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# A short overview on the latest updates on Cereal Crop Plant genome sequencing with an emphasis on Cereal Crops and their wild relatives

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**ABSTRACT**

The advent of next generation sequencing has brought a revolution in the sequencing and availability of whole genome data for numerous plant species. However the genome sequencing of major staple food crops has been noticeably obscure and till relatively recently majorly unaccomplished. The obstacles for sequencing of genomes of the Poaceae grasses including sugarcane and the Triticeae wheat, barley and rye has been largely ascribed to the complex polyploid nature of their genomes, having undergone numerous evolutionary changes duplications and additions resulting in their huge modern genomes of today. Undertaking their sequencing has been a daunting task however due to the sequencing of wild grass relatives such as *Brachypodium* and *Aegilops* has been an encouraging step providing an essential framework and reference for deciphering the complex genomes particularly Triticum aestivum. This paper discusses the major challenges involved, the approaches taken and the up to date accomplished tasks for sequencing a few of the major large grass crop genomes.

***Keywords:*** Next generation sequencing, whole genome sequencing, plant genomes, grass crop genomes, Triticeae, polyploid genomes

## Introduction

Since the introduction of next generation sequencing large and complex plant genome projects have been undertaken and their complex genomes sequences deciphered. Of considerable importance in today's world with a population expected to be greater than 9 billion by 2050 (Foley et al. 2011), cereal plants have attained special attention in having their genomes sequenced. It all began after the model plant *Arabidopsis* was sequenced in 2000 (The Arabidopsis Genome Initiative 2000), followed by one of the three major cereal plants harbouring the smallest genome, rice (*Oryza sativa*) (Yu et al. 2002). Due to the small genome size of rice its entire genome sequence was unravelled by BAC to BAC sequencing. However the large genome size and high repeat content of the other grasses posed obstacles in their genome sequencing. Only relatively recently due to the advancement and introduction of next generation sequencing has the rush towards crop genome sequencing been re-kindled. Several years after the genome sequencing of rice, with the improvement in technology other larger cereal grass species including sorghum (Paterson et al. 2009), *Brachypodium* (Vogel et al. 2010), barley (Mayer et al. 2012) and maize (Schnable et al. 2009) were sequenced and continues till today as a race for sequencing the larger genome grasses. This was the start of a difficult and laborious journey towards the initial steps towards genome sequencing of all the major cereal crops of the world notably the most important being bread wheat (*Triticum aestivum*) (Brenchley et al. 2012; Mayer et al. 2014). It is however interesting to note that the second cereal grass to be sequenced maize was incompletely sequenced in 2009 by BAC to BAC approach and not

whole genome shotgun sequencing, due to its high repeat content (Feuillet et al. 2011). This is similar to the problem posed by the bread wheat genome. This report sheds light on the very latest advancements of plant genome sequencing, its applications and milestones reached. For convenience only the latest research in cereal crop plants and the progress on the grass sugarcane will be discussed here.

## Crop genome sequencing and the impact of NGS

The ever increasing human population coupled with fluctuation in the global climate all pose threats to global annual crop yield and demand (Foley et al. 2011). Conventional molecular breeding techniques for crop improvements would alone prove insufficient for meeting the ever increasing demands of the world population. The breakthrough in efforts for increasing plant yield came with next generation sequencing. The advent of NGS and its use for sequencing plant genomes revolutionized the approach towards crop genetics and genomics. With sequencing multiple reads in parallel NGS changed the face of functional genomics with its massive amount of output data in the form of sequence reads (Pareek et al. 2011). With considerable reduction in cost, and the large scale of this technology plant food species were sequenced by the dozen (Bolger et al. 2014). Combining NGS with precise phenotyping techniques result in rapid and powerful tools for genetic identification of agriculturally significant traits and the prediction of the breeding value of plant individuals in a population (Varshney et al. 2014).

Whilst the genome sequencing of many non-cereal plant genomes underwent completion with the introduction of NGS, the main staple food crops remained hidden from mainstream sequencing efforts and initiatives. All three main food crops of the *Triticeae* namely, wheat and rye and until very recently barley, have not had their genomes readily sequenced and available for molecular breeding applications, contrary to the many non-plant species (Graph 1), (Bolger et al. 2014). As outstanding and popular as next generation sequencing has become in recent years undeniably due to its unique advantages and breakthrough technology, next generation sequencing platforms still have a long way to go before the final draft of the whole genome sequence of immensely essential staple crops such as bread wheat is completed. The second and third generation sequencers will have to undergo tremendous technological evolution similar to the way the cereal grasses underwent major evolutionary events to form into their giant present day genomes.

Barley (*Hordeum vulgare*) with a 5 GB genome was sequenced relatively recently in 2012 (Mayer et al. 2012). The diploidy of barley and three times smaller genome than *Triticum aestivum* are essentially contributing factors towards the availability of its genome sequence.

Rye (*Secale cereale*) a close relative of *Triticum aestivum* has an 8 Gb genome. Despite it also having a prominently vast genome, chromosome survey sequencing, high throughput transcript mapping alongwith exploiting the genome data of the sequenced grasses rice, sorghum and *Brachypodium,* resulted in a virtual linear gene order draft harbouring 31,008 rye genes. The application of sequenced grass genomes in syntenic analysis of huge plant genomes enables high-density genome wide comparative syntenic analysis. In rye this has enabled the identification of 17 conserved syntenic linkage blocks in both rye and barley and vivid dissimilarities in conserved syntenic gene content with an ancestral *Triticeae* genome (Martis et al. 2013).

Wheat with its gigantic allohexaploid genome consisting of 3 subgenomes A, B and D comprising a total of 17 Gb provides a huge obstacle in sequencing of its genome. Such a massive genome 5 times larger that of the human genome with an 80-90% repeat content (similar to rye and barley) is a daunting task. Still however efforts have been made to sequence the non-repetitive content of wheat to a 5X coverage. The sequence data of assembled Illumina reads of *Ae. tauschii* and *T. monococcum* were utilized for the gene assembly of the 5X coverage of *Triticum aestivum* cultivar Chinese Spring (Brenchley et al. 2012). Despite this, the sequencing and alignment of the uniform distribution of repetitive content in the wheat genome in long arrays and parallel copies is beyond the ability of next generation platforms and thus repetitive and intergenic remain un-assembled.

## Accomplished projects of NGS

Despite the cumbersome genome of wheat and the shortcomings of current next generation sequencers in terms of sequencing large repetitive genomes, progress has been made in terms of reading the genomes and gene content of *Triticeae*. One important aspect here has been of the chromosome sorting with the isolation of individual purified chromosomes used in shotgun sequencing or in creating BAC libraries (Bolger et al. 2014). As aforementioned creating a reference genome sequence for *Tritcum aestivum* has been unrealised owing to the repetitive nature of its genome. With the availability of the EST and unigene and cDNA database for *Triticum aestivum* studies on microarray gene expression and targeted gene association have been

facilitated. The availability of the genome sequence of *Triticum urartu, Ae. tauschii* the progenitors of A and D wheat genomes, through high throughput sequencing also proved to be a hallmark in the progress on unravelling bread wheat genome. Through the relentless efforts of the International Wheat Genome Sequencing Consortium of which we are a small part a draft sequence of prepared of *T. aestivum* has been prepared approximately more than 95% of the genes of Chinese Spring cultivar of bread wheat. However an indepth detailed sequence of only one chromosome 3B is available. This draft sequence of wheat was prepared through sequencing of the individual flow-sorted chromosome arms. 124,201 gene loci have been annotated throughout the homeologous subgenomes. For survey sequencing each chromosome arm of the genome was sequenced with Illumina platform to a depth between 30X and 241X. These sequence assemblies cover roughly 61% of the genome in the form of survey sequences. The repetitive DNA comprised of 24 to 26% of the sequence reads and contained high copy number repeats. From the raw reads 81% and from the assembled sequences 76.6% contained repeats. Notably genome A contained more retroelements (Class I elements) and a pronounced abundance of LTR retrotransposons in comparison to genome B or D. From the protein coding genes a total of 44%, (55,249) were termed as high confidence from those assigned to the chromosome. (Mayer et al. 2014).

One of the recently accomplished resequencing of genomes has been of sorghum. Although initially sequenced in 2009, lately a high coverage resequencing of genomes of 44 lines of sorghum from diverse geographical origins has been presented, depicting the primary gene pool. The genome of *S. propinquum* was resequenced for the first time and 8M high quality SNPs were identified along with 1.9M indels indicating distinctive events of gene loss and gain. From the representation of the largest high-quality indel and SNP data for sorghum intricate domestication events were observed along with a large pool of diversity (Mace et al. 2013).

Similar to the resequencing of diverse racial accessions of sorghum, deep sequencing of 6 divergent lines of *Brachypodium distachyon* was undergone to analyse polymorphisms and gene expression. mRNA-Seq was performed under normal conditions and drought stress through which 300 genotype dependent genes were identified. A de novo transcriptome assembly was created with the most divergent line with the mRNA-Seq dataset. This remarkably resulted in more than 2400 previously unannotated transcripts along with hundreds of newly discovered gene absent in the reference genome (Gordon et al. 2014).

Though not a cereal, but a major food, grass crop and a relative of sorghum nevertheless sugarcane is also a complex genome crop whose genome is too complex for the whole genome shotgun approach. Sugarcane also harbours a largely repetitive and complex genome with a monoploid genome size of 930Mb. Interspecific crosses generating hybrid cultivars of sugarcane having complex polyploidy and aneuploidy produce genomes with great variation in their repetitive content and regions. Therefore gene enrichment using methyl filtration in order to enrich euchromatic regions was used for genome sequencing and assembly preparation. The availability of the sorghum genome sequence has facilitated the sequencing of sugarcane genome with conserved sequences having greater than 85% similarity between orthologs and the methyl filtered assembly obtained covered 98.4% of the sorghum coding sequences. This highly novel sequencing approach opens doors for sequencing of complex genomes with hypomethylated gene regions (Grativol et al. 2014). A complete list of the major food grasses with the approaches and accomplished milestones in genome sequencing is listed in Table 1.

## Advantages and applications of plant genome sequencing

As mentioned earlier the whole genome sequence of crop species largely made possible due to NGS provide a not only a reference genome for unsequenced and/or large and complex grass genomes but also are reservoirs of genomic information to be manipulated for plant breeding strategies (Kurtoglu et al. 2014). The easy availability of relatively small noncomplex plant genome sequences by the progression in next generation sequencing has catapulted crop domestication studies, particularly in understanding the phenotype-genotype interaction. Genetic mapping of desirable traits has been facilitated by genotyoing by NGS through genome-wide SNP analysis. This has implications in GWAS studies, biparental crosses and intercrosses between parental lines of diverse origin. Genome resequencing can also identify genomic regions with low nucleotide diversity and linkage disequilibrium as genomic regions selected during domestication (Olsen and Wendel 2013).

Despite the limitations of next generation sequencing in sequencing the cumbersome wheat genome, the previous few years have witnessed a substantial increase in the amount of wheat genomic sequence data available publicly. Integrating whole genome sequencing and physical mapping will lead to a huge reservoir of wheat sequence data upon which a reliable reference genome sequence can be

Table 1. Chronological Order of Important Cereal Crop genomes sequenced (including the grass sugarcane).

| Year | Grass | Significance | Sequencing Platform | Seq-Approach | Genome Size | Protein Coding Genes | Total Coverage |
|---|---|---|---|---|---|---|---|
| 2002 | *Oryza sativa*ssp *japonica* (Goff et al., 2002) | Long grain rice | MegaBACE capillary DNA sequencers | (random fragment) whole-genome shotgun sequencing | 420 Mbp | 14,345 high evidence | 93% |
| 2002 | *Oryzasativa* ssp *indica* (Yu et al., 2002) | paternal cultivar of super-hybrid rice, Liang-You-Pei-Jiu (LYP9),short grain rice most widely cultivated in China | High throughput capillary machine MegaBACE 1000 | Whole-genome Shotgun sequencing | 466 Mb | 53,398 prediction | 92% functional coverage |
| 2009 | *Sorghum bicolor* (Paterson et al., 2009) | African grass related to maize and sugarcane | ABI 3730, Mega, Sanger | Whole genome sequencing, Sanger | 700-772 Mb | 27,640 bona fide protein-coding genes | 13.5X clone, 8.50x sequence, 11x BAC library coverage |
| 2009 | *Zea mays* ssp *mays* (Schnable et al., 2009) | Maize genome B73 | -----N/A---- | BAC-by-BAC shotgun sequencing | 2,300Mbp 2.3Gb | 109,563 annotated loci | ~38% |
| 2010 | *Brachypodium distachyon* (Vogel et al., 2010) | Small Foot, model organism for monocots, wild relative of wheat | Illumina GAIIx | Whole genome Shotgun, Deep sequencing | 272.1 Mb | 25,532 loci | 9.43 X |
| 2012 | *Hordeum vulgare* (cv.) *Morex* (Klaus F X Mayer et al., 2012) | Barely, food crop | Illumina GAIIx | Whole genome Shotgun, RNA-seq | 5.1 Gb | 14,481 low-confidence genes | 55.4 fold haploid genome coverage |
| 2012 | *Triticum aestivum* (Brenchley et al., 2012) | Bread wheat, main staple food of the world, hexaploid, landrace Chinese Spring | Roche 454 pyrosequencing/ Illumina | Whole genome sequencing, sanger | 17 Gb | 54,368 (~56%) | between 23X and 83X of non-repetitive region |
| 2013 | *Aegilops tauschii* (Jia et al., 2013) | Goat Grass Wild relative of T. aestivum, D genome progenitor, Accession AL8/78 | Roche 454 (long reads) Illumina | Whole genome Shotgun RNA-seq Sanger sequencing | 4.36 Gb | 34,498 | 76X |
| 2013 | *Oryza brachyantha* | Wild species of Oryza genus | Illumina GA II platform | Whole genome Shotgun | 297 Mb | 32038 | 104 fold |
| 2013 | *Triticum urartu* | Wild wheat relative donor of genome | Illumina HiSequation (2000) platform | Whole genome Shotgun sequencing | 4.94Gb | 34,879 protein-coding gene models | 94% |
| 2013 | *Sorghum bicolor, Sorghum propinquum* (Mace et al., 2013) | 44 lines of African Sorghum allopatric Asian species | HiSeq 2000 Illumina platform | Whole genome resequencing | 700-772 Mbp | 19348 | 16–45 |
| 2014 | *Saccharum. spontaneum* and *S. officinarum* (Grativol et al., 2014) | Wild sugarcane species | Illumina GAII machine, HiSeq2000 machin | genome sequencing by methylation filtration | 930 Mb (one monoploid genome) | 98.4% of sorghum protein sequences | 134X |
| 2014 | *Brachypodium distachyon* (Gordon et al., 2014) | 6 divergent lines | Illumina sequencing | Deep sequencing | 272 Mb | 33,626 | 92.6–96.8% of the reference genome |
| 2014 | *Triticum aestivum* (K. F. X. Mayer et al., 2014) | Chromosome based draft sequence of Chinese spring cultivar | Illumina sequencing | Individual chromosome arms | 17Gb | 124,201 gene loci | 61% of genome sequence |

drafted. Through the availability of wheat genomic data RNA-seq and exome capture have facilitated SNP identification and thus genome specific markers which can facilitate precise mapping of grain iron and zinc traits by marker assisted selection. This can result in availing all the genomic data resources in order to biofortify crops such as wheat with zinc and iron (Borrill et al. 2014).

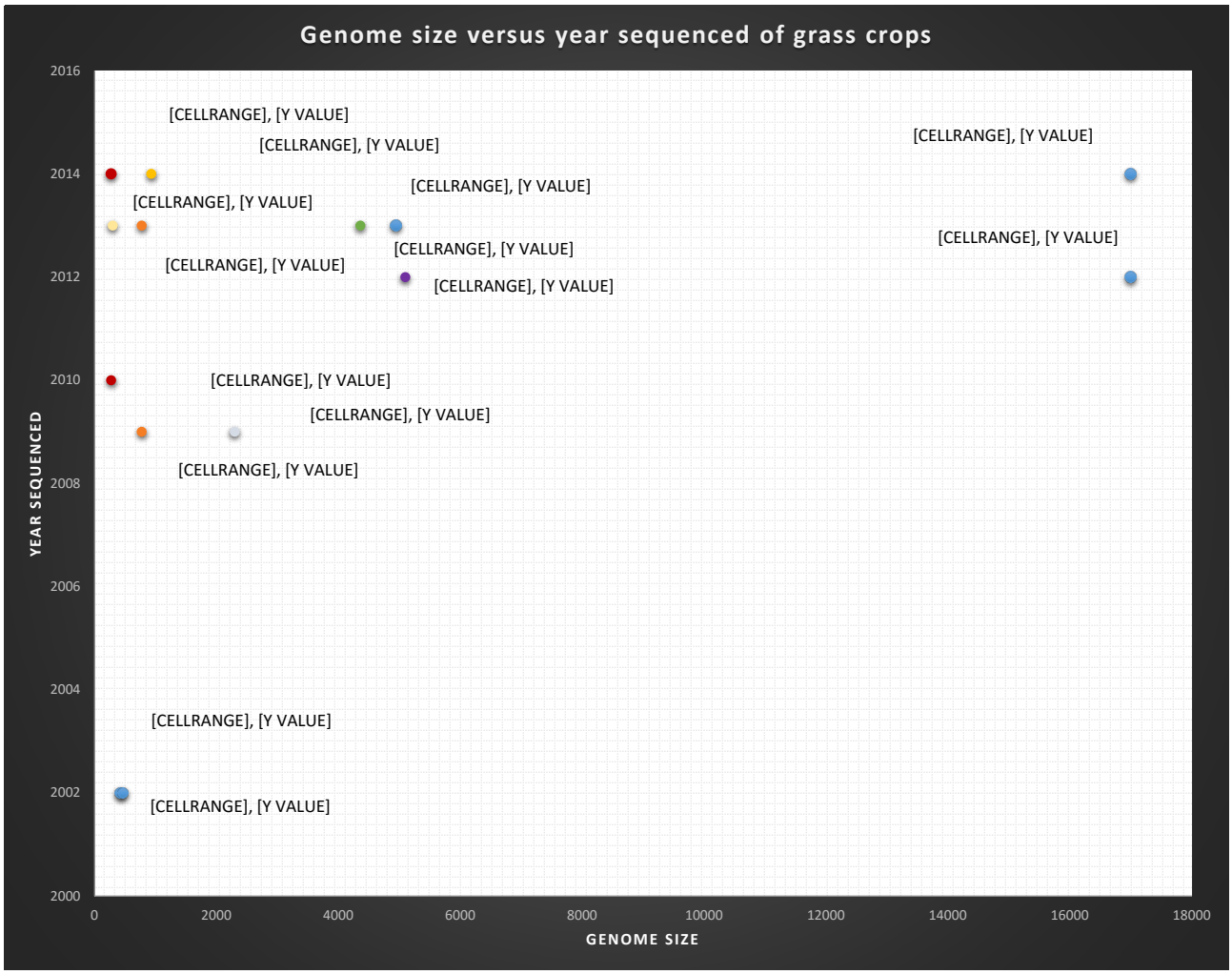
## Future prospects

This massive and continually increasing reservoir of plant genome sequence data is a huge step forwards in terms of speed and technology for plant breeders. They have become reliant on DNA marker assessment in seedlings for rapid elucidation of desired traits, rather than laboriously wasting time for a plant to mature. Although the progress is considerable in non-cereal plants and some cereals like rice, maize, *Brachypodium* and sorghum but still even the survey sequences of wheat provide a clear picture of DNA markers and genes in the vicinity of these markers and thus creating more precision for molecular breeding. Nevertheless the complete high quality genome sequence is essential for pin pointing the precise gene loci of a trait. This would facilitate in creating considerably tolerant and superior crop varieties (Pennisi 2014).

## Abbreviations

| | |
|---|---|
| BAC | Bacterial Artificial Chromosome |
| EST | Expressed Sequence Tag |
| Gb | Giga basepair |
| GWAS | Genome Wide Association Studies |
| LTR | Long Terminal Repeat |
| MB | Mega basepair |
| mRNA-Seq | mRNA Sequencing |
| NGS | Next Generation Sequencing |
| SNP | Single Nucleotide Polymorphism |

Graph 1.This graph depicts the progress over recent years with most of the progress in crop genome sequencing skewed between 2009-2014 (correlating with the progress in next generation sequencing). Note the size of wheat genome as compared to all the rest of the grasses. Identical grasses are depicted in the same colour.



**References**

Bolger ME, Weisshaar B, Scholz U, Stein N, Usadel B, Mayer KFX (2014) Plant genome sequencing - applications for crop improvement. Curr Opin Biotechnol 26:31–7. doi: 10.1016/j.copbio.2013.08.019

Borrill P, Connorton JM, Balk J, Miller AJ, Sanders D, Uauy C (2014) Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. Front Plant Sci 5:53. doi: 10.3389/fpls.2014.00053

Brenchley R, Spannagl M, Pfeifer M, Barker GLA, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo M-C, Sehgal S, Gill B, Kianian S, Anderson O, Kersey P, Dvorak J, McCombie WR, Hall A, Mayer KFX, Edwards KJ, Bevan MW, Hall N (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705–10. doi: 10.1038/nature11650

Feuillet C, Leach JE, Rogers J, Schnable PS, Eversole K (2011) Crop genome sequencing: lessons and rationales. Trends Plant Sci 16:77–88. doi: 10.1016/j.tplants.2010.10.005

Foley JA, Ramankutty N, Bennett EM, Brauman KA, Carpenter SR, Cassidy E, Gerber J, Hill J, Johnston M, Monfreda C, Mueller ND, O'Connell C, Polasky S, Ray DK, Rockström J, Sheehan J, Siebert S, Tilman D, West PC, Zaks DPM (2011) Solutions for a cultivated planet: Addressing our global food production and environmental sustainability challenges. Nature 478:337–342.

Goff S a, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296:92–100. doi: 10.1126/science.1068275

Gordon SP, Priest H, Des Marais DL, Schackwitz W, Figueroa M, Martin J, Bragg JN, Tyler L, Lee C-R, Bryant D, Wang W, Messing J, Manzaneda AJ, Barry K, Garvin DF, Budak H, Tuna M, Mitchell-Olds T, Pfender WF, Juenger TE, Mockler TC, Vogel JP (2014) Genome diversity in Brachypodium distachyon: deep sequencing of highly diverse inbred lines. Plant J 79:361–74. doi: 10.1111/tpj.12569

Grativol C, Regulski M, Bertalan M, McCombie WR, Da Silva FR, Zerlotini Neto A, Vicentini R, Farinelli L, Hemerly AS, Martienssen RA, Ferreira PCG (2014) Sugarcane genome sequencing by methylation filtration provides tools for genomic research in the genus Saccharum. Plant J 79:162–172. doi: 10.1111/tpj.12539

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W (2013) Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation.

Kurtoglu KY, Kantar M, Budak H (2014) New wheat microRNA using whole-genome sequence. Funct Integr Genomics. doi: 10.1007/s10142-013-0357-9

Mace ES, Tai S, Gilding EK, Li Y, Prentis PJ, Bian L, Campbell BC, Hu W, Innes DJ, Han X, Cruickshank A, Dai C, Frère C, Zhang H, Hunt CH, Wang X, Shatte T, Wang M, Su Z, Li J, Lin X, Godwin ID, Jordan DR, Wang J (2013) Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. Nat Commun 4:2320. doi: 10.1038/ncomms3320

Martis MM, Zhou R, Haseneyer G, Schmutzer T, Vrána J, Kubaláková M, König S, Kugler KG, Scholz U, Hackauf B, Korzun V, Schön C-C, Dolezel J, Bauer E, Mayer KFX, Stein N (2013) Reticulate evolution of the rye genome. Plant Cell 25:3685–98. doi: 10.1105/tpc.113.114553

Mayer KFX, Rogers J, Dole el J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski a. J, Sourdille P, Endo TR, Kubalakova M, Ihalikova J, Dubska Z, Vrana J, Perkova R, Imkova H, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu J, Ogihara Y, Handa H, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen O -a., Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Wright J, Pfeifer M, Spannagl M, Martis MM, Mascher M, Chapman J, Poland J a., Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Faccioli P, Colaiacovo M, Stanca a. M, Budak H, Cattivelli L, Glover N, Pingault L, Paux E, Sharma S, Appels R, Bellgard M, Chapman B,

Nussbaumer T, Bader KC, Rimbert H, Wang S, Knox R, Kilian A, Alaux M, Alfama F, Couderc L, Guilhot N, Viseux C, Loaec M, Keller B, Praud S (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science (80- ) 345:1251788–1251788. doi: 10.1126/science.1251788

Mayer KFX, Waugh R, Brown JWS, Schulman A, Langridge P, Platzer M, Fincher GB, Muehlbauer GJ, Sato K, Close TJ, Wise RP, Stein N (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711–6. doi: 10.1038/nature11543

Olsen KM, Wendel JF (2013) Crop plants as models for understanding plant adaptation and diversification. Front Plant Sci 4:290. doi: 10.3389/fpls.2013.00290

Pareek C, Smoczynski R, Tretyn A (2011) Sequencing technologies and genome sequencing. J Appl Genet 52:413–35. doi: 10.1007/s13353-011-0057-x

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev I V, Lyons E, Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein P, Kresovich S, McCann MC, Ming R, Peterson DG, Mehboob-ur-Rahman, Ware D, Westhoff P, Mayer KFX, Messing J, Rokhsar DS (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457:551–556. doi: 10.1038/nature07723

Pennisi El (2014) Agriculture. Harvest of genome data for wheat growers. Science 345:251. doi: 10.1126/science.345.6194.251

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves T a, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du F, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen W, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn MW, Ying K, Yeh C-T, Emrich SJ, Jia Y, Kalyanaraman A, Hsia A-P, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia J-M, Deragon J-M, Estill JC, Fu Y, Jeddeloh J a, Han Y, Lee H, Li P, Lisch DR, Liu S, Liu Z, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Bennetzen JL, Dawe RK, Jiang J, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen R a, Clifton SW, McCombie WR, Wing R a, Wilson RK (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–5. doi: 10.1126/science.1178534

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408:796–815. doi: 10.1038/35048692

Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol 12:e1001883. doi: 10.1371/journal.pbio.1001883

Vogel J, Garvin D, Mockler T (2010) Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature 463:763–8. doi: 10.1038/nature08747

Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L, Yang H (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science 296:79–92. doi: 10.1126/science.1068037

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# Heterosis and combining ability studies for quality protein maize

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**ABSTRACT**

Ten maize inbreds were crossed as lines to eight testers (Quality Protein Maize donors) in Line X Tester mating design to generate eighty F1 crosses. The ninety-nine genotypes including 80 F1 hybrids along with their 18 parents and a check were evaluated in Randomized Block Design to estimate the General Combining Ability (GCA), Specific Combining Ability (SCA) and Heterosis of F1 crosses. Analysis of Variance revealed significant differences among genotypes, parents and crosses for all the traits. The interaction of Line × Tester was highly significant for all the traits. Both, non-additive and additive types of gene action were observed to influence the expression of traits among the crosses. Among the lines, CM 141, V335 and V351 were promising as observed to be the superior general combiner. Cross CM 141 × CML 161 was among the best cross as the cross recorded positive and significant SCA effect, high heterosis and high per se performance for grain yield and other important traits. Standard heterosis for grain yield ranged from -56.45 to 53.31 %. Based on combining ability and hybrid vigour, the lines V335 and V351 figured to be potential lines to be converted in to QPM lines to develop local QPM hybrids. The QPM donor CML 141 based on its GCA, SCA and heterosis estimates seems to be most promising donor for conversion programme.

***Keywords*** **:** combining ability, grain yield, heterosis, maturity, quality protein maize *Zea mays* L.

***Abbreviations*** **:**
*GYP: Grain Yield Per Plot; QPM: Quality Protein Maize,*
*DMR: Directorate of Maize Research; BHU: Banaras Hindu University;*
*VPKAS: Vivekananda Institute of Hill Agriculture*

## Introduction

Maize *(Zea mays* L.) is the third most important cereal crop among the cereals grown in India and is one of the promising crops for food, feed, fodder and industrial utilization. However, its protein is deficit in essential amino acids particularly, lysine and tryptophan. To overcome this deficiency, Quality Protein Maize (QPM) donors with sufficiently higher quantity of lysine and tryptophan have been developed at CIMMYT Mexico (Vasal, 1999). The development of QPM donor stocks led to a large scale QPM germplasm development effort in different genetic backgrounds representing tropical, subtropical and highland maize germplasm involving different maturity as well as grain colour and texture. Potentially useful normal maize populations were identified for QPM conversion program. A number of advanced maize populations in CIMMYT maize program were converted to QPM using modified backcrossing-cum-recurrent selection procedure. Some of the QPM versions have given competitive performance in yield and other agronomic traits as compared to normal counterparts (Vasal, 1999).

The choice of QPM donor is just as critical as that of the recipient. The choice of a poor donor could prove to be very expensive and wasteful. In a QPM programme , a QPM line or OPV chosen as a donor for a conversion programme, by virtue of being elite, should possess good modifiers and should have high combining ability and the ability to pass them further when crossed (Vivek *et al.*, 2008). The QPM hybrid initiative at CIMMYT was introduced in 1985. Combining ability studies in QPM germplasm have been conducted and published. Inbred line development efforts have been strengthened and evaluated for combining ability. Several hybrid combinations have been tested internationally and some of them have performed equal or even better than some of the local checks (Vasal, 1999). The value of any inbred line in hybrid breeding ultimately depends on its ability to combine very well with other lines to produce superior hybrids. For development of superior QPM hybrids, the QPM lines should combine well with local inbred lines with high combining ability as well as heterosis. Heterosis has been extensively studied in maize because of (i) its large expression for grain yield (100-200 %), (ii) its intensive exploitation in hybrid breeding of maize, and (iii) the favourable biological prerequisites such as large multiplication coefficient and ease of both self and controlled cross-fertilization. Combining ability analysis and heterosis are useful to assess the potential inbred lines and also helps in identifying the nature of gene action involved in various quantitative characters. Hence, combining ability and heterosis are useful biometric tools to the plant breeders for formulating an efficient breeding programme (Jebaraj *et al*., 2010). A good number of inbreds developed recently are available in the maize breeding programme at Institute of Agricultural Sciences, BHU, Varanasi, However, combining ability of these inbred lines has not yet been studied for utilization in QPM inbred development programme. Most efficient use of such materials would be possible only when adequate information on the amount and type of genetic variation, combining ability effects and heterotic effects in the materials is available. In this context, L × T analysis (Kempthorne, 1957) has been widely used for evaluation of inbred lines by crossing them with testers. The present investigation was undertaken for estimation of combining ability and heterosis of normal inbred lines with QPM donors as tester for initiating a successful quality protein maize conversion programme.

## Materials and methods

### *The experimental materials*

A total of ninety-nine genotypes including 80 $F_1$ crosses, their 18 parents and one check were used for the present study. Ten maize inbreds *viz.* HUZM185, HUZM97-1-2, HUZM509, HKI 287, HUZM478, V336, V341, V351, CM 141 and V335 obtained from BHU(Banaras Hindu University),Varanasi, India; VPKAS(Vivekananda Institute of Hill Agriculture), Almora, India; were used as lines (female). Many of these lines were early and medium duration. Eight tropical and subtropical Quality Protein Maize (QPM) donor inbreds *viz.* CML 141, CML 193, DMRQPM 58, HKI 164-7-6, HKI 162, CML 169, CML 176 and CML 161 obtained from Directorate of Maize Research (DMR), New Delhi, India were used as testers (males). The tester used in present study are widely used QPM donors in many national maize breeding programme to convert local lines in to QPM version and study combining ability. These testers also have good ability to discriminate the inbred lines in to different heterotic groups. The characteristic features, origin and source of these parents (lines as well as testers) are given in Table 1.The check Malviya Makka 2 is medium maturing single cross local hybrid.

### *Field plot technique and layout*

Ten lines and eight testers were crossed in a line × tester fashion in the *Kharif* (rainy) season of 2012 and in the following *Rabi* (winter) season of 2012-13 all the $F_1$s along with their parents and check were grown in Randomized Block Design (RBD) with three replications at the Agriculture Research Farm, Institute of Agricultural Sciences, BHU, Varanasi, UP, India. Varanasi is situated at 25.2° N latitude and 83.0° E longitude with an altitude of 128.93 m above mean sea level. Each experimental plot comprised 3 m long two rows whereas, row to row and plant to plant spacing were 60 cm and 25 cm, respectively. One healthy seedling per hill was maintained. Fertilizers were applied @ 160, 80 and 60 kg/ha of N, P and K, respectively. One border row was maintained at end of each replication to minimize border effect. The recommended agronomic packages of practices were adopted to raise a good and healthy crop.

### *Data collection*

Ten competitive plants in each plot were randomly selected and tagged at tasseling to record observations for yield and maturity traits. Details of observational procedure for each trait are : *Days to 50 per cent tasseling* was recorded as the number of days from planting to the day on which 50 per cent of the plants in a plot showed full tassel emergence; *Days to 50 per cent silking* was recorded as the number of days from planting to the day on which 50 per cent of

the plants in a plot produces 2-3 cm long silk; *Days to 75 per cent brown husk* was recorded as the number of days from planting to the day on which 75 per cent of plants in a plot got first husk cover on the ear dried and turned brown and grain yield/ ha was computed from grain yield per plot and expressed in t/ha by the following formula (Elmyhum, 2013):

Grain Yield = [10 × GYP (kg)] / (3.6m$^2$)

### *Statistical analysis*

The mean data for yield and maturity traits were used for statistical analysis using Windostat 9.1 software program (Indostat Services, Hyderabad). Further analysis was done according to line × tester analysis to partition the mean square due to crosses into lines, tester and line × tester interaction (Singh and Chaudhary, 1985) using Windostat 9.1 software program. Further genetic analyses were carried out for traits that showed significant differences among the genotypes excluding the check according to line × tester analysis method (Kempthorne, 1957) to partition the mean square due to crosses in to lines effect, tester effect and line × tester effect using Windostat 9.1 software program. The mid-parent heterosis (MPH), heterobeltiosis (BPH) and standard heterosis (SH) were estimated as deviation of $F_1$ value from the mid-parent, better-parent and standard check values as suggested by Matzinger *et al.* (1962); Fonsecca and Patterson (1968); Turner(1953) and Hayes *et al.* (1955), respectively. Heterosis values were mathematically calculated by using the Windostat 9.1 software program. The following formulae were used for the estimation of MPH, BPH and SH for yield and maturity traits.

Heterosis over mid-parent (MPH %) = [(F1-MP)/MP × 100]
SE (F1-MP) = $(3\ Me/2r)^{1/2}$
Heterosis over better-parent (BPH %) = [(F1-BP/BP × 100]
SE (F1-BP) = $(2\ Me/r)^{1/2}$
Heterosis over standard check (SH %) = [(F1-SC)/SC × 100]
SE (F1-SC) = $(2Me/3r)^{1/2}$

where, Me = error mean squares for parents and $F_1$s; MP = mean mid-parent value = (P1+P2)/2; P1 = mean performance of parent one; P2 = mean performance of parent two; BP = mean better-parent value; SC = mean standard-check value; r = number of replications. The significance of MPH, BPH and SH were tested by 't' test using respective SE values in all the characters.

## Results and discussion

### *Analysis of variance*

The analysis of variance revealed that treatments, crosses and parents differed significantly for all the characters, indicating sufficient genetic variability present among them which is encouraging for selection of desirable genotypes (Table 2). The mean sum of square for crosses was highly significant, which indicated the diverse performance of different cross combinations for all traits viz. days to tasseling, days to silking, days to brown husk and grain yield. The parents *versus* hybrids mean sum of squares were highly significant for all traits, indicating the presence of heterosis due to the significant difference in the mean performance of hybrids and parents.

Analysis of variance for combining ability presented in Table 3, revealed that mean squares due to line effect showed significant differences for all the characters, whereas due to tester effect significant differences were revealed for days to tasseling, days to silking and days to brown husk. This indicated that there was a high level of genetic difference brought out by the lines for all the characters while testers had its impact on days to 50% tasseling, days to 50% silking and 75% brown husk. The significant difference in variances due to line × tester interaction effect indicated that the inbred lines performed differently in their respective hybrids depending on the type of testers used. The study revealed the importance of non additive gene action for grain yield and additive gene action for maturity traits in the expression of these traits. These results are in agreement with those of Joshi *et al.* (2002), Kanagarasu *et al.* (2010), Premlatha *et al.* (2011) and Kambe *et al.* (2013), whereas contrarily Sharma *et al.* (2004) reported preponderance of additive genetic effects. The grain yield was controlled by non-additive gene action since *SCA* variance was greater than *GCA* variance (Table 4), whereas the traits like days to tasseling, days to silking, days to brown husk were controlled by additive gene action. The importance of non additive gene action for grain yield and some other traits have been reported earlier by Singh and Singh (1998), Prasad and Pramod Kumar (2003), Subramaniyan and Subbraman (2006), Jayakumar and Sundram (2007), Vijayabharathi *et al.* (2009) and Kambe *et al.* (2013) whereas contrarily importance of additive gene effects was reported by Alamnie *et al.* (2006). So additive as well as non additive type of gene action prevails in expression of the grain yield per plant.

### *General combining ability (GCA) effects*

A wide range of variability for *GCA* effects was observed among the parents for different characters (Table 5). Estimates of GCA effects for grain yield showed that out of ten inbred lines studied, four expressed positive and highly significant *GCA* effect. Inbred line CM 141 exhibited the maximum *GCA*

effect (10.55 t/ha) whereas HUZM97-1-2 exhibited the lowest and negative *GCA* effect (-9.71 t/ha). Inbred line V351 exhibited desirable significant *GCA* effect for all the traits. Among the testers, CML 141 was the best as it expressed highest *GCA* effect (3.23 t/ha) whereas HKI 162 exhibited the lowest *GCA* effect (-2.30 t/ha) for grain yield. It was observed from the *GCA* effects that none of the parents individually showed good general combiner for all the characters. Both positive and negative *GCA* effects have been reported in maize by various studies (Fan *et al.*, 2008; Kambe *et al.*, 2013; Abrha *et al*, 2013 and Elmyhum, 2013). Both negative and positive *GCA* effects were observed for days to tasseling, silking and brown husk indicating possibilities of early as well as late hybrids. The V351 potential line for early hybrids as it exhibited highest negative and significant *GCA* effect (-3.26 days) followed by V335 (-1.47 days) and CM 141(-1.26 days) for days to tasseling. The similar trend was observed for days to silking, whereas for days to brown husk, V351 displayed maximum negative *GCA* effect (-2.98 days) followed by HUZM185 (-1.90 days) and HUZM97-1-2 (-1.31 days). The high *GCA* effect in negative direction indicates that they were good general combiner for earliness. Higher estimates of *GCA* effect in negative direction are desirable for days to brown husk. Among the testers, DMRQPM 58 was good general combiner for days to tasseling, silking and brown husk with *GCA* estimates of -1.10, -0.57 and -2.12 days, respectively. Xingming *et al*. (2002) found CML 161 as good general combiner in their study. Uddin *et al.* (2006) and Sundararajan and Kumar (2011) revealed the importance of negative *GCA* effect for days to tasseling and days to silking to develop early maturing varieties. Non QPM Parents *viz.*, V335, CM 141, V351 and V341; and QPM lines CML 141, CML 161 and DMRQPM 58, were identified as good general combiners and these parents could be used in hybridization programme to develop specific local hybrids.

### *Specific combining ability (SCA) effects*

For grain yield estimates due to *SCA* effect were observed in both, negative and positive directions (Table 6). High *SCA* estimates for yield of the crosses CM 141 × CML 161, HUZM509 × CML 176, V351 × CML 141 and V335 × CML 141 indicated high and desirable specific combining ability, whereas crosses HUZM478 × CML 161, HKI 287 × HKI 164-7-6, V335 × CML 176 and CM 141 × DMRQPM 58 were poor specific combiners for grain yield. The Cross CM 141 × CML 161 exhibited maximum significant and positive *SCA* effect of 21.64 t/ha followed by V351 × CML 141 (15.79 t/ha).The higher estimates of *SCA* effects in the present study is deviation from the prediction based on their parental performance. The crosses with significant and positive estimates of *SCA* effect are very useful for QPM maize hybrid development programme. The results of the current study are in agreement with the findings of Abrha *et al.* (2013) who reported high and significant *SCA* effects in most of the crosses they studied for grain yield in maize. In case of days to tasseling, cross HUZM185 × DMRQPM 58 expressed highest negative *SCA* effect (-3.36 days) followed by HUZM509 × HKI 164-7-6 (-3.08 days) and CM 141 × CML 161 (-2.71 days), whereas HUZM478 × DMRQPM 58 expressed high and positive *SCA* effect (2.47 days) followed by V 336 × CML 176 (2.22 days). For days to silking, cross CM 141 × CML 161 (-3.82 days) followed by V341 × CML 176 (-2.97 days) and HUZM509 × HKI 164-7-6 (-2.63 days) were promising for earliness, whereas crosses HKI 287 × HKI 164-7-6 (3.54 days) followed by HUZM509 × CML 141 (3.17 days) indicated their tendency for lateness. In case of days to brown husk, Cross V351 × DMRQPM 58 (-3.79 days) followed by HUZM509× CML 169 (-3.30 days) and CM 141 × CML 161(-2.76 days) were effective for earliness, whereas V335 × HKI 162 (2.30 days) was promising for developing late hybrids. In the present study we are looking for early QPM hybrids so the negative *SCA* estimates are desirable. Uddin *et al.* (2006) reported eleven and fourteen hybrids with negative *SCA* effects for days to tasseling and days to silking, respectively. The present results showed that, the crosses (CM 141 × CML 161 and V335 × CML 141) with higher estimates of *SCA* effect involved the parents with higher *GCA* effect for grain yield. Ivy and Howlader (2000) reported that *GCA* effect of the parents did not reflect in their *SCA* effect for all the traits. However, Amiruzzaman *et al*. (2011) pointed out that the *SCA* is a result of the interaction of G*CA* effects of the parents and that it can improve or deteriorate the hybrid vigour of a particular trait.

A critical evaluation of the results particularly for specific combining ability effects showed that few cross combinations exhibited desirable significant *SCA* effects for all the characters. The highest yielding cross CM 141 × CML 161 also revealed significant and positive *SCA* effects for grain yield along with significant negative *SCA* effects for early maturing traits and was the outcome of high (CM 141) × moderate (CML 161) general combining parents. Chaudhary *et al.* (2000) and Surya and Ganguli (2004) have also reported high positive specific combining ability effects along with high

*per se* performance for grain yield. The superiority of crosses involving high × low combiners could be explained as the result of interaction between positive alleles from good combiners and negative alleles for the poor combiners. The high yield of such crosses would be non-fixable and thus could be exploited for heterosis breeding. The superior cross combinations involving low × low general combiners could result from over dominance and epistasis.

