Interrelationship among cultivars and environments

Summary of the interrelationships among the environments for different cultivar is presented in Figure 1. The lines linking the biplot origin with the markers for the environments are called environment vectors. The angle between the vectors of two environments is related to the correlation coefficient between them. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Yan 2002; Yan *et al.* 2007; Brar *et al.* 2010). Based on the cosine of angles of environment vectors, the eight environments for grain yield were grouped into three groups. The presence of wide obtuse angles i.e. strong negative correlations among the environments is marker of strong cross-over genotype by environment interactions (Yan and Tinker 2006). The distance between two environments measures their dissimilarity in discriminating the genotypes. Therefore, eight environments for grain yield/ha were resolved into three groups. E1, E2, E5, E6, E7, E8, clustered in one group; E4 involved in second group; E3 included in third group. The concentric circles on the biplot help to visualize the length of the environment vectors, which is proportional to the standard deviation within the respective environments and is discriminating ability of the environments (Kroonenberg 1995). Thus, among the eight environments E2, E4 and E7 were the most discriminating (informative) while E3 and E5 were the less discriminating for grain yield. The test environment which have length vector and narrow angles environments that mean it is both discriminating and representative environment and good for selecting widely adaptive genotypes (Yan 2001). This concept showed that E1, E5 and E6, had very narrow angle with AEA, but the environmental conditions at these environments were not much discriminating as E2 and E4 have sufficient vector length. Thus, E2 and E4 are suitable environments for selecting high yielding cultivars having wider adaptability in South-eastern Anatolia Region (Figure 1 & Table 4).

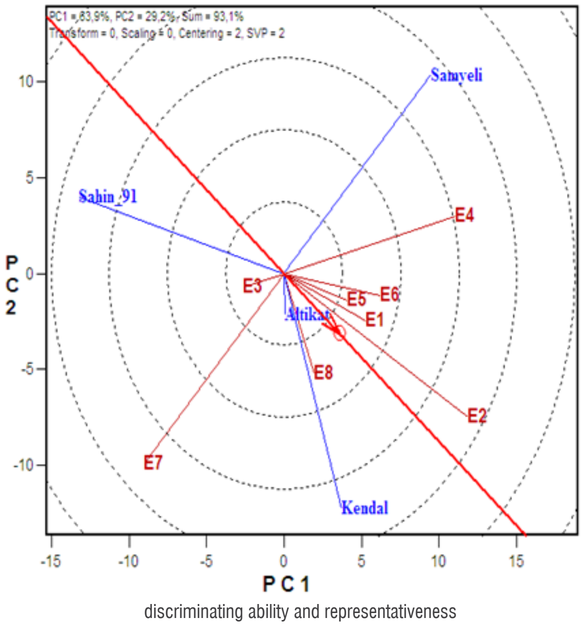


Figure 1: GGE biplot showing the performance of each cultivar at each environment

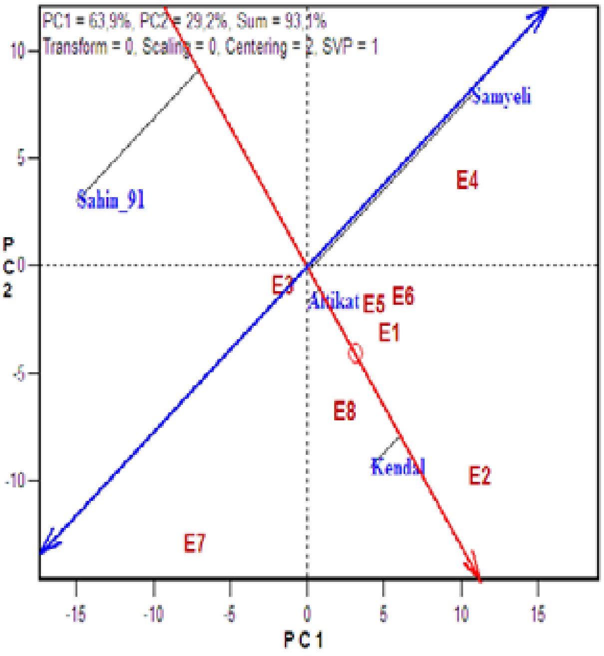
Mean performance of cultivars at different environments