### *Heterosis*

The crosses displayed heterosis in both negative as well as positive direction for all the characters (Table 7). For grain yield, fifteen, sixty two and seventy crosses exhibited positive heterosis over standard check, better parent and mid parent, respectively. The heterosis for grain yield over standard check, better parent and mid parent ranged from -56.45 to 53.31%, from -40.65 to 278.57% and from -30.11 to 294.68%, respectively. The maximum standard heterosis for grain yield was exhibited by the cross CM 141 × CML 161(53.31%) followed by V335 × CML 141 (34.71%). This may be mentioned here that the lines involve in development of best hybrids have come from diverse genetic background. The hybrids with over 20 per cent of Standard heterosis have high commercial value in almost all crops with special reference to maize. The result is in conformity with that of Saxena *et al*. (1998) who opined that hybrids produced from inbred lines having diverse origins tended to have greater consistent yield levels than hybrids of parental lines originating from the narrow source population. The present results particularly the parents of best yielding cross CM 141 × CML 161 have its origin from diverse maize population viz. Pool 33 and P 25 QPM (Table 1), respectively. These results are in agreement with Dagne (2008). In case of days to tasseling, negative estimates of heterosis are desirable in maize hybrids. Twenty two, forty and seventy crosses expressed negative standard, better parent and mid parent heterosis, respectively**;** for days to tasseling however, high and significant negative standard heterosis (-4.48%) was manifested by HUZM185 × DMRQPM 58 and V351 × HKI 164-7-6 followed by V351 × CML 161(-3.79%) for this trait. For days to silking, the maximum significant and negative standard heterosis was expressed by cross V351 × HKI 164-7-6 (-5.69%) followed by V351 × CML 161(-4.68%). In case of days to brown husk, the extent of standard heterosis was in positive direction, whereas better parent and mid parent heterosis were mostly in negative direction, however, eight crosses manifested significant and negative standard heterosis for this trait. The maximum significant and negative standard heterosis was recorded by cross V351 × HKI 164-7-6 (-5.00%) followed by cross V351 × DMRQPM 58 (-4.25%). Singh (1979) and Amiruzzaman *et al.* (2013) reported that earliness is associated with days to silking. Heterosis responses of hybrids largely depend on genetic diversity of parents and environmental conditions (Hallauer and Miranda, 1988).

### *Per se performance along with gca, sca effects and heterosis*

Five best crosses for grain yield per hectare, days to tasseling, days to silking and days to brown husk based on *per se* performance along with *SCA* effects, *GCA* effects and heterosis are presented in Table 8. The crosses selected on the basis of *per se* performance had high positive *SCA* effects and standard heterosis for grain yield. For days to tasseling, days to silking and days to brown husk, some of the crosses selected on the basis of *per se* performance had high negative *SCA* effects and standard heterosis. Out of eighty crosses, cross HUZM185 × DMRQPM 58 recorded minimum *per se* performance along with significant negative SCA effect and standard heterosis for days to tasseling followed by CM 141 × CML 161._Further, the cross CM 141 × CML 161 also recorded lower *per se* performance along with significant negative SCA effect and standard heterosis for days to silking, whereas, cross V351 × HKI 164-7-6 recorded minimum *per se* performance along with significant and negative SCA effect and standard heterosis for days to brown husk. None of the crosses was found desirable simultaneously for all the characters i.e., different crosses expressed desirable significant *SCA* effects and standard heterosis for different characters. However, out of eighty crosses, crosses CM 141 × CML 161 and V351 × HKI 164-7-6 were found desirable simultaneously for most of the characters with significant and negative *SCA* effects and standard heterosis for earliness. The results obtained in the present study are indicating similar trend as reported by Pal and Prodhan (1994), Rao et *al.* (1996), Mahto and Gunguli (2003), Malik *et al.* (2004) and Kanagarasu *et al.* (2010) for grain yield. It is evident that the best five crosses exhibiting high *per se* performance along with desirable *SCA* effects for grain yield had involvement of parents with high as well as low *GCA* estimates.

Based on the overall performance of the hybrids and parental lines, some of the lines could be used as parents of single cross hybrid maize with high quality and high yield potential. Hence, the information from this study may possibly be useful for researchers who would like to develop high yielding and high quality protein inbred lines and hybrids.

Table 1. Characteristic Features, Pedigree, Sources of Lines (10) and Testers (8) used in present study

| Inbred Name | Pedigree & Source | Characteristic Features |
|---|---|---|
| **Local Inbred Lines (Lines)** | | |
| HUZM185 | Seedtec-1250-1-2-2-1-# # BHU, Varanasi | Yellow, Flint kernel, Medium duration, Tassels and Leaf angle is small, Tall height and Good grain yield. |
| HUZM97-1-2 | Devaki × VCZ BHU, Varanasi | Yellow kernel, Early duration, Wide leaf angle. |
| HUZM509 | BHU, Varanasi | Yellow kernel, Late duration, Leaf angle small with narrow tassel angle. |
| HKI 287 | CML 287, Karnal | Yellow kernel, Late duration, Leaf and Tassel angle is wide, Tall height with high grain yield. |
| HUZM478 | BH-3427, BHU, Varanasi | Yellow, Flint kernel, Late duration, Leaf angle is wide with narrow tassel angle. |
| V336 | CML 145,P 63 CDHC 181-3-2-1-4 #2-BBBB #F-BBBBB # VPKAS, Almora | Yellow, Flint kernel, Medium duration, Leaf and Tassel angle is small, Straight leaf attitude. |
| V341 | Mexico Acc No. 3136@-3-2-3-8-1, VPKAS Almora | Yellow, Flint kernel, Early duration, Tall with drooping leaf attitude, straight tassel. |
| V351 | Shakti (So) HE 25,VPKAS, Almora | Orange yellow, Flint kernel, Early duration, Straight leaf attitude and better grain yield. |
| CM 141 | Pool 33 (Alm), VPKAS, Almora | Yellow kernel, Late duration, Curved tassel. |
| V335 | TZI-25, VPKAS, Almora | Orange, Flint kernel, Medium duration, Straight tassel. |
| **QPM Lines (Testers)** | | |
| CML 141 | Pop 62, CIMMYT | White, Flint kernel, Late duration, Dwarf height. |
| CML 193 | CY0162-B-1-1-B (S.Africa),CIMMYT | Yellow, Flint, Medium to late duration, Medium height |
| DMRQPM 58 | Shakti 1, DMR | Orange yellow, Flint kernel, Early duration, Tall height |
| HKI 164-7-6 | CML164, Karnal | Yellow, Semi Dent, Late duration, Medium height, Dark green plant, Sparse tassel. |
| HKI 162 | CML162, Karnal | Yellow, Flint kernel, Late duration, Tall plant, Small tassel, Erect and Narrow leaves. |
| CML 169 | P 26 QPM, CIMMYT | Yellow, Flint kernel, Medium duration, Curved tassel. |
| CML 176 | (P 63-12-2-1/P67-5-1-1)-1-2-B-B, CIMMYT | White kernel, Medium to Late duration. |
| CML 161 | P 25 QPM,CIMMYT | Orange yellow, Flint kernel, Late duration, Dwarf height with small leaf angle and straight leaf attitude. |

Table 2. Analysis of variance for parents and crosses for yield and maturity traits in maize

| Sources of Variation | DF | Mean Square | | | |
|---|---|---|---|---|---|
| | | Grain Yield | Days to 50% tasseling | Days to 50% silking | Days to 75% Brown Husk |
| Replications | 2 | 0.12 | 0.18 | 0.13 | 0.82 |
| Treatments | 97 | 3.56** | 21.65** | 25.96** | 21.53** |
| Parents | 17 | 1.75** | 35.41** | 42.82** | 42.58** |
| Parents (Line) | 9 | 2.24** | 32.46** | 42.36** | 63.93** |
| Parents (Testers) | 7 | 0.75** | 14.55** | 17.52** | 19.05** |
| Parents (L vs T) | 1 | 4.35** | 208.03** | 224.13** | 15.17* |
| Parents vs Crosses | 1 | 73.28** | 258.63** | 294.50** | 251.08** |
| Crosses | 79 | 3.07** | 15.69** | 18.93** | 14.09** |
| Error | 194 | 0.20 | 2.06 | 3.27 | 3.72 |
| Total | 293 | 1.31 | 8.53 | 10.76 | 9.59 |

* and **, significant at 5 and 1 per cent level of significance, respectively.

Table 3. Analysis of variance of combining ability for yield and maturity traits in maize

| Sources of Variation | D F | Mean Square | | | |
|---|---|---|---|---|---|
| | | Grain Yield (t/ha) | Days to 50% tasseling | Days to 50% silking | Days to 75% Brown Husk |
| Replications | 2 | 0.40 | 0.58 | 0.43 | 4.39 |
| Crosses | 79 | 3.07** | 15.69** | 18.93** | 14.09** |
| Line Effect | 9 | 11.23** | 73.91** | 76.71** | 59.13** |
| Tester Effect | 7 | 1.16 | 25.60** | 45.66** | 26.66** |
| Line × Tester Effect | 63 | 2.12** | 6.27** | 7.70** | 6.26** |
| Error | 158 | 0.23 | 2.11 | 3.17 | 3.22 |
| Total | 239 | 1.17 | 6.58 | 8.35 | 6.82 |

* and **, significant at 5 and 1 per cent level of significance, respectively.

Table 4. Estimates of components of variance (s2 A and s2 D ) and degree of dominance for yield and maturity traits in maize

| Traits | Components | | | | |
|---|---|---|---|---|---|
| | $\sigma^2$ gca | $\sigma^2$ sca | $\sigma^2$ D | $\sigma^2$ A | Degree of Dominance |
| **Grain Yield (t/ha)** | 0.22 | 0.63 | 0.63 | 0.44 | 1.20 |
| **Days to 50 % tasseling** | 1.77 | 1.41 | 1.41 | 3.53 | 0.63 |
| **Days to 50 % silking** | 2.15 | 1.48 | 1.48 | 4.29 | 0.59 |
| **Days to 75 % Brown Husk** | 1.45 | 0.85 | 0.85 | 2.90 | 0.54 |

Table 5. General combining ability (GCA) effects of parents for yield and maturity traits in maize

| S.No. | Inbreds | Grain Yield | Days to 50% tasseling | Days to 50% silking | Days to 75% Brown Husk |
|---|---|---|---|---|---|
| Lines | | | | | |
| 1 | HUZM185 | -0.22* | -0.68* | -0.30 | -1.90** |
| 2 | HUZM97-1-2 | -0.97** | -0.68* | 0.53 | -1.31** |
| 3 | HUZM509 | -0.66** | 1.41** | 1.20** | 0.77 |
| 4 | HKI 287 | -0.73** | 0.91** | 1.36** | -0.02 |
| 5 | HUZM478 | -0.04 | 2.49** | 1.95** | 1.19** |
| 6 | V336 | 0.01 | 1.62** | 1.45** | 1.10** |
| 7 | V341 | 0.30** | 0.91** | 0.40 | 1.52** |
| 8 | V351 | 0.26** | -3.26** | -3.85** | -2.98** |
| 9 | CM 141 | 1.06** | -1.26** | -0.85* | 1.56** |
| 10 | V335 | 0.99** | -1.47** | -1.89** | 0.06 |
| SE± GCA (Line) | | 0.09 | 0.29 | 0.37 | 0.39 |
| CD 5 % GCA (Line) | | 0.18 | 0.58 | 0.73 | 0.78 |
| CD 1 % GCA (Line) | | 0.24 | 0.76 | 0.96 | 1.03 |
| SE± Gi- Gj (Line) | | 0.13 | 0.41 | 0.52 | 0.56 |
| CD 5 % Gi- Gj (Line) | | 0.25 | 0.82 | 1.03 | 1.10 |
| CD 1 % Gi- Gj (Line) | | 0.33 | 1.08 | 1.36 | 1.45 |

*Continuing table 5*

| S.No. | Inbreds | Grain Yield | Days to 50% tasseling | Days to 50% silking | Days to 75% Brown Husk |
|---|---|---|---|---|---|
| **Testers** | | | | | |
| 11 | CML 141 | 0.32** | 0.1 | -0.34 | -0.15 |
| 12 | CML 193 | -0.21** | 1.20 ** | 1.43** | 0.75* |
| 13 | DMRQPM 58 | -0.04 | -1.10** | -0.57 | -2.12** |
| 14 | HKI 164-7-6 | 0.14 | -1.47** | -2.20** | 0.25 |
| 15 | HKI 162 | -0.23** | -0.07 | -0.04 | 0.45 |
| 16 | CML 169 | -0.14 | 0.80** | 1.43** | 0.35 |
| 17 | CML 176 | 0.00 | 0.70** | 0.93** | 0.81* |
| 18 | CML 161 | 0.16** | -0.17 | -0.64 | -0.32 |
| SE ±GCA(Tester) | | 0.08 | 0.26 | 0.33 | 0.35 |
| CD 5 % GCA (Tester) | | 0.16 | 0.52 | 0.65 | 0.70 |
| CD 1 % GCA (Tester) | | 0.21 | 0.68 | 0.86 | 0.92 |
| SE ±Gi – Gj (Tester) | | 0.11 | 0.37 | 0.47 | 0.50 |
| CD 5 % Gi- Gj (Tester) | | 0.23 | 0.73 | 0.92 | 0.98 |
| CD 1 % Gi- Gj (Tester) | | 0.30 | 0.97 | 1.22 | 1.30 |

* and **, significant at 5 and 1 per cent level of significance, respectively.

Table 6. Specific combining ability (SCA) effects of F1 crosses for yield and maturity traits in maize

| S.No. | Crosses | Grain Yield | Days to 50% tasseling | Days to50% silking | Days to 75% Brown Husk |
|---|---|---|---|---|---|
| **1** | HUZM185 × CML 141 | 0.58* | -1.56 | -1 | -1.47 |
| **2** | HUZM185 × CML 193 | 0.88** | -1.33 | -1.76 | -0.04 |
| **3** | HUZM185 × DMRQPM 58 | 0.74** | -3.36** | -1.76 | -0.5 |
| **4** | HUZM185 × HKI 164-7-6 | 0.54* | 1.67 | 0.87 | -0.87 |
| **5** | HUZM185 × HKI 162 | -0.94** | 1.94* | 0.7 | -0.07 |
| **6** | HUZM185 × CML 169 | -0.14 | -0.59 | -0.43 | -0.3 |
| **7** | HUZM185 × CML 176 | -0.69** | 1.84* | 1.4 | 1.56 |
| **8** | HUZM185 × CML 161 | -0.97** | 1.38 | 1.97 | 1.7 |
| **9** | HUZM97-1-2× CML 141 | -0.81** | 0.11 | -0.16 | 1.61 |
| **10** | HUZM97-1-2× CML 193 | 0.38 | -1.33 | -2.60* | -2.62* |
| **11** | HUZM97-1-2× DMRQPM 58 | -0.38 | -0.69 | 0.07 | -0.09 |
| **12** | HUZM97-1-2× HKI 164-7-6 | -0.13 | 0.68 | 0.37 | 1.88 |
| **13** | HUZM97-1-2× HKI 162 | 0.92** | -0.06 | -0.46 | -0.99 |
| **14** | HUZM97-1-2× CML 169 | 0.07 | 0.41 | 0.4 | 0.11 |
| **15** | HUZM97-1-2× CML 176 | -0.06 | 0.51 | 1.57 | -0.69 |
| **16** | HUZM97-1-2× CML 161 | 0.01 | 0.38 | 0.8 | 0.78 |
| **17** | HUZM509 × CML 141 | -1.2** | 2.03* | 3.17** | 2.2 |
| **18** | HUZM509 × CML 193 | 0.47 | 1.92 | 0.74 | 0.63 |
| **19** | HUZM509 × DMRQPM 58 | 0.10 | -0.77 | -0.26 | 0.16 |

*Continuing table 6*

| S.No | Crosses | Grain Yield | Days to 50% tasseling | Days to 50% silking | Days to75% Brown Husk |
|---|---|---|---|---|---|
| **20** | HUZM509 × HKI 164-7-6 | -0.33 | -3.08** | -2.63* | 0.13 |
| **21** | HUZM509 × HKI 162 | -0.38 | -0.81 | -0.46 | -0.07 |
| **22** | HUZM509 × CML 169 | -0.19 | 0.32 | -0.26 | -3.30** |
| **23** | HUZM509 × CML 176 | 1.46** | -0.57 | -1.1 | 1.23 |
| **24** | HUZM509 × CML 161 | 0.14 | 0.96 | 0.8 | -0.97 |
| **25** | HKI 287 × CML 141 | -0.03 | -0.14 | 0 | -2.01 |
| **26** | HKI 287 × CML 193 | -0.13 | 1.42 | 0.9 | 0.75 |
| **27** | HKI 287 × DMRQPM 58 | -0.46 | -0.61 | -0.1 | -1.38 |
| **28** | HKI 287 × HKI 164-7-6 | -1.34** | 2.09* | 3.54** | 1.59 |
| **29** | HKI 287 × HKI 162 | 0.20 | -0.64 | -0.96 | -0.28 |
| **30** | HKI 287 × CML 169 | 0.87** | -1.51 | -1.76 | 1.15 |
| **31** | HKI 287 × CML 176 | 0.68** | -0.08 | -0.93 | 0.69 |
| **32** | HKI 287 × CML 161 | 0.22 | -0.54 | -0.7 | -0.51 |
| **33** | HUZM478 × CML 141 | -0.96** | -0.06 | -0.58 | -0.22 |
| **34** | HUZM478 × CML 193 | 0.85** | 0.51 | 0.32 | -0.45 |
| **35** | HUZM478 × DMRQPM 58 | 0.00 | 2.47** | 1.32 | 0.75 |
| **36** | HUZM478 × HKI 164-7-6 | 0.41 | -1.16 | -0.38 | -0.29 |
| **37** | HUZM478 × HKI 162 | 0.47 | -0.89 | -1.55 | -1.49 |
| **38** | HUZM478 × CML 169 | 0.14 | -0.76 | 0.32 | 0.28 |
| **39** | HUZM478 × CML 176 | 0.55* | -0.32 | -0.18 | -0.52 |
| **40** | HUZM478 × CML 161 | -1.47** | 0.21 | 0.72 | 1.95 |
| **41** | V 336 × CML 141 | -0.13 | -0.18 | 0.25 | -0.47 |
| **42** | V 336 × CML 193 | -0.15 | 0.38 | 0.82 | -0.04 |
| **43** | V 336 × DMRQPM 58 | 0.03 | -2.65** | -2.51* | -0.84 |
| **44** | V 336 × HKI 164-7-6 | 0.89** | -0.28 | -1.21 | 0.13 |
| **45** | V 336 × HKI 162 | -0.17 | -1.35 | -1.38 | -0.74 |
| **46** | V 336 × CML 169 | 0.41 | 0.45 | 0.49 | 1.03 |
| **47** | V 336 × CML 176 | -0.78** | 2.22** | 1.65 | -1.1 |
| **48** | V 336 × CML 161 | -0.09 | 1.42 | 1.89 | 2.03 |
| **49** | V 341 × CML 141 | 0.57* | -0.14 | -0.7 | -0.89 |
| **50** | V 341 × CML 193 | -0.28 | 0.76 | 1.2 | 0.21 |
| **51** | V 341 × DMRQPM 58 | 0.30 | 1.39 | 0.2 | 1.75 |
| **52** | V 341 × HKI 164-7-6 | -0.68** | 2.09** | 3.16** | -0.62 |

*Continuing table 6*

| S.No | Crosses | Grain Yield | Days to 50% tasseling | Days to50% silking | Days to 75% Brown Husk |
|---|---|---|---|---|---|
| **53** | V 341 × HKI 162 | 0.93** | 0.03 | 0 | 0.85 |
| **54** | V 341 × CML 169 | 0.32 | -0.51 | -0.14 | 0.61 |
| **55** | V 341 × CML 176 | 0.02 | -2.41** | -2.97** | -1.52 |
| **56** | V 341 × CML 161 | -1.18** | -1.21 | -0.74 | -0.39 |
| **57** | V 351 × CML 141 | 1.57** | -0.64 | 0.55 | 1.61 |
| **58** | V 351 × CML 193 | -1.12** | -0.41 | -0.22 | 1.38 |
| **59** | V 351 × DMRQPM 58 | 0.85** | 0.89 | -0.22 | -0.42 |
| **60** | V 351 × HKI 164-7-6 | -0.51** | -0.41 | -0.92 | -3.79** |
| **61** | V 351 × HKI 162 | 0.29 | 0.52 | 0.58 | 0.01 |
| **62** | V 351 × CML 169 | -1.04** | 0.66 | -0.22 | 1.11 |
| **63** | V 351 × CML 176 | -0.93** | 0.43 | 1.95 | 1.98 |
| **64** | V 351 × CML 161 | 0.89** | -1.04 | -1.49 | -1.89 |
| **65** | CM 141 × CML 141 | -0.69** | 0.69 | -0.79 | 0.07 |
| **66** | CM 141 × CML 193 | -0.15 | 0.26 | 2.11* | 0.84 |
| **67** | CM 141 × DMRQPM 58 | -1.29** | 1.89* | 1.78 | 1.04 |
| **68** | CM 141 × HKI 164-7-6 | 0.44 | -0.41 | -1.92 | 1.67 |
| **69** | CM 141 × HKI 162 | -1.06** | 0.86 | 2.25* | 0.47 |
| **70** | CM 141 × CML 169 | -0.38 | 1.33 | 2.11* | 0.9 |
| **71** | CM 141 × CML 176 | 0.98** | -1.91* | -1.72 | -2.23* |
| **72** | CM 141 × CML 161 | 2.16** | -2.71** | -3.82** | -2.76* |
| **73** | V 335 × CML 141 | 1.16** | -0.1 | -0.75 | -0.43 |
| **74** | V 335 × CML 193 | -0.74** | -2.20** | -1.51 | -0.66 |
| **75** | V 335 × DMRQPM 58 | 0.09 | 1.43 | 1.49 | -0.46 |
| **76** | V 335 × HKI 164-7-6 | 0.71** | -1.2 | -0.88 | 0.17 |
| **77** | V 335 × HKI 162 | -0.23 | 0.4 | 1.29 | 2.30* |
| **78** | V 335 × CML 169 | -0.04 | 0.2 | -0.51 | -1.6 |
| **79** | V 335 × CML 176 | -1.22** | 0.3 | 0.32 | 0.6 |
| **80** | V 335 × CML 161 | 0.28 | 1.17 | 0.55 | 0.07 |
| SE± (SCA) | | 0.26 | 0.83 | 1.04 | 1.11 |
| CD 5 % | | 0.51 | 1.64 | 2.06 | 2.20 |
| CD 1 % | | 0.67 | 2.16 | 2.72 | 2.90 |
| SE± (Sij - Skl) | | 0.36 | 1.17 | 1.48 | 1.57 |
| CD 5 % | | 0.72 | 2.31 | 2.92 | 3.11 |
| CD 1 % | | 0.95 | 3.05 | 3.85 | 4.10 |

* and **, significant at 5 and 1 per cent level of significance, respectively.

Table 7. Percent (%) Standard heterosis (SH), better parent heterosis (BPH) and mid parent heterosis (MPH) for yield and maturity traits in Maize

| S. No. | Crosses | Grain Yield | | | Days to 50% tasseling | | | Days to 50% silking | | | Days to 75% Brown Husk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH |
| 1 | HUZM185×CML 141 | -2.48 | 67.14** | 101.97* * | -1.38 | -0.69 | -5.61** | -0.33 | 0.34 | -5.10** | -2.75* | -2.75* | -5.24** |
| 2 | HUZM185×CML 193 | -7.44 | 58.64** | 63.98 * * | 0.00 | 0.69 | -2.68** | 0.67 | 1.35 | -2.27 | -1.00 | -1.00 | -2.70** |
| 3 | HUZM185×DMRQPM 58 | -6.61 | 41.25** | 50.07 * * | -4.48** | -3.82** | -5.30** | -1.34 | -0.67 | -2.48 | -3.50** | -2.77* | -3.14** |
| 4 | HUZM185×HKI 164-7-6 | -7.02 | 59.35** | 69.68 * * | 0.34 | 1.04 | -1.02 | -0.33 | 0.34 | -1.65 | -2.00 | -2.00 | -2.61* |
| 5 | HUZM185×HKI 162 | -45.45** | -6.52 | 8.55 | 2.07 | 2.78* | 0.34 | 1.67 | 2.36 | -0.65 | -1.25 | -1.25 | -1.37 |
| 6 | HUZM185×CML 169 | -27.27** | 15.79 | 20.05 | 0.34 | 1.04 | -2.51* | 2.01 | 2.69 | -0.97 | -1.50 | -1.50 | -2.84** |
| 7 | HUZM185×CML 176 | -35.54** | 10.48 | 23.22 | 2.76* | 3.47** | 0.34 | 3.34* | 4.04** | 0.49 | 0.25 | 0.25 | -1.11 |
| 8 | HUZM185×CML 161 | -38.02** | 6.23 | 25.42 | 1.38 | 2.08 | -1.67 | 2.34 | 3.03 | -0.97 | -0.50 | -0.50 | -1.49 |
| 9 | HUZM97-1-2×CML 141 | -46.69** | -39.15** | -15.13 | 0.34 | 3.19* | -3.00** | 1.34 | 3.41* | -2.88* | 0.00 | 4.71** | -0.37 |
| 10 | HUZM97-1-2×CML 193 | -33.06** | -23.58** | -5.81 | 0.00 | 2.84* | -1.69 | 0.67 | 2.73 | -1.63 | -2.50* | 2.09 | -2.01 |
| 11 | HUZM 97-12×DMRQPM58 | -45.45** | -37.74** | -29.03 ** | -1.72 | 1.06 | -1.55 | 1.00 | 3.41* | 0.50 | -2.75* | 1.83 | -0.13 |
| 12 | HUZM97-1-2×HKI 164-7-6 | -36.43** | -27.44 | -8.43 | -0.69 | 2.13 | -1.03 | 0.00 | 2.05 | -0.66 | 0.50 | 5.24** | 2.16 |
| 13 | HUZM97-1-2×HKI 162 | -22.31** | -11.32 | 19.75 | 0.00 | 2.84* | -0.68 | 1.34 | 3.41* | -0.33 | -1.50 | 3.14* | 0.64 |
| 14 | HUZM97-1-2CML 169 | -38.02** | -29.25** | -17.58* | 1.38 | 4.26** | -0.51 | 3.68** | 5.80** | 1.31 | -0.75 | 3.93** | 0.13 |
| 15 | HUZM97-1-2×CML 176 | -38.02** | -29.25** | -7.41 | 1.38 | 4.26** | 0.00 | 4.35** | 6.48** | 2.13 | -1.00 | 3.66** | -0.13 |
| 16 | HUZM97-1-2×CML 161 | -33.06** | -23.58** | 4.52 | 0.34 | 3.19* | -1.69 | 2.01 | 4.10** | -0.65 | -0.75 | 3.93 | 0.51 |

| S. No. | Crosses | Grain Yield | | | Days to 50% tasseling | | | Days to 50% silking | | | Days to 75% Brown Husk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH |
| **17** | HUZM509×CML 141 | -49.72** | 15.43 | 23.27 | 4.48** | 0.66 | -2.10* | 5.35** | -0.63 | -2.78* | 2.00 | -0.49 | -1.81 |
| **18** | HUZM509 × CML 193 | -24.79** | 37.88** | 53.33** | 5.52** | 1.66 | 0.49 | 4.68** | -1.26 | -1.57 | 1.50 | -0.98 | -1.46 |
| **19** | HUZM509 × DMRQPM 58 | -28.93** | 7.50 | 29.62* | 0.34 | -2.02 | -2.68** | 1.67 | -1.30 | -2.72* | -1.00 | -0.25 | -1.86 |
| **20** | HUZM509×HKI 164-7-6 | -34.21** | 28.39 | 38.80** | -2.41* | -5.67** | -5.82** | -2.34 | -5.50** | -6.71** | 0.75 | -0.49 | -1.10 |
| **21** | HUZM509×HKI 162 | -42.98** | 30.93 | 33.08* | 1.38 | -2.33* | -2.49* | 2.01 | -3.17* | -3.48** | 0.75 | 0.50 | -0.62 |
| **22** | HUZM509×CML 169 | -37.19** | 0.00 | 18.10 | 3.45** | -0.33 | -1.64 | 3.68** | -2.21 | -2.52* | -1.75 | -4.15** | -4.26** |
| **24** | HUZM509 × CML 161 | -23.97** | 74.57** | 80.92** | 3.10* | -0.66 | -2.13* | 2.68 | -3.15* | -3.76** | -0.50 | -2.45* | -2.69* |
| **25** | HKI 287 × CML 141 | -25.62** | 1.35 | 33.28** | 1.72 | -1.99 | -4.68** | 2.34 | -0.65 | -4.23** | -1.75 | -5.07** | -5.87** |
| **26** | HKI 287 × CML 193 | -38.84** | -16.67 | -4.39 | 4.48** | 0.66 | -0.49 | 5.02** | 1.95 | 0.16 | 1.00 | -2.42* | -2.42* |
| **27** | HKI 287 × DMRQPM 58 | -42.15** | -21.17* | -17.06 | 0.00 | -2.36* | -3.01** | 2.01 | -0.97 | -0.97 | -2.75* | -2.02 | -4.07** |
| **28** | HKI 287 × HKI164-7-6 | -56.45** | -40.65** | -30.11** | 2.41* | -1.00 | -1.16 | 4.01** | 0.97 | 0.81 | 1.25 | 0.00 | -1.10 |
| **29** | HKI 287 × HKI 162 | -32.16** | -7.66 | 17.43 | 1.03 | -2.66* | -2.82** | 1.67 | -1.30 | -2.41 | 0.00 | -0.25 | -1.84 |
| **30** | HKI 287 × CML 169 | -16.39* | 13.74 | 22.78* | 1.03 | -2.66* | -3.93** | 2.34 | -0.65 | -2.39 | 1.00 | -1.70 | -2.06* |
| **31** | HKI 287 × CML 176 | -17.36* | 12.61 | 38.12** | 2.41* | -1.33 | -2.14* | 2.68 | -0.32 | -1.92 | 1.00 | -1.70 | -2.06* |
| **32** | HKI 287 × CML 161 | -23.55** | 4.17 | 34.25** | 1.03 | -2.66* | -4.09** | 1.34 | -1.62 | -3.66** | -0.75 | -2.70* | -3.41** |
| **33** | HUZM478 × CML 141 | -30.58** | -3.45 | 26.08* | 3.45** | -3.23** | -4.46** | 2.34 | -3.77** | -5.70** | 0.50 | -2.43* | -3.48** |

*Continuing table 7*

| S.No. | Crosses | Grain Yield | | | Days to 50% tasseling | | | Days to 50% silking | | | Days to 75% Brown Husk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH |
| 34 | HUZM478 × CML 193 | -4.13 | 33.33** | 51.63** | 5.17** | -0.97 | -1.29 | 5.02** | -1.26 | -1.41 | 1.00 | -1.94 | -2.18* |
| 35 | HUZM478×DMRQPM 58 | -18.18* | 13.79 | 18.56 | 4.83** | 2.36* | 0.16 | 4.01** | 0.97 | -0.64 | -0.25 | 0.50 | -1.36 |
| 36 | HUZM478 × HKI 164-7-6 | -5.79 | 31.03** | 53.02** | 0.69 | -2.67* | -4.26** | 0.67 | -2.59 | -3.99** | 0.75 | -0.49 | -1.35 |
| 37 | HUZM478 × HKI 162 | -12.40 | 21.84* | 53.62** | 2.41* | -1.66 | -2.94** | 1.67 | -3.49* | -3.95** | 0.00 | -0.25 | -1.60 |
| 38 | HUZM478 × CML 169 | -17.36* | 14.94 | 22.70* | 3.79** | -2.91* | -3.07** | 5.02** | -1.26 | -1.41 | 1.25 | -1.46 | -1.58 |
| 39 | HUZM478 × CML 176 | -5.99 | 31.03** | 59.09** | 3.79** | -1.63 | -2.27* | 4.01** | -2.20 | -2.20 | 1.00 | -1.70 | -1.82 |
| 40 | HUZM478 × CML 161 | -44.46** | -22.76* | -1.18 | 3.45** | -3.23** | -3.23** | 3.34* | -2.83* | -3.29** | 2.00 | 0.00 | -0.49 |
| 41 | V 336 × CML 141 | -12.40 | 10.42 | 49.03** | 2.41* | 0.68 | -3.10** | 2.68 | 1.32 | -3.15** | 0.25 | -2.43* | -3.61** |
| 42 | V 336 × CML 193 | -23.97** | -4.17 | 13.58 | 4.14** | 2.37* | 0.17 | 5.02** | 3.63* | 0.96 | 1.25 | -1.46 | -1.82 |
| 43 | V 336 × DMRQPM 58 | -16.53* | 5.21 | 14.77 | -1.38 | -3.05* | -3.38** | -0.33 | -1.65 | -2.45* | -1.50 | -0.76 | -2.48* |
| 44 | V 336 × HKI 164-7-6 | 4.96 | 32.29** | 60.76** | 0.69 | -1.02 | -1.85 | -0.67 | -1.98 | -2.94* | 1.00 | -0.25 | -0.98 |
| 45 | V 336 × HKI 162 | -24.79** | -5.21 | 23.81 * | 1.03 | -0.68 | -1.84 | 1.34 | 0.00 | -1.94 | 0.50 | 0.25 | -0.99 |
| 46 | V 336 × CML 169 | -10.74 | 12.50 | 25.58** | 3.79** | 2.03 | -0.33 | 4.68** | 3.30* | 0.64 | 1.75 | -0.97 | -0.97 |
| 47 | V 336 × CML 176 | -32.51** | -14.93 | 7.46 | 5.52** | 3.73** | 1.83 | 5.35** | 3.96 | 1.45 | 0.50 | -2.19 | -2.19* |
| 48 | V 336 × CML 161 | -14.88 | 7.29 | 42.07** | 3.79** | 2.03 | -0.50 | 4.01** | 2.64 | -0.32 | 2.00 | 0.00 | -0.37 |
| 49 | V 341 × CML 141 | 8.26 | 183.24** | 184.78** | 1.72 | 0.68 | -3.44** | 0.67 | -2.59 | -5.94** | 0.25 | -0.74 | -2.79** |

*Continuing table 7*

| S.No. | Crosses | Grain Yield | | | Days to 50% tasseling | | | Days to 50% silking | | | Days to 75% Brown Husk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH |
| 50 | V 341 × CML 193 | -20.66** | 45.45** | 71.43** | 3.79** | 2.73* | 0.17 | 4.35** | 0.97 | -0.64 | 1.75 | 0.74 | -0.49 |
| 51 | V 341 × DMRQPM 58 | -4.96 | 43.75** | 82.54** | 2.07 | 1.02 | 0.34 | 1.34 | -1.62 | -1.78 | 0.75 | 1.51 | 0.62 |
| 52 | V 341 × HKI 164-7-6 | -21.49** | 53.23** | 75.93** | 2.41* | 1.37 | 0.17 | 2.68 | -0.65 | -0.65 | 0.75 | -0.25 | -0.37 |
| 53 | V 341 × HKI 162 | 4.13 | 147.06** | 159.79** | 1.72 | 0.68 | -0.84 | 1.67 | -1.62 | -2.56* | 2.00 | 1.75 | 1.37 |
| 54 | V 341 × CML 169 | -6.61 | 48.68** | 85.25** | 2.07 | 1.02 | -1.66 | 3.01* | -0.32 | -1.91 | 1.75 | 0.74 | -0.12 |
| 55 | V 341 × CML 176 | -9.92 | 94.64** | 113.73** | 0.00 | -1.02 | -3.17** | -0.33 | -3.56* | -4.94** | 0.50 | -0.50 | -1.35 |
| 56 | V 341 × CML 161 | -31.40** | 69.39** | 74.74** | 0.34 | -0.68 | -3.48** | 0.33 | -2.91 | -4.76** | 0.50 | -0.50 | -0.99 |
| 57 | V 351 × CML 141 | 28.10** | 121.43** | 166.67** | -3.10* | 0.36 | -6.02** | -2.34 | 1.39 | -5.65** | -1.25 | 1.80 | -2.35* |
| 58 | V 351 × CML 193 | -38.84** | 5.71 | 8.82 | -1.72 | 1.79 | -3.06** | -1.34 | 2.43 | -2.80* | -0.75 | 2.32 | -1.00 |
| 59 | V 351 × DMRQPM 58 | 5.62 | 59.75** | 70.40** | -2.76* | 0.71 | -2.25* | -3.34* | 0.35 | -3.02* | -4.25** | -1.29 | -2.42* |
| 60 | V 351 × HKI 164-7-6 | -19.01* | 40.00** | 48.48** | -4.48** | -1.07 | -4.48** | -5.69** | -2.08 | -5.53** | -5.00** | -2.06 | -4.16** |
| 61 | V 351 × HKI 162 | -9.92 | 55.71** | 80.17** | -2.07 | 1.43 | -2.41* | -2.01 | 1.74 | -2.82* | -2.00 | 1.03 | -0.63 |
| 62 | V 351 × CML 169 | -35.74** | 2.30 | 6.51 | -1.03 | 2.50* | -2.55* | -1.34 | 2.43 | -2.80* | -1.25 | 1.80 | -1.13 |
| 63 | V 351 × CML 176 | -30.58** | 20.00 | 33.33** | -1.38 | 2.14 | -2.39* | 0.33 | 4.17** | -0.99 | -0.25 | 2.84* | -0.13 |
| 64 | V 351 × CML 161 | 10.74 | 91.43** | 125.21** | -3.79** | -0.36 | -5.42** | -4.68** | -1.04 | -6.40** | -4.00** | -1.03 | -3.52** |
| 65 | CM 141 × CML 141 | -2.48 | 155.14** | 158.63** | 0.34 | -4.59** | -6.58** | -0.67 | -7.48** | -8.90** | 1.00 | -4.04** | -5.16** |
| 66 | CM 141 × CML 193 | -2.48 | 79.04** | 112.91** | 1.03 | -3.93** | -4.40** | 4.01** | -2.51 | -2.81* | 2.25 | -1.21 | -3.20** |
| **67** | CM 141×DMRQPM 58 | -22.45** | 17.29 | 50.13** | 0.34 | -2.02 | -3.32** | 1.67 | -1.30 | -3.34** | 0.25 | 1.01 | -3.14** |
| **68** | CM 141×HKI 164-7-6 | 17.36* | 129.03** | 165.42** | -2.41* | -5.67** | -6.45** | -3.68* | -6.80** | -8.57** | 2.50 * | 1.23 | -1.91 |

*Continuing table 7*

| S.No. | Crosses | Grain Yield | | | Days to 50% tasseling | | | Days to 50% silking | | | Days to 75% Brown Husk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH | SH | BPH | MPH |
| **69** | CM 141 × HKI 162 | -21.49* | 86.27** | 97.92** | 0.34 | -3.64** | -4.12** | 2.68 | -2.54 | -3.46** | 1.75 | 1.50 | -2.16* |
| **70** | CM 141 × CML 169 | -5.79 | 50.00** | 88.71** | 1.72 | -3.28** | -3.91** | 4.01** | -2.51 | -2.81* | 2.00 | -0.73 | -3.09** |
| **71** | CM 141 × CML 176 | 25.62** | 171.43** | 200.99** | -1.72 | -6.56** | -6.71** | -0.33 | -6.29** | -6.73** | 0.00 | -2.68* | -4.99** |
| **72** | CM 141 × CML 161 | 53.31** | 278.57** | 294.68** | -3.45** | -8.20** | -8.94** | -4.01** | -10.59** | -10.59** | -0.75 | -3.19** | -5.84** |
| **73** | V 335 × CML 141 | 34.71** | 84.18** | 141.93** | -0.69 | -0.35 | -5.11** | -1.67 | -1.34 | -6.52** | -0.50 | -0.75 | -3.16** |
| **74** | V 335 × CML 193 | -15.87* | 15.03 | 31.78** | -1.72 | -1.38 | -4.52** | -0.67 | -0.34 | -3.73** | 0.00 | -0.25 | -1.84 |
| **75** | V 335 × DMRQPM 58 | 4.96 | 43.50** | 50.74** | -0.34 | 0.00 | -1.37 | 0.33 | 0.67 | -0.99 | -2.00 | -1.26 | -1.75 |
| **76** | V 335 × HKI 164-7-6 | 21.49* | 66.10** | 95.35** | -3.45** | -3.11* | -4.92** | -3.68* | -3.36* | -5.11** | 0.25 | 0.00 | -0.50 |
| **77** | V 335 × HKI 162 | -5.79 | 28.81* * | 63.44** | -0.34 | 0.00 | -2.20* | 0.67 | 1.01 | -1.79 | 2.00 | 1.75 | 1.75 |
| **78** | V 335 × CML 169 | 0.00 | 36.72* * | 47.11** | 0.34 | 0.69 | -2.68** | 0.33 | 0.67 | -2.76* | -1.00 | -1.25 | -2.46* |
| **79** | V 335 × CML 176 | -21.49* | 7.34 | 31.49** | 0.34 | 0.69 | -2.18* | 0.67 | 1.01 | -2.27 | 1.00 | 0.75 | -0.49 |
| **80** | V 335 × CML 161 | 13.22 | 54.80** | 99.27** | 0.34 | 0.69 | -2.84** | -0.67 | -0.34 | -4.04** | -0.25 | -0.50 | -1.36 |
| SE± | | 0.36 | 0.36 | 0.31 | 1.17 | 1.17 | 1.01 | 1.48 | 1.48 | 1.28 | 1.57 | 1.57 | 1.36 |
| CD 5 % | | 0.72 | 0.72 | 0.62 | 2.31 | 2.31 | 2.00 | 2.92 | 2.92 | 2.53 | 3.11 | 3.11 | 2.69 |
| CD 1 % | | 0.95 | 0.95 | 0.83 | 3.05 | 3.05 | 2.64 | 3.85 | 3.85 | 3.33 | 4.10 | 4.10 | 3.55 |
| Mean Heterosis (%) | | -16.58 | 35.48 | 55.49 | 0.83 | -4.75 | -2.63 | 1.30 | -4.87 | -2.69 | -0.10 | -3.61 | -2.01 |
| Crosses with positive heterosis | | 15 | 62 | 70 | 58 | 40 | 10 | 58 | 35 | 9 | 46 | 30 | 7 |
| Crosses with negative heterosis | | 65 | 18 | 10 | 22 | 40 | 70 | 22 | 45 | 71 | 34 | 50 | 73 |
| Range | | -56.45 to 53.31 | - 40.65 to 278.57 | -30.11 to 294.68 | -4.48 to 5.52 | -8.2 to 4.26 | -8.94 to 1.83 | -5.69 to 5.35 | -10.59 to 6.48 | -10.59 to 2.13 | -5 to 2.5 | -5.07 to 5.24 | -5.87 to 2.16 |

* and **, significant at 5 and 1 per cent level of significance, respectively.

Table 8. Top ranking (First five) cross combinations based on Per se performance, SCA, GCA effects and Heterosis

| Traits | Significant Crosses | *Per se* performance | | | sca effect | gca effects | | Standard Heterosis |
|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | Line | Tester | | Line | Tester | |
| **Grain Yield** | CM 141 × CML 161 | 7.42 t/ha | 1.80 t/ha | 1.96 t/ha | 2.16** | 1.05** | 0.16** | 53.31** |
| | V 335 × CML 141 | 6.52 t/ha | 3.54 t/ha | 1.85 t/ha | 1.16** | 0.99** | 0.32** | 34.71** |
| | V 351 × CML 141 | 6.20 t/ha | 2.80 t/ha | 1.85 t/ha | 1.57** | 0.26** | 0.32** | 28.10** |
| | CM 141 × CML 176 | 6.08 t/ha | 1.80 t/ha | 2.24 t/ha | 0.98** | 1.05** | 0.00 | 25.62** |
| | V 335 × HKI 164-7-6 | 5.88 t/ha | 3.54 t/ha | 2.48 t/ha | 0.71** | 0.99** | 0.13 | 21.49* |
| **Days to 50 % tasseling** | HUZM185 × DMRQPM 58 | 92.33 | 96.00 | 99.00 | -3.36** | -0.68* | -1.10** | -4.48** |
| | V 351 × HKI 164-7-6 | 93.00 | 93.33 | 100.00 | -0.41 | -3.26** | -1.47** | -4.48** |
| | V 351 × CML 161 | 93.33 | 93.33 | 103.33 | -1.04 | -3.26** | -0.17 | -3.79** |
| | V 335 × HKI 164-7-6 | 93.33 | 96.33 | 100.00 | -1.20 | -1.47** | -1.47** | -3.45** |
| | CM 141 × CML 161 | | 101.67 | 103.33 | -2.71** | -1.26** | -0.17 | -3.45** |
| **Days to 50 % silking** | V 351 × HKI 164-7-6 | 94.00 | 96.00 | 103.00 | -0.92 | -3.85** | -2.20** | -5.69** |
| | V 351 × CML 161 | 95.00 | 96.00 | 107.00 | -1.49 | -3.85** | -0.64 | -4.68** |
| | CM 141 × CML 161 | 95.67 | 107.00 | 107.00 | -3.82** | -0.85* | -0.64 | 4.01** |
| | V 335 × HKI 164-7-6 | 96.00 | 99.33 | 103.00 | -0.88 | -1.89** | -2.19** | -3.68* |
| | CM 141 × HKI 164-7-6 | 96.00 | 107.00 | 103.00 | -1.92 | -0.85** | -2.20** | -3.68* |
| **Days to 75 % Brown Husk** | V 351 × HKI 164-7-6 | 126.67 | 129.33 | 135.00 | -3.79** | -2.98** | 0.25 | -5.00** |
| | V 351 × DMRQPM 58 | 127.67 | 129.33 | 132.33 | -0.42 | -2.98** | -2.12** | -4.25** |
| | V 351 × CML 161 | 128.00 | 129.33 | 136.00 | -1.89 | -2.98** | -0.32 | -4.00** |
| | HUZM185 × DMRQPM 58 | 128.67 | 133.33 | 132.33 | -0.50 | -1.90** | -2.12** | -3.50** |
| | HUZM185 × CML 141 | 129.67 | 133.33 | 140.33 | -1.47 | -1.90** | -0.16 | -2.75* |

* and **, significant at 5 and 1 per cent level of significance, respectively.

**References**


Abrha SW, Zeleke HZ and Gissa DW (2013). Line × tester analysis of maize inbred lines for grain yield and yield related traits. Asian Journal of Plant Science and Research 3(5):12-19.

Alamnie A,Wali MC, Salimath PM and Jagadeesha BC (2006). Combining ability and heterosis for grain yield and ear characters in maize. Karnataka J Agric Sci 19:13-16.

Amiruzzaman Md, Islam Md A, Hasan L, Kadir M and Rohman Md M (2011). Heterosis and combining ability in a diallel among elite inbred lines of maize (*Zea mays* L.). Emir J Agric 23: 204-208.

Amiruzzaman Md, Islam Md A, Hasan L, Kadir M and Rohman Md M (2013). Heterosis and combining ability in a diallel among elite inbred lines of maize (*Zea mays* L.) Emir. J. Food Agric. 25 (2): 132-137.

Chaudhary AK, Chaudhary LB and Sharma KC (2000). Combining ability estimates of early generation inbred lines derived from two maize populations. Indian J Genet 60: 55-61.

Dagne W (2008). Genotypic variability and combining ability of quality protein maize inbred lines under stress and optimal conditions. Ph D Thesis University of the Free State, South Africa.

Elmyhum M (2013). Estimation of combining ability and heterosis of quality protein maize inbred lines. Afr J Agric Res 8(48): 6309-637.

Fan XM, Chen HM, Tan J, Xu CX, Zhang YD, Luo LM, Huang YX and Kang MS (2008). Combining abilities for yield and yield components in maize. Maydica 53:39-46.

Fonsecca S, and FL Patterson (1968). Hybrid vigour in a seven-parent diallel cross in common wheat (*Triticum aestivum* L.). Crop Sci 2: 85-88.

Hallauer AR and Miranda JB (1988). Quantitative Genetics in Maize Breeding. Iowa State University Press Iowa.

Hayes HK, Immer FR and Smith DC (1955). Methods of Plant Breeding. Mc Graw Hill Book Inc NewYork USA.

Ivy NA and Howlader MS (2000). Combining ability in maize. Bangladesh J Agril Res 25: 385-392.

Jayakumar J and Sundram T (2007). Combining ability studies for grain yield and other yield components in maize. Crop Res 33: 179-186.

Jebaraj S, Selvakumar A and Shanthi P (2010). Study of gene action in maize hybrids. Indian J Agric Res 44(2):136-140.

Joshi VN, Dubey RB and Marker S (2002). Combining ability for polygenic traits in early maturity hybrids of maize (*Zea mays* L.). Indian J Genet 62: 312-315.

Kambe GR, Kage Udaykumar, Lohithaswa HC, Shekara BG and Shobha D (2013). Combining ability studies in maize (*Zea mays* L.). Molecular Plant Breeding 4(14): 116-127.

Kanagarasu S, Nallathambi G and Ganesan KN (2010). Combining ability analysis for yield and its component traits in maize (*Zea mays* L.). Electronic J Plant Breeding 1(4): 915-920.

Kempthorne O (1957). An Introduction to Genetic Statistics. John Willy and Sons Inc New York.

Mahto RN and Ganguli DK (2003). Combining ability analysis in inter varietal crosses of maize (*Zea mays* L.). Madras Agric J 90: 29-33.

Malik SI,Malik HN, Minhas NM and Munir M (2004). General and Specific combining ability studies in maize. Int J Agri Biol 6: 856-859.

Matzinger DF,Mannand TJ and Cockerham CC (1962). Diallel cross in *Nicotiana tabacum*. Crop Sci 2: 238-286.

Pal AK and Prodhan HS (1994). Combining ability analysis of grain yield and oil content along with some other attributes in maize (*Zea mays* L.). Indian J Genet 54: 376-380.

Prasad SK and Kumar P (2003). Line × tester analysis for combining ability in maize. J Res (RAU) 13: 68-72.

Premlatha M, Kalamani A and Nirmalakumari A (2011). Heterosis and combining ability for grain yield and quality in maize (*Zea mays* L.). Advances in Environmental Biology 5(6): 1264-1266.

Rao GP, Rai B, Singh SV and Sahi JP (1996). Heterosis and combining ability in inter- varietal crosses of maize. Madras Agric J 83: 291-295.

Saxena VK, Mathi NS, Singh NN and Vasal SK (1998). Heterosis in maize: Grouping and patterns. In: Vasal SK, FC Gonzalez and F Xingming (ed) Proc 7th Asian Reg Maize Workshop Los Banos, Philippines February pp: 124-133.

Sharma S, Narwal R, Kumar MS and Dass S (2004). Line x tester analysis in maize (*Zea mays* L.). Forage Res 30: 28-30.

Singh DN and IS Singh (1998). Line × tester analysis in maize (*Zea mays* L.). J Res (BAU) 10: 177-182.

Singh RK and BD Chaudhary (1985). Biometrical Methods in Quantitative Genetic Analysis. Kalyani, New Delhi, India.

Singh SB (1979). Genetic analysis for grain yield and other quantitative traits in inbred lines of maize (*Zea mays* L.). Ph D Dissertation, Banaras Hindu University Varanasi, India.

Subramaniyan A and N Subbraman (2006). Combining ability analysis for yield and its contributing traits in maize. Indian J Agric Res 40:131-134.

Sundararajan R and Kumar PS (2011). Studies on combining ability through line × tester analysis in maize (*Zea mays* L.). Plant Archives 11(1): 75-77

Surya P and Ganguli DK (2004). Combining ability for various yield component and characters in maize. J Res (BAU) 16: 55-60.

Turner JH Jr (1953). A study of heterosis in upland cotton. II Combining ability and inbreeding effects. Agron J 45:487-490.

Uddin MS, Khatun F, Ahmed S, Ali MR and Bagum SA (2006). Heterosis and combining ability in corn (*Zea mays* L.) Bangladesh J Bot 35(2): 109-116.

Vasal SK (1999). Quality Protein Maize Story. In: Improving Human Nutrition Through Agriculture:The Role of International Agricultural Research. A Workshop. October 5-7, 1999 Los Banos, Philippines .pp 1-16.

Vijayabharathi A, Anandakumar CR and Gnanamalar RP (2009). Combining ability analysis for yield and its components in popcorn (*Zea mays var. everta* Sturt). Electronic J Plant Breed 1:28-32.

Vivek BS, Krivanek AF, Palacios-Rojas N, Twumasi-Afriyie S and Diallo AO (2008). Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars.Mexico, D F: CIMMYT.

Xingming F, Tan J, Chen Z and Yang J (2002). Combining ability and heterotic grouping of ten temperate, tropical and subtropical quality protein maize. In: Srinivasan G, Zaidi PH, Prasanna BM, Gonzalez FC and Lesnick K (ed). Proc 8th Asian Reg Maize Workshop Bangkok, Thailand.

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# Genotypes X environment interaction effect on nutritional quality of sorghum lines in Indonesia

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**ABSTRACT**

The adoption of sorghum as alternative souce of food in Indonesia depends on consummer acceptance of sorghum grain which is determine by the nutritional and anti-nutirtional values and the quality of sorghum grain. The objectives of this study were to obtain information on genetic variability of sorghum genotypes with diverse backgroud for nutritional values and the effect of genetic x environment on the variablity in sorghum grain nutritional values. The study was conducted on 24 sorghum lines consisted of breeding lines, introduced lines and a local variety. The lines were planted in a randomized complete block design with tree replications in two locations differing in soil fertility. The results showed that the protein, fat, amylose and tannin contents of the sorghum lines are effected by the genotype x environment interaction with different magnitutes. The presence of G X E interaction reduced the genetic variances and estimates of heritability of the characters.

***Keywords:*** Sorghum grain, protein content, tannin content, genetic variance, heritability

## Introduction

Indonesia has a large areas of dryland with a total of 148 Million ha which are prone to drought, and a total of 102.8 million ha of the area are acid soils (Mulyani *et al*, 2011). In addition to the low nutrient content, acid soils are also characterized by high concentrations of Al, especially $Al3^{+}$, which is the form of Al considered to be most toxic to plants. Aside from Al stress, constraints on acid soils is P deficiency caused by binding of P by Al, which makes P unavailable (Marschner, 1995). A large areas of dryland are still under utilized with cropping index less than two. A crop with good adaptation to the condition, could improve the productivity of Indonesian dry lands and improve farmer income.

Sorghum is a drought tolerant crop and suitable for cultivation in dought prone areas in Indonesia. According to Assefa *et al* (2010) a medium-to-late maturing sorghum cultivar requires approximately 450 to 650 mm of water during a growing season. Water stress or drought reduces biomass, yield and harvest index more in maize than in sorghum, giving higher yields for sorghum under limited water. Sorghum has great ability to extract water from deep soil layers due to its deep root system (Farre & Faci, 2004). In addition, several sorghum varieties are tolerant to acid soil and able to maintain growth and yield under high Al toxicity and low P found in many acid soil in Indonesia (Agustina, et al, 2010).

Sorghum grains are important source of dietary proteins, carbohydrates, minerals, and B group vitamins with some values higher than rice. Sorghum is a good candidate to be promoted to improve human health and reduce rice consumption in Indonesia as a mean to sustain national food security. However, sorghum is still considered as a minor crop in Indonesia and still underutilized. To improve sorghum adoption by farmers, sorghum varieties should have good grain quality, high nutrional values and low in anti nutritional values.

There is variability for grain nutritional contents among sorghum genotypes as shown in the total protein, total starch and mineral contents among the South African sorghum accession (Ngu'ni *et al*, 2012) and in many cases also effected by the interaction of genotypes with the environment as shown in wheat (Kiliç and Yağbasanlar, 2010) and oats (Doehlert *et al*, 2001). The objectives of this study was to obtain information on genetic variability of sorghum germ plasm with diverse backgroud for nutritional and antinutritional values and the effect of genotype x environment on the variablity in sorghum grain nutritional values.

## Materials and methods

This study was conducted in two locations (1) A farmer field in Jasinga, West Bogor District, Bogor Indonesia with soil pH of 4.1-4.4 and exchangable Al of 2.3-5.8 cmol/kg (2) The University Farm of Bogor Agricutlural University with soil pH of 5.5 and 0 cmol/kg of exchangeable Al. The grain analysis was conducted in the Laboratory of Post Harvest Research Center of the Ministry of Agriculture. The field experiments were conducted from April-July 2012 and the grain analysis was conducted in August 2012.

The genetic materials used in this study were 17 $F_7$ breeding lines from the cross of Numbu x UPCA-S1 developed by the Laboratory of Plant Breeding and Genetics, Department of Agronomy and Horticulture, Bogor Agricultural University, four introduced lines from ICRISAT and one local variety. Two national varieties UPCA-S1 and Numbu were used for comparison.

The experiment was conducted in a Randomized Complete Block Design with three replicates nested in location. The planting was conducted as direct seeding with 2 seeds per hole at 70 x 10 cm planting distance in a 4 m x 5 m plot. Fertilizers of Urea, SP36 and KCl were applied at the rate of 100 kg/ha, 100 kg/ha and 60 kg/ha, respectively. Two third of the urea was applied as base fertilizers at planting with SP-36 and KCl. The rest of the urea was applied at seven weeks after planting. Plot maintenance and pest and disease control was conducted accoring to standard practices.

After harvesting, the seeds were dehulled and analyzed for fat content, protein content, amylose content and tannin content The study was concuted at the Laboratory of Grain Quality of the Center for Postharvest Research the Ministry of Agriculture, in Bogor. The amylose content was determined using iodo-colorimetry method , protein content was analyzed by Kjeldahl method (AOAC, 2007) and tannin content was analyzed by the vanillin in acidic methanol method (Price *et al*, 1978).

Analysis of variance for randomized complete blocks design was carried out for each location using SAS version 9.2 (SAS Institutes, NC) where locations were considered as random and all genotypes were considered as fixed. Homogeny test of varinces was conducted by Bartlet's test and the combined analysis of variances was conducted for genotypes under two locations. The estimated variances of each components were partitioned into variace due to genotypes ($\sigma^2_g$ ), variance due to environment ($\sigma^2_e$) and the interation ($\sigma^2_{g\,x\,e}$) and broad sense heritablity was estimated for eah location and for the combined conditioned.

## Results and discussion

Nutritional and anti-nutritional value of sorghum grains is important to accelarate consummers acceptance of sorghum in Indonesia. This study was conducted to evaluate nutritional values of grains of sorghum lines of diverse backgrounds. The lines consisted of introduced lines from ICRISAT, national varieties and breeding lines. The analysis of variance from each location showed that genotypes significantly effects the protein, fat, tannin and amylose content (Table 1 and Table 2). Genetic variability in nutritive content of sorghum grain has been reported among Southern African sorghum accessions (Ng'uni et al, 2012), and among Indian sorghum varieties used for Roti (Chavan *et al*, 2009).

The protein content of sorghum variety is important if the variety is to be designated as grain sorghum.The genotypic means showed that the protein content of the lines evaluated ranged from 8.0-11.41 % when grown in Jasinga and 8.83-9.83 % when grown in Leuwikopo. This is within the range for sorghum as reported in some inbred and hybrid lines of sorghum in Kansas, whrere the range was 10.3-16.5 % (Hicks *et al*, 2002). A local variety, Watar Hammu Puti (WHP) had the highest protein content (11.4%) compared to introduced lines and breeding lines when grown in

acid soil of Jasinga with high Al content (Table 4). Aba *et al* (2005) reported that the protein content of ten African sorghum varieties ranged from 10 – 16.45%. The lines tested have different grain color from pearly white to pale red color. Ng'uni *et al* (2012) reported that there is no significant difference in protein content between red and white sorghum grains. The genotypic means for fat content of the evaluated lines ranged from 2.75-4.06 %. The introduced lines PI-10-90-A has the highest fat content of 4.06 %. This value is higher than reported by Hicks et al (2002) among sorghum inbred lines and hybrid which ranged from 3.17-3.63%. The fat content is important if sorghum grain is going to be used as feeds, because fat produces higher energy than carbohydrate.

The main storage carbohydrate in sorghum grain is starch, which consist of amylose and amylopectin. Sorghum is classified into three groups based on the amylose content, namely waxy (<1%), heterowaxy (10-20%) and normal (>20%) (Shelton *et al*, 2004). The amylose content of the sorghum lines evaluated ranged from 18.82-23.44 % in Jasinga and 18.83-24.98 % in Leuwikopo. Based on the amylose content, the sorghum lines were classified as normal sorghum. For food and industrial purposes, lower amylose content is needed, because lower amylose content increases carbohydrate digestibility (Lichtenwarner *et al*, 1978) and improve ethanol fermentation (Yan *et al*, 2011).

Sorghum grains contain tannin, a phenolic compound, which could reach up to 6%, the highest among grain cereals. Tannin can reduce protein and carbohydrate digestibility . Many consummers also prefer sorghum food with low tannin content because of the bitter taste of tannin. Our study showed that there is variability in tannin content among the sorghum lines. The introduced lines from ICRISAT have higher tannin content compared to IPB breeding lines and the national variety Numbu. Numbu has the lowest tannin content of only 0.11% (Table 1). Puspitasari *et al* (2011) reported that the the tannin content of sorghum mutant lines and national varieties grown in acid soil ranged from 0.38-3.66 %, and national variety Mandau grown in acid soil has high tannin content of 3.66 %. Ebadi et al (2005) classified sorghum varieties as low tannin (< 0.10%, LTS), medium tannin (0.10-0.3%, MTS), and high tannin (>0.3%, HTS). According to this classification, two IPB breeding lines were classified as high tannin content, and two lines, N/UP-48-2 and N/UP-156-8 were classified as medium tannin content with tannin content of 0.15% and 0.14%, respectively.

Environment conditions effect chemical composition, physical properties and food quality of sorghum. The nutritional content of sorghum grain is affected by environmental conditions such as drought, soil fertility, pest and diseseas (Roony and Murty, 1982). Pale et al (2010) reported that the both water management and fertilizer applications affected grain physicochemical characteristics and malting quality in two sorghum varieties. The results of the combined analysis of variances showed that sorghum genotypes differed significantly for protein, fat, amylose and tannin content , while locations were significant for fat and tannin content . Genotypes x Locations interaction was significant for all the traits. (Table 3).

The combined analysis showed that the nutritional and anti-nutritional content of sorghum lines were effected by the genotype x locations with different magnitutes. The variances due to genotypes were higher for protein content and amylose content, but the variability observed for fat content and tannin content were mostly due to locations. The magnitute of variance due to genotype x locations was high for protein and amylose, but for fat and tannin content, the magnitutes of the genotype x location interaction were lower than variance due to genotype and environment alone.

The combined analysis showed that locations was the main source of variation in tannin content of sorghum lines (Table 3). Taleon et al (2012) reported that the total flavonoid content of black sorghum was effected strongly by environment, mainly due to the differential effect of abiotic factors such as light and temperature and also by the differential intensity of fungal infection.

The genetic x environment interaction qualitatively affect the protein content of sorghum genotypes causing a change in the ranking of genotypes. The intoduced lines PI-150-21-a which was ranked as the genotypes with the highest protein content in Jasinga was only third in Leuwikopo, and the local variety Watar Hamu Putih which produced second highest protein content in Jasinga was dropped to number thirteen in Leuwikopo. This type of interaction will complicate selection for protein content in sorghum.

The broad sense hertability estimates for a single environment were high for fat and tannin content. However, presence of genotype x environment interaction reduced the magnitutes of the genetic variances and the estimate of heritability as compared to the estimates of a single environment (Table 5). The reduction of the heritability estimates are propotional to the magnitute of the variace of genotypes x

environment interaction. This findings indicated that for nutritional content, estimation of heritability should include variablity due to genotype x invironment interaction to avoid upward bias of the estimate for one or the other environment.

Genotype by environment interaction effects sorghum grain nutritional and anti nutritional content. The presence of genotypes x environemntal interaction resulted in differential nutritional values of a genotypes over environments. The result indicated that while conducting yield stability trials, breeders should not only focused on agronomic characters and yield potential. Observation should be made on important nutritional content of sorghum grain over environments in order to select for superior sorghum genotypes with good grain quality.

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Table 1. Mean Squares (MS) for seed content of sorghum introduced and breeding lines grown in acid soil in Jasinga

| Source | Mean Squares | | | | |
|---|---|---|---|---|---|
| | df | Protein | Fat | Amylose | Tannin |
| **Replicaate** | 2 | 0.0459 | 0.0568 | 1.7123 | 0.0352 |
| **Genotypes** | 16 | 2.3954* | 0.4888* | 6.4912* | 0.0773* |
| **Error** | 32 | 0.6670 | 0.0520 | 3.3005 | 0.0167 |
| **Total** | 50 | | | | |

Table 2. Mean Squares (MS) of seed content of introduced and breeding lines of sorghum grown in non acid soil in Leuwikopo

| Source | df | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Protein | Fat | Amylose | Tannin |
| **Replicaate** | 2 | 0.1246 | 0.1274 | 0.3544 | 0.0329 |
| **Genotypes** | 16 | 0.3149* | 0.3288* | 10.7089* | 0.0293* |
| **Error** | 32 | 0.2045 | 0.1100 | 4.1850 | 0.0122 |
| **Total** | 50 | | | | |

Table 3. Combined Analysis of Variances for grain nutrition and anti nutritional content of introduced and breeding lines of sorghum over two environment.

| Source | df | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Protein | Fat | Amylose | Tannin |
| **Replicates** | 4 | 0.04348 | 0.00753 | 0.36281 | 0.02149 |
| **Location** | 1 | 0.39221 | 0.55663* | 0.46812 | 3.29042** |
| **Genotypes** | 16 | 1.44126* | 0.52815* | 9.86565* | 0.05323* |
| **Genotypes x Locations** | 16 | 1.26897* | 0.28940* | 7.33445* | 0.05342* |
| **Error** | 64 | 0.43833 | 0.08629 | 3.78463 | 0.01519 |
| **Total** | 101 | | | | |

Table 4. Nutritional and anti-nutritional content of sorghum lines in two locations in Indonesia

| No | Genotypes | Protein (%) | | Fat (%) | | Amylose (%) | | Tannins | |
|---|---|---|---|---|---|---|---|---|---|
| | | JSG | LEU | JSG | LEU | JSG | LEU | JSG | LEU |
| 1 | PI-150 21 a | 11.22 | 9.74 | 2.68 | 2.87 | 20.57 | 20.68 | 0.80 | 0.75 |
| 2 | PI-5 193 C | 9.88 | 9.33 | 2.61 | 2.99 | 20.30 | 19.51 | 0.42 | 0.75 |
| 3 | PI-10 90 A | 10.33 | 9.50 | 4.06 | 2.99 | 18.82 | 18.83 | 0.32 | 0.81 |
| 4 | PI-150 20 A | 9.38 | 9.58 | 2.97 | 2.65 | 21.48 | 21.70 | 0.29 | 0.78 |
| 5 | WHP | 10.82 | 9.22 | 2.91 | 2.43 | 23.90 | 21.74 | 0.14 | 0.77 |
| 6 | N/UP-166-6 | 10.65 | 9.59 | 2.76 | 3.14 | 23.44 | 19.48 | 0.25 | 0.41 |
| 7 | N/UP-48-2 | 10.32 | 9.56 | 2.85 | 2.69 | 21.05 | 24.98 | 0.15 | 0.72 |
| 8 | N/UP-82-3 | 9.20 | 9.83 | 2.45 | 2.49 | 23.76 | 18.62 | 0.25 | 0.66 |
| 9 | N/UP-118-3 | 8.86 | 9.20 | 2.61 | 3.05 | 22.85 | 21.17 | 0.40 | 0.66 |
| 10 | N/UP-156-8 | 9.56 | 9.55 | 2.83 | 2.24 | 21.80 | 22.63 | 0.14 | 0.65 |
| 11 | N/UP-89-3 | 9.36 | 8.92 | 2.85 | 2.21 | 22.97 | 24.61 | 0.20 | 0.75 |
| 12 | N/UP-39-10 | 9.51 | 8.83 | 2.65 | 2.30 | 20.99 | 22.63 | 0.21 | 0.73 |
| 13 | N/UP-118-7 | 8.68 | 9.98 | 2.19 | 2.28 | 21.59 | 20.70 | 0.28 | 0.71 |
| 14 | N/UP-139-1 | 9.19 | 9.23 | 2.60 | 2.62 | 22.82 | 24.17 | 0.41 | 0.56 |
| 15 | N/UP-124-7 | 8.04 | 9.04 | 2.61 | 2.85 | 21.34 | 21.10 | 0.38 | 0.64 |
| 16 | Numbu | 8.20 | 9.74 | 2.89 | 2.17 | 19.60 | 21.02 | 0.45 | 0.62 |
| 17 | UPCA-S1 | 9.11 | 9.31 | 2.23 | 2.26 | 20.56 | 21.95 | 0.40 | 0.60 |

JSG = Location 1 (Jasinga pH 4.1-4.4, 2.3-5.8 cmol/kg Al), LEU = Location 2 (Leuwikopo, pH 5.5, 0 cmol/kg Al), WHP = Watar Hamu Puti

Table 5. Partition of variances and estimates of heritability for nutritional content of sorghum in each location and over two environemnts

| Characters | Jasinga | | Leuwikopo | | Combined | | |
|---|---|---|---|---|---|---|---|
| | $\sigma^2_g$ | $h^2$ | $\sigma^2_g$ | $h^2$ | $\sigma^2_g$ | $\sigma^2_{gxe}$ | $h^2$ |
| Protein | 0.576 | 46.346 | 0.037 | 15.257 | 0.167 | 0.277 | 18.944 |
| Fat | 0.146 | 73.677 | 0.073 | 39.867 | 0.074 | 0.068 | 32.351 |
| Amylose | 1.064 | 24.371 | 2.175 | 0.006 | 1.014 | 1.183 | 16.944 |
| Tannin | 0.020 | 54.812 | 34.195 | 32.022 | 0.006 | 0.013 | 18.498 |

**References**


[AOAC] Official Methods of Analysis of AOAC International. 2007. 18th ed, AOAC International, Maryland .

Aba D A, E. Abu, P S Chindo, P S Marle, D N Maigida, A O Ogungbile. 2005. Characterization of Some Released Sorghum Varieties for Food and Industrial Utilization In Nigeria. Agricultura. Tropica Et Subtropica Vol. 38(2): 1-6

Agustina K, D Sopandie, Trikoesoemaningtyas, D Wirnas. 2010. Physiological Response of Sorghum (*Sorghum bicolor* L. Moench) Roots to Aluminium Toxicity and Phosphorous Deficiency. (in Indonesian). Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy) Vol 38, No 2.

Assefa Y, A Scott, Staggenborg, V P V Prasad. 2010. Grain Sorghum Water Requirement and Responses to Drought Stress: A Review. Plant Management Network. Crop Management doi:10.1094/CM-2010-1109-01-RV

Chavan V D, J V Patil, M S Shinde. 2009. Nutritional and Roti Quality of Sorghum Genotypes. Indonesian Journal of Agriculture Sciences 10 (2): 80-85.

Doehlert D C, M S McMullen, J J Hammond. 2001. Genotypic and Environmental Effects on Grain Yield and Quality of Oat Grown in North Dakota Crop Sci. 41:1066-1072.

Ebadi M R, J Pourreza, J Jamalian, M A Edriss, A H Samie, S A Mirhadi. 2005. Amino Acid Content and Availability in Low, Medium and High Tannin Sorghum Grain for Poultry. International Journal of Poultry Science 4 (1): 27-31, 2005

Farre I and J M Faci. 2004. Comparative response of maize (*Zea mays* L.) and sorghum (S*orghum bicolour* L. Moench) to irrigation deficit in a Mediterranean climate. Proceedings of the 4th International Crop Science Congress Brisbane, Australia, 26 Sep-1 Oct 2004 | ISBN 1 920842 20 9 | www.cropscience.org.au

Hicks C, M R Tuinsra, J F Pederssen, K D Kofoid. 2002. Genetic analysis of feed quality and seed weight of sorghu inbred lines and hybrids using analytical methods and NIRS. Euphytica 127: 31-40.

Kiliç H, T Yağbasanlar. 2010. Genotype x Environment Interaction and Phenotypic Stability Analysis for Grain Yield and several Quality Traits of Durum Wheat in the South-Eastern Anatolia Region. Not. Bot. Hort. Agrobot. Cluj 38 (3) 2010, 253-258.

Lichtenwalner E, B Ellis, L W. Rooney. 1978. Effect of incremental dosage of waxy genes of sorghum on digestibility. Journal of Animal Sciences 46: 1113-1119.

Marschner H. 1995. Mineral Nutrition in Higher Plant. San Diego: Acad Press.

Mulyani, A, S Ritung, I. Las. 2011. Potential and availability of land resources to support food security. (In Indonesian). Journal Litbang Pertanian (3) 2: 73-80.

Ng'uni D, M Geleta, P Hofvander, M Fatih, T Bryngelsson. 2012. Comparative genetic diversity and nutritional quality variation among some important Southern African sorghum accessions [*Sorghum bicolor* (L.) Moench]. Australian Journal of Crop Science 6(1): 56-64

Palé S, S J B. Taonda, B Bougouma S C Mason. 2010. Water and fertilizer influence on sorghum grain quality for traditional beer (*dolo*) production in Burkina Faso. African Journal of Food Science Vol. 4(11) : 723-734. http://www.academicjournals.org/ajfs

Price, ML, S Van Scoyoc, L G Butler. 1978. A critical evaluation of vanillin reaction as an assay for tannin in sorghum. Journal of Agricultural and Food Chemistry 26, 1214-1218.

Puspitasari W, S Human, D Wirnas and Trikoesoemaningtyas. 2011. Evaluating Genetic Variability of Sorghum Mutant Lines Tolerant To Acid Soil. Atom Indonesia Vol. 38 No. 2

Rooney , L W, D S Murty. 1982. Evaluation of Sorghum Food Quality. Sorghum in the Eighties: Proceedings Symposium on Sorghum, 2-7 Nov 81, Patancheru, India: ICRISAT.

Shelton J L, J O Mattews, L L Southern, A D Higbie, T D Bidner, J M Fernandez and J E Pontit. 2004. Effect of non-waxy and waxy sorghum on growth, carcass traits and glucose and insulin kinetics of growing and finishing barrows and gilts. J. Animal Sciences 82: 1699-1706.

Taleon V, L Dykes, W L Rooney , L W Rooney., Effect of genotype and environment on flavonoid concentration and profile of black sorghum grains, *Journal of Cereal Science* (2012), doi: 10.1016/j.jcs.2012.05. 001.

Yan S, X Wu, S R. Bean, J F. Pedersen, T Tesso, Y R. Chen, D Wang. 2011. Evaluation of waxy grain sorghum for ethanol production. Cereal Chemistry 88 (6) : 589-595

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# KASIB spring common wheat genotype identification on glutenin and gliadin subunits

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**ABSTRACT**

Based on the electrophoretic spectrum, analysis, 216 genotypes of spring wheat were identified as belonging to 25 types including 18 types of HMW-glutenin for 188 homogeneous cultivars. Most of the spring wheat KASIB could be characterized by the subunits 2 * 7+9 5+10 (40% genotypes), and subunits 2* 7+9 2+12– (30%) and subunits 1*7+9 5+10– (8%). The diversity appears to be determined by varying chromosome 1A and 1B at the 5 subunits and 1D-4 subunits HMG. Judging from the distribution of genotypes KASIB nurseries are mostly HMW-glutenin determining high quality baking by subunits: 1A-2 *(77-79%) and 1 (14-15%), at 1B-7+9 (81-82%) and 7+8(13-15%), by 1D –5+10 to 60%. A new subunit is to be included to this set ie., "4+10" for 1D for samples from Erythrospermum 55/94-01-20 (Pavlodar Research Institute, KASIB8-9), Fiton 41 (KASIB 8-9) relative to genofund of high-quality genotypes found in Kazakhstan. Cultivar "Iridost" is identified with alleles 5.5+10 on chromosome 1D, previously identified for cultivars Tselinogradka, Tselinnaya 24, Akmola 3. In cultivar Chelyaba, chromosome 1B set possessed only 6+8 subunit and in the mix for cultivars Lutescens 29-94; for genotypes: E-607 and E-757 - a rare characteristic subunit7*+8 was found. Genotypes OK -1, Fiton 25 and GVK 1916-9 carrying wheat-rye 1B/1R-translocation clustered distantly from all other genotypes. Wheat-rye translocation (IB/ IR) were identified more in samples for Omsk breeding and West Kazakhstan in each KASIB (exceptKASIB4-5), for all samples submitted "Kurgansemena" (except Lutescens415/00) and "Agrosemkonsalt" (KASIB8-9 and 10-11), as well as for LLP "Fiton" breeding samples (Fiton 41 and Fiton 43, including CIMMYT shuttle breeding material.

***Keywords:*** spring wheat, the HMW and the LMW glutenin, gliadin, wheat-rye translocation, hardness, varieties identification, UPOV.

## Introduction

The UPOV embarks on having clear criteria for DUS-testing. For this purpose, a series of special research on the suitability and expediency of using the researched results of electrophoresis protein markers - first of all the grain prolamins were suggested (Cooke, 1995). In recent years, in the UPOV method for identifying varieties, electrophoresis analysis of seed storage proteins (27-29 sings) is used.

Information on HMW glutenin and gliadin data content offers to answer to the 3 DUS criteria: homogeneity, distinctness and stability. According to the UPOV recommendation, the results of HMW glutenin describe the 1A, 1B and 1D chromosomes for containing specific subunits, as it has been realized in context of Kazakhstan variety genofund (Abugalieva and, Pena, 2010; Abugalieva, and Morgounov, 2004).

Kazakhstan-Siberian nursery improvement programmes of spring wheat are being conducted under the auspices of the CIMMYT. It is important to identify and investigate these samples for distance, uniformity and stability. The main part of spring wheat is characterized by the subunit "2* 7+9 5+10" to 40% of all homogeneous; subunits "2* 7+9 2+12" - up to 30% and by the subunits "1 7+9 5+10"- up to 8% of variety samples. Relative to Kazakhstan's cultivars some genotypes were with new subunit 4+10 (1D) in Erythrospermum 55/94-01-20, Fiton 41 samples. Iridost cultivars were marked to posses relatively rare alleles 5.5 +10 on the chromosome 1D, previously identified for Tselinogradka, Tselinnaya 24, Akmola 3 cultivars. For genotypes: E-607 and E-757 - a rare subunit 7 * +8 was noticed. Uniformity level increased for the last 3 KASIB blocks 8-9; 10-11; 12-13 to 74-84%. Wheat-rye translocation were identified for all samples of SibSRIA (Omsk) and EastSRIA (Ust-Kamenogorsk) breeding lines in each KASIB for all samples submitted by "Kurgansemena" and "Agrosemkonsalt" for sample of LLP "Fiton" breeding.

## Materials and methods

The material comprised cultivars and samples (KASIB 4-13) of Kazakhstan-Siberian network of spring common wheat improvements – 216 genotypes from 17 originators, grown up in 2 replications in 3-8 locations/ conditions of Kazakhstan and Siberia. During the decade five investigated sets (blocks) were identified for the composition of gliadin (1B/1R translocation), the composition of the HMW and the LMW glutenin and the hardness class. HMW glutenin composition is given in accordance with the UPOV rules on 27, 28 and 29 featured system testing for uniformity, distinctness and stability for. Content of high-molecular and the low molecular glutenin subunits determined by the method used in CIMMYT laboratories (J.R. Pena), gliadin component content analyzed according to Peruanski et al (1996). Hardness was determined by SKCS 4100 (Perten Instrument).

A variety of blocks was defined by a variation of 5 subunits on 1A and 1B to a chromosome and 4 subunits of HMG on 1D-(figure 1). Judging by distribution of genotypes the nurseries of KASIB are presented generally to HMW-glutenin which determines high baking quality: on 1A – 2* (77-79%) and 1 (14-15%); on 1B – 7+9 (81-82%) and 7+8 (13-15%); on 1D – 5+10 to 60% (Table 1).

In this set of rather high-quality genofund of Kazakhstan genotypes with new subunits 4+10 on 1D in Erithrospermum 55/94-01-20 samples (the Pavlodar SRIA, KASIB 8-9), Fiton 41 (KASIB 8-9) were found.

The Iridost cv is noted as the carrier of rather rare subunit 5.5+10 on the chromosome 1D as revealed earlier for cvs Tselinogradka, Tselinnaya 24, Akmola 3 (Abugalieva, Morgounov, 2004). All set of the Chelyaba cultivars figured to possess on 1B chromosome a part of HMW glutelin subunits 6+8 and in mix for Lutescent 29-94. For genotypes: E-607 and E-757 – a characteristic rare subunits 7 * + 8 (1B) was noticed.

KASIB blocks consist of cultivars-mixes from 16% (KASIB 12-13) to 60% (KASIB 6-7). The later is probably connected with that wherein, the analysis of HMW-glutelin was carried out for samples from each region separately whereas in other blocks – only in one district. The level of uniformity recorded for the last three KASIB 8-9 blocks; 10-11; 12-13 to 74-84%. In a section of originator the percent of polymorphism on HMW-glutenin cultivars fluctuates from 14% (East SRIS) to 50-60% (Kurgan SRIA, the Pavlodar SRIA and the Chelyabinsk SRIA). Low percentage of the mixed genotypes is noted for genotypes of East SRIA breeding, also for the Aktyubinsk RAES (20%), KazRIAPG (21%), Altai SRIA (25%). Thus, on HMW-glutenin 40-84% of genotypes depending on the KASIB block, 14% of genotypes as carriers of 1B/1R wheat and rye translocation can be identified.

The variability of HMW glutenin subunits on 1A, 1B and 1D are presented as follows on KASIB blocks from different originator (Figure 1).

Attention is needed on cultivars, presented as a mixture in terms of bringing them to the homogeneity in composition of HMW-glutenin subunits during the primary seed-based selection method according to the seed storage protein electrophoresis (Kozhemjakin et al, 1995; Abugaliyeva and Pena 2010). Information on the electrophoretic spectrum of seed storage proteins is also important as it has a technological relevance as a basis for gluten complex.

Using polyacrylamide gel electrophoresis for gliadin , 52 cultivars of wheat from 6 locations; 1-Akmola; 2-Pavlodar; 3-Kostanay; 4-Karaganda; 5-East Kazakhstan region; 6-Kazakh Institute of Agriculture for a KASIB 4-5 block (297 samples) were analyzed. Three cultivars: Sonata, Lutescens 574 and Lutescens 424 appeared homogeneous over

the spectrum of gliadin. Sufficiently homogeneous (have on 1 biotype) cultivars Irene, Chelyaba and №18 were also identified. The cultivars which have 3 types of spectrum are: Krasnoufimskaya 90, Sibirskaya12, Sibirskaya 123, Omskaya 34, Novosibirskaya 15, Lutescens 53-95, Altayakaya 50, Fora, Lutescens 219-94 and GVK 1860-80.

Concerning the analysis of gliadin electrophoretic spectrum: basically on 4 origins of KASIB 6, 7 (Fiton, Karabalik, Aktobe, Pavlodar) cultivars were found to be homogeneous along the spectrum of gliadin. Cvs Lutescens 94, Lutescens 1300, Altaiskaya 10 has a different range as compared to Pavlodar.

Many cultivars have the same subunits for the spectrum of gliadin: 1) GVK 1526-2, GVK 1860-12, Lutescens 1350, 53-90-98-2, Kurganskaya 5, 110 Malcevskaya; 2) Stepnaya 2, Stepnaya 15, 53-88-94-12, Altayskaya 105, Altayskaya 530 Chelyaba 2; 3) Zhenis, Lutescens 166-SP-94, Lutescens20, Lutescens 94; 4) Fiton 42, Fiton 156, Stepnaya 16, Lutescens 196/94-6; 5) Pamyati Ryuba, Omskaya 36, 27-90-98-2 (Figure 2).

Three genotypes; OK -1, Fiton 25, GVK 1916-9 were placed most distantly from all other clusters genotypes, of which two have wheat-rye 1B/1R-translocation.

Wheat-rye translocation were identified for samples from 1) SibSRIA (Omsk) breeding and East-Kz SRIA in each KASIB set (except KASIB 4-5), 2) for all samples submitted by “Kurgansemena” (except Lutescens 415/00), 3) presented by “Agrosemkonsalt”, also for the LLP “Fiton” breeding sample (Fiton 41, Fiton 43), including material based on the shuttle breeding (Lutescens 19 ChS). Out of 216 genotypes total detected 1B/1R translocation genotypes were 30 only (Table 2).

For Omskaya 37 cultivar originators showed also wheat-agropyron translocation (Belan et al, 2012) that describes the pedigree of Omsk breeding cultivars, presented also by “Kurgansemena” company.