Both vectors for genotype and environment, as drawn in Fig 1, are helpful to visualize the specific interactions between a genotype and an environment, as well as, the performance of each genotype in each environment (Yan and Tinker 2006). The performance of a genotype at a specific environment is better, when the angle is <90° between genotype vector and environment vector; it is poorer than average if the angle is >90° ; and it is near average if the angle is about 90°, which is based on the "inner product property" principle of biplot (Gabriel 1971). Therefore, the potential grain yield of Sahin 91 is under average at majority environments without E3 and E7. However the performance of Samyeli is above average at E4 and E6, while it was near average at E2. Similarly Kendal gave better yield than average at E5 and E7, also it was adapted in E2 and E8 environments. Altikat was well adapted to E1 and E3, while it took place above average for grain yield in E2, E4, E5 and E7 environments (Fig 1 and Table 4).

Stability of cultivars through the environments

The ideal genotype should have high mean performance coupled with high stability to give wide adaptability in the target region, As shown in Figure 2, the single-arrowed line called average–environment coordination abscissa (or AEA) points to higher mean yield through the environments. Thus, Kendal had the highest mean yield, followed by Altıkat and Samyeli. Sahin 91 had low yield for overall mean yield through all environment. The double-arrow line is the AEC ordinate and it points to greater variability (poor stability) in either direction.

The instability index calculated as *per* Eberhart and Russel (1966) model has the same magnitude as depicted by GGE biplot (Fig. 2). Therefore, to rows barley cultivars Altikat and Kendal are highly stable genotypes, whereas two rows cultivar of



Average Tester Coordination for Teseter Evaluation

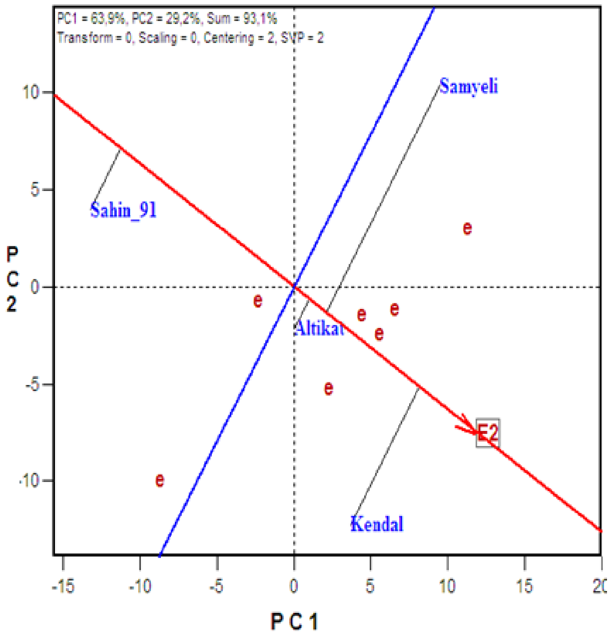
Figure 2: Average- environment coordination (AEC) show the mean performance and stability of cultivars

Samyeli and Sahin 91 were most unstable through all environments for grain yield. Kendal cultivar is stable for grain yield as it has performed better than average at E2 and E8 environments. Samyeli strain is also unstable as its performance was opposite to Sahin 91 at different sites; also it has performed better than average at E4, E6 and E5 and poor at E8 and E7 (Fig. 2).

Ranking of cultivars based on performance in a specific environment and across environments

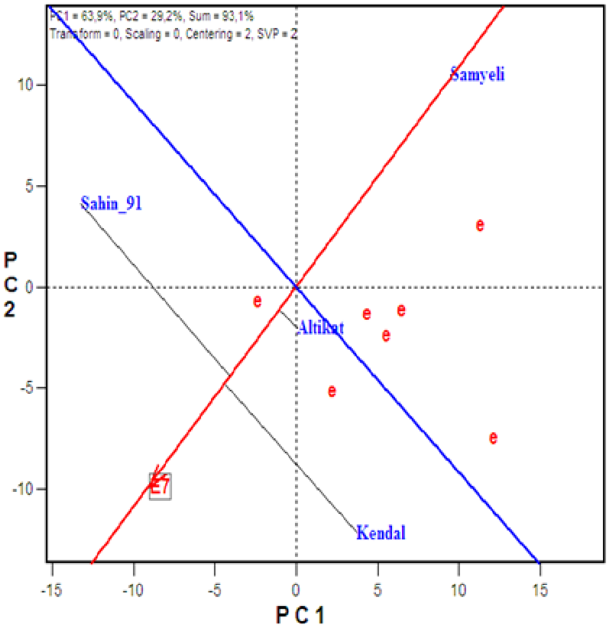
Conjecture, we wanted to see the yield potential of different genotypes at E2 environment, the line will be drawn that passes through the biplot origin and E2 environment (Fig 3). The Kendal, Altıkat and Samyeli gave highest yield, while Sahin 91 provided the lowest yield. On the other hand, some environments (E3 and E7 as well as E8) the ranking of cultivars were just across to especially E2 and other environments. The graph indicates the clear-cut presence of cross-over interaction (COI). This warrants exploitation of GEI (Yan *et al.* 2000). It is pertinent to mention that these environments are conducting breeding program of spring barley in South-eastern Anatolia Region of Turkey.

The adaptability of cultivars to these environments showed opposite points on Figures 3, 4 & 5. The conditions of these environments are different; because these environments consist of three Sub-regions in South-eastern Anatolia Region The environment of E8 located in south of region which is very dry ; E2, E1, E4 and E5 located in central of region which are normal; E3and E7 located in north of region is colder than other environments. This means that specific adaptability of cultivars at these environments is entirely different and GEI can be exploited for selecting cultivars rather than ignoring it. We can also visualize biplot for best adaptability of cultivar in a specific environment or sub-region as well.



Examining the performance of/relative to E2

Figure 3: Ranking of cultivars performance based on *E2*



Examining the performance of/relative to E7

Figure 4: Ranking of cultivars performance of based on *E7*

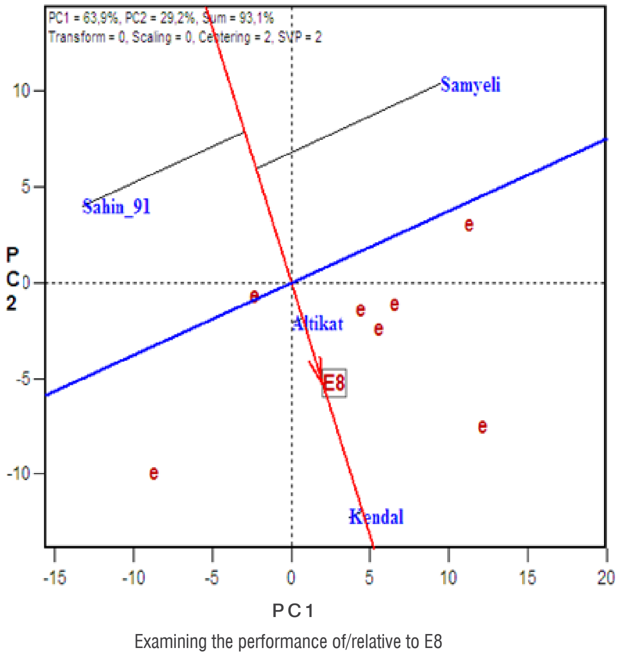


Figure 5: Ranking of cultivars performance based on *E8*

Ranking of environments in terms of the relative performance of cultivars

Based on the relative performance of the selected cultivar, the environments are ranked along the cultivar axis, with the arrow pointing to a better relative adaptation of cultivar. On this basis the adaptability of Kendal was highest at E2 followed by E7, E8, E1, E6, E5, E4 and least at E3 (Fig. 6). Similarly, Altikat possessed extreme adaptability at E1, E6, E5 and E3 environments, while it showed bad adaptation to E4 on grain yield (fig. 7). Samyeli was the best cultivar for E4, while it was least at E3, E8 and E7 on grain yield (Fig. 8). Whereas other cultivars, Sahin 91 showed good adaptation to E7 and E4, while it had not good adaptation at environments which Kendal, Altikat and Samyeli cultivars showed good performance (Fig 9). Moreover, Kiliç (2014) explained that the study consist of 25 advanced line displayed that G17 (Altikat) and G21 (Samyeli) had high or moderate stability with high grain yield and desirable quality with acceptable morphological