## Discussion

Cultivars classification by hardness in the testing and registration process is the key in the grain marketing system from the cultivar creation to commercial production, as it determines the cultivar belonging to specific technological class “end use” and requirements for its quality. According to the strict and strong standards of leading wheat exporting countries, only 203 out of 212 cultivars appeared to belong to hard and middle hard classification.. Block KASIB 6-7 included the highest percentage of cultivars and lines with unstable grain hardness index (up 15.2%), which was accompanied by a transition in the class “mixture” and “semi soft” for Altayskaya 105, Kurganskaya 5, Lutescens 1300, Fiton 42, Lutescens 53/95-98-1 Lutestsens 53/88-94-12 and in different growing conditions.

In block KASIB 4-5 the Lutescens 54 cultivar; Lutescens 30-94 and Erythrospermum 607 were characterized by a full range of variability in grain hardness from semisoft to hard depending on growing conditions (because of the heterogeneity of the initial ratio of soft / hard grains), in the KASIB 8-9 block up to 5% (Lutescens 53/95-98-1 and Lutescens 53/88-94-12) were soft and in KASIB 10-11 block – one genotype Aktobe 1574 was soft.

Kazakhstan-Siberian nursery for improvements of spring wheat works under the auspices of the CIMMYT. During the decade five investigated clusters have been identified on the composition of gliadin (1B/1R translocation), the composition of the HMW and the LMS glutenin and the hardness class. HMW glutenin composition is given in accordance with the rules of the UPOV on 27, 28 and 29 featured system testing for homogeneity, distinctness and stability for 188 homogeneous samples.

The main part of spring wheat could be characterized by the subunits “2* 7+9 5+10” to 40% of all homogeneous; subunits “2* 7+9 2+12” - up to 30% and by the subunits “1 7+9 5+10”- up to 8% of variety samples. Relative to varieties of the Kazakhstan genofund, genotypes with new subunit 4+10 to 1D in Erythrospermum 55/94-01-20, Fiton 41 samples were identified . Iridost variety was marked as carrier of relatively rare alleles 5.5 +10 on the chromosome of 1D, previously identified for Tselinogradka, Tselinnaya 24, Akmola 3 varieties. For genotypes: E-607 and E-757 - a rare subunit 7 * +8 was found . Uniformity level increased for the last 3 KASIB blocks 8-9; 10-11; 12-13 to 74-84%. Wheat-rye translocation were identified for all samples of SibSRIA and EastSRIA breeding in each KASIB for all samples submitted by “Kurgansemena”, “Agrosemkonsalt” and for sample of LLP “Fiton” breeding.

Table 1. Distribution of spring common wheat genotypes of five KASIB blocks 4-5; 6-7; 8-9; 10-11; 12-13 on the HMW-glutenin subunits frequency, %.

| Chromosome | HMW-glutenin subunits | К-4-5 | К-6-7 | К-8-9 | К-10-11 | К-12-13 |
|---|---|---|---|---|---|---|
| 1A | 2* | 70 | 84 | 69 | 74 | 73 |
| | 1 | 16 | 10 | 17 | 12 | 15 |
| | 0 | 4 | 6 | 7 | 4 | 6 |
| | 2*/1 | 6 | - | 5 | 4 | 4 |
| | 1/2* | 2 | - | 2 | 6 | - |
| | 0/2* | 2 | - | - | - | 2 |
| 1B | 7+9 | 66 | 84 | 79 | 80 | 80 |
| | 7+8 | 20 | 11 | 12 | 10 | 10 |
| | 17+18 | 2 | 5 | 5 | - | 2 |
| | 7*+8 | 4 | - | - | 2 | 2 |
| | 6+8 | 2 | - | - | - | - |
| | 7+9/6+8 | 2 | - | - | - | - |
| | 7+9/17+18 | 2 | - | 2 | - | - |
| | 17+18/7+8 | 2 | - | - | - | - |
| | 7+9/7+8 | - | - | 2 | 8 | 6 |
| 1D | 5+10 | 43 | 56 | 50 | 48 | 50 |
| | 2+12 | 45 | 35 | 32 | 34 | 42 |
| | 5,5+10 | - | - | 5 | - | - |
| | 4+10 | - | - | 2 | - | - |
| | 4+10/2+12 | - | - | 2 | - | - |
| | 2+12/5+10 | 6 | 4 | 5 | 6 | 6 |
| | 5+10/2+12 | 6 | 5 | - | 12 | 2 |
| | 5,5+10/2+12 | - | - | 2 | - | - |
| | 4+10/5+10 | - | - | 2 | - | - |

Figure 1. The genetic potential of spring wheat KASIB 4-13 block quality from different originators on HMW-glutenin, 1B/1R translocation and hardness.

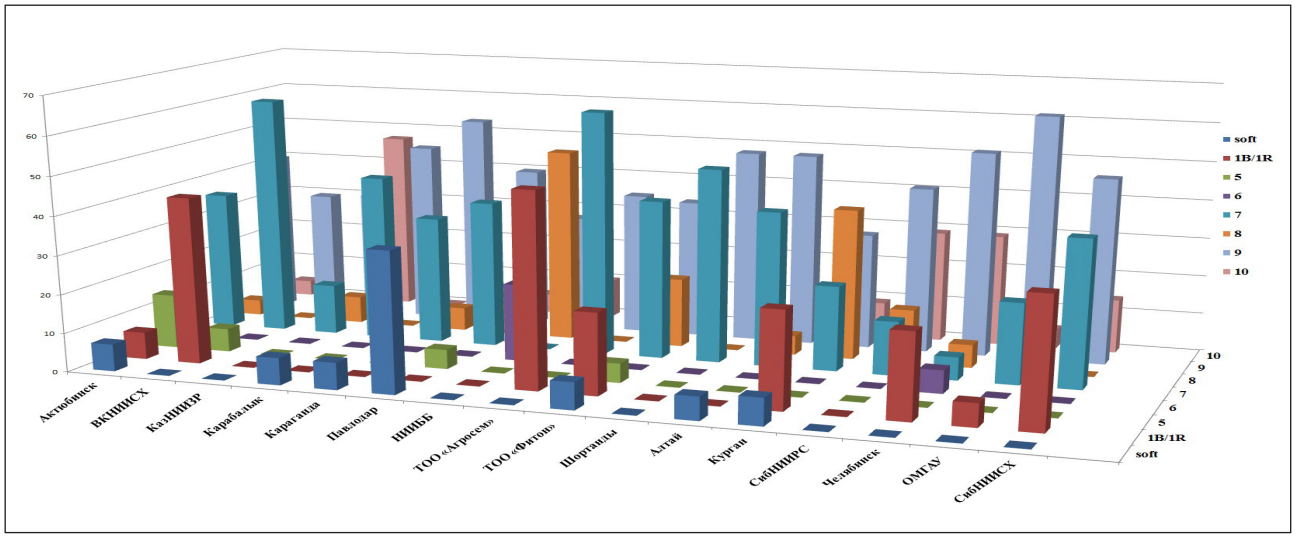

Figure 2 - The dendrogramme of similarity-differences (Mere Hamming) of spring common wheat KASIB-6 samples on gliadin components

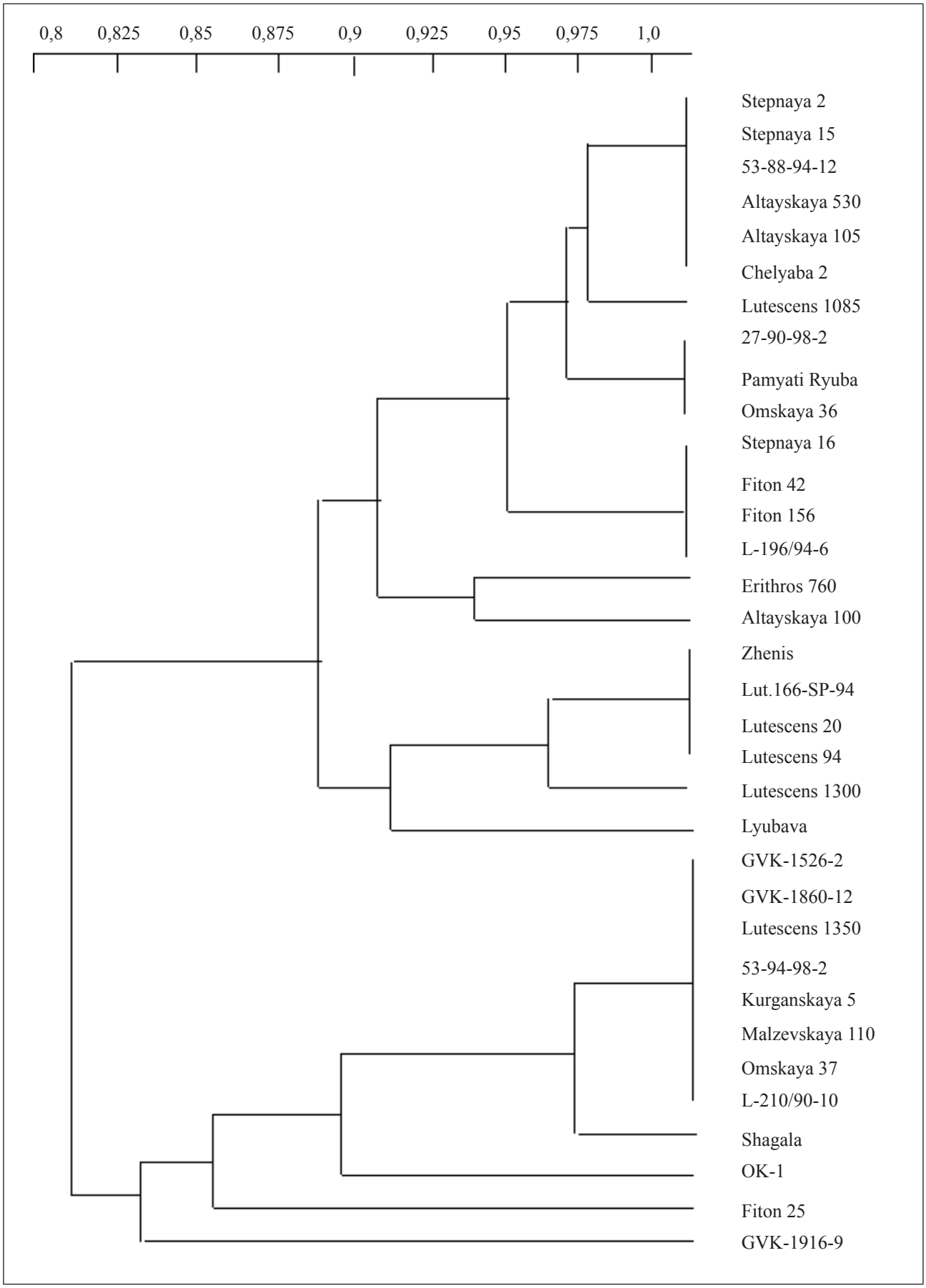

Table 2. Cultivars of spring wheat KASIB network with 1B/1R translocation

| KASIB block | Cultivar | Originator |
|---|---|---|
| **4-5** | Chernyava 13 | OMGAU, RU |
| | Chebarkulskaya | Chelyabinsk SRIA, RU |
| | Erithrospemum-746 | RIBS (Otar, KZ) |
| **6-7** | GVK 1916-9 | East Kazakhstan SRIA |
| | Lutescens 210/99-10, Omskaya 37 | Siberian SRIA (Omsk) |
| | OK-1 | Kurgan SRIA, RU |
| | Stepnaya 15 - mix | Aktobe SRIA, KZ |
| **8-9** | Predgornaya 70 – mix, GVK 1914-15 | East Kazakhstan SRIA |
| | Fiton 41 | Fiton, RU |
| | Severyanka, Lutescens 801 | Agrosemconsalt, KZ |
| | Lutescens 529/00-10 C,<br>Lutescens 307/97-23 | Siberian SRIA, OMSK<br>Sibernina SRIA, Novosibirks |
| **10-11** | Zaulbinka, Velyutinum 15 | East Kazakhstan SRIA |
| | Severyanka 2 | Agrosemconsalt, KZ |
| | Lutescens 363/96-4, Lutescens 360/96-6, Lutescens 290/99-7 | Kurgansemena, RU |
| | Omskaya 39 | Siberian SRIA, Omsk, RU |
| **12-13** | GVK 2033-7 | East Kazakhstan SRIA |
| | Fiton 43, Lutescens C 19 ЧВ | Fiton, RU |
| | Line 96-99-14, Line 241-00-4 | Kurgansemena, RU |
| | Omskaya 41, Lutescens 311/00-2(2)-6 | Siberian SRIA, Omsk, RU |
| | Erithrospermum 23390 | Chelyabinsk SRIA, RU |

**Reference**

Abugalieva A., and Pena R.J. (2010) Grain Quality Spring and Winter Wheat in Kazakhstan. Asian and Australian of Plant Science & Biotechnology 4:87-90.

Abugalieva A.I., and Morgounov A.I. (2004) . Grain quality of spring wheat in Kazakhstan and Central Asia. Thesis, In Inter. Wheat Quality Conference "From Molecular Improvement to Consumer needs", Beijing, China.

Belan I.A., (2012) Rosseeva L.P., Badeeva E.D., Zelenski Y.I., Blohina N.P., Shepelev S.S. and Pershina L.A. Izuchenie hozyiastvenno cennih i adaptivnih priznakov u linii sorta yarovoj miyagkoj pshenici Omskaya 37, nesushih translokacii 1RS. 1BL and 7DL-7Ai. Vavilov journal 1:178-186.

Cooke R.J. (1995) Gel electrophoresis for the identification of plant varieties. *J.Chrom A*.698: 281-299.

Kozhemyakin E.V., (1995) Abugalieva A.I., Savin V.N. and Abugalieva S.I. Systemni podhod i geneticheskie marker v selekcii semenovodstve na primere hlebopekarnoi pshenici Triticum Aestivum L. Monography 4:63.

Peruanski Y.V. (1996), Abugalieva A.I., and Savin V.N. Metodi biohimicheskoi ocenki kollekcioonogo i selekcionnogo materiala. Almaty,123p.

Research Article

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# A new semidwarf cultivar "Uruq" developed from irradiated stored seeds of soft wheat cv. "Inia-66"

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**ABSTRACT**

A new semidwarf cultivar Uruq was developed by irradiated storage seeds of Inia-66 cultivar. Cultivar Uruq has many quantitative and qualitative characters which surpassed its parent (Inia-66), first of all, by reduction of height by 25 cm, which leads to lodging resistant character and, the locally desirable amber seeds color instead of red seeds color which characterize the seeds of parent plant (Inia-66).
Uruq cultivar also surpassed its origin in yield components at different environmental conditions, resistant to brown leaf rust, and suitable for bread making according to their physical and chemical characters and baking test.
The techniques at the molecular level showed that Uruq cultivar has the dwarf gene Rht-D1b which is responsible for the semidwarfism.

***Keywords:*** Bread wheat, semidwarf, ınduced mutation, breeding.

## Introduction

The semidwarfism in wheat has an important role in selection for new cultivars. This character is usually associated with stiffer straw (Djelepov 1976). Short stem is considered to be one of the most efficient means for further increase in yield capacity of wheat (Konzak *et al*., 1984).

A tremendous success in wheat breeding was achieved in early 80's, with the introduction of semidwarf genes: Rht1 and Rht2 (Sial, *et al*, 2010). Norman Borlaug, who is known as the father of "the green revolution", was awarded the Nobel prize in 1970 for developing new strains of wheat in Mexico. He began working with wheat in Rockefeller-Mexico in 1945 (Stern *et al*., 2008), accelerate his wheat breeding on disease resistance; later he sought to reduce lodging.

The semidwarf cultivars utilize more efficiently the soil moisture and the applied fertilizers, this being especially true in the areas of higher rainfall or irrigation production (Kubba and Ishu, 1989). Most of the genetic sources of semidwarfism for wheat breeding programs were originated from induced mutations. About 16 semidwarf mutant cultivars have been released directly, and 11 cultivars were modified by cross-breeding (Konzak *et al*. 1984).

The genes associated with a semidwarf growth habit in wheat are known as reduced height (Rht) and many of them are dominant or semi-dominant. Two genes in particular, Rht-B1b and Rht-D1b are used in much commercial wheat.

This study aims to through a light on how to produce cultivar Uruq from irradiated stored seed of soft wheat cultivar Inia-66.

## Materials and methods

Seeds of bread wheat Triticum aestivum L. cv. Inia-66 were stored for 0,5 and 10 years under

prevailing room conditions of Grain Department-State Board for applied Agriculture Research, Abu-Ghraib, Baghdad. Their moisture contents at the end of storage periods were 8.1%, 7.5% and 6.3% respectively.

Samples of 1500 seeds were irradiated with doses of 5, 10 and 15 k rad of gamma rays emitted from Co-60 source using gamma cell-220 (Atomic Energy of Canada Ltd.) at a dose rate of 81 rad/sec.

The experiment was arranged in a split plots design with four replications. The main plots were the storage periods and the sub plots were the doses of gamma rays.

The plot dimensions were 2.5x4m., and the space between rows was 30 cm. The electric conductivity (Ec) of the soil was 5.4 mmhos/cm.

Super phosphate and urea fertilizer were added at a rate of 200 kg/ha. Urea fertilizer was added at the time of planting and at tillering.

Germination, plant height, spike length, number of kernels per spike and weight of 1000 seeds for M1 plants were recorded.

In the M2 plants, all variant plants during the developmental stages were isolated and labeled.

The seeds of the semidwarf plants were planted with their parent Inia-66 for 3 successive generations (M3, M4, and M5) in comparative studies. At maturity stage: plant height, spike length, stem diameter and number of kernels per spike were measured (Kubba, et al., 1988).

Lately, we have performed DNA extraction, PCR assay and RAPD-PCR assay techniques to detect the genes responsible for reducing heights of semidwarf wheat cultivar Uruq and its parents, cultivar Inia-66 (Kubba, et al, 2013).

## Result and discussion

Table 1 showed that the storage periods and gamma rays and their interaction had significantly influence by all tested parameters of the M1 plants. These results indicated that germination reduced with longer storage time and higher dose of gamma rays.

The reduction in the height of M1 plants increased with the increase of storage periods and dose of gamma rays. This reduction can be used as a parameter to measure the alteration and has direct relationship with the increase of mutation frequency in the next generations.

Table 2 showed that the two plants with reduced height accompanied by change in seed color (from red to amber) have been isolated and used as genetic source to breed semidwarf plants which are mostly stiff straw.

The seeds of the two semidwarf plants (variants): ZB103 and ZC115 were planted in order to study some of their morphological characters in M3 plants. It appears from Table 3 that there was significant reduction in the height of the two mutant plants ZB103 and ZC115 by 31.5 cm. and 29.7 cm respectively, compared with their parent (cv. Inia-66).

In M4 generations, Table 4 showed those mutants showed the same behavior as in M 3 generation. This indicates that the two mutants, ZB103 and ZC115 have genetic stability in the studied characters (Kubba, et al., 1988).

The results of the techniques at the molecular level (Kubba, et al, 2013) showed that the allelic difference between cultivars Inia-66 and Uruq is that Uruq has the dwarf gene Rht-D1b and it is responsible for the semi dwarfism (Table 5). This result agreed with (Knopf, et al, 2008) who found out that wheat varieties with the Rht-D1b were shorter and produces higher yield than varieties without this allele (figure 1 and 2).

ZB103 mutant has been registered in the National Committee for Registration and Release of Agricultural Varieties / Ministry of Agriculture in the name of URUQ cultivar. Lately, Uruq cultivar has been taken patent and release from the National Committee for patent and release of Agriculture Varieties / Ministry of Agriculture.

Table 1. The storage periods, gamma rays and their interaction on some characteristics of M1 plants

| Storage periods (years) | Treatments (Doses of gamma rays) | Germination percent | Plant height (cm) | Spike length (cm) | No. of kernels per spike | Weight of 1000 kernels (g) |
|---|---|---|---|---|---|---|
| 0 (seeds harvested from the same year) | Control (untreated seeds) | 80.8 | 101.2 | 12.8 | 69.3 | 34.0 |
| | 5 krad | 65.0 | 89.0 | 12.2 | 58.0 | 41.7 |
| | 10 krad | 58.3 | 86.8 | 11.7 | 50.2 | 43.1 |
| | 15 krad | 48.5 | 84.2 | 11.0 | 39.8 | 46.6 |
| 5 (Five years) | Control (untreated seeds) | 75.5 | 96.3 | 11.5 | 62.1 | 35.8 |
| | 5 krad | 56.3 | 85.3 | 11.0 | 52.0 | 40.2 |
| | 10 krad | 50.8 | 82.7 | 10.1 | 46.4 | 42.9 |
| | 15 krad | 42.0 | 79.0 | 9.3 | 36.0 | 48.5 |
| 10 (Ten years) | Control (untreated seeds) | 68.0 | 87.6 | 10.3 | 48.1 | 37.4 |
| | 5 krad | 52.5 | 80.4 | 9.5 | 36.5 | 44.0 |
| | 10 krad | 33.0 | 79.1 | 8.8 | 33.2 | 48.3 |
| | 15 krad | 22.3 | 76.8 | 8.1 | 25.0 | 50.1 |
| L. S. D. (5% level) | | 4.8 | 3.2 | 0.5 | 2.2 | 2.5 |

Table 2: No. of observed variants in M2 plants

| Storage periods (years) | Treatments | Number of selected variants in M2 plants | | | |
|---|---|---|---|---|---|
| | | Chlorophyllous mutation | Earliness | Semidwarf (70cm-80cm) | Spike with more than 98 seeds |
| 0 | Control (untreated seeds) | 0 | 0 | 0 | 0 |
| | 5 krad | 0 | 0 | 0 | 0 |
| | 10 krad | 0 | 1 | 0 | 0 |
| | 15 krad | 1 | 1 | 0 | 1 |
| 5 | Control (untreated seeds) | 0 | 0 | 0 | 0 |
| | 5 krad | 1 | 0 | 0 | 0 |
| | 10 krad | 2 | 4 | 0 | 2 |
| | 15 krad | 2 | 2 | 0 | 2 |
| 10 | Control (untreated seeds) | 0 | 0 | 0 | 1 |
| | 5 krad | 1 | 1 | 1 | 2 |
| | 10 krad | 1 | 1 | 1 | 2 |
| | 15 krad | 3 | 3 | 0 | 5 |
| Total | | 11 | 13 | 2 | 15 |

Table 3: Some morphological characters of the two mutants: ZB 103 and ZC 115 in M3 generation

| Characters | Parent (Inia-66) | Semidwarf mutants | | L. S. D. (5% level) |
|---|---|---|---|---|
| | | Inia-ZC115 | Inia-ZB103 | |
| **Plant height (cm)** | 103.8 | 74.1 | 72.3 | 15.62 |
| **Spike length (cm)** | 14.0 | 15.1 | 15.6 | 0.38 |
| **Stem diameter (m.m)** | 4.2 | 5.0 | 5.2 | 0.18 |
| **No. of kernels per spike** | 71.0 | 78.5 | 80.9 | 6.42 |

Table 4: Some morphological characters of the two mutants: ZB 103 and ZC 115 in M4 generation

| Characters | Parent (Inia-66) | Semidwarf mutants | | L. S. D. (5% level) |
|---|---|---|---|---|
| | | Inia-ZC115 | Inia-ZB103 | |
| **Plant height (cm)** | 104.5 | 76.2 | 73.0 | 15.24 |
| **Spike length (cm)** | 13.7 | 14.9 | 15.3 | 0.32 |
| **Stem diameter (m.m)** | 4.3 | 5.2 | 5.5 | 0.29 |
| **No. of kernels per spike** | 69.6 | 80.2 | 81.3 | 5.26 |

Table 5: Rht-alleles found in Inia-66 and Uruq cultivars

| Genotype | | Inia-66 | Uruq |
|---|---|---|---|
| Tall | Rht-B1a | + | + |
| | Rht-D1a | + | + |
| Dwarf | Rht-B1b | + | + |
| | Rht-D1b | - | + |

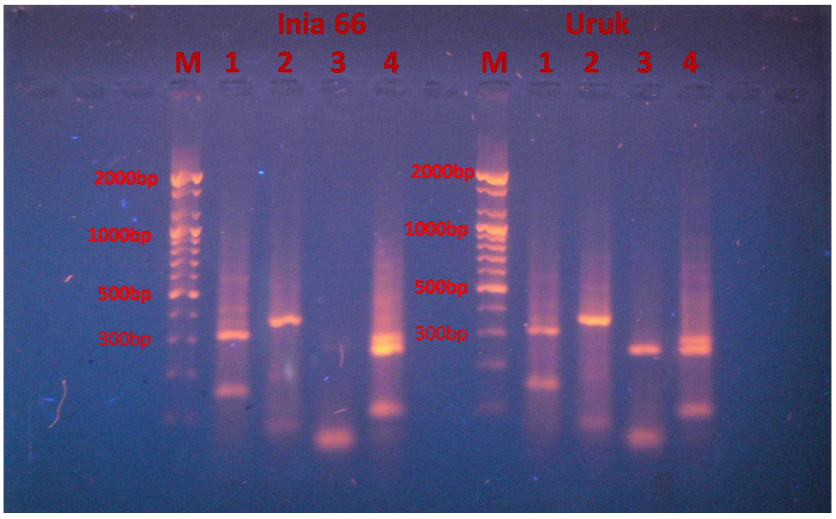


Figure 1. Ethidium bromide stained agarose gel electrophoresis of the PCR detection of Rht allele using specific primers: 1-Rht B1a 2-Rht B1b 3-Rht D1a 4-Rht D1b M DNA ladder 100bp.

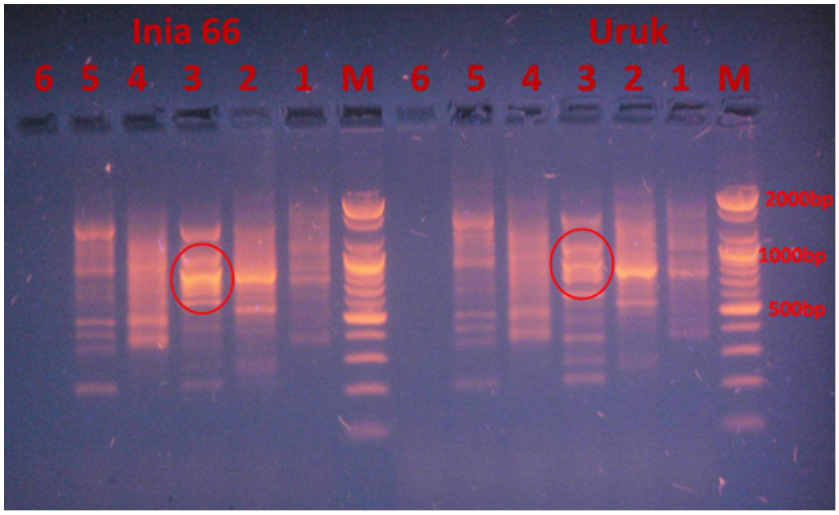


Figure 2. Ethidium bromide stained agarose gel electrophoresis of the RAPD-PCR product banding pattern obtained from six rapd primer as in table1, separated on 1.8% agarose gel, 5V/cm at 3hr., lane M represented the molecular marker 100bp DNA Ladder.

**References**

Djelepov K (1976), Induced short-stemmed winter common wheat mutant. Int. Symp. Experimental Mutagenesis in Plants, 177-185.

Konzak. CF, Wilson M.R and Frank PA (1984) Progress in the evaluation. Use in breeding and genetic analysis of semidwarf mutants of wheat, semidwarf cereal mutants and their use in cross breeding, 11, IAEA:39-50.

Sial M.A, Dahot MU, Laghari KA, Arain MA, Mangrio SM and Pirzada AJ (2010) "Agronomic Performance of Semidwarf and Dwarf Wheat Genotypes", World Applied Sciences Journal 8, 30-33.

Stern KR, Bidlack JE and Jansky SH 2008, "Introductory Plant Biology". Mc Graw Hill, Higher Education, 11th Edition.

Kubba AJ, Ishu RM (1989), Characteristics of two semidwarf mutants obtained from bread wheat Triticum aestivum L., J. Agric. Water Reso. Res., 8 (1): 27-33.

Kubba AJ, Ibrahim IF and Ishu RM (1988), Induction of Semidwarf Mutation in Triticum Aestivum L. cv. Inia-66, J. Agric. Water Reso. Res., 7 (2): 95-105.

Kubba AJ, Munir AIM and Sabbah MA (2013), Rht-B1b and Rht-D1b analysis of semidwarf wheat cv. Uruq produced by Induced Mutation, Iraqi J. of Biotechnology,12 (2): 101-106.

Knopf C, Becker H, Ebmeyer E and Korzun V (2008). Occurrenece of three dwarfing Rht genes in German winter wheat Varieties: Cereal Research Communications, 36(4): 553-560.

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# Effect of growth regulators on tissue culture parameters in rice (*Oryza sativa L.*)

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**ABSTRACT**

This study was conducted in Ankara University, Faculty of Agriculture, Department of Field crops, Biotechnology Laboratory. The objective of the present study was to determine the effects of growth regulators on tissue parameters in rice. In this study, mature embryos of three rice cultivars (Aromatik-1, Baldo and Karadeniz) and different growth regulators (2,4-Dichlorophenoxyacetic acid (2,4-D) and picloram) were used as material. For callus induction, mature embryos were placed with scutellum upwards on three different medium (hormone-free MS-0, MS + 2 mg/l 2,4-D and MS + 2.5 mg/l picloram) in sterile Petri dishes for two weeks at 25±10C in darkness. After incubation; obtained calli were transferred to hormone-free MS-0 medium for regeneration. According to results, the effect of growth regulators and genotypes on callus induction and plant regeneration in rice were found to be statistically significant

***Keywords:*** Rice, *oryza sativa* L., callus induction, 2, 4-D, picloram.

## Introduction

Rice (*Oryza sativa* L.) is the grain with the third-highest cultivated area, after wheat and maize. Rice can be grown in all types of soils with sufficient water holding capacity and suitable temperature, in Turkey its grown intensively especially in Marmara and Karadeniz Regions. However, domestic production is not enough for the internal consumption. In recent years, the amount of import has outpaced the amount of production. According to data of year 2012, approximately 880.000 tones of rice had been obtained from 118.720 ha area in Turkey (FAO, 2012). Rice breeders in Turkey should improve the yield and quality of rice to decrease the amount of import. In this context, beside the classical plant breeding, genetic engineering and biotechnological methods should also be utilized. However, plant breeders developed different types of rice cultivars successfully in recent years and showed that gene transferring techniques can be used as supporting tool for classical plant breeding methods (Koyuncu et al. 2005).Recently, particle bombardment technique (Christou et al. 1991; Li et al. 1993;Christou 1997) and protoplast culture (Moura et al. 1997; Tsugawa and Suzuki, 2000) methods have also been used in the rice gene transfer studies.

On the other hand, plant tissue culture is the most important step of plant regeneration and gene transfer among modern methods. Embryogenic calli, rather than direct tissues such as shoot spices, immature inflorescences, roots and leaves are used for genetic transformation and regeneration of rice plants because the callus culture, compared with organogenesis, is much more suitable for the gene delivery and regeneration of transgenic rice plants (Ananthi et al. 2010).

As known, callus induction and plant regeneration potential are affected by the genotypes, carbohydrate metabolism-source, plant growth regulators, culture medium and conditions etc. In particular genotype,

and explants are important factors for a successful embryogenic callus induction and regeneration of the rice plants (Rueb et al. 1994). In the plant tissue culture studies, embryos are mostly used as source of explant in cereal crops. For embryo culture, mostly embryos obtained from mature and immature seeds are used. Mature embryos, which are always available without time limitation, are widely used rather than immature embryos according to Özgen et al., (1996).

In this study, the effects of some plant growth regulators such as 2,4-D and picloram, on callus induction and plant regeneration were obeserved and determined by using mature embryo culture method.

## Materials and methods

In this study, 3 rice (*Oryza sativa* L.) genotypes, Aromatik-1, Baldo and Karadeniz were used as sources of mature embryos. A completely randomized design with three replications per seed group for each genotype was used. Rice seeds were dehulled mechanically and they were surface-sterilized with 70% (v/v) ethanol for 5 min., washed 3 times with sterile distilled water, immersed in commercial bleach (containing 5% sodium hypoclorite) for 30 min, and rinsed at least 7 times with sterile distelled water. Then, the seeds were imbibed in sterile distilled water for 2 h at 33°C in submarine. Afterwards, the embryos were seperated from the endosperm in imbibed seeds and scutellum were placed on 3 types of culture media containing 20 g/l sucrose+ 4,43 g/l MS + 2 mg/l 2,4-D + 7 g/l agar, 20 g/l sucrose + 4,43 g/l MS + 2.5 mg/l picloram + 7 g/l agar and hormone-free MS medium 20 g/l sucrose + 4,43 g/l MS + 7 g/l agar and incubated for callus induction at 25±1°C for 14 days in darkness. Based on preliminary work and literature survey; 2 mg/l 2,4-D and 2,5 mg/l picloram doses were used in this study which have provided high callus induction and regeneration capacity in mature embryo culture of cereals (Raina et al, 1987; Barro et al, 1999; He and Lazzeri 2001) At the end of this stage, callus induction ratio (%) and callus weight (g) parameters were determined.

After the incubation, the calli were transferred to hormone-free MS medium for initiating root and shoot and maintained for 4 weeks at 25±1°C in 16-h light and 8-h dark photoperiod. After 4 weeks, by counting the regenerated calli regeneration capacity and culture efficiency data were obtained.

Petri dishes containing 10 embryos were considered the units of replication. All obtained data were subjected to statistical analyses using MSTAT statistical software and comparison of means was based on a LSD test (Düzgüneş et al. 1983)

## Results

### *Callus induction*

Callus formation from mature embryos started after 4-5 days of culture. At the end of 14 days, callus induction rate (%) and callus weight (g) data were obtained (Figure 1). Considering overall avarages in examined parameters; the medium including 2,4-D gave higher callus weight and callus induction values than the medium containing picloram (Table 1). Our experimental results revealed that in the medium with 2,4-D, Aromatik-1 and Baldo cultivars gave higher callus induction frequency (100% and -100%) and callus weight (0,310 g and 0,403 g), respectively as compared to the medium with picloram . Also , it was observed that Karadeniz gave highest results in callus induction (100%) and callus weight (0,325 g) in medium containg picloram compared to 2,4-D (Table 2).

According to the results of the analysis of variance; Genotype x Hormone interaction was found statistically significant in terms of callus induction frequency ($P<0.05$) and callus weight ($P<0.01$) parameters. These effects indicated that used genotypes are affected differently by hormones.

Similarly, the correlation between callus induction and callus weight found statistically significant ($r=0,946$, $P<0.01$) (Table 3). This situation shows that, when callus weight is increasing regeneration capacity also increases significantly.

### *Plant regeneration*

At the end of 14 days, green spots and shoots were observed after 3-4 days in the calli which were transferred to hormone-free MS-0 medium (Figure 2). The calli of Aromatik-1 developed in 2,4-D medium gave the highest regeneration capacity (80%). The calli of Baldo developed in picloram medium had higher results (95,3%) in regeneration capacity (Table 2). On the other hand, the calli of Karadeniz, developed in 2,4-D and picloram media gave the same regeneration capacity (93,3%) result (Table 2).

In terms of culture efficiency; the calli of Aromatik-1 and Baldo cultivars developed in 2,4-D medium gave higher results (80% and 86,7%, respectively ), however the calli of Karadeniz developed in picloram medium gave the highest culture efficiency result (93,3%) (Table 2).

According to the results of the analysis of variance; Genotype x Hormone interaction was found statistically significant in terms of the regeneration capacity and culture efficiency ($P<0.01$). It means that the increment in regeneration capacity increases the culture efficiency significantly in the used cultivars. Additionally, the

correlation coeffficents between regenaration and culture effect were found statistically significant (r=0,990, P<0.01) and they are presented at Table 3.

## Discussion

According to the data of this research; it figured out that the active ingredient 2,4-D is more effective than picloram in terms of tissue culture parameters. Also, the statistical performed showed significant Genotype x Hormone interaction and the increment of callus weight increases the regenaration capacity and it was seen that culture efficiency was increased parallelly. It can be concluded that using appropriate genotype and hormonal selection and its application can provide increase in tissue culture parameters in rice plant and enhance success.

Table 1. The effects of growth regulator on tissue culture parameters in 3 rice genotypes

| Hormones | Callus induction frequency (%) | Callus weight (g) | Regenaration capacity [a] (%) | Culture Efficiency [b] (%) |
|---|---|---|---|---|
| **Control** | 2,2 | 0,008 | 0 | 0 |
| **2,4-D** | 98,9 | 0,337 | 86,7 | 85,6 |
| **Picloram** | 90,0 | 0,304 | 74,5 | 68,9 |

[a] Regenarated callus number/ Induced callus number x100
[b] Regenarated callus number/Cultured embryo numberx100

Table 2. The effects of 2,4-D and picloram on tissue culture parameters in mature embryos of rice genotypes

| Genotypes | Hormones | Callus induction (%) | Callus weight (g) | Regeneration capacity (%) | Culture efficiency (%) |
|---|---|---|---|---|---|
| **Aromatik** | MS 0 | 0 **c** | 0 **e** | 0 **c** | 0 **c** |
| | 2,4-D | 100 **a** | 0,310 **bc** | 80 **a** | 80 **a** |
| | Picloram | 86,7 **b** | 0,234 **d** | 35 **b** | 33,3 **b** |
| **Baldo** | MS 0 | 6,7 **c** | 0,024 **e** | 0 **c** | 0 **c** |
| | 2,4-D | 100 **a** | 0,423 **a** | 86,7 **a** | 86,7 **a** |
| | Picloram | 83,3 **b** | 0,352 **b** | 95,3 **a** | 80 **a** |
| **Karadeniz** | MS 0 | 0 **c** | 0 **e** | 0 **c** | 0 **c** |
| | 2,4-D | 96,7 **a** | 0,276 **cd** | 93,3 **a** | 90 **a** |
| | Picloram | 100 **a** | 0,325 **bc** | 93,3 **a** | 93,3 **a** |

Table 3. The correlation coeffficents of mature embryogenic calli of rice genotypes

| | Callus induction (1) | Callus weight (2) | Regeneration capacity (3) | Culture efficiency (4) |
|---|---|---|---|---|
| **1** | - | 0,946 ** | 0,907 ** | 0,922 ** |
| **2** | - | - | 0,925 ** | 0,922 ** |
| **3** | - | - | - | 0,990 ** |
| **4** | - | - | - | - |

Significantly different from zero at ** P<0,01

Figure 1. The callus induction from mature embryos of rice genotypes after 14 days (a: Aromaik-1, b:Baldo, c: Karadeniz)

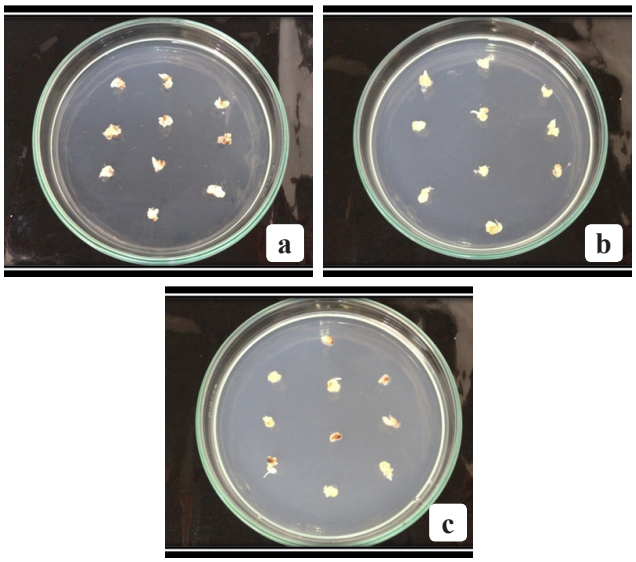
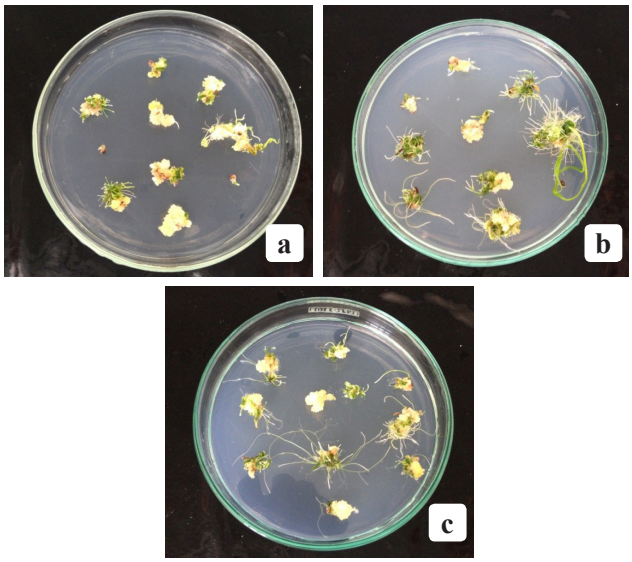



Figure 2. Plant regeneration in rice genotypes after 4 weeks (a: Aromatik-1, b:Baldo, c: Karadeniz)




**References**

Ananthi N, Anandakumar CR, Ushakumari R and Shanti P(2010) Regeneration Study of Some Indica Rice Cultivars Followed by Agrobacterium- Mediated Transformation of Highly Regenerable Cultivar, Pusa Basmati 1. Electronic Journal of Plant Breeding.1249-1256.

Barro F, Martin A, Lazzeri PA and Barcelo P(1999) Medium optimization for efficient somatic embryogenesis and plant regeneration from immature inflorescences and immature scutella of elite cultivars of wheat, barley and Tritordeum. Euphytica, 1.8, 161-167.

Christou P, Ford TL and Kofron M(1991) Production of Transgenic Rice (*Oryza sativa* L.) Plants from Agronomically Important Indica and Japonica Varieties via Electric Discharge Particle Acceleration of Exogenous DNA from Immature Zygotic Embryos. Nature Biotechnology. 9, 957-962.

Christou P(1997) Rice transformation: bombardment. *Plant Molecular Biology,* vol: 35, p. 197-203.

Düzgüneş O, Kesici T ve Gürbüz F (1983) İstatistik metodları I. Ankara Üniv. Ziraat Fak. Yayınları. 861. Ders Kitabı, 229, Ankara.

FAOSTAT J (2012) http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 25 September 2013.

He GY and Lazzeri PA (2001) Improvement of somatic embryogenesis and plant regeneration from Durum wheat *(*Triticum turgidum var. durum Desf.) scutellum and inflorescense cultures. Euphytica. 119, 369-376.