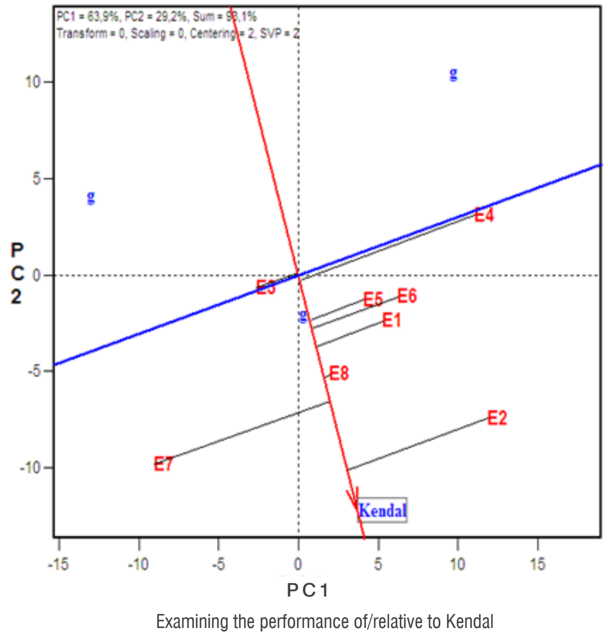


Figure 6: Ranking of environments in terms of the relative performance of *Kendal* variety

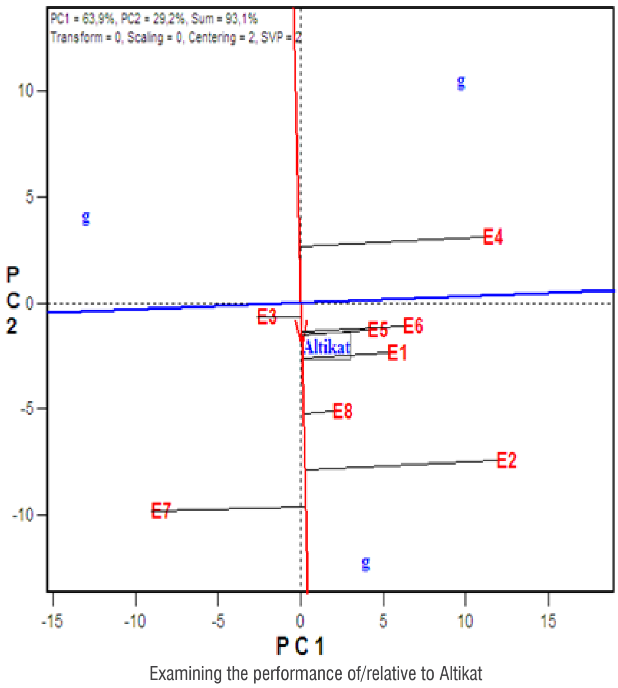


Figure 7: Ranking of environments in terms of the relative performance of *Altikat* variety

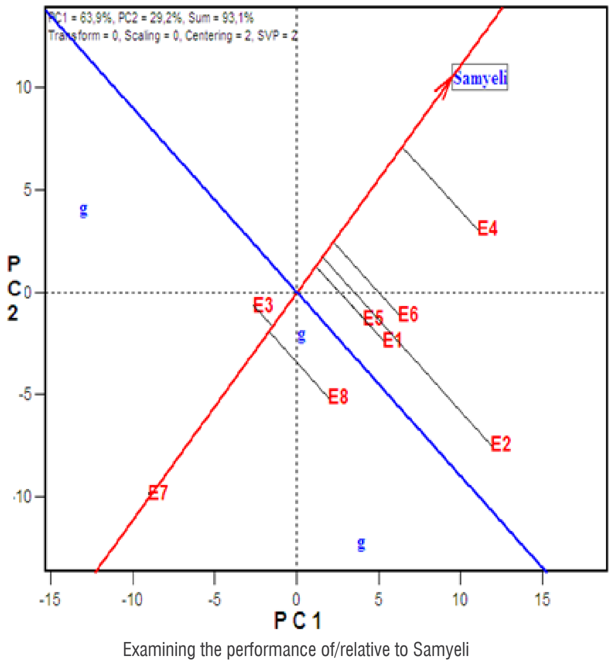


Figure 8: Ranking of environments in terms of the relative performance of *Samyeli* variety

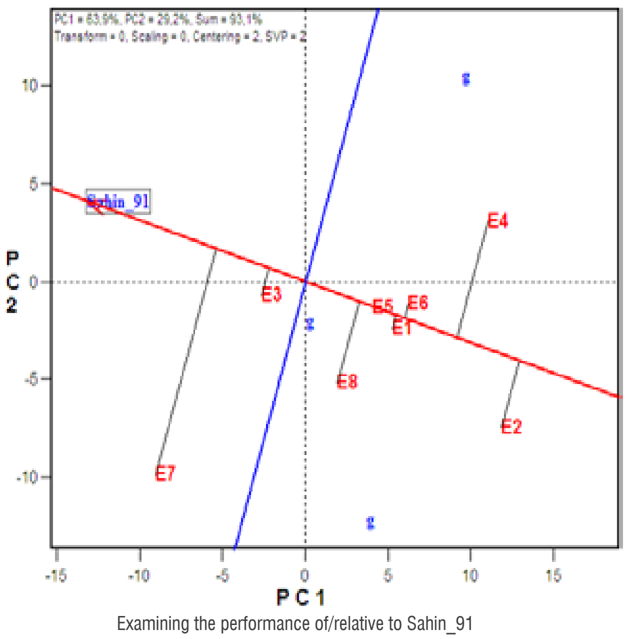


Figure 9: Ranking of environments in terms of the relative performance of *Sahin 91* variety

traits. When we rank cultivars across environments it should be done with respect to an ideal cultivar that lies on AEA (absolutely stable) in the positive direction and has a vector length equal to the longest vector of the cultivars on the positive side of AEA i.e., highest mean performance. Thus, cultivars which are closer to "ideal cultivar" are more desirable than others (Yan and Tinker 2006) and so, Kendal was high yielding with consistent of performance across the environments (Fig. 10). Altikat though moderate yielder, indicated highest stability among overall cultivars. Yan and Tinker (2006) are of the view that when we are interested to transfer "stability gene" to other genotypes it should be desirable to use a donor having high mean performances along with stability. Therefore, Kendal or Altikat can prove to be a better donor than Sahin 91 as far "stability genes" are concerned. On the other hand, new cultivars (Kendal, Altikat and Samyeli) had good stability than old cultivar (Sahin 91).Similarly, Kendal is the last cultivar which was registered by GAP International Agricultural Research and Training Center, it showed best performance among cultivars at majority environments.

Comparison among the cultivars

The distance between two genotypes approximates the Euclidean distance between them and hence, is measure of dissimilarity among the genotypes (Kroonenberg 1995). Therefore, Kendal and Samyeli and Sahin 91 are quite different in their genetic make-up with respect to grain yield. In that context Kendal and Altikat are very close to each other (Fig. 11). The biplot center also represents a "virtual" cultivar with grand mean value and zero contribution of additive effect of genotype (G) as well as multiplicative interactions (GE). The vector length of a cultivar of the center of biplot is due to the contribution of G and/or GE. The cultivar which located near to the biplot center have less contribution to G or GE (Altikat), while cultivars having longer vectors show the most contribution

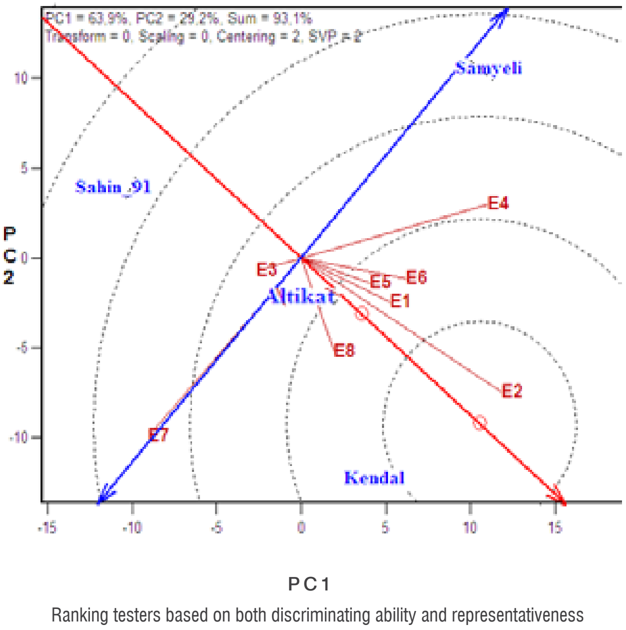


Figure 10: The average-environment coordination (AEC) view to rank cultivars relative to an ideal cultivar for grain yield per/ ha in SEA.