Koyuncu N, Ulukan H and Özgen M(2003) Türkiye'de yetiştirilen çeltik çeşitlerinin olgun embriyolarında kallus oluşumu ve bitki rejenerasyonu. XIII. Biyoteknoloji Kongresi, Bildiriler:111-116, 25-29 Ağustos 2003, Çanakkale.

Li L, Qu R, Kockho de A, Faquet C and Beachy RN(1993) An improvement rice transformation system using the biolistic method. Plant Cell Reports, vol: 12, p: 250-255.

Moura DS, Zapata-Arias FJ, Ando A and Neto AT(1997) Plant regeneration from protoplast isolated from primary calli using mature embryos of two Brazilian rice cultivars. Euphytica, vol: 94 p. 1-5.

Özgen M, Türet M, Özcan S and Sancak C(1996). Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breeding. 115, 455-458.

Raina SK, Sathish P and Sarma KS(1987) Plant regeneration from in vitro cultures of anthers and mature seeds of rice (*Oryza sativa* L.) cv. Basmati-370. Plant Cell Reports. Volume 6, Issue 1, pp 43-45.

Rueb S, Leneman M, Schilperoot RA and Hensgens LAM(1994) Efficient Plant Regeneration Through Somatic Embriyogenesis from Callus Induced on Mature Rice Embryos (Oryza sativa L.). Plant Cell, Tissue and Organ Culture. 36, 259-264.

Tsugawa H and Suzuki M(2000) A low temperature method for maintaining plant regeneration activity in embryogenic callus of rice (*Oryza sativa* L.). Plant Cell Reports, vol: 19, p. 371-375.

Research Article

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# Developing confectionery sunflower hybrids and determination of their yield performances in different environmental conditions

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## ABSTRACT

Confectionery type sunflower grows generally in Eastern and Middle Anatolia in Turkey but there is no certified seed in the production which have white color with grey stripe. The study covered the confectionery sunflower hybrids developed in Confectionery Sunflower Breeding Project conducted by Trakya Agricultural Research Institute, Edirne, Turkey. The candidate confectionery hybrids were tested and evaluated in the regional yield trials in Trakya Region in 2009-2010. Seed yield, 1000 seed weight, flowering and physiological maturity period, plant height, head diameter, oil content were measured. Based on trial results; some experimental hybrids exhibited higher performance than controls for both for seed yield and seed weight and promising candidate hybrids were selected to send to registration trials. From these promising hybrids, 09 TRÇ 003 and 09 TRÇ 004 confectionery hybrid were sent to registration trials in 2011 2012, respectively and production permission were obtained for them. After evaluation of candidate hybrids in these years, having higher general combining inbred lines were also sent to National Registration Office for registration too. In 2010; two female line and three restorer (male) lines were sent to registration. In 2011; one female and one male line sent for DUS tests and then registration in 2012.

***Keywords:*** Sunflower, confectionery hybrid, inbred line, seed yield, seed quality

## Introduction

Although sunflower grows mainly for producing vegetable oils in the world, it is one of the most preferred confectionery seed in Turkey, in Eastern Europe, US, Canada and also in some Asian countries such China, Pakistan, Iran, Middle East countries, etc.. They generally are white color with grey stripes (however black ones are also popular in Slavic countries), and larger than the oil-type, with a lower oil percentage (Hladni et al., 2012; Kaya, 2002, 2004; Kaya et al., 2008, 2009, 2013; Gontcharov, 2011; Kholghi, 2011; Nabloussi, 2011; Crnobarac et al., 2014; Gholinezhad et al., 2014; Velasco et al., 2014). However, bigger size is the most preferable character by customers in confectionery sunflower so 1000 seed weight and seed sizes are higher interests in the confectionery breeding in addition to seed yield. In the confectionary sunflower sectors; highest quality seeds including the largest and cleanest seeds are used for snack and hulled sunflowers are seeds that are still food-grade quality, but they do not possess the characteristics to be in the food-grade category and smaller and lower quality seeds are used for birdseed (Evci et al., 2011; Nabloussi, 2011; Velasco et al., 2014).

Sunflower which is the most consuming snack in Turkey is growing both for producing vegetable oil and also for confectionery seed in manly Middle and Eastern Anatolia, Southern Marmara and Agean Regions of Turkey. However, there is

big problem on high quality seed because there is no certified seed and not more registered hybrids in the market. Turkish people prefer to consume commonly confectionery sunflower seed as bigger sizes, white color with grey stripes and the price is higher in recent years due to higher demand both for domestic market and also export potential to Europe and Middle East. Therefore Turkish confectionery sunflower production reached to 100,000 MT in recent years (Kaya et al., 2009; 2013). The aim of the study was to determine of yield and seed quality performances of hybrids in confectionery sunflower to supply promising cultivars for sector.

## Materials and methods

Confectionery sunflower hybrid breeding research was started in National Sunflower Project conducted by Trakya Agricultural Research Institute, Edirne, Turkey in 2001. The experiments in the study were conducted in Lüleburgaz and Edirne locations under this project to develop confectionery sunflower hybrids and lines in 2009 and 2010. In the trials, the obtained hybrids were also tested to evaluate general and specific combining ability of inbred lines. The trials were conducted in rain fed irrigated conditions in Lüleburgaz and irrigated conditions in Edirne location. In each year and each location, two regional yield trials were conducted in the study. Total 64 candidate hybrids were included in 2009 and 56 candidates in 2010 with three controls. Suriye population- village population, Çiğdem- an open pollinated variety, Palanci-1- first registered confectionery hybrid in Turkey, and Marker -a commercial hybrid were included as controls in the yield trials.

The experimental design was a Randomized Complete Block Design with four replications. The four rows plots were 7,5 m long with the 70 x 45 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 harvested mostly in mid September by hand in each year. Seed yield (kg $ha^{-1}$), 1000 seed weight (g), flowering and physiological maturity (days), plant height (cm), head diameter (cm), oil content (%) were measured. The data were analyzed statistically with JUMP program.

## Results

Many candidate confectionery sunflower hybrids exhibited higher yield and quality performances in both years and locations in the study (Tables 1 to 12). They were analyzed not only for seed yield, but also seed weight, diameter and height and color too because Turkish market request as much as bigger size and whiter color. Based on the study results, similar performances were attained by same candidate hybrids both under irrigated and non-irrigated conditions in the regional experiments. For instance, while 09 TRC 30, 09 TRC 32, 09 TRC 36, 09 TRC 32 and 09 TRC 41 hybrids existed in first five ranks in the Edirne location and same candidates kept higher performances with ranking in top positions in Luleburgaz location too (Tables 1 and 2).

While some candidates revealed higher seed yield performance over 40% than control in the trials, many candidates had also higher 1000 seed weight than controls too. Furthermore, most of the candidates had over 100 g weights which was acceptable point for confectionery market. The study results indicated that the candidates could attain enough seed weight and higher quality for preferable level by customers and also higher seed yield potential for growers, if they could be grown in suitable conditions. On the other hand, almost all candidates had lower oil contents such as around 30% as requested for confectionery sector (less than 30%). Promisingly, many candidates had not higher plant heights which are characteristics of confectionery types and had higher head diameters than controls.

## Discussion

The promising results indicated that the success of selection in the National program. After analyzing all seed yield and quality and morphological data of trials, promising hybrids were selected. From these promising hybrids, 09 TRÇ 003 (96171 A X 9892 R) (09TR51 in Tables 3 and 4) were sent in 2011 and 09 TRÇ 004 (9707 A X 9881 R) (09 TRC 30 in Tables 1 and 2) confectionery hybrid were sent registration trials in 2012 production permission were obtained for them. After evaluation of candidate hybrids in these years, hybrids involving higher general combining inbred lines as parents were also sent to National Registration Office for registration too. In 2010; 08-9322-29-A-ÇRZ female line and, 08-9640-1-R-ÇRZ, 08-9717-4-R-ÇRZ, 08-9775-23-R-ÇRZ restorer (male) lines were sent to registration. In 2011; 96171-A ÇRZ female line and 9892-R ÇRZ male line and, 9707-A female and 9881-R male lines sent for DUS tests and then registration in 2012.

Table 1: Confectionery type hybrids in Yield Trial-2 at Edirne in 2009

| Hybrids | Seed Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 45 | 2443.0 | 1 | 140.3 | 29.0 | 97.16 | 70 | 94 | 152 | 15 |
| 09 TRC 33 | 2339.0 | 2 | 134.3 | 28.7 | 110.70 | 64 | 93 | 148 | 17 |
| 09 TRC 30 | 2325.0 | 3 | 133.5 | 26.7 | 106.40 | 67 | 100 | 153 | 15 |
| 09 TRC 41 | 2298.0 | 4 | 132.0 | 34.4 | 74.48 | 68 | 99 | 135 | 17 |
| 09 TRC 32 | 2233.0 | 5 | 128.3 | 30.4 | 85.67 | 69 | 96 | 149 | 13 |
| 09 TRC 36 | 2174.0 | 6 | 124.9 | 31.7 | 106.58 | 63 | 97 | 139 | 18 |
| 09 TRC 39 | 2158.0 | 7 | 124.0 | 31.4 | 98.87 | 64 | 93 | 152 | 16 |
| 09 TRC 27 | 2051.0 | 8 | 117.8 | 28.0 | 71.83 | 65 | 98 | 134 | 14 |
| 09 TRC 31 | 2022.0 | 9 | 116.1 | 32.0 | 92.87 | 68 | 94 | 153 | 16 |
| 09 TRC 35 | 2017.0 | 10 | 115.9 | 30.6 | 96.66 | 64 | 96 | 136 | 15 |
| 09 TRC 23 | 1976.0 | 11 | 113.5 | 31.1 | 85.13 | 68 | 95 | 169 | 17 |
| 09 TRC 25 | 1975.0 | 12 | 113.4 | 33.8 | 80.55 | 71 | 100 | 116 | 15 |
| ÇİĞDEM-1(C) | 1901.0 | 13 | 109.2 | 29.3 | 89.06 | 70 | 102 | 169 | 18 |
| 09 TRC 37 | 1886.0 | 14 | 108.3 | 30.2 | 89.44 | 67 | 92 | 137 | 18 |
| 09 TRC 24 | 1863.0 | 15 | 107.0 | 34.9 | 90.00 | 67 | 94 | 129 | 14 |
| PALANCI 1(C) | 1698.0 | 18 | 97.5 | 27.6 | 97.10 | 74 | 104 | 175 | 13 |
| SURİYE NEV(C) | 1626.0 | 19 | 93.4 | 32.2 | 90.52 | 72 | 99 | 159 | 14 |

CV (%) =10.53 LSD=287.9 kg ha$^{-1}$ for seed yield.

Table 2: Confectionery type hybrids in Yield Trial-2 at Lüleburgaz in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 42 | 3118.0 | 1 | 121.9 | 30.7 | 125.49 | 65 | 106 | 174 | 21 |
| 09 TRC 32 | 3093.0 | 2 | 120.9 | 28.3 | 137.36 | 67 | 108 | 170 | 17 |
| 09 TRC 30 | 3063.0 | 3 | 119.8 | 31.3 | 130.15 | 67 | 102 | 150 | 18 |
| 09 TRC 29 | 3050.0 | 4 | 119.3 | 24.0 | 138.90 | 66 | 104 | - | 21 |
| 09 TRC 41 | 3036.0 | 5 | 118.7 | 30.3 | 106.40 | 65 | 104 | 177 | 22 |
| 09 TRC 36 | 2966.0 | 6 | 116.0 | 29.4 | 127.88 | 60 | 103 | 150 | 20 |
| 09 TRC 45 | 2919.0 | 7 | 114.1 | 27.7 | 130.33 | 66 | 109 | 164 | 20 |
| 09 TRC 33 | 2909.0 | 8 | 113.7 | 29.6 | 141.76 | 59 | 109 | 192 | 20 |
| 09 TRC 23 | 2876.0 | 9 | 112.5 | 28.8 | 108.5 | 68 | 107 | 183 | 16 |
| 09 TRC 34 | 2838.0 | 10 | 111.0 | 32.0 | 126.44 | 59 | 106 | 163 | 19 |
| SURİYE NEV(C) | 2803.0 | 11 | 109.6 | 30.9 | 96.92 | 67 | 105 | 183 | 17 |
| 09 TRC 44 | 2714.0 | 12 | 106.1 | 32.6 | 95.38 | 62 | 107 | 163 | 20 |
| 09 TRC 37 | 2622.0 | 13 | 102.5 | 29.0 | 121.04 | 64 | 107 | 136 | 19 |
| PALANCI 1(C) | 2534.0 | 16 | 99.1 | 28.4 | 103.98 | 67 | 104 | 170 | 22 |
| ÇİĞDEM-1(C) | 2336.0 | 20 | 91.3 | 31.6 | 82.49 | 65 | 105 | 164 | 17 |

CV (%) =7.35 LSD=274.0 kg ha-1 for seed yield.

Table 3: Confectionery type hybrids in Yield Trial-3 at Edirne in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 51 | 2556.0 | 1 | 115.2 | 29.9 | 111.80 | 69 | 100 | 181 | 17 |
| 09 TRC 54 | 2422.0 | 2 | 109.1 | 30.6 | 95.18 | 67 | 106 | 164 | 16 |
| 09 TRC 62 | 2401.0 | 3 | 108.2 | 33.2 | 97.75 | 65 | 93 | 143 | 14 |
| 09 TRC 57 | 2371.0 | 4 | 106.8 | 31.3 | 99.66 | 67 | 94 | 166 | 13 |
| ÇİĞDEM-1(C) | 2314.0 | 5 | 104.3 | 29.1 | 94.66 | 70 | 102 | 169 | 14 |
| 09 TRC 55 | 2313.0 | 6 | 104.3 | 32.5 | 107.91 | 63 | 98 | 160 | 15 |
| 09 TRC 52 | 2310.0 | 7 | 104.1 | 31.1 | 106.71 | 63 | 104 | 159 | 14 |
| 09 TRC 64 | 2307.0 | 8 | 104.0 | 29.0 | 97.78 | 69 | 102 | 174 | 15 |
| 09 TRC 49 | 2249.0 | 9 | 101.4 | 28.6 | 115.89 | 68 | 107 | 169 | 15 |
| 09 TRC 60 | 2248.0 | 10 | 101.3 | 27.3 | 122.62 | 67 | 103 | 164 | 14 |
| 09 TRC 56 | 2246.0 | 11 | 101.2 | 31.7 | 102.70 | 66 | 94 | 123 | 14 |
| 09 TRC 58 | 2184.0 | 12 | 98.4 | 30.9 | 84.85 | 68 | 104 | 168 | 15 |
| SURİYE NEV(C) | 2178.0 | 13 | 98.2 | 29.7 | 115.45 | 69 | 102 | 169 | 12 |
| 09 TRC 68 | 2169.0 | 14 | 97.8 | 32.1 | 96.48 | 62 | 103 | 161 | 16 |
| PALANCI 1(C) | 2165.0 | 15 | 97.6 | 27.7 | 102.81 | 74 | 94 | 161 | 14 |
| 09 TRC 65 | 2156.0 | 16 | 97.1 | 32.9 | 98.40 | 64 | 97 | 154 | 17 |

CV (%) =7.39 LSD=225.2 kg ha-1 for seed yield.

Table 4: Confectionery type hybrids in Yield Trial-3 at Lüleburgaz in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 54 | 3075.0 | 1 | 139.3 | 27.8 | 155.42 | 64 | 106 | 182 | 13 |
| 09 TRC 64 | 3027.0 | 2 | 137.1 | 28.2 | 111.54 | 63 | 104 | 163 | 17 |
| 09 TRC 55 | 3022.0 | 3 | 136.9 | 28.9 | 145.85 | 59 | 102 | 150 | 17 |
| 09 TRC 51 | 2953.0 | 4 | 133.7 | 28.3 | 109.02 | 61 | 106 | 179 | 19 |
| 09 TRC 49 | 2905.0 | 5 | 131.6 | 30.3 | 121.06 | 58 | 105 | 168 | 19 |
| 09 TRC 62 | 2903.0 | 6 | 131.5 | 32.8 | 119.24 | 58 | 106 | 133 | 15 |
| 09 TRC 59 | 2886.0 | 7 | 130.7 | 25.0 | 165.90 | 57 | 105 | 170 | 20 |
| 09 TRC 58 | 2884.0 | 8 | 130.6 | 29.3 | 127.39 | 63 | 104 | 187 | 18 |
| 09 TRC 53 | 2855.0 | 9 | 129.3 | 28.1 | 147.45 | 65 | 108 | 190 | 20 |
| 09 TRC 60 | 2850.0 | 10 | 129.1 | 27.2 | 139.73 | 61 | 106 | 160 | 20 |
| 09 TRC 48 | 2816.0 | 11 | 127.5 | 27.6 | 136.16 | 62 | 107 | 170 | 17 |
| 09 TRC 57 | 2813.0 | 12 | 127.4 | 30.5 | 126.47 | 62 | 105 | 180 | 21 |
| 09 TRC 66 | 2792.0 | 14 | 126.4 | 30.9 | 121.65 | 62 | 100 | 166 | 17 |
| SURİYE NEV(C) | 2581.0 | 21 | 116.9 | 27.3 | 136.14 | 65 | 104 | 179 | 23 |
| ÇİĞDEM-1(C) | 2033.0 | 24 | 92.1 | 31.9 | 105.77 | 66 | 106 | 173 | 20 |
| PALANCI 1(C) | 2012.0 | 25 | 91.1 | 25.3 | 130.38 | 66 | 105 | 185 | 15 |

CV (%) =10.15 LSD=385.8 kg ha$^{-1}$ for seed yield.

Table 5: Confectionery type hybrids in Yield Trial-5 at Edirne in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 93 | 3037.0 | 1 | 116.2 | 34.5 | 96.8 | 62 | 100 | 159 | 22 |
| 09 TRC 97 | 2969.0 | 2 | 113.6 | 41.2 | 70.8 | 62 | 103 | 146 | 18 |
| 09 TRC 95 | 2919.0 | 3 | 111.7 | 40.6 | 68.4 | 62 | 101 | 154 | 19 |
| 09 TRC 87 | 2910.0 | 4 | 111.4 | 32.8 | 78.5 | 56 | 99 | 145 | 20 |
| PALANCI 1(C) | 2863.0 | 5 | 109.6 | 30.6 | 117.0 | 67 | 106 | 160 | 21 |
| ÇİĞDEM-1(C) | 2841.0 | 6 | 108.7 | 33.0 | 79.10 | 68 | 108 | 135 | 18 |
| 09 TRC 92 | 2695.0 | 7 | 103.1 | 31.1 | 74.6 | 59 | 102 | 156 | 22 |
| 09 TRC 88 | 2671.0 | 8 | 102.2 | 29.6 | 81.3 | 56 | 96 | 143 | 20 |
| 09 TRC 86 | 2629.0 | 9 | 100.6 | 31.4 | 87.7 | 57 | 97 | 172 | 19 |
| 09 TRC 96 | 2615.0 | 10 | 100.1 | 40.0 | 83.3 | 61 | 104 | 142 | 20 |
| 09 TRC 89 | 2436.0 | 11 | 93.2 | 28.2 | 80.30 | 58 | 97 | 139 | 18 |
| 09 TRC 94 | 2337.0 | 12 | 89.4 | 40.1 | 80.7 | 62 | 99 | 163 | 23 |
| 09 TRC 90 | 2257.0 | 13 | 86.4 | 35.4 | 76.0 | 60 | 106 | 153 | 22 |
| 09 TRC 85 | 2158.0 | 14 | 82.6 | 31.1 | 81.4 | 56 | 96 | 160 | 18 |
| SURİYE NEV(C) | 2136.0 | 15 | 81.7 | 28.3 | 102.6 | 62 | 102 | 180 | 23 |

CV (%) =8.46 LSD=263.8 kg ha-1 for seed yield.

Table 6: Confectionery type hybrids in Yield Trial-5 at Lüleburgaz in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 09 TRC 96 | 3191.0 | 1 | 108.5 | 33.9 | 110.50 | 65 | 106 | 176 | 24 |
| SURİYE NEV(C) | 3135.0 | 2 | 106.6 | 28.3 | 103.24 | 66 | 104 | 189 | 20 |
| 09 TRC 93 | 3050.0 | 3 | 103.7 | 27.1 | 141.76 | 59 | 105 | 163 | 18 |
| 09 TRC 87 | 3043.0 | 4 | 103.4 | 28.6 | 118.13 | 58 | 105 | 178 | 22 |
| ÇİĞDEM-1(C) | 2990.0 | 5 | 101.6 | 29.8 | 93.16 | 67 | 105 | 141 | 19 |
| 09 TRC 92 | 2949.0 | 6 | 100.2 | 28.9 | 89.68 | 58 | 106 | 172 | 17 |
| 09 TRC 85 | 2883.0 | 7 | 98.0 | 26.3 | 121.60 | 58 | 101 | 147 | 20 |
| 09 TRC 94 | 2878.0 | 8 | 97.8 | 35.3 | 107.87 | 66 | 104 | 169 | 15 |
| 09 TRC 86 | 2871.0 | 9 | 97.6 | 27.1 | 111.14 | 57 | 104 | 165 | 21 |
| PALANCI 1(C) | 2702.0 | 10 | 91.8 | 27.7 | 133.74 | 68 | 105 | 154 | 18 |
| 09 TRC 91 | 2624.0 | 11 | 89.2 | 24.1 | 130.30 | 59 | 103 | 180 | 26 |
| 09 TRC 88 | 2597.0 | 12 | 88.3 | 30.4 | 119.09 | 57 | 104 | 181 | 24 |
| 09 TRC 97 | 2577.0 | 13 | 87.6 | 36.7 | 102.84 | 64 | 104 | 171 | 20 |
| 09 TRC 89 | 2570.0 | 14 | 87.4 | 29.4 | 114.23 | 57 | 103 | 150 | 25 |
| 09 TRC 95 | 2556.0 | 15 | 86.9 | 36.1 | 108.28 | 66 | 105 | 179 | 22 |
| 09 TRC 90 | 2497.0 | 16 | 84.9 | 27.7 | 115.74 | 57 | 101 | 156 | 27 |

CV (%) =10.83 LSD=434.7 kg ha-1 for seed yield.

Table 7: Confectionery type hybrids in Yield Trial-1 at Edirne in 2010

| Hybrids | S Yield (kg ha$^{-1}$) | Rank | Rate to Std (%) | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-015 | 2930.0 | 1 | 126.5 | 35.2 | 129.3 | 71 | 116 | 154 | 21 |
| MARKER (C) | 2774.0 | 2 | 119.7 | 27.8 | 140.8 | 71 | 132 | 165 | 21 |
| 10-TR-Ç-011 | 2597.0 | 3 | 112.1 | 37.5 | 107.2 | 70 | 117 | 143 | 20 |
| 10-TR-Ç-004 | 2503.0 | 4 | 108.0 | 36.9 | 111.3 | 70 | 123 | 155 | 20 |
| 10-TR-Ç-009 | 2396.0 | 5 | 103.4 | 26.2 | 142.8 | 68 | 131 | 164 | 16 |
| 10-TR-Ç-007 | 2365.0 | 6 | 102.1 | 26.5 | 143.6 | 67 | 124 | 102 | 15 |
| 10-TR-Ç-002 | 2333.0 | 7 | 100.7 | 30.0 | 138.1 | 68 | 120 | 167 | 19 |
| 10-TR-Ç-010 | 2295.0 | 8 | 99.1 | 29.8 | 138.6 | 70 | 121 | 146 | 18 |
| 10-TR-Ç-005 | 2286.0 | 9 | 98.7 | 26.7 | 142.2 | 67 | 130 | 180 | 19 |
| 10-TR-Ç-012 | 2256.0 | 11 | 97.4 | 30.7 | 136.4 | 65 | 117 | 129 | 20 |
| 10-TR-Ç-006 | 2255.0 | 12 | 97.3 | 29.4 | 137.9 | 66 | 119 | 135 | 17 |
| ÇİĞDEM-1(C) | 2234.0 | 13 | 96.4 | 32.5 | 156.5 | 66 | 122 | 156 | 18 |
| 10-TR-Ç-014 | 2160.0 | 14 | 93.2 | 36.0 | 124.7 | 66 | 119 | 123 | 19 |
| PALANCI 1(C) | 1942.0 | 16 | 83.8 | 30.4 | 119.9 | 69 | 121 | 176 | 20 |

CV (%) =12.23 LSD=384.4 kg ha$^{-1}$ for seed yield.

Table 8: Confectionery type hybrids in Yield Trial-1 at Lüleburgaz in 2009

| Hybrids | S Yield (kg ha$^{-1}$) | Rate to Std (%) | Rank | Oil C (%) | 1000 S. W. (g) | Flower (Day) | P M (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-017 | 3626.0 | 128.5 | 1 | 37.3 | 156.5 | 66 | | | |
| 10-TR-Ç-015 | 3614.0 | 128.1 | 2 | 33.7 | 119.9 | 67 | | | |
| 10-TR-Ç-004 | 3266.0 | 115.8 | 3 | 34.6 | 140.8 | 75 | | | |
| MARKER (C) | 3103.0 | 110.0 | 4 | 27.5 | 126.1 | 67 | | | |
| 10-TR-Ç-014 | 3095.0 | 109.7 | 5 | 35.0 | 138.1 | 68 | | | |
| ÇİĞDEM-1(C) | 3045.0 | 107.9 | 6 | 31.2 | 112.4 | 68 | | | |
| 10-TR-Ç-010 | 3027.0 | 107.3 | 7 | 28.0 | 111.3 | 68 | | | |
| 10-TR-Ç-016 | 2966.0 | 105.1 | 8 | 35.2 | 142.2 | 70 | | | |
| 10-TR-Ç-008 | 2940.0 | 104.2 | 9 | 27.9 | 137.9 | 65 | | | |
| 10-TR-Ç-002 | 2890.0 | 102.4 | 10 | 28.8 | 143.6 | 70 | | | |
| 10-TR-Ç-005 | 2867.0 | 101.6 | 11 | 23.4 | 110.5 | 68 | | | |
| 10-TR-Ç-006 | 2855.0 | 101.2 | 12 | 32.2 | 142.8 | 74 | | | |
| 10-TR-Ç-007 | 2834.0 | 100.5 | 13 | 27.3 | 138.6 | 72 | | | |
| 10-TR-Ç-009 | 2810.0 | 99.6 | 14 | 25.6 | 107.2 | 68 | | | |
| 10-TR-Ç-012 | 2709.0 | 96.0 | 15 | 32.0 | 136.4 | 65 | | | |
| 10-TR-Ç-001 | 2433.0 | 86.2 | 16 | 31.2 | 134.6 | 64 | | | |
| PALANCI 1(C) | 2315.0 | 82.1 | 17 | 28.5 | 124.7 | 67 | | | |

CV (%) =11.00 LSD=457.4 kg ha$^{-1}$ for seed yield.

Table 9. Confectionery type hybrids in Yield Trial-2 at Edirne in 2010

| Hybrids | S Yield (kg ha$^{-1}$) | Rate to Std (%) | Rank | Oil C (%) | 1000 S.W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-041 | 2648.0 | 121.5 | 1 | 28.5 | 153.2 | 66 | 120 | 166 | 22 |
| 10-TR-Ç-037 | 2577.0 | 118.3 | 2 | 27.7 | 156.4 | 67 | 122 | 153 | 19 |
| MARKER (C) | 2332.0 | 107.1 | 3 | 28.8 | 145.4 | 71 | 129 | 168 | 24 |
| 10-TR-Ç-033 | 2312.0 | 106.1 | 4 | 26.4 | 174.5 | 66 | 124 | 147 | 20 |
| 10-TR-Ç-032 | 2301.0 | 105.6 | 5 | 38.3 | 116.4 | 69 | 115 | 137 | 19 |
| 10-TR-Ç-039 | 2291.0 | 105.2 | 6 | 29.7 | 131.8 | 67 | 126 | 143 | 21 |
| 10-TR-Ç-030 | 2278.0 | 104.6 | 7 | 39.4 | 104.8 | 66 | 116 | 150 | 18 |
| 10-TR-Ç-035 | 2276.0 | 104.5 | 8 | 27.6 | 165.6 | 65 | 127 | 151 | 19 |
| ÇİĞDEM-1(C) | 2261.0 | 103.8 | 9 | 31.1 | 155.2 | 65 | 116 | 141 | 16 |
| 10-TR-Ç-029 | 2195.0 | 100.7 | 10 | 25.8 | 159.1 | 67 | 120 | 140 | 18 |
| 10-TR-Ç-027 | 2188.0 | 100.5 | 11 | 25.0 | 151.4 | 67 | 118 | - | - |
| 10-TR-Ç-031 | 2167.0 | 99.5 | 12 | 26.9 | 163.6 | 65 | 120 | 159 | 21 |
| 10-TR-Ç-042 | 2130.0 | 97.8 | 13 | 27.6 | 149.0 | 69 | 127 | - | - |
| 10-TR-Ç-040 | 2124.0 | 97.5 | 14 | 35.8 | 124.4 | 68 | 121 | 127 | 20 |
| 10-TR-Ç-038 | 2072.0 | 95.1 | 15 | 31.5 | 135.3 | 68 | 118 | 133 | 20 |
| 10-TR-Ç-026 | 1944.0 | 89.3 | 17 | 29.1 | 146.4 | 67 | 120 | 134 | 22 |
| PALANCI 1(C) | 1942.0 | 89.1 | 18 | 31.6 | 120.2 | 69 | 118 | 164 | 21 |

CV (%) =14.00 LSD=436.4 kg ha$^{-1}$ for seed yield.

Table 10. Confectionery type hybrids in Yield Trial-2 at Lüleburgaz in 2010

| Hybrids | S Yield (kg ha$^{-1}$) | Rate to Std (%) | Rank | Oil C (%) | 1000 S.W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-031 | 3112.0 | 115.1 | 1 | 30.0 | 113.9 | 65 | | | |
| 10-TR-Ç-029 | 3033.0 | 112.2 | 2 | 29.1 | 108.4 | 66 | | | |
| 10-TR-Ç-027 | 3020.0 | 111.7 | 3 | 29.0 | 113.0 | 67 | | | |
| MARKER (C) | 2915.0 | 107.9 | 4 | 25.3 | 119.5 | 73 | | | |
| 10-TR-Ç-033 | 2892.0 | 107.0 | 5 | 29.8 | 113.8 | 65 | | | |
| ÇİĞDEM-1(C) | 2787.0 | 103.1 | 6 | 30.9 | 125.6 | 65 | | | |
| 10-TR-Ç-034 | 2787.0 | 103.1 | 7 | 30.2 | 91.00 | 64 | | | |
| 10-TR-Ç-038 | 2757.0 | 102.0 | 8 | 31.2 | 102.6 | 66 | | | |
| 10-TR-Ç-041 | 2756.0 | 102.0 | 9 | 31.1 | 108.3 | 66 | | | |
| 10-TR-Ç-037 | 2754.0 | 101.9 | 10 | 29.9 | 101.4 | 67 | | | |
| 10-TR-Ç-039 | 2740.0 | 101.4 | 11 | 32.9 | 93.20 | 68 | | | |
| 10-TR-Ç-042 | 2725.0 | 100.8 | 12 | 27.1 | 102.0 | 68 | | | |
| 10-TR-Ç-035 | 2707.0 | 100.2 | 13 | 29.3 | 113.5 | 67 | | | |
| 10-TR-Ç-026 | 2704.0 | 100.0 | 14 | 30.3 | 117.0 | 66 | | | |
| PALANCI 1(C) | 2406.0 | 89.0 | 16 | 29.1 | 85.72 | 67 | | | |

CV (%) =10.06 LSD=380.8 kg ha$^{-1}$ for seed yield.

Table 11: Confectionery type hybrids in Yield Trial-8 at Edirne in 2010

| Hybrids | S Yield (kg ha$^{-1}$) | Rate to Std (%) | Rank | Oil C (%) | 1000 S. W. (g) | Flower (Day) | P M (day) | P Hgt (cm) | H D (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-025 | 4189.0 | 139.8 | 1 | 34.6 | 134.7 | 68 | 119 | 155 | 20 |
| 10-TR-Ç-019 | 3918.0 | 130.7 | 2 | 35.7 | 114.6 | 68 | 120 | 141 | 18 |
| 10-TR-Ç-045 | 3737.0 | 124.7 | 3 | 31.9 | 122.6 | 67 | 117 | 152 | 18 |
| 10-TR-Ç-020 | 3730.0 | 124.5 | 4 | 30.5 | 139.1 | 69 | 118 | 120 | 23 |
| 10-TR-Ç-021 | 3698.0 | 123.4 | 5 | 27.2 | 132.6 | 68 | 117 | 143 | 19 |
| 10-TR-Ç-044 | 3678.0 | 122.7 | 6 | 36.9 | 115.7 | 68 | 124 | 151 | 19 |
| 10-TR-Ç-287 | 3675.0 | 122.6 | 7 | 31.6 | 140.6 | 70 | 118 | 155 | 23 |
| 10-TR-Ç-049 | 3653.0 | 121.9 | 8 | 27.8 | 152.4 | 67 | 120 | 123 | 21 |
| 10-TR-Ç-050 | 3551.0 | 118.5 | 9 | 23.4 | 156.2 | 69 | 120 | 164 | 19 |
| 10-TR-Ç-023 | 3499.0 | 116.8 | 10 | 32.7 | 136.2 | 67 | 117 | 137 | 20 |
| PALANCI 1(C) | 3266.0 | 109.0 | 11 | 31.5 | 113.7 | 69 | 119 | 176 | 24 |
| 08-TR-Ç-001 | 3231.0 | 107.8 | 12 | 30.0 | 133.1 | 69 | 119 | 154 | 19 |
| MARKER (C) | 3230.0 | 107.8 | 13 | 27.2 | 140.0 | 71 | 127 | 146 | 23 |
| 08-TR-Ç-002 | 3167.0 | 105.7 | 14 | 28.2 | 159.9 | 68 | 118 | 158 | 22 |
| 10-TR-Ç-048 | 3047.0 | 101.7 | 15 | 21.5 | 144.8 | 69 | 126 | 137 | 21 |
| 10-TR-Ç-046 | 3033.0 | 101.2 | 17 | 25.6 | 150.7 | 68 | 121 | 136 | 20 |
| 10-TR-Ç-047 | 2917.0 | 97.3 | 18 | 25.6 | 146.2 | 67 | 120 | 138 | 21 |
| ÇİĞDEM-1(C) | 2495.0 | 83.3 | 21 | 32.4 | 158.6 | 67 | 118 | 132 | 22 |

CV (%) =13.60 LSD=626.9 kg ha$^{-1}$ for seed yield.

Table 12: Confectionery type hybrids in Yield Trial-8 at Lüleburgaz in 2010

| Hybrids | S Yield (kg ha$^{-1}$) | Rate to Std (%) | Rank | Oil C (%) | 1000 S. W. (g) | Flower (Day) | PM. (day) | P Hgt (cm) | HD (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 10-TR-Ç-046 | 3069.0 | 115.7 | 1 | 27.6 | 106.3 | 68 | | | |
| 10-TR-Ç-021 | 2987.0 | 112.6 | 2 | 30.7 | 98.72 | 73 | | | |
| 10-TR-Ç-048 | 2782.0 | 104.9 | 3 | 26.9 | 98.72 | 70 | | | |
| 10-TR-Ç-023 | 2765.0 | 104.2 | 4 | 31.6 | 101.6 | 65 | | | |
| ÇİĞDEM-1(C) | 2726.0 | 102.8 | 5 | 31.6 | 103.6 | 65 | | | |
| MARKER (K) | 2718.0 | 102.5 | 6 | 28.1 | 96.44 | 74 | | | |
| 08-TR-Ç-002 | 2713.0 | 102.3 | 7 | 28.5 | 97.44 | 68 | | | |
| 10-TR-Ç-025 | 2707.0 | 102.0 | 8 | 32.3 | 93.52 | 69 | | | |
| 08-TR-Ç-001 | 2701.0 | 101.8 | 9 | 32.4 | 97.20 | 65 | | | |
| 10-TR-Ç-044 | 2682.0 | 101.1 | 10 | 34.1 | 86.08 | 69 | | | |
| 10-TR-Ç-050 | 2650.0 | 99.9 | 11 | 27.1 | 87.44 | 69 | | | |
| 10-TR-Ç-043 | 2644.0 | 99.7 | 12 | 30.2 | 86.36 | 64 | | | |
| 10-TR-Ç-047 | 2573.0 | 97.0 | 13 | 28.6 | 82.68 | 64 | | | |
| PALANCI 1(C) | 2514.0 | 94.8 | 15 | 29.9 | 79.84 | 69 | | | |

CV (%) =13.00 LSD=471.8 kg ha$^{-1}$ for seed yield.

**References**

Crnobarac, J., I. Balalić, B. Marinković, G. Jaćimović, D. Latković. (2014) Influence of stand density on yield and quality of NS sunflower confectionary hybrids. Research Journal of Agricultural Science, 46 (1), 178-183.

Evci, G., Pekcan, V., Yilmaz, M.I., Kaya, Y, (2011) The genetic diversity of confectionery sunflower on seed types and some yield traits. Proceeding Abstracts of International Symposium on Sunflower Genetic Resources. 16 - 20 October, Kusadası, Turkey. 55.

Gholinezhad, E., Darvishzadeh, R., Bernousi, I. (2014) Evaluation of Drought Tolerance Indices for Selection of Confectionery Sunflower (Helianthus annuus L.) Landraces under Various Environmental Conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 42(1): 187-201.

Gontcharov, S.V. and Beresneva, N.D. (2011) Confectionery Hybrid Sunflower Breeding in Russia. Journal of Agricultural Science and Technology. B 1: 919-924.

Hladni, N., Miklič, V., Jocić, S., Jocković, M., Radeka, I. Lečić, N. (2012) Determining the influence of yield components on the confectionary sunflower seed yield. Proc. 53rd Conference of Oils Industry. Production and Processing of Oilseeds. Herceg Novi, Montenegro, June 3-8. p. 55-62.

Kaya, Y. (2002) Ülkemizde Çerezlik Ayçiçeği Tohumluk Üretimi ve Karşılaşılan Sorunlar ve Çözüm Yolları. Türkiye 1. Tohumculuk Kongresi - IZMIR, 11-13 Eylül. 75-86.

Kaya, Y. (2004) Confectionery Sunflower Production in Turkey. Proceeding of 16th International Sunflower Conference. August 29-September 2. Fargo, US. 817-822.

Kaya, Y., Evci, G., Pekcan, V., Gucer, T., Yilmaz, I.M. (2008) Yield Relationships in Confectionery Sunflower (Helianthus annuus L.). Annual conference of the University of Rousse. Bulgaria 31 October - 01 November. 7-11.

Kaya, Y., Evci, G., Pekcan, V., Gucer, T., Yilmaz, I.M. (2009) Bazı Çerezlik Ayçiçeği Hibritlerinin Verim ve Verim Öğelerinin Karşılaştırılması. Türkiye 8. Tarla Bitkileri Kongresi, Hatay. 19-22 Ekim. 1: 154-158.

Kaya, Y., Evci, G., Pekcan, V., Yilmaz, I.M. (2013). Determining yield and quality performances of confectionery sunflower hybrids. 4th International Conference "Research People and Actual Tasks on Multidisciplinary Sciences". June 12–16. Lozenec, Bulgaria. 16-20.

Kholghi, M., Bernousi, I., Darvishzadeh, R., Pirzad, A. (2011) Correlation and path-cofficient analysis of seed yield and yield related trait in Iranian confectionery sunflower populations. African Journal of Biotechnology 10 (61): 13058-13063.

Nabloussi, A., Fernández-Cuesta, Á, El-Fechtali, M., Fernández-Martínez, J.M. and Velasco, L. (2011) Performance and seed quality of Moroccan sunflower varieties and Spanish landraces used for confectionery and snack food. Helia 34 (55): 75-82.

Sincik, M., Goksoy, A. (2014) Investigation of Correlation between Traits and Path Analysis of Confectionary Sunflower Genotypes. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 42(1):227-231

Velasco L., Fernández-Cuesta Á., Fernández-Martínez J. M. (2014) Variability of seed quality traits in a collection of Spanish landraces of confectionery sunflower. Crop and Pasture Science, 65: 242–249

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# Determination of morphological variability of local pea genotypes

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**ABSTRACT**

This study was conducted to determine morphological variability of 40 pea genotypes obtained from Plant Gene Bank of Aegean Agricultural Research Institute and collected from five districts of Black Sea Region and divided according to seed colour and shape. Genotypes were sowed in the field area of Agricultural Faculty of Ondokuz Mayıs University in the autumn rearing period. 45 different traits were observed taking into account the list for identification suggested by UPOV and EU-CPVO. Principal Component Analysis (PCA) was done in order to determine morphological variability. 13 principal component axis were obtained by the analysis. These components represented 85.61 % of total variation among genotypes. Eigen value of the first 13 principal components ranged between 1.12-7.60 and 41.97 % of the variation was explained. Properties of seed coat colour and leaf colour on the varieties with anthocyanin at the second main component axis and characteristics of dry leaf weight, dry stem weight, dry leaf / dry stem ratio at the third main component have larger values than ±0.3. As the eigen value of the genotypes was greater than 1, dendrogram was created by using Cluster analysis. Genotypes could be clustered in 8 groups based on Cluster analysis. Group A was found to be having the most genotypes with 14 numbers in these 8 groups. In this study it was determined that plant height varied between 57.5-173.2 cm, branch number per plant 1.4-7.8, pod number per plant 10.6-43.0, pod length 4.9-9.9 cm, seed number per pod 4.0-7.6, seed yield per plant 5.3-30.0 g, 100 seed weight 10.3- 36.4 g and crude protein rate 16.3-23.6 %. The range of variation in observed traits suggested the usability of the genotypes in the variety development and breeding studies.

***Keywords:*** Local pea, morphological variability, cluster, principal component analysis

## Introduction

Pea is a plant which is accepted as cool climatic plant among legumes. Although pea has the opportunity of being cultivated in lots of area including coastal segments and interior regions of our country surrounded on three sides by the sea pea's sowing area is quite a little. In fact, our country is the gene center of pea (Akçin 1988). In accordance with FAOSTAT 2012, the sowing area of dry pea is 1219 ha, with average yield 2173 kg/ha and total production 2650 t in Turkey. Whereas, in our country, there is no registered variety intended for the use of pea as dry seed purpose, until today. Out of 11 registered or production permitted variety oriented for fresh consumption has got involved in the market, only one is registered in our country.