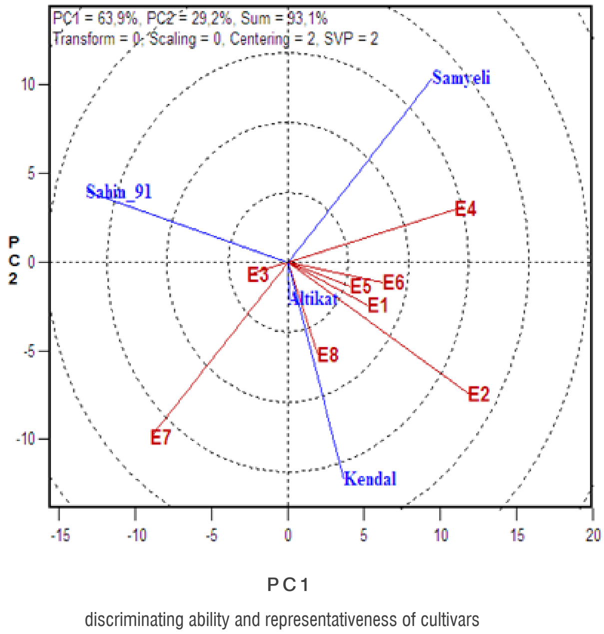


Figure 11: The cultivars-vector show similarities in their performance in individual environment for grain yield per/ ha.

of G and/or GE. So, cultivars with the longest vectors are either the best (Samyeli) or the poorest (Sahin 91) or most unstable (Kendal). Samyeli can be considered as the best cultivar as its angle is very close to the ideal cultivar coupled with longer vector length. Moreover, the angle between vector of a cultivar and the AEA partitions the vector length into components of G and GE. (Fig. 11). Therefore, there is major contribution of G for Samyeli and Sahin 91 and Kendal for grain yield, because they have opposite direction, so they can make up different genetic contribution. As Altikat took place of Center biplot, so it can't make up different genetic contribution.

Mega environments "which-won-where or which is best for what"

Dividing the target environment into meaningful mega-environments and deploying different cultivars for different mega-environments is the only way to utilize positive GE and avoid negative GE and the sole purpose for genotype by environment interaction analysis (Yan *et al.* 2007). A mega-environment is defined as a group of environments that consistently share the same best cultivar(s) (Yan and Rajcan 2002). This definition explain the following biplot based on the multi-environment trials (MET) data of barley yield which illustrates two points: 1) A mega-environment may have more than one winning cultivar (sector 1), and 2) even if there exists a universal winner (Kendal), it is still possible, and beneficial, to divide the target environments into meaningful mega-environments (Fig 12). Mainly, these three lines divide the biplot into three sectors. Five environments fall in the one sector. Cultivars located on the vertices of the polygon reveal the best or the poorest in one or other environment (Gauch & Zobel 1997). Consequently, Kendal was high yielding at five environments (E1, E2, E5, E7 and E8), while Samyeli at E4 and E6, Sahin 91 at E3.

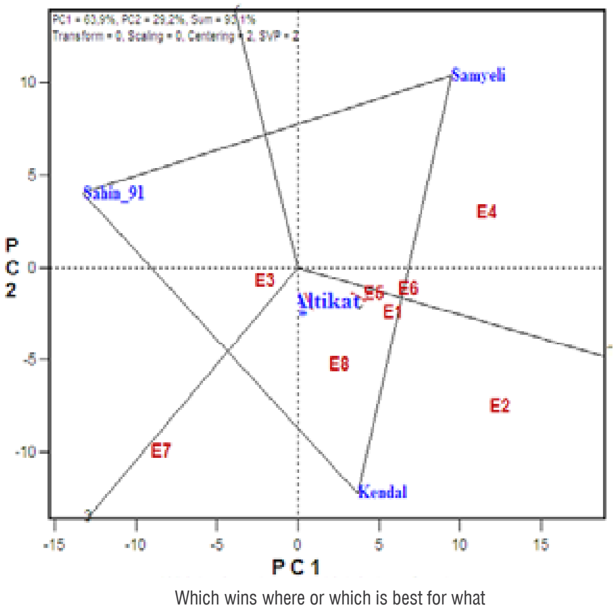


Figure 12: The which-won-where view of the GGE biplot to show which cultivars performed better in which Environment for grain yield of barley