Collection, characterization and conservation of the plant gene sources come at the beginning of the highest priority of research and development studies in terms of the agricultural sustainability. Our country's the wealth of herbal bio-diversity is the one most important advantage for us to catch the developments in this sector in the world. Thus, natural genetic materials in a region, are also important sources especially for the studies of resistance breeding.

To determine of genetic variability among plant materials (genotypes) selected for the variety breeding studies is essential. Recent statistical tools are required to identify variation among genotypes which otherwise is difficult based on morphological variation as it is impacted by genotype x environment interactions. Principal Component, Discriminant and Factor analysis called as multivariate analysis methods give a chance to analyze more than one property together. Cluster analysis has been accepted as one of the multivariate methods to analyze a large number of variables collectively (Rencher 1995). Using cluster analyses, Sözen et al. (2013) found wide variation for qualitative and quantitative traits in local bean population collected from West Black Sea Region. This wide variation built a rich genetic base enables selection of genotypes for developing varieties of sugar grain type which are preferred especially by the majority of our consumers.

We believe that Black Sea Region has an important potential to generalize the agriculture considering the ecological request of pea. To be able to carry out this, suitable sorts should be developed for the region. This study, aimed at identification of pea's gene sources picked from our region and provided by national plant gene bank and to determine morphological variability for finding out its agricultural properties, will throw light on possibilities of breeding and development of a variety in future.

## Materials and methods

In this study, material comprised 40 pea genotypes procured from National Plant Gene Bank included in Aegean Agricultural Research Institution and collected from local sources from 5 districts in Black Sea Region. The material then was distinguished with regard to seed colour and type. Among these, 24 belong to Black Sea Region, 10 Marmara Region and 1 Mediterranean Region (Karayel and Bozoğlu 2008). These materials were sown in 5m long rows at 50x15cm density at Ondokuz Mayıs University, Agricultural Faculty research and application field in 2004-2005 period. CAN fertilizer was applied with 4 kg/da N in February. In the trial, harvest time was determined considering that plants's stems and leaves have dried and seeds have ripened. 45 different traits were determined on each genotypes (Table 1). 13 were quantitative and 32 qualitative from among these traits. These traits were identifies from morphological characterization list determined by UPOV (The International Union for the Protection of New Varieties of Plants) and EU-CPVO (Communıty Plant Variety Office) (URL 2003). In order to characterize pea genotypes, obtained data was subjected to Principal Components Analysis first to determine morphological variability and then to clustering analysis to compose dendogram and to see classification. JMP 5.0.1 software was used for Cluster and ABA analysis.

## Results and discussion

First Principal Components Analysis was carried out using obtained data to characterize and determine morphological variation among pea genotypes. Variances for scatter around principal components were calculated separately for every component. These are called eigen value. In the conclusion of ABA obtained PC axis and eigen values belonging them, varience and cumulative varience ratios with factor coefficient indicating weighted factor values at principal components occuring on the basis of trait as given at Table 2. 13 principal component axes which were independent from each other were obtained related to traits observed in the conclusion of principal component analysis. 13 principal component axes accounted for 85.61 % of total variation in respect of local pea genotypes. The initial 3 of principal component's eigen value ranged between 1.12-7.60, the third principal component axis explained 41.97 % of the variation (Table 2). If the weight values at the principal components of traits observed at the Principal Component Analysis are over ±0.3, they are accepted to have a significant weight (Brown 1991). When weight degrees at second principal component axis were observed, pink or purple spots on testa at varieties with anthocyanin and the colour of testa at the varieties with anthocyanin were determined to attain greater than ± 0.3 value. Besides, when weight degrees were observed at the third principal component axis, leaf area, the weight of dry leaf and the weight of dry stem; at the fourth principal component axis, the maximum wideness of vexillum; at the fifth principal component axis, dry leaf/ dry stem ratio, curvature degree of pod, pod colour and density of green colour at pod; at the sixth principal component axis, the length of stipule, the wideness of stipule and the density of green colour at pod properties have gotten greater value than ±0.3 (Table 3). Because of this, traits which have been mentioned above are represented for the initial six principal component axis.

Eigen values of Local pea genotypes picked from our region and procured from National Plant Gene Bank were bigger than 1(1.12) which shows that principle component weight degrees dealt are reliable and can be carried out on Cluster Analysis (Mohammadi and Prasanna 2003). In the conclusion of Cluster Analysis, genotypes have been accumulated into 8 groups at dendogram. The A group has had the most genotypes with 14 genotypes. The E and G groups have followed

it with 6 genotypes. Dendogram obtained in the conclusion of the Cluster Analysis is given at Figure 1 and the distribution of groups and sub-groups composed in the conclusion of the dendogram are given at Table 4.

By determining genotypes included in main and sub-groups indicated in the conclusion of the Cluster Analysis, severities of proximity between genotypes were found. It was determined that the relationship severities of Bz23 and Bz38 genotypes were higher than other genotypes, Bz29 and Bz35 genotypes followed them, Bz1 and Bz10 genotypes were the farthest in terms of relationship severities.

For plant height 40 genotypes ranged between 57.5-173.2 cm. Gülümser et al. (2008) have qualified that the ones shorter than 75cm are short, the ones between 75-125 cm are medium and the ones higher than 125 cm are long for pea. The plant height average of 3 genotypes represented for the group H that they have the shortest plant height average (63 cm) among 8 groups composed through Cluster Analysis and these genotypes comprise short group. Group G has 6 genotypes and follows group H with 68.4 plant height average. On the contrary, it has been determined that group F has 1 genotype and the longest plant height average with 173.2 cm.

The pod number per plant is one of the most important traits affecting the yield for legumes positively (Tiwari et al. 2001; Gülümser et al. 1994; Karayel and Bozoğlu 2009), It ranged between 10.6-43.0 number/plant for observed pea genotypes in this study. It was observed that the pod number on pea ranged between 6-14 number in the similar ecological studies (Gülümser et al. 1994). Group C included 4 genotypes took place on the top with 28.7 pod number average, the maximum pod number, among all groups. However, group H took place at the bottom as having the fewest pod number with 13.8 number/plant of average of 3 genotypes' for pod number per plant.

Seed number per pod is among the important traits affecting yield. Karayel and Bozoğlu (2009), determined that there is very important and positive relationship ($r=0.363^{**}$) between seed yield and seed number per pod of pea. In our study, seed number per pod ranged between 4.0 and 7.6 number/pod. Toğay et al. (2006) found that seed number per pod of pea ranged between 3.69-5.23 number under Van conditions, Alan and Geren (2012) reported that it ranged between 4.9 and 7.2 under İzmir conditions. Two groups which have the highest average of seed number per pod (7.0), were obtained upon conclusion of the Cluster Analysis. These groups were group C that had 4 genotypes and Group G that had 6 genotypes.

There is a wide variation in pea from small grained types to big grained types. In variety and cultural application studies for pea, thousand seed weight ranged between 139.5 and 147.0 g as reported by Toğay et al. (2006), between 153.3 and 189.7 g by Öz and Karasu (2010) and between 150.7 and 335.1 g by Alan and Geren (2012), hundred seed weight changed between 15.06 and 31.09 g reported by Gülümser et al. (1994), between 10.8 and 17.3 g by Demirci and Ünver (2005) and between 14.01 and 17.84 g by Kaya (2000). In our study hundred seed weight of genotypes ranged between 10.3 and 36.4 g. Group H had 3 genotypes and the most 100 seed weight average in the conclusion of the Cluster Analysis. Genotypes in this group have been seen as short type. On the contrary, group C included 4 genotypes having the lowest 100 seed weight average (12.4 g). From these genotypes, Bz4 is Aydın's material and Bz7, Bz15 and Bz16 are Muğla's materials.

One of the most important properties discriminating legumes family from other cultivated plants is the high protein ratio in their dry seeds. In studies, the protein ratio of pea ranged between 20.3 and 37.9 % as reported by Perez et al. (1993), between 17.56 and 25.24 % by Kaya (2000) and between 17 and 23.5 % by Timuroğlu et al. (2004). Raw protein ratios of 40 genotypes was used in our study ranged between 16.3 and 23.6 %. It was determined that group H included 3 genotypes having the highest average of raw protein ratio. This group has the highest average of 100 seed weight and pod lenght at the same time and genotypes in this group are short.

Leaves are the most important assimilation organs of a plant. Area of leaves was measured to their areas as well as their numeric values and to determine their relationship with yield as it is beneficial and especially on the grounds that to be an important criterion for pea types that can be a crib. Bhatt and Chanda (2003) reported that area of leaves' should be determined for plant growth analysis and envapotranspiration studies, and also they reported leaves' area is required because of eclipsing of the light, the activity of radiation usage and find the index of leaf area which is an important value for the plant growth. Garnier et al. (2001) have repoted the property of leaf area can be used to compare species. Leaf area of genotypes in our study ranged between 1577.4-16984.6 cm/plant. Group B had 3 genotypes having the highest average of leaf area.

Quantitative as well as qualitative traits were considered for the Cluster Analysis carried out to group 40 local pea genotypes. 17 from 40 genotypes have been with anthocyanin and could be included in part in A, B, C groups in Cluster Analysis. In 4 genotypes testa did not have pink or purple spots in variety with anthocyanin, one of the qualititive properties and they were grouped in group C. It was seen that scala value (1=reddish brown, 2=brown, 3=brownish green)

belonging to colour of testa variation with anthocyanin was also encountered in materials of ours study

Pea is grown to meet mainly house and local market need at small areas in our region, but it is grown almost everywhere from east to west of our region. Pea is a product which bring the industry to areas being cultivated widely depending upon its agriculture, because of that especially its frozen fresh ones and fresh and dry seed are raw material of canned food processing industry. Because of this reason, on the purpose of also bringing agricultural industry to our region, pea's cultivation should be promoted in large areas and at the commercial level. Suitable varieties should be developed for the region for realization of it. So, the properties of pea genotypes were found and their variability were determined in this study.

Dendrogram composed in the conclusion of clustering analysis showed rather wide variation in terms of 45 qualitative and quantitative traits investigated. The wideness of this variation put forward that we have a material which forms a rich genetic base for selection studies onwards. Selection for pea genotypes will be continued regarding pink or purple spots on testa among the varieties with anthocyanin, for testa colour with anthocyanin, leaf area, dry leaf weight, dry stem weight, the maximum wideness of vexillum on flower, dry leaf/dry stem rate, curvature degree of pod, pod colour, density of green colour of pod, stipule lenght and stipule width properties that these have got higher value than ±3 at the initial six principal component.

Table 1. Qualitative and quantitative traits considered for Cluster and ABA analysis

| Trait number | Morphologic properties | Trait number | Morphologic properties |
|---|---|---|---|
| 1 | Plant height (cm) | **24** | Wideness of stipule (3-5-7) |
| 2 | Branch number per plant | **25** | Density of spot on stipule (1-3-5-7-9) |
| 3 | Pod number per plant | **26** | Time of flowering (1-3-5-7-9) |
| 4 | Pod length (cm) | **27** | Max number of flowers per node (1-2-3-4-5-6-7) |
| 5 | Seed number per pod | **28** | Density of alea colour in the red-pink flowers varieties (3-5-7) |
| 6 | Leaf number per plant | **29** | Density of vexillum colour in the red-pink flowers varieties (3-5-7) |
| 7 | Leaf area per plant ($cm^2$) | **30** | Colour of vexillum in the anthocyanin varieties (1-2-3) |
| 8 | Dry leaf weight per plant (g) | **31** | Max wideness of vexillum (3-5-7) |
| 9 | Dry stem weight per plant (g) | **32** | Shape of base of vexillum (1-3-5-7-9) |
| 10 | Dry leaf weight/Dry stem weight | **33** | Density of waving of vexillum (1-3-5-7-9) |
| 11 | Seed yield per plant (g) | **34** | Wideness of sepal (3-5-7) |
| 12 | 100 seed weight (g) | **35** | Shape of upper of sepal (1-2-3) |
| 13 | Crude protein rate (%) | **36** | Size of pod (1-3-5-7-9) |
| 14 | Seed shape (1-2-3-4-5-6)* | **37** | Max wideness of pod (1-3-5-7-9) |
| 15 | Pink or purple spots on testa of varieties with anthocyanin (1-2-3) | **38** | Curvature degree of pod (1-3-5-7-9) |
| 16 | Colour of testa of varieties with anthocyanin (1-2-3) | **39** | Pod colour (1-2-3-4) |
| 17 | Foliage colour (1-2-3) | **40** | Density of green colour of pod (3-5-7) |
| 18 | Intensity of foliage colour (3-5-7) | **41** | Number of ovules in pod (3-5-7) |
| 19 | Leaflet size (1-3-5-7-9) | **52** | Density of green colour of immature seed (3-5-7) |
| 20 | Leaflet length (3-5-7) | **43** | Seed maturation time (1-3-5-7-9) |
| 21 | Leaflet wideness (3-5-7) | **44** | Degree of wrinkling of cotyledon (3-5-7) |
| 22 | Distance of broadest from the bottom of leaflet (3-5-7) | **45** | Seed weight (1-3-5-7-9) |
| 23 | Length of stipule (3-5-7) | | |

* Scale values of UPOV and EU-CPVO

Table 2. Factor coefficients of examined traits in the conclusion of principal components analysis

| | Principal Companent Axis | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **PCA1** | **PCA2** | **PCA3** | **PCA4** | **PCA5** | **PCA6** | **PCA7** | **PCA8** | **PCA9** | **PCA10** | **PCA11** | **PCA12** | **PCA13** |
| **Eigen Value** | 7.60 | 6.55 | 4.74 | 3.52 | 3.35 | 2.25 | 2.18 | 1.71 | 1.57 | 1.42 | 1.31 | 1.21 | 1.12 |
| **Variance (% )** | 16.88 | 14.55 | 10.53 | 7.83 | 7.44 | 4.99 | 4.84 | 3.80 | 3.50 | 3.15 | 2.91 | 2.68 | 2.48 |
| **Cumulative Variance (% )** | 16.88 | 31.44 | 41.97 | 49.80 | 57.24 | 62.24 | 67.09 | 70.89 | 74.38 | 77.54 | 80.44 | 83.13 | 85.61 |

Table 3. Principal companent values of examined traits

| Trait number | PCA1 | PCA2 | PCA3 | PCA4 | PCA5 | PCA6 | PCA7 | PCA8 | PCA9 | PCA10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.21257 | 0.20241 | 0.04503 | -0.08225 | -0.23182 | -0.00208 | -0.05576 | 0.18932 | -0.15801 | 0.09969 |
| 2 | -0.17626 | 0.03965 | 0.25770 | 0.15776 | 0.00741 | 0.06438 | 0.11961 | 0.01532 | 0.21024 | -0.08940 |
| 3 | -0.18073 | -0.05006 | 0.20591 | 0.10485 | 0.06051 | 0.12778 | -0.15885 | -0.08657 | 0.00706 | -0.03851 |
| 4 | 0.26603 | 0.08709 | 0.10966 | -0.16181 | 0.15340 | 0.07309 | 0.02504 | 0.10151 | 0.05321 | 0.02272 |
| 5 | -0.08903 | -0.03473 | 0.16101 | -0.00228 | 0.28678 | -0.04426 | 0.06007 | 0.13832 | 0.31431 | -0.32656 |
| 6 | -0.22257 | 0.09603 | 0.26629 | 0.14531 | -0.02877 | 0.04550 | 0.13937 | -0.03344 | 0.13358 | 0.03680 |
| 7 | -0.12504 | 0.19841 | 0.33528 | 0.05204 | -0.05182 | -0.06646 | 0.11819 | -0.02870 | -0.05135 | 0.00982 |
| 8 | -0.13203 | 0.17259 | 0.33442 | 0.10684 | -0.00433 | -0.05547 | 0.08302 | -0.05096 | -0.07147 | 0.06110 |
| 9 | -0.14810 | 0.17394 | 0.30948 | 0.03626 | -0.12171 | -0.02114 | 0.08732 | -0.01869 | -0.04902 | 0.12941 |
| 10 | 0.05039 | -0.07428 | 0.14330 | 0.23720 | 0.32783 | -0.18028 | 0.10640 | -0.02133 | 0.07139 | -0.13630 |
| 11 | 0.05572 | -0.03038 | 0.24300 | -0.00323 | 0.03684 | 0.16083 | -0.34681 | -0.08419 | -0.06333 | -0.31588 |
| 12 | 0.26653 | 0.18323 | 0.03177 | -0.14314 | -0.02863 | 0.12395 | -0.05522 | 0.02179 | -0.03184 | -0.00516 |
| 13 | 0.06548 | -0.12885 | 0.07878 | -0.12219 | -0.08831 | 0.07537 | 0.38325 | 0.30676 | -0.07626 | 0.03808 |
| 14 | 0.04998 | 0.17942 | -0.01366 | -0.16056 | 0.29693 | 0.11797 | 0.11855 | -0.09560 | 0.17389 | -0.08398 |
| 15 | -0.08276 | 0.31442 | -0.16925 | 0.00766 | 0.00918 | -0.06736 | -0.06873 | -0.01405 | 0.09020 | -0.05747 |
| 16 | -0.04923 | 0.31507 | -0.10831 | 0.02929 | 0.03592 | 0.01593 | -0.03240 | -0.01265 | 0.06197 | -0.06680 |
| 17 | 0.07089 | -0.25042 | 0.09361 | -0.05978 | 0.06498 | 0.01625 | -0.09210 | 0.06937 | 0.29960 | 0.14486 |
| 18 | -0.11398 | -0.08911 | -0.04167 | 0.11400 | 0.01781 | 0.01858 | 0.06780 | 0.05865 | -0.01951 | 0.45635 |
| 19 | 0.10962 | 0.11854 | 0.20299 | -0.14490 | 0.01184 | -0.11548 | -0.12006 | -0.19227 | -0.25266 | 0.09870 |

*Continuing table 3*

| Trait number | PCA1 | PCA2 | PCA3 | PCA4 | PCA5 | PCA6 | PCA7 | PCA8 | PCA9 | PCA10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | -0.12936 | -0.13208 | -0.02927 | 0.02503 | -0.20384 | 0.09810 | -0.00675 | -0.14327 | -0.02668 | -0.28949 |
| 21 | 0.19451 | 0.11893 | 0.16377 | 0.01891 | 0.10381 | -0.24485 | -0.07909 | 0.16457 | 0.03450 | 0.13528 |
| 22 | -0.15575 | -0.05212 | -0.06916 | -0.00778 | 0.13967 | 0.10890 | 0.12685 | 0.25253 | -0.00993 | 0.13214 |
| 23 | -0.14015 | 0.11631 | -0.03424 | -0.04348 | -0.04936 | 0.33452 | 0.27778 | -0.03021 | -0.04182 | -0.01178 |
| 24 | 0.12631 | 0.01772 | 0.16066 | 0.04153 | -0.00283 | -0.40255 | -0.22539 | 0.02956 | -0.05645 | 0.13521 |
| 25 | -0.02782 | 0.17821 | -0.02492 | -0.16642 | 0.07919 | -0.00917 | 0.31111 | -0.14021 | -0.18444 | 0.02272 |
| 26 | -0.17509 | 0.02549 | 0.07288 | -0.23447 | 0.00390 | 0.09401 | -0.28893 | 0.00424 | 0.29044 | -0.02616 |
| 27 | 0.10057 | -0.00945 | -0.11320 | 0.21801 | -0.09992 | 0.11879 | 0.08331 | -0.34176 | -0.11954 | -0.18038 |
| 28 | -0.09923 | 0.26041 | -0.23234 | 0.17475 | 0.06125 | -0.05657 | -0.04284 | 0.02909 | 0.13110 | 0.00629 |
| 29 | -0.09755 | 0.26288 | -0.21117 | 0.17856 | 0.05428 | -0.05472 | -0.05614 | 0.05122 | 0.16990 | 0.01241 |
| 30 | 0.18976 | -0.16768 | 0.24882 | -0.00432 | -0.05151 | 0.11453 | 0.05396 | -0.16614 | -0.03053 | 0.10062 |
| 31 | 0.20720 | 0.05567 | 0.02092 | 0.35675 | -0.07738 | 0.12955 | -0.04569 | 0.01743 | 0.00522 | 0.01589 |
| 32 | 0.15284 | 0.05422 | 0.06029 | 0.28026 | -0.14746 | 0.14876 | -0.01497 | 0.13664 | 0.07833 | 0.00033 |
| 33 | 0.15099 | 0.06032 | -0.09228 | 0.27975 | -0.03925 | -0.03298 | -0.00094 | -0.09130 | 0.23533 | 0.27631 |
| 34 | 0.21464 | 0.10956 | 0.03329 | 0.25148 | -0.09830 | 0.19781 | -0.00816 | 0.17206 | 0.03937 | 0.03431 |
| 35 | 0.20173 | -0.01358 | 0.10239 | 0.14913 | -0.10435 | 0.17788 | 0.00004 | 0.10968 | 0.05611 | -0.00811 |
| 36 | 0.17061 | 0.23917 | -0.00445 | -0.15404 | 0.09824 | 0.14362 | 0.08787 | -0.04739 | 0.04209 | 0.05994 |
| 37 | 0.14854 | 0.29593 | 0.09346 | -0.12764 | 0.05795 | 0.03376 | 0.01774 | 0.02738 | -0.03366 | -0.08663 |
| 38 | -0.04356 | 0.03963 | -0.06075 | 0.10550 | 0.35044 | -0.14829 | -0.02002 | -0.24005 | -0.12962 | 0.14033 |
| 39 | -0.07242 | -0.00140 | -0.00712 | 0.14956 | 0.33406 | 0.23706 | -0.16526 | 0.19883 | -0.33295 | 0.00139 |
| 40 | -0.04976 | -0.02136 | -0.02314 | 0.14058 | 0.32646 | 0.30394 | -0.15317 | 0.14733 | -0.28228 | 0.04402 |
| 41 | -0.06369 | -0.06396 | 0.05466 | -0.05634 | 0.19449 | 0.25612 | -0.06754 | -0.39453 | 0.05129 | 0.33383 |
| 42 | 0.00883 | -0.04078 | 0.01560 | 0.04133 | 0.14125 | -0.19579 | 0.08171 | 0.29020 | -0.25690 | -0.16300 |
| 43 | -0.14872 | 0.02421 | 0.03996 | -0.22608 | -0.04054 | 0.18505 | -0.27029 | 0.23158 | 0.12794 | 0.20938 |
| 44 | 0.21810 | -0.09527 | 0.01385 | -0.09672 | 0.19004 | 0.03420 | 0.26655 | -0.00840 | 0.20090 | 0.04315 |
| 45 | 0.25152 | 0.19008 | 0.01732 | -0.11472 | -0.08658 | 0.09986 | -0.11815 | 0.01328 | -0.02488 | -0.03920 |

Figure 1. Dendogram obtained from cluster analysis

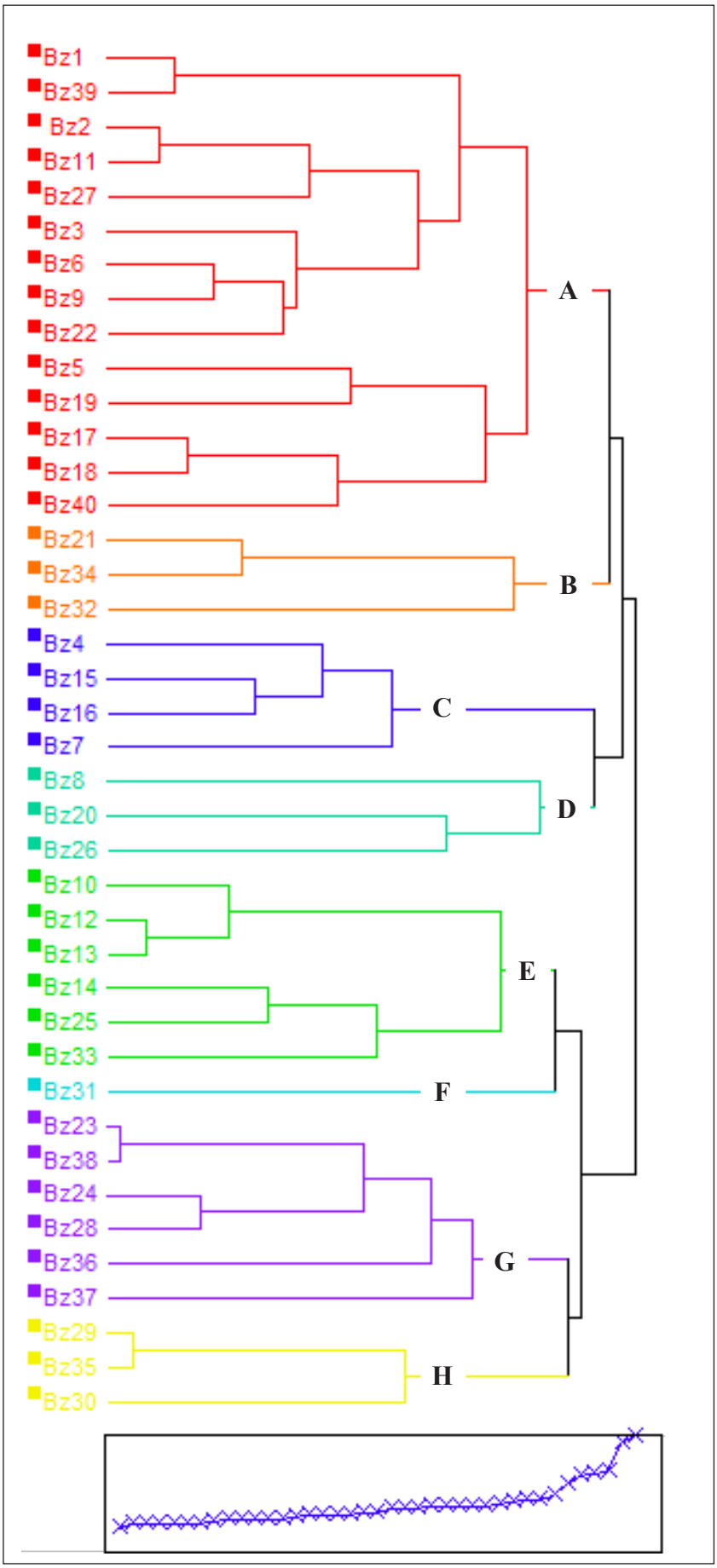

Table 4. Genotypes owned by groups and sub-groups as a result of cluster analysis

| Groups | Sub-groups | Genotypes | Genotype number |
|---|---|---|---|
| **A** | A1 | Bz1, Bz39 | 2 |
| | A2 | Bz2, Bz11, Bz27 | 3 |
| | A3 | Bz3, Bz6, Bz9, Bz22 | 4 |
| | A4 | Bz5, Bz19 | 2 |
| | A5 | Bz18, Bz17, Bz40 | 3 |
| **B** | B1 | Bz21, Bz34 | 2 |
| | B2 | Bz32 | 1 |
| **C** | C1 | Bz4, Bz15, Bz16 | 3 |
| | C2 | Bz7 | 1 |
| **D** | D1 | Bz8 | 1 |
| | D2 | Bz20, Bz26 | 2 |
| **E** | E1 | Bz10, Bz12, Bz13 | 3 |
| | E2 | Bz14, Bz25, Bz33 | 3 |
| **F** | F1 | Bz31 | 1 |
| **G** | G1 | Bz23, Bz38 | 2 |
| | G2 | Bz24, Bz28 | 2 |
| | G3 | Bz36 | 1 |
| | G4 | Bz37 | 1 |
| **H** | H1 | Bz29, Bz35 | 2 |
| | H2 | Bz30 | 1 |

**References**


Akçin A (1988). Yemeklik Dane Baklagiller. Selçuk Üniversitesi Yayınları: 43, Ziraat Fakültesi Yayınları: 8, Konya.

Alan Ö and Geren H (2012). Bezelye'de (*Pisum sativum* L.) farklı ekim zamanlarının tane verimi ve diğer bazı tarımsal özellikler üzerine etkisi. Ege Üniversitesi Ziraat Fakültesi Dergisi 49 (2): 127-134.

Bhatt M and Chanda SV (2003). Prediction of leaf area in *Phaseolus vulgaris* by nondestructive method. Bulg J Plant Physiol 29 (1-2): 96-100.

Brown JS (1991) Principal component and cluster analysis of cotton cultivar variability a cross the U.S. cotton belt. Crop Sci. 31: 915-922.

Demirci G and Ünver S (2005). Ankara koşullarında bezelyede (*Pisum sativum* L.) farklı ekim zamanlarının verim ve verim öğelerine etkileri. Anadolu J of AARI 15 (1): 49-60.

FAOSTAT (2012). http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Accessed 30 October 2013.

Garnier E, Shipley B, Roumet C and Laurent G (2001). A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15: 688-695.

Gülümser A, Seyis F and Bozoğlu H (1994). Samsun ekolojik şartlarında kışlık ve yazlık olarak ekilen bezelye çeşitlerinin konservecilik özellikleri ile tane veriminin tespiti. E.Ü.Z.F. Tarla Bitkileri Bölümü Tarla Bitkileri Bilim Derneği TUBİTAK ve ÜSİGEM. Tarla Bitkileri Kongresi, 25-29 Nisan 1994 Cilt I Agronomi Bildirileri, İzmir, 87-90.

Gülümser A, Bozoğlu H and Peşken E (2008). Yemeklik Baklagiller. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi, Ders Kitabı No:27, 2. Baskı, Samsun.

Karayel R and Bozoğlu H (2008). Türkiye'nin farklı bölgelerinden toplanan yerel bezelye polulasyonunun bazı agronomik özellikleri. OMÜ Zir Fak Dergisi 23 (1): 32-38.

Karayel R and Bozoğlu H (2009). Bezelye (*Pisum sativum* L.) genotiplerinde korelasyon ve path analizi. Türkiye VIII. Tarla Bitkileri Kongresi, Cilt II, Poster Bildiriler, 19-22 Ekim 2009, Hatay. 712-716.

Kaya M (2000). Winner bezelye (*Pisum sativum* L.) çeşidinde farklı aşılama yöntemleri, azotlu gübre dozları ile ekim zamanlarının verim ve verim öğelerine etkisi. Doktora Tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Ankara.

Mohammadi SA and Prasanna BM (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Science 43: 1235-1248

Öz M and Karasu A (2010). Bazı bezelye (*Pisum sativum* L.) çeşitlerinin tohum verimi ve verim kompenentlerinin belirlenmesi. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi 5 (1): 44-49.

Perez MD, Chambers SJ, Bacon JR, Lambert N, Hedley CL and Wang TL (1993). Seed protein content and composition of near-isogenic and induced mutant pea lines. Seed Science Research 3: 187-194.

Rencher AC (1995). Methods of Multivariate Analysis. John Willey&Sons Inc.

Sözen Ö, Özçelik H and Bozoğlu H (2013). Determination of morphological variability at domestic bean (*Phaseolus vulgaris* L.) populations collected from west black sea region. Soil-Water Journal 2(2): 1543-1552.

Timuroğlu KA, Genç A and Altınok S (2004). Ankara koşullarında yem bezelyesi hatlarında yem ve tane verimleri. Ankara Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi 10(4): 457-461.

Tiwari SK, Sing HL, Kumar R, Nigam HK and Singh AP (2001). Apostportem of selection parameters in pea (*Pisum sativum* L.). Research on Crops 2(2): 237- 242.

Toğay N, Toğay Y, Erman M and Yıldırım B (2006). Kışlık iki bezelye hattı (*Pisum sativum* ssp. *arvense* L.)'nda farklı bitki sıklıklarının bazı tarımsal özellikler üzerine etkisi. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi (J. Agric. Sci.) 16 (2): 97-103.

URL (2003). Protokol for Ditsinctness, Uniformity and Stability Tests, Pea (*Pisum sativum* L. sensu lato), Europan Union, Communıty Plant Variety Office.

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# Screening of new varieties of sainfoin with a high potential nitrogen fixation

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**ABSTRACT**

The most common legume in Northern Kazakhstan is sainfoin. Sainfoin has the unique ability to enter into symbiosis with rhizobia, forming nitrogen-fixing nodules which have the ability to absorb 125-480 kg/ha of nitrogen from the air annually. These high yields of cheap vegetable protein are achieved without the use of mineral fertilizers. However, in the arid steppes of northern Kazakhstan root system nodules are not formed due to the moisture deficit in the soil. The objective of the current research is to identify new and promising varieties and sainfoin lines with high nitrogen-fixing ability. To increase the nitrogen-fixing capacity of promising new sainfoin lines, plant seeds were inoculated before sowing with an experimental biopreparation based on nodule bacteria (nitragin) obtained in microbiology laboratories from the local strains of rhizobia to Sainfoin *Rhizobium simplex* (Rs.–5 is for the sainfoin). These studies showed that new lines seeds inoculated with rhizobia promotes more active nitrogen fixation compared to the plants not treated with nitragin. Using the chromatographic method of determination of the nitrogen-fixing ability in new Sainfoin lines K-185 and K-209 it was found that during the growing season fixed nitrogen balance in the atmosphere was respectively 491 and 458.7 mg/ha of the total nitrogen balance of 84 %. This shows that new Sainfoin lines have a more active ability to fix atmospheric nitrogen after inoculation rhizobia than the standard variety of sainfoin. Sainfoin lines K-209 and K-185 proved to be the best forms of sainfoin for nitrogen-fixing conditions in Northern Kazakhstan.

***Keywords:*** sainfoin, rhizobium simplex, nitrogen fixation, nitrogen, nitragin.

## Introduction

The problem of nitrogen deficiency will continue to be one of the main problems of agriculture. Based on a thorough analysis of the history of agricultural development by Pryanishnikov, it was established that the nitrogen level of plants was the main condition determining the average height of the legumes. In the last 2-3 decades, interest in biological nitrogen fixation significantly increased. This is due not only to the determining role of this process in the nitrogen balance of the biosphere, but also the possibility of reductions in the use of mineral nitrogen while reducing energy costs for production, which is very important in the light of current trends in biological farming (Kozhemyakov and Chebotar 2005; Schott 2010; Trepachev 2009). Despite the considerable progress made in the research on this problem, the practical use of techniques enhancing the life of diazotrophes remains scanty, due to the underestimation of the practical significance of the process by production workers, because of insufficient knowledge of many physiological, biochemical and genetic features of the process of nitrogen fixation.

It is especially essential for Kazakhstan conditions since the climate peculiarities, a short vegetative period and short active growth cycle, which lead to more deep undesirable changes in soil

properties while using resource saving techniques (Vorobev 1999).

Intensification of the process of symbiotic nitrogen fixation is an urgent priority in creating new and promising varieties of legumes. One of the promising ways to achieve this is to increase the portion of nitrogen symbiotrophic agrocenosis by expanding the range and area of cultivation of legumes and create conditions for the establishment and effective functioning of their symbiosis with appropriate species of nodule bacteria. During the growing season and due to symbiotic nitrogen fixation, legumes herbs are able to accumulate up to 125-480 kg/ha of nitrogen from the air and generate high yields of high-quality environmentally safe food and feed protein, without the use of expensive, energy-intensive and environmentally hazardous mineral nitrogen fertilizers (Zavalin 2005). About 50 % of fixed atmospheric nitrogen is left in the soil with stubble root residues of perennial legumes, which significantly increases the yield of subsequent crops (Bazilinskaya 1988). Taking the above mentioned into account, our task was to evaluate the microbiological studies of nitrogen-fixing ability of new and promising varieties and lines of perennial legumes after rhizobia inoculation.

## Materials and methods

Research was done on different crop varieties and lines of perennial legumes (clover, sainfoin) inoculated with nitragin. Soil samples and plants were collected in triplicate in stooling, budding and flowering periods. An experimental nitragin obtained in microbiology laboratories from local strains of rhizobia for clover and sainfoin was used in these experiments. Seeds of plants promising new lines were inoculated in order to increase the nitrogen fixing capacity of sainfoin. An experimental biopreparation of nodule bacteria (nitragin) obtained in the Laboratory of Microbiology from the local strains of nodule bacteria, sainfoin *Rhizobium* simplex (Rs.-5 is. for sainfoin) was used. The amount of symbiotically fixed nitrogen was measured by the chromatographic method. The principle of the method is based on the ability of root nodule bacteria to restore not only the molecular nitrogen, but also a number of other compounds, in particular acetylene ($C_2H_2$) to ethylene ($C_2H_4$). It was found that the amount of ethylene formed per unit time is in proportion to the amount of fixed nitrogen of approximately 3:1. The amount of ethylene was calculated by micro gas chromatograph Agilent 3000 and, using the specified ratio, the amount of fixed nitrogen was determined. Multiplying this value by the weight of nodules per unit area, we find the absolute value of fixed nitrogen. Since this figure varies according to the phases of the growing season and time of day, the value of nitrogen fixation for the whole period of vegetation was carried out by multiple measurements in relation to each class. The nitrogen content in the plant mass was determined by the Kjeldahl method (GOST 13496.4-93) (using the unit UDK-142). Field studies were carried out in competitive strain testing. The objects of research are the well-known varieties of sainfoin, “Sandy improved” and promising new lines of sainfoin, K-185, K-209, which were inoculated with nitragin.

## Results and discussions

Research done by A. I. Barayev SPCGF breeders of different sainfoin varieties and lines has shown that inoculating new lines seeds with rhizobia promote more active nitrogen fixation than non-treated plants. In order to study the influence of root nodule bacteria biomass on sainfoin nitrogen-fixing ability, the number of nodules formed on the roots of plants was counted. The maximum number of nodules occurred in the stooling phase, in the flowering stage lysis of nodules began due to lack of moisture in the soil. During the sainfoin growing season the maximum number of nodules on the roots of plants was observed on the K-185 line which was treated with nitragin and was up to 31 pcs. per plant (Table 1). The K-185 line uptake of atmospheric nitrogen was 192.8 mg or 83.4 % of total amount of nitrogen. Sandy Improved and K-209 sainfoin both formed more nodules after being inoculated with rhizobia compared to untreated plants. Using the method of determining the balance of nitrogen-fixing ability in Sainfoin new lines K-185 and K-209 fixed 491 and 458.7 mg/ha atmospheric nitrogen, respectively, which was 84 % of the total nitrogen balance, indicating the new lines have a more active ability to fix atmospheric nitrogen than the standard variety of sainfoin. In lines K-209 and K-185 nitragin significantly increased the nitrogen content in the plant mass, which also stimulated the fixation of atmospheric nitrogen.

Observations of the legume-*rhizobium* complex formation in different clover lines showed intense nodulation on the roots of plants during germination and branching. On average during the growing season their number varied from 1.8 to 15.8 nodules per plant. Maximum numbers of nodules were 12.4 and 15.8 pcs per plant from lines D-10 and D-12, respectively. Legumes which grow in a particular area which

lacks the specific bacteria needed by the host plant, fail to be a nitrogen accumulator from the air and start to feed on nitrogen from the soil and fertilizers (Kozhemyakov 1988). In addition, nodule bacteria that remains in the soil without the host plant for a prolonged period, as well as in adverse environmental conditions like high soil acidity, drought or flooding, lack of mineral nutrients, energy sources, material, etc. show a reduction in their nitrogen-fixing activity (Buyankin 2005; Gamzikov 2006).

Using nitragin along with the active *Rhizobium* strains stimulates inactive and less active nodule bacteria to provide biological nitrogen to legumes. Less active and inactive strains of nodule bacteria constitute one third or more of nodule bacteria. In areas where this is a problem, the use of nitragin containing high tiers of active breeding strains of nodule bacteria is one of the main methods of increasing not only the yield of legumes, but also the level of accumulation of general and biologically fixed nitrogen in plants and soil.

The highest protein content and dry weight was observed in all lines of clover inoculated with rhizobia and the value ranged from 3.63 to 3.74 % protein and 3.9 to 5.2 % by dry weight of plants. Biological nitrogen fixation from the atmosphere can be the main instrument to solve the problem of producing sufficient vegetable protein. Use of additional atmospheric nitrogen in the biological cycle will produce additional protein. The protein production capacity of a crop capable of symbiotic nitrogen fixation under favourable conditions of symbiosis exceeds many folds the protein productivity of crop plants that don't have this property. Intensive nodulation on the clover roots contributed to the active fixation of atmospheric nitrogen, a high percentage of fixed nitrogen was observed in lines D-2 and D-12 at a rate of 80.2 % and 82 %, respectively.

The amount of fixed nitrogen in the air at the symbiosis period on all variants of the experiment demonstrates the great possibilities of symbiotic systems of studied cultures to provide plants with nitrogen without the use of mineral nitrogen fertilizers. Due to symbiotic air nitrogen fixation energy costs per unit of output is reduced, for example, 1 kg vegetable protein of smooth brome obtained by the use of nitrogen fertilizers is 65 mJ whereas 1 kg of sainfoin protein obtained with biological nitrogen - 21, yellow sweet clover is 14 mJ. Air nitrogen fixation by using nitrogen fertilizers is a very energy-intensive process. Technically, one ton of nitrogen fixation into mineral nitrogen fertilizers requires about 80 gJ of energy. Application of nitragin based on local strains of nodule bacteria provides an increase in fixed nitrogen up to 82 % of atmospheric nitrogen in new varieties of sweet clover and sainfoin. Fixed nitrogen concentration in the soil contributes to the accumulation of nitrogen in plant mass. In promising Sainfoin lines K-209 and K-185, nodule bacteria significantly stimulate the processes of nitrogen fixation. Inoculation with nitragin on clover seeds helped increase the fixation of atmospheric nitrogen, which amounted to 174.7 mg (82%).

Studies suggest that in places of systematic cultivation of perennial legumes (host plants), the introduction of appropriate rhizobia populations in the soil will contribute to further fixation of atmospheric nitrogen. Biological nitrogen fixation of air can be the main instrument to solve the problem of vegetable protein production (Mishustin et al. 1980; Kozhemyakov et al. 1989).

Production of additional protein can be obtained by including atmospheric nitrogen into the biological cycle through symbiotic nitrogen fixation under favourable symbiosis conditions. Best of all, production of protein through appropriate bacteria, many times exceeds the protein crop produced using nitrogen fertilizers.

Table1. Effect of rhizobia inoculation of different sainfoin lines on the atmospheric nitrogen fixation, the average value for the vegetation period of 2009 - 2011.

| Treatment | The amount of nodules | Dry weight, g | Content of N in seed oil of mass, % | The nitrogen was assimilated, mg | | |
|---|---|---|---|---|---|---|
| | | | | The total | Atmospheric | % |
| **Sandy improved control** | 7.8 | 5.3 | 3.82 | 202.5 | 164.0 | 81.0 |
| **Sandy improved nitragin** | 10.6 | 5.5 | 3.85 | 211.7 | 173.2 | 81.8 |
| **К-185 control** | 3.4 | 5.1 | 3.85 | 196.3 | 157.8 | 80.4 |
| **К-185 nitragin** | 31.0 | 5.9 | 4.92 | 231.3 | 192.8 | 83.4 |
| **К-209 control** | 7.0 | 5.1 | 3.71 | 189.2 | 150.7 | 79.6 |
| **К-209 nitragin** | 10.2 | 5.9 | 5.81 | 224.8 | 186.3 | 82.9 |

Table 2 – The atmospheric nitrogen fixation during clover stooling phase by sowing clover different lines of the second year of life, 2010.

| Treatment | The amount of nodules | Dry weight, g | Content of N in seed oil of mass, % | The nitrogen was assimilated, mg | | |
|---|---|---|---|---|---|---|
| | | | | The total | Atmospheric | % |
| **Д.1 control** | 1.8 | 3.1 | 3.61 | 111.9 | 73.4 | 65.6 |
| **Д.1 nitragin** | 4.8 | 4.1 | 3.75 | 153.7 | 115.2 | 74.9 |
| **Д.2 control** | 1.8 | 3.0 | 3.55 | 106.5 | 68.0 | 63.8 |
| **Д.2 nitragin** | 4.0 | 5.2 | 3.75 | 195.0 | 156.5 | 80.2 |
| **Д.5 control** | 2.2 | 2.4 | 3.64 | 87.4 | 48.9 | 55.9 |
| **Д.5 nitragin** | 3.6 | 3.9 | 3.68 | 143.5 | 105.0 | 73.2 |
| **Д.10 control** | 2.6 | 2.6 | 3.50 | 91.0 | 52.5 | 57.7 |
| **Д.10 nitragin** | 12.4 | 4.2 | 3.63 | 152.5 | 114.0 | 74.7 |
| **Д.11 control** | 0.4 | 2.4 | 3.44 | 82.6 | 44.1 | 53.4 |
| **Д.11 nitragin** | 8.4 | 4.1 | 3.72 | 152.5 | 114.0 | 74.7 |
| **Д.12 control** | 0.4 | 3.2 | 3.67 | 117.4 | 78.9 | 67.2 |
| **Д.12 nitragin** | 15.8 | 5.7 | 3.74 | 213.2 | 174.7 | 82 |

**References**

Bazilinskaya MV (1988). Associative nitrogen fixation of cereal crops. Russian ed. “Kolos”.

Buyankin NI. (2005). A biological farming and crop production of a promising direction. Mocow, Russia 3rd Ed.

Gamzikov GP. (2006 ). Nitrogen in the agriculture of Western Siberia. Russia.

Kozhemyakov AP. (1988). Sources of lupine nitrogen nutrition with regard to the dose and timing of nitrogen fertilizer application. Mocow, Russia. 2nd Ed.

Kozhemyakov AP and Chebotar VK (2005). Biological science for agriculture. In the book: Biology in agriculture (Methodology and practice of microorganisms in crop and forage production), Russia, pp: 18 -54.

Kozhemyakov AP, Dorosinsky, LM and Berestetskyi OA. (1989). The efficiency of biopreparation of fixing microorganisms in agriculture. Publishing house “Kolos”.

Mishustin EN, Vostrov IS and Petrov AN (1980). Methods of soil microbiology and biochemistry. Russian publishing house “Mir”.

Schott PR. (2010). Opportunities and prospects of energy and resource optimization with nitrogen and nutrition of crops. Sat energy and resources in agriculture in arid areas, Moscow, Russia.

Trepachev EP. (2009). Agrochemical aspects of biological nitrogen in modern agriculture, Russia.

Vorobev VA (1999). Symbiotic nitrogen fixation and temperature. 2nd Ed.

Zavalin AA (2005). Biologicals, fertilizers and cropping. Publishing house, Russia.

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# The formation and study in the culture of genetic resources of forage crops by the expeditionary collection of wild forms from the natural landscapes of Kazakhstan

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**ABSTRACT**

Diversity of plant genetic resources is the main basis of breeding. Gene pool of crops, including fruit, in Kazakhstan consists of 79,400 samples. In this, the proportion of forage crops is 12.8 thousand samples. Experiments were carried out by the method of scientific research institute of agriculture and crop N.I. Vavilov. The questions replenish the gene pool of forage grasses by collecting expedition in Kazakhstan and the results of the study on the productivity of forage alfalfa 1578 collection samples representing 12 of its species, including wild.

***Keywords:*** The gene pool, forage crops, alfalfa, the wild specimens.

## Introduction

Biological diversity in the form of genetic resources, cultivated plants, is the primary basis of breeding, to improve agronomic traits, product quality, stress resistance and adaptability to specific agro-ecologies of new varieties as well as adapt to climate change.

Currently, the gene pool of crops in Kazakhstan consists of 79.4 thousand samples. Out of these, the proportion of forage crops is 16.2 % (12, 834 samples). In the structure of the gene pool of forages alfalfa accounts for (60%) followed by wheat grass (32%). It should be noted that the exclusive role of forming the gene pool of forage crops belongs to by Russian Scientific Research Institute of plant production named N.I. Vavilov .

Wild gene pool of other forage crops consists of 70 species belonging to 29 genera. Flora of Kazakhstan has a unique variety of species composition and ecotypes of forage crops that are of interest for use in breeding. The main ones are: ***Medicago L****: M.coerulea, M. difalcata, M. falcata, M.sativa, M.tianchanic, M. Trautvetteri; Melilotus: M. albus Dest. , M. officinalis* Dest.,sp.vilgicus*, M. varia;* Trifolium*: M. medium, M. pretense L ;* ***Onobrychis:*** *O. arenaria, O. inermis, O.viciafolia* scop*, O.antasiatic;* ***Astragalus:*** *A.alopecias, A. anungdalinus, A.chionantus, A. flexus, A.globiceps, A.sieversianus, A. turszaninovii, A. unifolatus, A. vulpinu* ***Vicia. L****.: V.sativa L., V. Villosa, V. kronenburgii, V. Juncea, V. Lanuginose,* ***Phleum L****: L. Paniculatum, L. phleoides., L. Alpinum, L. Pretense,* ***Roshevitzii****; Agropyron: A.cristatum, A.cristatum subsp., A. desertorum, A. fragile sups.;* ***Dastylis L****.: L. glomerata;* ***Bromus L.:*** *B. inermi, B. occidentalis, B. turkestanicus, B.gracillimus, B.sterilis, B. tectorum,B.secalinum, B. Danthoniae, B. popovii Drob, B.severtzovii., B. sepparius, B.macrostachys Dest. and others* (Meyirman et al., 2013).

For expansion of existing gene pool; the wild forms of species are the valuable source of genetic resources for further expansion of the gene pool of forage crops. In evolutionary terms, many forage crops have wild relatives and, of course, these can be used as a donor of important characteristics for improvement of cultivated ones. Those valuable features and their inclusion in the selection process can be a starting point to achieve breakthrough success in breeding.

The territory of Kazakhstan, unlike other countries, covering different ecological zones, subzones of steppe , semi-desert landscape , as well as powerful mountain ranges - Tarbagatai, Tien Shan, Altai, Mugaljar and various soil - climatic conditions with its environmental pressure, which contributed to the formation of highly diverse ecotypes. On the other hand, the industrial civilization: the development of large areas under agricultural crops, production of hydrocarbons, construction of various facilities, geological exploration studies, as well as global climate change cause extinction of some species and limit their distribution in nature.

During the period (1969-1978) by territory of Kazakhstan was the expedition researchers of Russian Research Institute of plant production named N.I. Vavilov to collection of wild species of plants. World collection of Russian Research Institute of plant production was enriched with in 2446 by Kazakhstan samples of forage plants, among which - 209 samples are of various types of alfalfa (Ivanov and Kolos, 1980).

In order to confirm the significance of the problem it is relevant to note the participation of one of the author of this article Meyirman GT expedition in Almaty region with Canadian scientists Lorenz for collection of nodule bacteria that settled in the root system of cultivated and wild legumes. This approach is very important in the selection of the nitrogen-fixing ability to raise pulses, thereby stabilize the environmental situation in agriculture by reducing the consumption of such mobility in soil mineral nitrogen. This approach is very important in breeding to enhance nitrogen-fixing ability of legumes, thereby stabilize the environmental situation in agriculture by reducing the consumption of such mobility in soil mineral nitrogen.

There are many examples in the world where wild collected specimens (ecotypes) or local ecotypes in Kazakhstan became the ancestor of many commercial varieties. Thus, the known varieties of cultivated alfalfa in America, originated from Turkestan lucerne. Collected samples (ecotypes) of yellow alfalfa (*Medicago falcata* L.), lomkokolosnik Sitnikov (*Psathyrostachys juncea*) and wheatgrass (*Agropyron desertorum*) from the territory of the former Semipalatinsk region by Canadian scientists in the last century (1930) became the basis of genetic plasma in breeding varieties of alfalfa type Rambler, drought-resistant varieties of lomkokolosnik Sitnikov -Bozoysky

Of perennial forage grasses greatest fame and distribution received alfalfa. Its gene pool in Kazakhstan refers to one of the richest regions of - Central Asia, which is considered the primary focus of origin alfalfa: of the Alatau mountains, eastern Tien Shan and Jungar Alatau.

Variety of original forms of alfalfa, especially local varieties generated by factors morphogenesis and differentiation, increased dramatically thanks to the many separate geographical areas, each of which has its own set of sorts.

Genetic composition of species and varietal potential alfalfa directly related to habitat ecology, methods of cultivation and use. In nature, the localization characteristics and properties depending on the ecological and geographical areas of origin of the samples.

Currently, based on the generalization of the results of studying an extensive set of world collection of alfalfa Scientific Research Institute of Agriculture and crop production by N.I. Vavilov (VIR) mapped the localization of geneplasms important characteristics and properties of alfalfa perennial species of subgenus Falcago the centers of origin of plants (Ivanov, 1976).

## Materials and methods

Bookmark technique of field experience

Objects of study were 1078 samples of alfalfa. These types and samples of alfalfa were studied in nurseries during different years of sowing by comparing them with standard variety. As standard variety ; local Semirechinsk that is widely cultivated in Kazakhstan, occupying an area of about 1 million hectares, was used for comparisons.. Bookmark nurseries carried out by the method of All-Russian Institute of Plant named after N.I. Vavilov (Methodological guidance on perennial grasses, 1981) with the placement of standard variety after every 6 to 10 studied samples, with 1-3 replicates and high bay arrangement plots.

Plots dimensions 1m$^2$ - (length 1.7 m, width 0.6 m). Sowing - Terraced row spacing of 15 cm with norms a sowing of 2 g of seeds per 1 m$^2$. The distance between plots - 0.6 m study and evaluation of the samples were carried out 2-4 hay harvest for economically - valuable attributes and properties of interest for breeding: the productivity of green and

dry weight, height, tillering, number of leaves of plants, symbiotic nitrogen fixation activity chemical composition of forage, resistance to major diseases. This article presents the results of studies on dry weight yield of alfalfa.

Agro-technical measures - recommended for the area (cropping system in the Almaty region, 2005.). Phenological observations were carried out in the morning. Structural analysis. Korsakov Nikolai, Makasheva AD, Adam OP Method of study collections legumes - A ,: WRI, 1968. - 175s. Harvesting was done with mini harvesters SAMPO or Hege following the procedure with ICG / crop variety trials // Methods public from / crops. Vol. 2. Grains, oilseeds and fodder crops. M., 1956. -229 p.

P. Chumakov AE, Zakharova TI Disease severity crops. - M .: Agroprom izdat, 1990. - 123 p. VA Megalov. Identification of pests of field crops. M. Kolos, 1968. pp 15-19, 43-46.

Determination of the main nutrients in the soil under the relevant state standards: Total nitrogen, total phosphorus, total potassium in plants was determined after the wet incineration plant material from a single sample. Further, total nitrogen content by Kjeldahl, phosphorus by colorimetric method and , potassium by flame photometer were measured following methodology described by // Mineev ( 2001)

Ratios of nitrogen and phosphorus fertilizer difference was calculated by the formula: C = (VNR - B0) / N. 100, where the BNP - Nutrient on plots with N or P; B0 - Tap on plots without fertilizer; N, P - application rate of fertilizer nitrogen or phosphorus.

The total grain nitrogen was determined by Kjedahl method and the protein content in grain was estimated by multiplying total grain nitrogen by a factor of 5.71 as descibed by Pleshkou BP, Workshop on Plant Biochemistry, Moscow, (1976)

## Results

The long-term studies on the collection of samples from different years in the nursery seeding at 2-4 mowing hay annually for 3-4 years it was possible to identify high-yield samples that served as the original forms in breeding synthetic varieties. They have been used in breeding populations by partitioning on genotypes and bookmark inbred lines to the second and third generations. On the basis of inbred lines with high general combining ability high productive new varieties such as Darkhan 90, Turkestan 15, Kokoray, Osimtal and Kokbalausa were synthesized .

Breeding - genetic basis of studying the problem and the choice of the starting material of alfalfa should be ecological principles to better use in the selection of a single advantage of eco - geographical groups, each ecotype.

Given the importance of proper selection of the starting material in the creation of new varieties, 1078 samples of alfalfa (table 1) related in 12 species, which cover 32 eco-geographical groups of 34 were studied according to the classification of Russian Research Institute of plant production named N.I. Vavilov. Samples were grown on irrigation (961) and without irrigation (117) (Meyirman *et al*., 1979). Standard varieties were - Semirechinskaya a local type.

Yellow alfalfa in the collection was represented by 58 specimens belonging to 7 ecotypes, and other wild species - 35 samples.

Using fodder as criterion in alfalfa and volatile 47 samples and wild species only 2 samples with efficiency exceeding the standard variety ( spot of the Semirechinsk) more than 20%, 49 samples - from 6 to 20%, 85 samples at the level of the standard could be identified , while for the 754 sample productivity was below standard. More productive samples were isolated more often from plain - Turkestan, Semirechinsk, Southern European and North African ecology - geographical groups.

In one of the collection of nursery a large set of alfalfa gene pool consisting of 500 accessions belonging to alfalfa and changeable was studied. Based on the study for the first three years of use, 31 accessions were isolated on yield of green mass, and 4-6 years of use - 16 accessions, exceeding the standard by more than 20% were selected . In accordance with the scheme of crop rotation in irrigated agriculture alfalfa, as a rule, use no more than 3 years by leveraging its biological features. The samples were studied by the yield of green mass and hay, foliage, weight and height of vegetation, plants infection by brown and yellow spotted askohitozom, mildew and other viral diseases, the rate of regrowth and growth, the passage of the main phases of plant development.

Many farmers working in the south and south-east of Kazakhstan tend to keep as long as possible stands of alfalfa that is to use old-growth crops with reduced harvest.

In our experiments (Meyirman *et al*., 2012) we tracked productivity of green mass from the first to the sixth year of use in order to allocate more productive perennial alfalfa accessions (Table 2). Virtually all high-yielding accessions, subsequently reduced yield of green mass.

If at the end of the study in the first three years they exceeded standard - Semirechensk local average by 15-35% with yields 7,63-8,96 kg/m2, then the further use of the grass in 4-6 years of use, the excess over standard in some samples was 5-32% with a yield of 2,48-3,14 kg/m2, and many accessions sharply reduced productivity.

## Discussions

In Kazakhstan, for conducting research on the formation of the gene pool of forage crops: 12834 samples have been registered, of them alfalfa - 60%, wheatgrass - 32% and for other types of herbs - 8%. Win forage crops in the volume of the gene pool of crops including fruit is 16.2%. In yield of alfalfa forage allocated 78 accessions at three annual herbage use and 16 accessions with many years' use (4-6 years), which exceeded the standard variety of more than 20%. They are used in the program to create synthetic varieties.

The results of tests on samples of alfalfa confirmed low productivity of wild species, although they differ in individual securities characteristics and properties important for breeding. Thus, according to the salt tolerance of alfalfa samples highlighted blue (*M. coerulea Less.),* For drought tolerance - *(M. difalcata Sin., M. falcata L.)* and others, resistance to disease - almost all kinds of wild alfalfa. Therefore, the wild species are of interest as sources and donors to improve alfalfa cultivars based backcross crosses given the ploidy level and the need to transfer from diploid species to tetraploid level to overcome uncrossability among species, or by the use of genetic engineering techniques.

Table 1. Level of harvest of alfalfa samples in the context of eco - geographical groups

| **Ecological- geographic group** | **Number studied samples** | **Of these samples compared with the harvest standard grade** | | | |
|---|---|---|---|---|---|
| | | **exceeding over 20%** | **guides exceeding 6-20%** | **at the level 95-100%** | **yield** |
| 1 | 2 | 3 | 4 | 5 | 6 |
| **Alfalfa (*M. sativa L.*) sowing and variable (*M. varia Mart.*)** | | | | | |
| Khiva | 11 | - | 1 | - | 10 |
| Plain- Turkestan | 85 | 6 | 10 | 16 | 53 |
| Semirechinskaya | 66 | 9 | 6 | 12 | 39 |
| Turkmenskaya | 21 | 2 | 3 | 2 | 14 |
| North Kazakhstanskaya | 17 | 1 | 4 | 4 | 8 |
| China Plain | 18 | 1 | 2 | 3 | 12 |
| Chinese foothill | 9 | 1 | 1 | 2 | 5 |
| Kashgarskaya | 10 | - | 1 | 3 | 6 |
| Kandahar- kabulskaya | 11 | - | - | 2 | 9 |
| Transcaucasian flat | 38 | 2 | 2 | 10 | 24 |
| Asia Minor | 9 | - | 3 | 1 | 5 |
| West European | 136 | 6 | 12 | 32 | 86 |
| South European | 80 | 2 | 7 | 24 | 47 |
| Ukrainian | 59 | 1 | 6 | 9 | 43 |
| North Caucasus | 50 | 2 | 3 | 4 | 41 |
| North Caucasian | 11 | - | 1 | 1 | 9 |
| South East | 17 | 2 | 2 | 3 | 10 |

*Continuing table 1*

| Ecological- geographic group | Number studied samples | Of these samples compared with the harvest standard grade | | | |
|---|---|---|---|---|---|
| | | exceeding over 20% | guides exceeding 6-20% | at the level 95-100% | yield |
| 1 | 2 | 3 | 4 | 5 | 6 |
| **Alfalfa (*M. sativa L.*) sowing and variable (*M. varia Mart.*)** | | | | | |
| Northwestern | 7 | - | - | - | 7 |
| Fair Russian | 19 | 1 | 1 | 1 | 16 |
| East Siberian | 21 | 1 | 1 | 1 | 18 |
| West Siberian | 15 | - | 1 | - | 14 |
| C North American | 95 | 1 | 4 | 17 | 73 |
| Canada | 8 | - | - | 2 | 6 |
| Chilean- peruvian | 28 | - | 2 | 5 | 21 |
| Mexico -Brazilian | 25 | - | 2 | 3 | 20 |
| Argentine | 9 | - | - | 3 | 6 |
| Indian | 28 | 2 | 4 | 3 | 19 |
| North | 42 | 4 | 3 | 10 | 25 |
| Mesopotamian | 9 | 1 | - | 3 | 5 |
| Syrian | 7 | 1 | 2 | - | 4 |
| Yemen | 7 | - | 1 | 2 | 4 |
| Ladakhi | 17 | 1 | - | 1 | 15 |
| **Total** | 985 | 47 | 85 | 179 | 674 |
| Ecotypes of wild yellow alfalfa (*M. falcata L.*) | | | | | |
| Ukrainian steppe | 3 | - | - | - | 3 |
| North Caucasian | 16 | 1 | - | - | 15 |
| De Sales steppe | 4 | - | - | - | 4 |
| South-east | 16 | - | - | 2 | 14 |
| North Russian | 1 | - | - | - | 1 |
| West Siberian | 7 | - | - | - | 7 |
| East Kazakhstan | 11 | - | - | - | 11 |
| **Total** | 58 | 1 | - | 2 | 55 |
| Other wild alfalfa | | | | | |
| Alfalfa blue (*M. coerulea Less.*) | 12 | - | - | - | 12 |
| A.polutsiklicheskaya (*M. hemicycla Grossh*) | 3 | - | - | 1 | 2 |
| A.adhesive (*M. glutinosa M.B.*) | 2 | - | - | - | 2 |

*Continuing table 1*

| Ecological- geographic group | Number studied samples | Of these samples compared with the harvest standard grade | | | |
|---|---|---|---|---|---|
| | | exceeding over 20% | guides exceeding 6-20% | at the level 95-100% | yield |
| 1 | 2 | 3 | 4 | 5 | 6 |
| Other wild alfalfa | | | | | |
| A.colored (Subsp. Poluchroa Sinsk.) | 10 | 1 | - | 2 | 7 |
| A.Tien Shan (M. tianshanica Vass.) | 2 | - | - | 1 | 1 |
| A.Lavrenko (M. Lavrenko Vass.) | 1 | - | - | 1 | - |
| A.pyreynaya (M. agropyretorum Vass.) | 2 | - | - | 2 | - |
| A.Trautfettera ( M. Trautvetteri Sumn.) | 2 | - | - | 2 | - |
| A. glandular (M. glandulosa David) | 1 | - | - | - | 1 |
| **Total** | 35 | 1 | - | 9 | 25 |
| **Total** | 1078 | 49 | 85 | 190 | 754 |

Table 2. The change of productivity of green mass of high-yielding examples of alfalfa with many years use of grass

| № Catalogy of VIR | Origin | Green weight from 1 kg / m² | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | years of use | | | average for the first 3 years of use | in % of standard | years of use | | | average for the 4-6 years of use | in % of standard |
| | | 1 | 2 | 3 | | | 4 | 5 | 6 | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 45335 | Kyrgyzstan | 3.5 | 15.1 | 7.9 | 8.82 | 132 | 4.0 | 3.5 | 1.3 | 2.94 | 124 |
| 46528 | Estonia | 2.6 | 14.7 | 9.6 | 8.96 | 135 | 3.5 | 3.0 | 1.7 | 2.73 | 115 |
| 36049 | Kazakhstan | 3.4 | 15.3 | 8.4 | 9.0 | 135 | 2.8 | 3.0 | 1.2 | 2.33 | 98 |
| 43782 | Ukraine | 2.2 | 14.7 | 8.7 | 8.49 | 127 | 3.5 | 2.7 | 1.9 | 2.7 | 114 |
| 43821 | Georgia | 1.6 | 15.0 | 9.5 | 8.71 | 131 | 5.2 | 2.1 | 0.2 | 2.5 | 105 |
| 44568 | Russia | 3.3 | 13.3 | 8.5 | 8.36 | 126 | 3.7 | 3.6 | 1.1 | 2.8 | 118 |
| 43784 | Russia | 3.4 | 12.6 | 9.6 | 8.51 | 128 | 3.1 | 3.4 | 1.3 | 2.6 | 110 |
| 47050 | Russia | 2.7 | 14.4 | 8.5 | 8.52 | 128 | 3.0 | 3.7 | 1.1 | 2.59 | 109 |
| 47049 | Russia | 2.9 | 13.9 | 9.1 | 8.63 | 130 | 2.5 | 3.5 | 1.5 | 2.48 | 105 |
| 43777 | Russia | 2.3 | 12.2 | 10.1 | 8.19 | 123 | 3.8 | 3.6 | 1.2 | 2.85 | 120 |
| 43779 | Russia | 2.3 | 13.4 | 9.5 | 8.39 | 126 | 3.5 | 2.6 | 1.9 | 2.66 | 112 |

*Continuing table 2*

| № Catalogy of VIR | Origin | Green weight from 1 kg / m² | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | years of use | | | average for the first 3 years of use | in % of standard | years of use | | | average for the 4-6 years of use | in % of standard |
| | | 1 | 2 | 3 | | | 4 | 5 | 6 | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 44419 | USA | 2.2 | 12.9 | 9.2 | 8.1 | 122 | 4.3 | 3.4 | 1.2 | 2.95 | 124 |
| 45369 | Kazakhstan | 3.4 | 14.4 | 8.5 | 8.74 | 131 | 2.4 | 2.7 | 1.8 | 2.29 | 97 |
| 45036 | Armenia | 3.0 | 12.7 | 8.0 | 7.9 | 119 | 4.1 | 3.5 | 1.2 | 2.93 | 123 |
| 6231 | Russia | 1.9 | 13.3 | 8.1 | 7.76 | 117 | 4.1 | 4.1 | 1.1 | 3.1 | 131 |
| 62097 | Kazakhstan | 3.4 | 11.4 | 9.1 | 7.95 | 119 | 3.9 | 3.0 | 1.9 | 2.93 | 123 |
| 47492 | Kazakhstan | 3.3 | 10.4 | 9.3 | 7.63 | 115 | 4.4 | 3.0 | 2.0 | 3.14 | 132 |
| 22571 | Russia | 3.0 | 11.1 | 9.3 | 7.82 | 117 | 4.1 | 2.8 | 1.9 | 2.92 | 123 |
| 44566 | Russia | 3.4 | 11.6 | 9.8 | 8.25 | 124 | 4.4 | 1.6 | 1.6 | 2.51 | 106 |
| 33481 | Finland | 3.0 | 14.3 | 8.1 | 8.45 | 127 | 3.1 | 2.4 | 1.4 | 2.27 | 96 |
| 39952 | Russia | 2.9 | 12.5 | 9.0 | 8.13 | 122 | 4.0 | 2.3 | 1.2 | 2.51 | 106 |
| 6015 | Malaysia | 2.1 | 12.8 | 9.3 | 8.05 | 121 | 3.0 | 2.7 | 2.0 | 2.53 | 107 |
| 44032 | Russia | 3.4 | 10.9 | 8.8 | 7.71 | 116 | 3.4 | 2.8 | 2.0 | 2.75 | 116 |
| 30830 | Ukraine | 2.6 | 13.1 | 8.8 | 8.17 | 123 | 3.2 | 3.0 | 0.8 | 2.34 | 99 |
| 46529 | Ukraine | 3.1 | 13.3 | 9.5 | 8.62 | 129 | 2.9 | 0.9 | 1.3 | 1.67 | 70 |
| 46249 | USA | 2.6 | 15.9 | 6.8 | 8.44 | 127 | 3.0 | 1.2 | 1.3 | 1.82 | 77 |
| 30071 | Russia | 3.2 | 13.8 | 8.0 | 8.31 | 125 | 2.1 | 2.8 | 0.8 | 1.9 | 80 |
| 6014 | Malaysia | 2.5 | 13.6 | 9.1 | 8.37 | 126 | 3.1 | 1.3 | 0.9 | 1.75 | 74 |
| 45860 | Russia | 1.6 | 15.7 | 7.9 | 8.4 | 126 | 2.8 | 0.9 | 1.3 | 1.65 | 70 |
| 47705 | USA | 3.2 | 14.0 | 7.0 | 8.05 | 121 | 3.5 | 0.7 | 1.5 | 1.91 | 80 |
| 45712 | USA | 2.6 | 15.3 | 6.4 | 8.1 | 122 | 3.1 | 0.9 | 1.3 | 1.73 | 73 |
| 45081 | Georgia | 2.8 | 12.9 | 8.6 | 8.12 | 122 | 2.7 | 0.8 | 1.1 | 1.53 | 64 |
| 28460 | Ukraine | 3.6 | 11.3 | 9.4 | 8.07 | 121 | 3.1 | 1.5 | 0.3 | 1.62 | 68 |
| 34627 | Kazakhstan | 2.3 | 13.0 | 9.0 | 8.1 | 122 | 2.3 | 1.9 | 0.4 | 1.53 | 64 |
| 46270 | Ukraine | 3.0 | 13.2 | 7.8 | 8.01 | 120 | 2.5 | 0.7 | 1.3 | 1.45 | 61 |
| Standard | | 2.3 | 10.2 | 7.5 | 6.66 | 100 | 3.2 | 2.4 | 1.5 | 2.37 | 100 |

**References**

Meyirman G., Yerzhanova S., Yessimbekova M., Masonichich-Shotunova R. (2013) Formation of the Genetic Resources of Forage Crops in Kazakhstan: Luzerne and Wheatgrass // Journal of Entomology and Zoology Studies ISSN 2320-7078. **1**(4): 141-144.

Meyirman G.T, Masonichich - Shotunova R. Lucerne. -Almalybak: Asil kitap (2012).-p.415.

Meyirman G.T. , Rakisheva Ch. S., Sadvakasov S.S., (1979) Source material for breeding alfalfa / / Breeding and Seed Production of forage grasses in the south and south-east of Kazakhstan: themes. Sat articles \ Alma-Ata: IN Agricultural Sciences. **3:** 47-53.

Ivanov A.I. and Kolos, M. (1980) Alfalfa. 350p.

Ivanov A.I. (1976) Alfalfa spread across countries and continents / / Proc. by prikl. botany, genetics and breeding. -T.56. MY. 2. P.151-152.

Methodological guidance on the study collection of perennial grasses, VIR: L (1981). – p.17.

Korsakov Nikolai, Makasheva AD, Adam OP Method of study collections legumes - A ,: WRI, 1968.-175s.

Methods public from / crops. Vol. 2. Grains, oilseeds and fodder crops. M., 1956.-229 p.

Chumakov A.E., Zakharova T.I. Disease severity crops. -M.: Agropromizdat, 1990.-123 p.

Megalov V.A. Identification of pests of field crops. M. Kolos, 1968. pp 15-19, 43-46.

Mineev V.G. Workshop on Agricultural Chemistry, Moscow, 2001, pp. 355-356. 409;

Pleshkou B.P. Workshop on Plant Biochemistry, Moscow, 1976, pp. 3-7.

Research Article

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# Morphological and seed yield characteristics of orchardgrass ecotypes of Eastern Anatolia Region

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**ABSTRACT**

Present research was carried out to assess the genetic diversity in orchardgrass ecotypes of Eastern Anatolia region and to determine the genotypes available for breeding. Morphological and seed yield characteristics of 25 orchardgrass ecotypes, collected from natural vegetations of Ağrı, Ardahan, Artvin, Bayburt, Bingöl, Erzurum, Kars and Muş provinces of the region, were determined. After germination, the orchardgrass seedlings were transferred to the experimental field area of Eastern Anatolia Agricultural Research Institute in early spring of 2010. The 10 plants of each ecotype were investigated regarding plant height, number of nodes, node spacing, leaf length, leaf width, length of panicle axis, seed yield parameters of orchardgrass (Dactylis glomerata L.) in 2011-2012. Of all investigated parameters, seed yield (g) and plant height (cm) exhibited a greatest variation. Based on the Principal Components Analysis, orchardgrass genotypes were divided into 3 principal groups. Specified parameters were able to explain 72,666 % of current variation. The first component representing 34,611 % of total variation was composed of leaf length and leaf width; the second component representing 21,101% of total variation was plant height, and third component representing 16,953% of total variation was number of nodes.

***Keywords:*** Orchardgrass, Eastern Anatolia, Morphological Biodiversity.

## Introduction

Turkey exhibits characteristics of a small continent with regard to biological diversity since there are three different bio-climates and three bio-geographical regions (Europe-Siberia, Mediterranean and Iran-Turan) in Turkey (Aytepe and Varol, 2007; Anonymous, 2007).

Cultivation of several common field and forage crops has successfully been performed under field conditions in Turkey, which has various soil, climate and cropping patterns. However, very few forage crops are actually cultivated in Turkey and it is hard to improve forage crop cultivation. Therefore, in addition to current ones, new species and genotypes should be included in forage crop cultivation (Canbolat and Karaman 2009).

Natural pastures are significant genetic resources in development of new plant species and such lands constitute almost one-fifth of country surface area. Eastern Anatolia Region has about 35% of total pasture lands of the country and pastures of the region constitute gene source of various plants used in pasture and meadows of the country. Despite the significantly rich natural flora, plant species and diversity of the region, especially with regard to forage crops, a number of economically valuable plant species, such

as orchardgrass (*Dactylis glomerata* L.), have not been identified in detail. Orchardgrass is a long-life, perennial cool season bunchgrass adapted to cool regions of the world. It is highly adaptive to various environmental conditions and has well re-grow characteristics. Orchardgrass is widespread in most European countries, Northern and southern American countries, Australia, New Zealand and Asia. It yields proper mixtures for dry hay, silage and pasture. It is usually used with alfalfa (*Medicago sativa* L.) or red clover (*Trifolium pratense* L.) for dry hay and with white clover (*Trifolium repens* L.) for pastures (Sanada *et. al*., 2010).

There are limited studies on orchardgrass (*Dactylis glomerata* L.) populations which are very common over pasture and meadows of Turkey (Tuna *et al.,* 2004). Such a rich genetic diversity has not sufficiently been valued and commercial species have not been developed yet.

Genetic resource preservation and plant breeding programs mainly depend on the use of current genetic diversity to a large extend (Ahmad *et al*., 2008). There are several methods in genetic analysis of breeding lines and population germplasm. These methods include pedigree data, morphological data, agronomic performance data, biochemical data and recently the molecular data (Mohammadi and Prasanna, 2003). Determination of genetic diversity via morphological data is among the traditional methods (Tuna *et al*., 2004).

Determination of agronomic and morphological characteristics of high yielding orchardgrass genotypes well-adapted to the regional conditions and determination of genetic diversity based on these characteristics are of significant issues in utilization of current population into breeding programs.

Objectives of present study were to determine the morphological, phenological and agronomic characteristics of orchardgrass ecotypes collected from Eastern Anatolia Region to determine current diversity and to select available genotypes for the use in advanced breeding programs.

## Materials and methods

### *Material*

A total of 25 orchardgrass (*Dactylis glomerata* L.) ecotypes, collected from natural pastures of Ağrı, Ardahan, Artvin, Bayburt, Bingöl, Erzurum, Kars and Muş Provinces of Eastern Anatolia Region of Turkey, constituted the plant material of this study. Experiments were carried out in Pasinler experimental station of Eastern Anatolia Agricultural Research Institute during the years 2010-2012. The information on orchardgrass ecotypes used as material is provided in Table 1.

## Methods

Orchardgrass seeds, obtained from single plants in different years were sown into the pots in early spring of 2010. The seedlings with certain level of growth were transplanted into field area providing 10 seedlings per row with 50 cm x 50 cm spacing. The observations were obtained from 10 seedlings of each ecotype during the years 2011 and 2012. The genotypes yielded two cuts under ecological conditions of Erzurum. Morphological and phonological characteristics were determined at the first cut. Total fresh and dry hay yield were taken as the total of two cuts. Plant height, number of nodes, node spacing, leaf length, leaf width, length of panicle axis parameters of orchardgrass (*Dactylis glomerata* L.) were investigated.

The methods and principals given for orchardgrass (*Dactylis glomerata* L.) in the "Technical Directives for Agricultural Experiments" issued by the General Directorate of Seed Registration and Certification Center of Ministry of Food, Agriculture and Livestock of Turkey were used (Anonymous, 2001) and observations were made on 10 plants of each ecotype.

## Data analysis

All data were standardized before the principal components and cluster analyses. Standardization was conducted to eliminate the unit effect and carried out through dividing entire data by standard deviation and subtracting from the mean value. The descriptive statistical analysis was carry out using SPSS (IBM-SPSS statistic for windows version 20.0). Statgraphics Centurion XV (Statpoint Inc 2006) was used for principal components analysis.

Moreover, Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.1 software) was used to generate dendrogram (Rohlf, 2000). Euclidian distances were calculated by using standardized data to determine the differences among genotypes. Couple methods were tried to generate clusters and EUCLID distances with the highest cophenetic correlation coefficient (r = 0.77453) and UPGMA method were used as the cluster method.

## Results and discussion

### *Descriptive statistics*

The statistics value defined for investigated ecotypes are given in Table 2. While the highest variation with 51.8% was observed in number of seed yield, it was followed by total fresh leaf length with 27.3%, then length of panicle axis with 26,7%. Plant height yielded the lowest variation (9.9%) among the parameters examined.

In general, the population used in the present study exhibited a wide range of variation which can be considered as an advantage since it provides opportunity in multipurpose breeding studies.Leaf lengths of ecotypes varied between 7.0-26 cm. Various observations were reported between 14.99- 27.40 cm, 7.0–20.5 cm and 2.00 - 36.00 cm in respective order by Tosun and Sağsöz (1994), Aygün *et al* (2009) and Ayan *et al* (2010).

Similarly, Aygün *et al* (2009), Tosun and Sağsöz (1994) and Ayan *et al* (2010) reported different leaf widths varying between 5- 11 mm, 5.18-7.19 mm and 2.7 -10 mm, respectively. Leaf widths varied between 7-10 mm in the present study.

Although plant heights varied between 70-111 cm in this study, in previous studies it varied between 74.7-101.47 cm (Tosun and Sağsöz, 1994), 59.8-64.5 cm (Mika *et al.,* 2002), 49.1- 95 cm (Aygün *et al.,* 2009) and 63.00 -160.00 cm (Ayan *et al.,* 2010).

Number of nodes varied between 3-5 per plant. This parameter was determined between 2.7-4.0 nodes/plant by Tosun and Sağsöz (1994) and between 3-6 nodes/plant by Ayan *et al.* (2010). Field experiments were found to be in harmony with them but the value reported by Tosun and Sağsöz (1994) for greenhouse conditions were found to be lower than field experiments.

Node spacing in present study was found to be between 15-27 cm. Ayan *et al.* (2010) investigated the same parameter on the seeds collected from natural floras of Ordu, Samsun and Sinop Provinces and reported between 1.50 - 29.30 cm.

Length of panicle axis of investigated ecotypes varied between 8 - 23 cm. Mika *et al.* (2002) reported the lengths of seed head axis of ecotypes that varied between 10.5-10.3 cm.

### *Principal components analysis*

Principal components analysis revealed that 3 components had Eigen values greater than 1 (Table 3). The factors with Eigen values greater than 1 were taken into consideration to determine the number of factors (Kaiser 1960). An Eigen value greater than 1 indicates that weighted values of the relevant principal component are reliable (Mohammadi and Prasanna 2003). Scree test, developed by Cattell (1966), is another graphical method to determine number of factors. According to scree test, 3 principal components had a value greater than 1 (Table 3). The factor groups and corresponding PC axis values based on scree test and principal components analysis carried out on investigated orchardgrass ecotypes are presented in Table 3 and Figure 1.

Principal components represented 72.66% of total variation observed in orchardgrass ecotypes (Table 3). While determining number of principal components, it is reported that it should be in number to explain at least 67% of total variation (Karaağaç and Balkaya 2010). Considering all these criteria, number of principal components was determined to be 3 (Table 3).With regard to parameters investigated in principal component analysis, the observations with the highest component weight values had a large interval. Analyses revealed that the first principal component, representing 34.61% of total variation, was composed of length of leaf, leaf width, the second principal component, representing 21,10% of total variation, was the plant height; third principal component, representing 16.95% of total variation, was due to node numbers

### *Cluster analysis*

UPGMA dendrogram (Figure 2) was drawn and evaluated as four groups to present the relationships among ecotypes. Cluster analysis is usually used for grouping the collected germplasm. Groups can be formed either randomly or in a way to maximize genetic distance (Tuna *et al.,* 2004).

Orchardgrass ecotypes were divided into 4 groups based on cluster analysis. The ecotypes 1,3,4,5,6,7, 8,9,11,12,13,14,16,17,18,19,22 and 23 were placed into the first group and they had the highest mean node spacing (21.6 cm) and the lowest mean leaf length (15.3 cm). The ecotypes of 10, 15 and 21 were placed into the second group and these ecotypes had the highest plant height (21.7 cm) and panicle length axis (19.3 cm) as well as with the lowest distance of internodes (17 cm) and number of nodes (3.3 cm). The third group consisted of 20 and 24 ecotypes with highest mean values of plant height (106.5cm) and seed yield (51.03 g). In fourth group a sole ecotype (2) was found with mean lowest values of plant height (70 cm) and seed yield (15.23 g).

## Conclusion

Genetic diversity assessment of collected materials and selection of plants available for breeding programs were the main objectives of this study. Experimental results revealed a large variation among collected ecotypes. This may be considered as an advantage since it provides opportunity in multipurpose breeding studies. Determination of genetic diversity of these materials with large variations and grouping them accordingly may help in decision making process of breeding line selection phase.

In general, the plants collected in present study exhibited a broad range of variation. Regarding seed

yield prominent ecotypes were determined and they were transferred to the breeders in the institute for detailed analysis while studies will continue on other ecotypes.