Conclusion

The results indicated that yield performance of barley cultivars were highly influenced by environment followed by GE interaction effect and genotype with the least effects. Because of the changing conditions of environments in SEA, the magnitude of environment effect was very high than that of cultivar effect. The Kendal cultivars, which are newly registered, showed best performance among genotypes tested across environments, while the oldest cultivar (Sahin 91) had least grain yield and adaptability. So, the new cultivars were desirable in terms of high mean yield and stability, this means that the study provided an indication of the genetic progress. According to the results, the specific cultivar was appropriate for specific environment (Samyeli-E4, Sahin 91-E3, Kendal-E2) and E1 was the best yielding, while E8 least. The GGE biplot analysis allowed a meaningful and useful summary of GE interaction data and assisted in examining the natural relationships and variations in genotype performance across test environments. As our results indicated, GGE biplot is illuminant methods to discover stability and adaptation pattern of varieties in practical recommendations.

Acknowledgements

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Table 1. The information’s about varieties, used in experiment.

Name	Pedigree of cultivar	Origin	Approved year and institution	Spike rows
Kendal	Lent/Bllu//Pinon CBSS97M00698T-C-2M-1Y-0M	ICARDA	2013 GAPIARTC	6
Altikat	Arta/4/Arta/3/Hml-02//Esp/1808-4L ICB96-0601-0AP-10AP-0AP	ICARDA	2011 GAPIARTC	6
Samyeli	Hml-02//WI2291/Bgs ICB83-1554-1AP-1AP-6AP-0AP-22AP-0AP	ICARDA	2011 GAPIARTC	2
Sahin 91	YEA 1553-1/Eskişehir	TURKEY	1993 GAPIARTC	2

GAPIARTC: GAP International Agricultural Research and Training Center

Table 2. Years, sites, codes, coordinate status of environment long term of precipitation

Years	Sites	Code of sites	Altitude (m)	Latitude	Longitude	Annual rainfall (mm)
2011/12	Diyarbakir	E1	496	36° 97’ N	38°42’ E	550.6
	Kiziltepe	E2	483	37° 20’ N	40^{0} 56’ E	217.0
	Hazro	E3	895	38° 15’ N	40° 49’ E	891.9
2012/13	Diyarbakir	E4	496	36° 97’ N	38°42’ E	405.0
2013/14	Diyarbakir	E5	496	36° 97’ N	38°42’ E	363.0
	Adiyaman	E6	685	37° 46’ N	380 17’ E	592.0
	Hazro	E7	895	38° 15’ N	40° 49’ E	743.9
	Ceylanpinar	E8	363	36° 51’ N	40° 20’ E	260.3

Table 3. Combined analysis variance grain yield of barley cultivar tested across environments

Source	DF	SS	MS	F	LSD	Explained (%)
Environment(E)	7	3702452	528922	135.4261	45.6**	82.89
Rep(E)	24	93734.7	3905.61	1.0997		
Genotype(G)	3	196406	65468.8	18.4338	29.7**	4.39
GEI	21	567355	27016.9	7.607	84.0**	12.7
Error	72	255712.9	3551.6			
Total	127	4815661	37918.6			
CV(%)				13.53		

**Value significant for 0.01 probability level.

Table 4. Grain yield performance at different environment, average over environments (kg ha^{-1})

Cultivars	**E1**		**E2**		**E3**		**E4**		**E5**		**E6**		**E7**		**E8**		**Mean**	
Kendal	7070	bc	8180	a	3100	km	5240	ef	3880	jk	4740	fi	4250	gj	2520	m	4870	A
Altikat	7540	ab	6770	bc	3610	jl	5510	ef	3860	jk	4220	gj	3980	hj	1160	n	4580	A
Samyeli	6960	bc	7140	bc	2870	lm	6610	cd	3850	jk	4810	fh	1550	n	1340	n	4390	B
Sahin 91	5890	de	4930	fg	3490	jl	3900	ık	2990	lm	3480	jl	4220	gj	1260	n	3770	C
Mean	6870	A	6750	A	3270	D	5320	B	3650	D	4310	C	3500	D	1570	E	-	-

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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.

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Abbreviations

Abbreviations should be defined at first mention and used consistently

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