## Acknowledgements

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Table 1. List of orchardgrass (Dactylis glomerata L.) ecotypes used in this study

| Plant number | Origin | Plant number | Origin | Plant number | Origin |
|---|---|---|---|---|---|
| 1 | Erzurum | 10 | Erzurum | 19 | Artvin |
| 2 | Ardahan | 11 | Bingöl | 20 | Erzurum |
| 3 | Ağrı | 12 | Artvin | 21 | Kars |
| 4 | Muş | 13 | Erzurum | 22 | Bayburt |
| 5 | Erzurum | 14 | Bayburt | 23 | Ardahan |
| 6 | Kars | 15 | Erzurum | 24 | Erzurum |
| 7 | Bingöl | 16 | Muş | 25 | Ardahan |
| 8 | Erzurum | 17 | Ağrı | | |
| 9 | Bingöl | 18 | Erzurum | | |

Table 2. Descriptive Statics value for investigated ecotypes

| | Minimum | Maximum | Mean | Std. Deviation | Variance | % CV |
|---|---|---|---|---|---|---|
| Plant height (cm) | 70 | 111 | 91.16 | 9.012 | 81.223 | 9.9 |
| Number of nodes (nodes/plant) | 3 | 5 | 3.44 | 0.583 | 0.340 | 16.9 |
| Length of panicle axis (cm) | 8 | 23 | 12.76 | 3.407 | 11.607 | 26.7 |
| node spacing (cm) | 15 | 27 | 20.68 | 3.772 | 14.227 | 18.2 |
| Leaf length (cm) | 7 | 26 | 16.44 | 4.491 | 20.173 | 27.3 |
| Leaf width (cm) | 0.7 | 1.0 | 0.804 | 0.0978 | 0.010 | 12.2 |
| Seed yield (g) | 12.90 | 93.55 | 34.6332 | 17.931 | 321.529 | 51.8 |

Figure 1. Screen plot of principal component analysis of investigated parameters

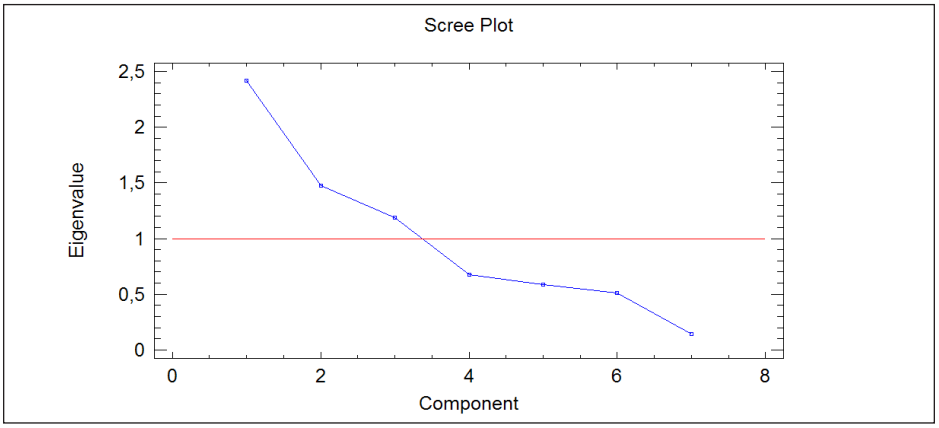
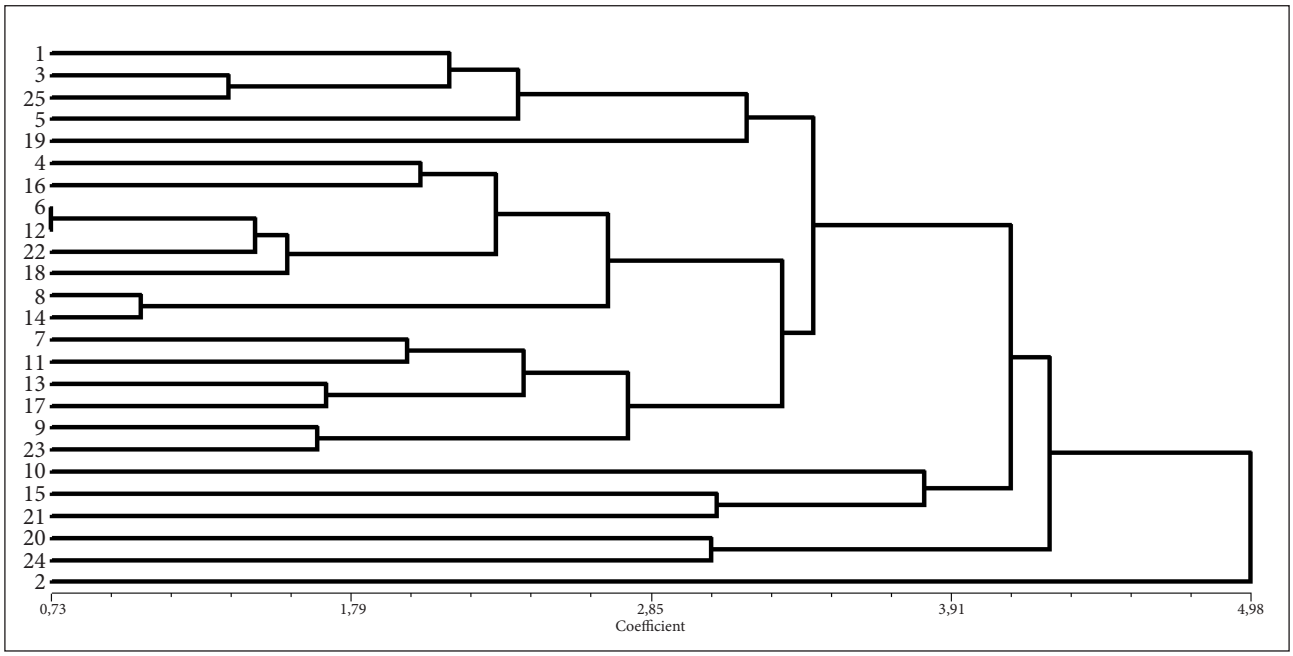


Table 3. Principal components and factors groups based on principal component analyses investigated in orchardgrass ecotypes

| | **1** | **2** | **3** |
|---|---|---|---|
| Eigen value | 2.42279 | 1.47707 | 1.18674 |
| Proportion of variance % | 34.611 | 21.101 | 16.953 |
| Cumulative variance, % | 34.611 | 55.712 | **72.666** |
| | | | |
| Observation | 1 | 2 | 3 |
| Plant height (cm) | 0.0394297 | **0.666219** | -0.120285 |
| Number of nodes (nodes/plant) | -0.0371103 | -0.211208 | **-0.820891** |
| Length of panicle axis (cm) | 0.414892 | -0.0962776 | 0.460501 |
| node spacing (cm) | -0.36789 | 0.369574 | 0.0548325 |
| Leaf length (cm) | **0.559125** | 0.0957526 | -0.0136107 |
| Leaf width (cm) | **0.570966** | -0.0794078 | -0.271273 |
| Seed yield (g) | 0.225759 | **0.591789** | -0.151084 |

Figure 2. UPGMA dendrogram of orchardgrass ecotypes



**References**

Ahmad, Z., Ajmal, S.U., Munır, M., Zubair, M. and Masood, M.S. (2008) Genetic diversity for morpho-genetic traits in barley germplasm. Pak J Bot **40** (3): 1217-1224.

Anonymous, (2001) T.C. Tarım ve Köyişleri Bakanlığı. Tohumluk Tescil ve Sertifikasyon Merkezi Müdürlüğü. Tarımsal Değerleri Ölçme Denemeleri Teknik Talimatı Domuz Ayrığı (*Dactylis glomerata* L.) Ankara.

Anonymous, (2007) T.C. Çevre ve Orman Bakanlığı Ulusal Biyolojik Çeşitlilik Stratejisi ve Eylem Plant.

Ayan, I., Mut, H., Onal, O., Basaran, U. and Tongel, O. (2010) Morphological traits of orchard grass accessions in black sea region of Turkey. Options Méditerranéennes - The Contributions of Grasslands to the Conservation of Mediterranean Biodiversity A No **92** : 121-124.

Aygün, C., Çakal, S. and A, Kara. (2009) Characterization of some coksfoot (*Dactylis glomerata* L.) lines from the natural rangelands of Eastern Anatolia BioDiCon 2/2: 57-64.

Aytepe, A.H and Varol, Ö. (2007) Bencik Dağı (Yatağan-Muğla) Florası. Ekoloji 16, **63** : 41-61.

Canbolat, Ö. and Karaman, Ş. (2009) Bazı baklagil kaba yemlerinin in vitro gaz üretimi, organik madde sindirimi, nispi yem değeri ve metabolik enerji içeriklerinin karşılaştırılması. Tarım Bilimleri Dergisi **15**(2): 188-195.

Cattell, D.R.B. (1966) The screen test for the number of factors. Multivariate Behav. Res. **1**: 245-76.

IBM Corp Released (2011) IBM SPSS Statistics for Windows Version 20.0. Armonk NY: IBM Corp.

Kaiser, H.F. (1960) The application of electronic computers to factor analysis Educ. Psychol. Meas. **20**:141-51.

Karaağaç O and A Balkaya (2010) Bafra kırmızı biber populasyonlarının [*Capsicum annuum* L. var. *conoides* (Mill.) Irish] tanımlanması ve mevcut varyasyonun değerlendirilmesi. Anadolu J. Agric. Sci. **25**(1):10-20.

Míka, V., Kohoutek, H. and Odstrčilová, V. (2002) Characteristics of important diploid and tetraploid subspecies of *Dactylis* from point of view of the forage crop production. Rostlınná Výroba **48** (6): 243-248.

Mohammadi, S.A and Prassana, B.M. (2003) Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci. **43** : 1235-1248.

Rohlf, F.J. (2000) NTSYS-PC Numerical Taxonomy. Multivariate Analysis System version 2.1. *Exeter Software New York.*

Sanada, Y., Gras, M.C. and Santen, E. (2010) Fodder crops and amenity grasses. Handbook of Plant Breeding Edition : **1**(5) : 317-328.

Statpoınt Inc (2006) Statgraphics centurion XV, version 15.1.02

Tosun, M. and Sağsöz, S. (1994) Erzurum yöresinde doğal olarak yetişen domuz ayrığı (*Dactylis glomerata ssp hispanica (roth) nyman*) bitkilerinde bazı morfolojik ve fenolojik özelliklerin belirlenmesi. Türkiye II Tarla Bitkileri Kongresi Cilt III 25-29 Nisan 1994 İzmir 39-43.

Tuna, M, Khadka, D.K., Shrestha, M.K., Arumuganathan, K. and Golan-Goldhirsh, A. (2004) Characterization of natural orchardgrass (*Dactylis glomerata* L.) populations of the Thrace Region of Turkey based on ploidy and DNA polymorphisms Euphytica **135** (1): 39-46.

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# Development of BAC-End based simple sequence repeat (SSR) markers in apple

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**ABSTRACT**

A genome-wide bacterial artificial chromosome (BAC) physical map of the apple, *Malus domestica* Borkh., has been recently developed. This study addresses development of SSR markers from the BAC-end sequences in apple. Previously designed 187 BAC-SSR primer pairs were subjected firstly to gradient-PCR for amplification and to determine their optimum annealing temperatures. Then, the amplified primer pairs were tested in two F1 segregating populations: 'Kasel-37' x 'Delbarestivale', and 'Kasel-41' X 'Williams Pride'. The PCR reactions were performed in 12 F1 progenies and in the parents to determine segregation types of the primer pairs. There was no amplification in 20 SSR primer pairs, and 87 of them were monomorphic in the two populations. As a result, 80 of the primer pairs showed segregation in each of the two F1 populations.

***Keywords:*** SSR, apple, polymorphism, microsatellite.

## Introduction

Apples have been grown for thousands of years in Asia and Europe, and have been cultivated for more than 4000 years ago (Özçağıran et al. 2004). Apple, *Malus domestica,* is one of the most popular tree fruit species in the rose family (Rosaceae) which belongs to the Maloideae subfamily of the Rosaceae. It is one of the most widely cultivated tree fruits; and the most widely known of genus *Malus*. Although it is functionally diploid (2n=2x=34); it has been suggested that the Maloideae are of allopolyploid origin.

According to FAO data, Turkey is among the top ten apple producers in the world. Apples are grown in many regions in Turkey, and approximately 50 percent of all commercial apple production comes from three provinces; Isparta, Karaman and Nigde. These provinces are located in the southern part of Central Anatolia and the Northern Mediterranean Regions. In addition to them; commercial apples are also grown in Antalya, Eregli, Denizli, Yalova and Amasya.

Amasya apple is Turkey's premier apple cultivars that its taste and aroma are more attractive among all other commercial varieties. In addition to aroma compounds; sugars and organic acids along with other pleasant fruit characteristics are noteworthy. Trade value of this cultivar in international market

is very low; because of low yield, alternate bearing and fruit size. Therefore, it is necessary to select the best ones with high quality and yield.

Bacterial artificial chromosome (BAC) libraries have been used in genomics research due to their large DNA inserts, high cloning efficiency, and stable maintenance of foreign DNA. In plants, BAC libraries have been constructed for a variety of species such as *Arabidopsis* (Choi et al. 1995), rice (Wang et al. 1995), maize (Yim et al. 2002), sorghum (Woo et al. 1994), soybean (Shoemaker et al. 1996; Salimath and Bhattacharyya 1999; Tomkins et al. 1999; Meksem et al. 2000), papaya (Ming et al 2001), and apple (Vinatzer et al. 1998; Xu et al 2001). These libraries have made invaluable contributions to plant genomic studies including map-based or positional cloning of genes, genome-wide physical map construction (Mozo et al. 1999; Klein et al. 2000; Chen et al. 2002; Xuand Korban 2002; Shultz et al. 2006; Han et al. 2007).

SSR markers have several advantages over other molecular markers. They are infact neutral, co-dominant, highly polymorphic markers, widely used in genetic mapping, fingerprinting and diversity studies.Furthermore, the high information content of microsatellites can be fully applied to QTL mapping and in general to apple breeding, enormously.

BAC-SSR primer pairs designed by Han et al. (2009) were used in this study.Here, we report segregation types of these BAC-end sequence derived SSR primers in two segregating $F_1$ populations in apple.

## Material and method

### *Plant material and DNA extraction*

Twelve progenies of two $F_1$ populations were used for this study. The first population (A) was derived from 'Kaşel-41' x 'Williams Pride' cross, and the second population (B) was derived from a cross between 'Kaşel-37' and 'Delbarestivale'. All crosses were made in Eğirdir Fruiticulture Research Station in Isparta province of Turkey.

DNAs were isolated according to the CTAB-based protocol (Doyle and Doyle, 1990) with minor modifications (Kafkas et al. 2006). After calculating concentration of DNA with QubitFluorometre (Invitrogen) and diluted to a concentration of 10 ng/µl for SSR-PCR reactions. 187 previously designed SSR primers by Han et al. (2009) were used to test in two $F_1$ populations in this study.

### *PCR conditions*

Firstly, gradient PCR was performed in a 1.5% agarose gel to determine optimum annealing temperatures of the primer pairs. M13 universal primer 5'-TGTAAAACGACGGCCAGT-3' is attached to the forward primer at the 5' end, were synthesised by labelling with 6-FAM, VIC, NED and PET fluorescent dyes (Schuelke 2000).

PCRs were carried out in 12.5- µL volumes containing 10 ng of DNA, 75 mMTris-HCl (pH: 8.8, 20 mM $(NH_4)_2SO_4$), 2.0 mMMg$Cl_2$, %0.01 Tween 20, 200mM dNTP, 10nM forward primer that added M13 Universal (5 `-TGTAAAACGACGGCCAGT-3 ') primer and 200 nM FAM, VIC, NED and PET, 200 nMreverse primer and 0.6 U of *Taq* polymerase (Fermentas).

The PCR conditions comprised an initial denaturation step at 94°C for 5 min, followed by 30 cycles at 94°C for 30s, 52-60°C for 45s and 72°C for 60s, plus 10 cycles at 94°C for 30s, annealing temperature 52°C for 45 s and 72°C for 60s, plus and a final extension at 72°C for 10 min. (Schuelke, 2000).

The products of gradient PCR reactions were stained with ethidium bromide in 1.5% agarose gel, and were photographed under UV light. Segregation types of the primer pairs were determined by testing them using 12 $F_1$ progenies in two populations.

In order to identify the allele sizes of SSR primer pairs, the electrophoresis of PCR reactions were done on automatic sequencing device, the model of ABI 3130xl. In the consequence of capillary electrophoresis, the allele sizes produced by SSR primer pairs were defined on the Genemapper 4.0 software.

In this study, a total of 187 SSR primer pairs from Han et al. (2009), were used to determine segregation types of the primer pairs in 'Kaşel-41' x 'Williams Pride' and 'Kaşel-37' x 'Delbarestivale' $F_1$ segregating populations.

The possible segregation types in $F_1$ populations using SSR markers are given in Table 1. The segregation types considered 'abxcd', 'efxeg' and 'hkxhk' as heterozygous in both parents, are called as common markers. 'nnxnp' and 'lmxll' as heterozygous in the male and female, respectively (Van Ooijen and Voorrips, 2001).

SSR alleles (band) can also be scored as presence/absence, but some genetic information will be lost. In this case, three segregation types ('lmxll', 'nnxnp', 'hkxhk') will be available. The single allele is present in one of the parents and available in 50% of individuals was scored as 'lmxll' 'nnxnp'. If one allele is present in both parents and also available in 75% of individual was scored as 'hkxhk' (Table 2).

## Results

All 187 SSR primer pairs used in this study were firstly tested by gradient PCR and 20 of them did not have amplification in both populations. After gradient PCR, all the primer pairs were screened using 12 $F_1$ individuals in each of two segregated populations. After capillary electrophoresis of the PCR reactions, 87 SSR primer pairs were monomorphic, and 80 of them were polymorphic in two populations (Table 3).

In 'Kaşel-41' x 'Williams Pride' $F_1$ population; 25 SSR primer pairs scored as dominant whereas 55 primer pairs had co-dominant segregating patterns: fourteen of them had 'abxcd', 11 of them had 'efxeg', 4 of them had 'hkxhk', 16 of them had 'lmxll', and 11 of them had 'nnxnp' segregation (Table 2).

In 'Kaşel -37' x 'Delbarestivale' $F_1$ population; 24 SSR primer pairs scored as dominant whereas 56 co-dominant markers were produced: 10 markers had 'abxcd' segregation, 13 markers had 'efxeg', 3 markers had 'hkxhk', 20 marker had 'lmxll', and 13 markers had 'nnxnp' segregation (Table 2).

The list of primers which showed polymorphism in two populations is given in Table 3. In both of the populations, 91 primers showed polymorphism. Some of primer pairs were monomorphic in population 'A' and was polymorphicin population 'B' or *vice versa*. According to the results, segregation patterns of polymorphic primer pairs were different in each population. Segregating patterns were determined for each primer as co-dominant (abxcd-efxeg- hkxhk-lmxll- nnxnp) or dominant (hkxhk- lmxll- nnxnp). In addition, some of primer pairs had amplification in more than one locus. Some primer pairs showed two co-dominant segregating loci such as 'BACSSR187' primer in both 'A' and 'B' populations, some of them showed co-dominant and dominant loci such as 'BACSSR40' and 'BACSSR155' loci in 'B' population (Table 4).

## Discussion

Molecular markers can be used to study the relationship between an inherited trait and its genetic cause. It is known that pieces of DNA that lie near each other on a chromosome tend to be inherited together. This property enables the use of a marker, which can be used to determine the precise inheritance pattern of the gene that has not yet been exactly localized.

Genetic markers are employed in genealogical DNA testing for genetic genealogy to determine genetic distance between individuals or populations. Genetic markers have to be easily identifiable, associated with a specific locus, and highly polymorphic, because homozygotes do not provide any information. Some of the methods used to study for these purposes are RFLP, Amplified fragment length polymorphism (AFLP), RAPD, and SSR markers. SSR markers are rapid and relatively simple to use, and their banding pattern is almost always easy to interpret. (Smeets et al. 1989). Finding a set of highly polymorphic BAC-SSR markers may help future studies in Amasya apple genome.

A total of 187 BAC-SSR primers were tested in two apple $F_1$ populations in this study. The screening of BAC-SSR over in two populations allowed determining level of polymorphism in two $F_1$ segregating populations. The role of polymorphic assay procedures in plant breeding was quickly realized for cultivar and parental identification, gene identification and selection (Ainsworth and Sharp 1989; Soller and Beckmann, 1983).

For assessing the size of the amplified amplicons of cultivars, M13 universal labeled primers which have been already discussed by Schuelke (2000) were used. The absolute fragment size could be determined in the model of ABI 3130xl. The differences of the allele sizes in some primer pairs between the parents in this study were ±1 or ±2 bases. This range of allele sizes can't determine in other platforms such as polyacrylamide gel electrophoresis (This et al. 2004). The results of SSR electrophoresis are reproducible and exchangeable between laboratories (Jones et al. 1997).

All the tested SSR primer pairs in two populations in this study showed different segregating patterns. The primer pairs with different segregating patterns or having different allele sizes can be used in the characterization of apple germplasm or genetic mapping studies in different populations in apple. Moreover, these markers correspond to physical location of DNA on their chromosomes and marker loci in genetic mapping studies and allow the detection of difference between the individuals.

As a result of this study, polymorphism levels and segregation types of 91(51.1%) newly developed BAC-SSR primer pairs were determined they showed a high degree of polymorphism in two $F_1$ populations.

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Table 1.Co-dominant Segregation types in $F_1$ populations.

| Segregation types | Female parent | | Male Parent | |
|---|---|---|---|---|
| | Allele size(1) | Allele size(2) | Allele size(1) | Allele size(2) |
| **abxcd (1:1:1:1)** | a | b | c | d |
| **efxeg (1:1:1:1)** | e | f | e | g |
| **hkxhk (1:2:1)** | h | k | h | k |
| **lmxll (1:1)** | l | m | l | l |
| **nnxnp (1:1)** | n | n | n | p |

Table 2. Dominant segregation types in $F_1$ populations and information about scoring system .

| Segregation types | Female parent | Male Parent | Present allele size In individuals | Absent allele size In individuals |
|---|---|---|---|---|
| | Present allele size | Absent allele size | | |
| **hkxhk (1:3)** | h- | h- | h- | kk |
| **lmxll (1:1)** | lm | ll | lm | ll |
| **nnxnp (1:1)** | nn | np | nn | np |

Table 3. The results of screened SSR primer pairs in two population: (Pop A) 'Kaşel-41' x 'Williams Pride', (Pop B) 'Kaşel-37' x 'Delbarestivale'

| Explanation of testing primers | K-41 x WP (Pop A) | K-37 x Delbarestivale (Pop B) |
|---|---|---|
| **Number of tested primer pairs** | 187 | 187 |
| **Number of primer pairs not amplified in gradient PCR** | 20 | 20 |
| **Number of primer pairs amplified monomorphic** | 87 | 87 |
| **Number of primer pairs amplified polymorphic** | 80 | 80 |
| **Number of primer pairs amplified co-dominant** | 55 | 56 |
| **Number of primer pairs amplified dominant** | 25 | 24 |
| **Number of primer pairs had abxcd segregation** | 14 | 10 |
| **Number of primer pairs had efxeg segregation** | 11 | 13 |
| **Number of primer pairs had hkxhk segregation** | 4 | 3 |
| **Number of primer pairs had lmxll segregation** | 16* | 20* |
| **Number of primer pairs had nnxnp segregation** | 11* | 13* |

*one primer had two loci (lmxll and nnxnp)

Table 4. The information of SSR primer pairs which amplified polymorphisms in two population: (Pop A) 'Kaşel-41' x 'Williams Pride', (Pop B) 'Kaşel-37' x 'Delbarestivale'.

| No | Primer | 'Kaşel-41' x 'Williams Pride' | 'Kaşel-37' x 'Delbarestivale' |
|---|---|---|---|
| | | (Pop A) | (Pop B) |
| | | Segregation patterns of markers | Segregation patterns of markers |
| 1 | BACSSR2 | hkxhk | efxeg |
| 2 | BACSSR6 | lmxll*/nnxnp* | lmxll*/nnxnp*/nnxnp* |
| 3 | BACSSR9 | Monomorphic | nnxnp |
| 4 | BACSSR10 | lmxll | efxeg |
| 5 | BACSSR11 | lmxll | hkxhk |
| 6 | BACSSR12 | lmxll | lmxll |
| 7 | BACSSR14 | lmxll*/nnxnp*/nnxnp* | Monomorphic |
| 8 | BACSSR16 | lmxll**/nnxnp** | lmxll*/nnxnp*/nnxnp* |
| 9 | BACSSR18 | lmxll | lmxll |
| 10 | BACSSR19 | hkxhk*/nnxnp*/nnxnp* | hkxhk*/lmxll*/nnxnp* |
| 11 | BACSSR20 | abxcd | efxeg |
| 12 | BACSSR22 | nnxnp | Monomorphic |
| 13 | BACSSR24 | lmxll* | lmxll*/nnxnp* |
| 14 | BACSSR29 | nnxnp* | nnxnp |
| 15 | BACSSR30 | lmxll*/nnxnp* | lmxll*/nnxnp* |
| 16 | BACSSR32 | lmxll*/lmxll*/nnxnp* | lmxll*/hkxhk* |
| 17 | BACSSR34 | abxcd | efxeg |
| 18 | BACSSR35 | efxeg | lmxll |
| 19 | BACSSR37 | efxeg | efxeg |
| 20 | BACSSR39 | lmxll*/nnxnp*/nnxnp* | lmxll |
| 21 | BACSSR40 | Monomorphic | lmxll//nnxnp* |
| 22 | BACSSR42 | abxcd | abxcd |
| 23 | BACSSR43 | lmxll*/nnxnp* | lmxll*/nnxnp* |
| 24 | BACSSR45 | Monomorphic | lmxll*/nnxnp* |
| 25 | BACSSR46 | abxcd | lmxll |
| 26 | BACSSR47 | efxeg | lmxll |
| 27 | BACSSR48 | lmxll*/lmxll*/nnxnp* | Monomorphic |
| 28 | BACSSR51 | efxeg | lmxll |
| 29 | BACSSR53 | lmxll*/nnxnp* | lmxll*/nnxnp* |

*Continuing table 4*

| No | Primer | 'Kaşel-41' x 'Williams Pride' | 'Kaşel-37' x 'Delbarestivale' |
|---|---|---|---|
| | | (Pop A) | (Pop B) |
| | | Segregation patterns of markers | Segregation patterns of markers |
| 30 | BACSSR57 | nnxnp | Monomorphic |
| 31 | BACSSR59 | abxcd | efxeg |
| 32 | BACSSR61 | Monomorphic | nnxnp |
| 33 | BACSSR62 | lmxll*/lmxll*/nnxnp* | lmxll*/nnxnp* |
| 34 | BACSSR63 | lmxll*/nnxnp* | lmxll*/nnxnp*/nnxnp* |
| 35 | BACSSR64 | lmxll | abxcd |
| 36 | BACSSR65 | lmxll | lmxll |
| 37 | BACSSR67 | abxcd | lmxll |
| 38 | BACSSR68 | abxcd | abxcd |
| 39 | BACSSR69 | nnxnp | nnxnp |
| 40 | BACSSR70 | lmxll*/lmxll*/nnxnp* | efxeg |
| 41 | BACSSR71 | efxeg | abxcd |
| 42 | BACSSR72 | abxcd | abxcd |
| 43 | BACSSR75 | abxcd | abxcd |
| 44 | BACSSR78 | Monomorphic | lmxll*/lmxll* |
| 45 | BACSSR79 | lmxll | abxcd |
| 46 | BACSSR82 | abxcd | efxeg |
| 47 | BACSSR83 | abxcd | abxcd |
| 48 | BACSSR84 | abxcd | lmxll*/nnxnp* |
| 49 | BACSSR87 | Monomorphic | efxeg |
| 50 | BACSSR88 | nnxnp | Monomorphic |
| 51 | BACSSR90 | lmxll*/lmxll*/nnxnp*/nnxnp* | lmxll*/nnxnp*/nnxnp* |
| 52 | BACSSR91 | nnxnp | nnxnp |
| 53 | BACSSR92 | lmxll | lmxll |
| 54 | BACSSR93 | Monomorphic | lmxll*/nnxnp* |
| 55 | BACSSR94 | lmxll | lmxll*/nnxnp*/nnxnp* |
| 56 | BACSSR96 | lmxll*/nnxnp* | lmxll*/nnxnp*/nnxnp* |
| 57 | BACSSR98 | lmxll | abxcd |
| 58 | BACSSR99 | efxeg | efxeg |
| 59 | BACSSR101 | lmxll*/lmxll*/nnxnp* | efxeg |
| 60 | BACSSR105 | Monomorphic | lmxll |

*Continuing table 4*

| No | Primer | 'Kaşel-41' x 'Williams Pride' | 'Kaşel-37' x 'Delbarestivale' |
|---|---|---|---|
| | | (Pop A) | (Pop B) |
| | | Segregation patterns of markers | Segregation patterns of markers |
| 61 | BACSSR108 | lmxll*/nnxnp* | nnxnp* |
| 62 | BACSSR112 | efxeg | lmxll*/nnxnp*/nnxnp* |
| 63 | BACSSR116 | lmxll*/nnxnp*/nnxnp* | Monomorphic |
| 64 | BACSSR117 | nnxnp | Monomorphic |
| 65 | BACSSR118 | nnxnp | nnxnp* |
| 66 | BACSSR119 | efxeg | Monomorphic |
| 67 | BACSSR120 | lmxll*/nnxnp*/nnxnp* | Monomorphic |
| 68 | BACSSR122 | abxcd | efxeg |
| 69 | BACSSR123 | lmxll*/hkxhk* | Monomorphic |
| 70 | BACSSR128 | efxeg | llxlm |
| 71 | BACSSR132 | hkxhk | lmxll*/hkxhk* |
| 72 | BACSSR133 | abxcd | abxcd |
| 73 | BACSSR136 | nnxnp | nnxnp |
| 74 | BACSSR137 | Monomorphic | nnxnp |
| 75 | BACSSR139 | efxeg | lmxll |
| 76 | BACSSR143 | Monomorphic | nnxnp |
| 77 | BACSSR145 | efxeg | lmxll |
| 78 | BACSSR149 | hkxhk* | lmxll* |
| 79 | BACSSR153 | hkxhk* | nnxnp |
| 80 | BACSSR155 | lmxll | lmxll /hkxhk*/nnxnp* |
| 81 | BACSSR156 | lmxll | lmxll |
| 82 | BACSSR162 | lmxll | lmxll |
| 83 | BACSSR164 | lmxll | efxeg |
| 84 | BACSSR169 | lmxll*/nnxnp*/nnxnp* | Monomorphic |
| 85 | BACSSR173 | nnxnp | nnxnp |
| 86 | BACSSR174 | Monomorphic | nnxnp |
| 87 | BACSSR178 | nnxnp | nnxnp |
| 88 | BACSSR180 | lmxll | lmxll |
| 89 | BACSSR181 | hkxhk | hkxhk |
| 90 | BACSSR182 | hkxhk | hkxhk |
| 91 | BACSSR187 | nnxnp/lmxll | nnxnp/lmxll |

*Dominantly scored markers

**References**


Ainsworth CC, Sharp PJ (1989) The potential role of DNA probes in plant variety identification. Plant Var Seed 2: 27- 34.

Jones PD,OsbornT.J and BriffaKR. (1997) Estimating sampling errors in large-scale temperature averages. *Journal of Climate* 10:2548-2568.

Chen M, Presting G, Barbazuk WG, Goicoechea JL, Blackmon B, Fang G, Kim H, Frisch D, Yu Y, Sun S, Higingbottom S, Phimphilai J, Phimphilai D, Thurmond S, Gaudette B, Li P, Liu J, Hatfield J, Main D, Farrar K, Henderson C, Barnett L, Costa R, Williams B, Walser S, Atkins M, Hall C, Budiman MA, Tomkins JP, Luo M, Bancroft I, Salse J, Regad F, Mohapatra T, Singh NK, Tyagi AK, Soderlund C, Dean RA, Wing RA (2002) An integrated physical and genetic map of the rice genome. Plant Cell 14:537–545.

Choi S, Creelman RA, Mullet JE, Wing RA (1995) Construction and characterization of a bacterial artificial chromosome library from *Arabidopsis thaliana*. Weed World 2:17–20.

Doyle JJ and Doyle JL(1987) A Rapid isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11-15.

Han Y, Gasic K, Marron B, Beever JE, Korban SS (2007) A BAC-based physical map of the apple genome. Genomics 89:630–637.

Han, Y, Chagne D, Gasic K, Rikkerink EHA, Beever JE, Gardiner SE and Korban SS(2009) BAC-end sequence-based SNPs and Bin mapping for rapid integration of physical and genetic maps in apple. Genomics, 93: 282-288.

Kafkas S, (2006) Phylogenetic analysis of the genus Pistacia by AFLP markers. Plant Systematics and Evolution, 262: 113-124.

Klein PE, Klein RR, Cartinhour SW, Ulanch PE, Dong J, Obert JA, Morishige DT, Schlueter SD, Childs KL, Ale M, Mullet JE (2000) A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. Genome Res 10:789–807.

Meksem K, Zobrist K, Hyten D, Quangzhou T, Zhang H, Lightfoot DA (2000) Two large-insert soybean genomic libraries constructed in a binary vector: applications in chromosome walking and genome wide physical mapping. TheorAppl Genet 101:747–755.

Ming R, Moore PH, Zee F, Abbey CA, Ma H, Paterson AH (2001) Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome. TheorAppl Genet 102:892–899.

Mozo T, Dewar K, Dunn P, Ecker JR, Fischer S, Kloska S, Lehrach H, Marra M, Martienssen R, Meier-Ewert S et al (1999) A complete BAC-based physical map of the *Arabidopsis thaliana* genome. Nat Genet 22:271–275.

Özçağiran R, Ünal A, Özeker E and İsfendiyaroğlu M(2004) Ilıman iklim meyve türleri, yumuşak çekirdekli meyveler, Cilt: II. Ege Üniversitesi Ziraat Fakültesi Yayınları, No: 556, Bornova, İzmir.

Salimath SS, Bhattacharyya MK (1999) Generation of a soybean BAC library, and identification of DNA sequences tightly linked to the *Rps1-k*disease resistance gene. TheorAppl Genet 98:712–720.

Shoemaker RC, Polzin K, Labate J, Specht J, Brummer EC, Olson T, Young N, Concibido V, Wilcox J, Tamulonis JP, Kochert GA, Boerma HR (1996) Genome duplication in soybean (*Glycine* subgenus *soja*). Genetics 144:329–338.

Scheulke M (2000)An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology, 18: 233-234.

Shultz JL, Kurunam D, Shopinski K, Iqbal MJ, Kazi S, Zobrist K, Bashir R, Smeets H J M, Brunner H G, Ropers H H and Wieringa B (1989) Use of variable simple sequence motifs as genetic markers: application to study of myotonic dystrophy. Hum Genet 83: 245–251.

Soller M., Beckmann J.S. (1983) Genetic polymorphism in varietal identification and genetic improvement. Theor. Appl. Genet. 67, 25-33.

This P, Jung A, Boccacci P, Borrego J, Botta R, Costantini l, Crespan M, Dangl GS, Eisenheld C, Ferreira-Monteiro F, Grando S, Ibáñez J, Lacombe T, Laucou V, Magalhães R, Meredith CP, Milani N, Peterlunger E, Regner F, Zulini L, Maul E (2004) Development of a standard set of microsatellite reference alleles for identification of grape cultivars. TheorAppl Genet 109:1448–1458.

Tomkins JP, Mahalingam R, Smith H, Goicoechea JL, Knap HT, Wing RA (1999) A bacterial artificial chromosome library for soybean PI 437654 and identification of clones associated with cyst nematode resistance. Plant MolBiol 41:25–32.

Tóth G, Gáspári Z, Jurka J (2000) Microastellites in different eukaryotic genomes: survey and analysis. Genome Res 10:967–981.

Vandijk T, Dubos T, Noordijk Y, Pikunova A, Yilmaz-temel H, and Van de weg E., (2010) Increased effectiveness of mapping in octoploid strawberry through quantitative interpretation of SSR data. In Plant and Animal Genomes XVIII Conf. Fruit and Nut Crops, San Diego.

Van ooijen JW(2011)JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands.

Vinatzer BA, Zhang H-B, Sansavini S (1998) Construction and characterization of a bacterial artificial chromosome library of apple. TheorAppl Genet 97:1183–1190

Wang GL, Holsten TE, Song WY, Wang HP, Ronald PC (1995) Construction of a rice bacterial artificial chromosome library and identification of clones linked to the *Xa-21* disease resistance locus. Plant J 7:525–533.

Woo SS, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucleic Acids Res 22:4922–4931.

Xu M, Korban SS (2002) A cluster of four receptor-like genes resides in the *Vf* locus that confers resistance to apple scab disease. Genetics 162:1995-2006.

Xu M, Song J, Cheng Z, Jiang J, Korban SS (2001) A bacterial artificial chromosome (BAC) library of *Malus floribunda* 821 and contig construction for positional cloning of the apple scab resistance gene *Vf*. Genome 44:1104-1113.

Yaegashi S, Lavu N, Afzal AJ, Yesudas CR, Kassem MA, Wu C, Zhang HB, Town CD, Meksem K, Lightfoot DA (2006) The Soybean Genome Database (SoyGD): a browser for display of duplicated, polyploid, regions and sequence tagged sites on the integrated physical and genetic maps of Glycine max. Nucleic Acids Res 34:D758–D765.

Yim YS, Davis GL, Duru NA, Musket TA, Linton EW, Messing JW, McMullen MD, Soderlund CA, Polacco ML, Gardiner JM, Coe EH Jr (2002) Characterization of three maize bacterial artificial chromosome libraries toward anchoring of the physical map to the genetic map using high-density bacterial artificial chromosome filter hybridization. Plant Physiol 130:1686–169

Research Article

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# Developing new microsatellite markers in walnut (*Juglans regia L.*) from *Juglans nigra* genomic GA enriched library

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**ABSTRACT**

We attempted to develop new polymorphic SSR primer pairs in walnut using sequences derived from *Juglans nigra* L. genomic enriched library with GA repeat. The designed 94 SSR primer pairs were subjected to gradient PCR in 12 walnut cultivars to determine their optimum annealing temperatures and to determine whether they produce bands. Then, the primer pairs which had amplification in agarose gel were analyzed in capillary electrophoresis to determine their allele sizes. According to the gradient PCR and capillary electrophoresis results, 60.6 % of the SSR primer pairs did not amplify any bands in agarose gel. Rest of the 37 primer pairs produced bands and their annealing temperatures and allele sizes were determined. From the amplified primer pairs, 18 of them were monomorphic, while 19 of them were polymorphic. As a result, 20.2 % polymorphism was obtained from 94 SSR primer pairs tested in this study which had lower ratio when compared to the literature.

***Keywords:*** SSR, walnut, polymorphism, PCR.

## Introduction

There are 21 species in the genus *Juglans* of which *Juglans regia* L. is the species with major economical importance (Manning 1978). Turkey has an considerable quantity of walnut production in the world. The major producing countries in the world are China, USA, Turkey, Iran and Ukraine (FAOSTAT, 2014). Walnut has monoecious feature, so clusters of male and female flowers are on the same tree, but located in different places and is pollinated by wind (Şen 1986). The diversity within this species is low and the cultivars are mostly from natural selections. Walnut has a long juvenility period, therefore, its breeding takes a few years. It is very important to characterize the cultivars for breeding purposes. However, morphological, physiological and biochemical methods used in the characterization are time consuming and are influenced by the environment. Development of DNA-based marker may overcome these problems.

Among the PCR-based DNA molecular marker techniques, simple sequence repeat markers (SSRs) are a perfect polymorphism source for eukaryotic genomes. Because SSRs have more genetic information and are co-dominant, and it is preferred in the areas of genetic mapping and population genetics (Powell et al. 1996). In walnut, biochemical methods have been first used to identify the cultivars (Arulsekar et al. 1985, 1986;

Aleta et al. 1990, ; Germain et al. 1993; Malvolti et al. 1993; Solar et al. 1994; Fornari et al. 2001; Vyas et al. 2003). Then, RFLP (Fjellstrom et al. 1994), RAPD (Nicese et al. 1997; Malvolti et al. 1997, 2001; Woeste et al. 1996), ISSR (Potter et al. 2002), SSR (Woeste et al. 2002; Dangl et al. 2005; Foroni et al. 2005, 2007; Victory et al. 2006; Robichaud et al. 2006; Ross-Davis and Woeste 2008a,b), Pollegioni et al. 2008; Wang et al. 2008; Hoban et al. 2008, Zhang ve ark. 2010) have been used in the characterization of genetic resources, genetic mapping and population genetic studies.

By now 56 SSR primer pairs from *J. nigra* genomic DNA, 13 microsatellite primer pairs from *J. cinerae* species and 41 EST-SSR primer pairs were developed from *J. regia* species (Woeste et al. 2002; Dangl et al. 2005; Foroni et al. 2005,2007; Victory et al. 2006; Robichaud et al. 2006; Ross-Davis and Woeste 2008a,b; Pollegioni et al. 2008, Wang et al. 2008, Zhang ve ark. 2010). So, there are 110 SSR primer pairs reported so far that can be used in genetic studies of *Juglans* species.

Studies on the genetic linkage map construction in *Juglans* species are limited and SSRs are powerful tools in genetic mapping studies because of their co-dominant nature. SSRs are very useful markers especially for reference genetic map construction in a plant species. However, there have to be enough SSR markers developed to use in the construction of a reference genetic map in walnut. Because of the limited number of SSR markers in the literature, there is no reference map in walnut. Therefore, we conducted a study to develop new SSRs in walnut.

## Materials and methods

### *Plant material and DNA isolation*

As plant materials, 'Maraş-12', 'Kaplan-86', 'Chandler', 'Franquette', 'Serr', 'Pedro', 'Van-4', 'Yalova-1', 'Bilecik', 'Şebin', 'Karabodur' and 'Maraş-18' cultivars were used in this study. Ninety-four SSR primer pairs developed by Dr. Woeste (Department of Forestry and Natural Resources, Purdue University) were used.

Genomic DNA was extracted using the CTAB method of Doyle and Doyle (1987) with minor modifications (Kafkas et al. 2006). DNA concentration was determined by gel electrophoresis (0.8 % agarose gel) and adjusted to 5 ng/µl for SSR reactions (Figure 1).

### *SSR analysis*

PCR reactions and cycling condition in SSR analysis were done according to Zaloglu (2008) by using M13 tailed primer in accordance with the method developed by Schuelke (2000). M13 universal primer 5'-TGTAAAACGACGGCCAGT-3' is attached to the forward primer at the 5' end and synthesized. Forward primers became 38-42 base-length. At the same time, 5' end of M13 primer of which base sequence is given are synthesized by labelling with 6-FAM, VIC, NED and PET fluorescent dyes. Therefore, SSR reaction included reverse primer, forward primer with M13 primer tailed at the 5' end and labelled M13 primer. The optimum annealing temperatures of SSR primer pair were determined by gradient PCR by applying six different temperatures.

12.5 µl PCR amplification reaction includes 75 mM Tris-HCl, pH = 8.8, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1 % Tween 20, 0,2 mM dNTP, 10 nM forward primer with an M13 tail at the 5' end, 200 nM reverse primer, 200 nM universal M13 primer labelled with one of the following dyes (6-FAM,VIC, NED, PET), 0.6 unit Taq DNA Polimerase and 10 ng DNA. Denaturation was 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 45 s at 50-60 °C and 1 min at 72 °C, and then followed by 10 cycles of 30 s at 94 °C, 45 s at 52 °C and 1 min at 72 °C. Final extension was included a cycle of 5 min at 72 °C.

### *Electrophoresis of PCR products*

The products of gradient PCR reactions were stained with etidium bromide in 3%-agarose gel, and their photos were taken under UV transsimilator. 50 base pair size standard was used to identify DNA band size in the gel. In order to identify exact allele size of SSR primer pairs, the electrophoresis of PCR reactions was done on automatic base sequencing device using the model of ABI 3130xl having 16 capillary array (capillary electrophoresis), and the allele sizes were defined using Genemapper 4.0 software.

## Results

### *Gradient PCR analysis*

Ninety-four SSR primer pairs were screened by using DNAs of 'Maraş-12' and 'Kaplan-86' cultivars. An example of the agarose gel image in gradient PCR is shown in Figure 2**.**

37 (39.4 %) of 94 SSR primer pairs produced DNA band and, therefore, their annealing temperatures were determined (Table 1). In the conclusion of the gradient PCR, the annealing temperatures of the SSR primer pairs varied from 50°C to 60°C.

### *Determination of allele sizes and polymorphism level of the SSR primer pairs*

The PCR reactions were done using 37 SSR

primer pairs which produced band in agarose gel in 12 *Juglans regia* cultivars and the results are given in Table 1. According to the results of capillary electrophoresis (Figure 3), 18 of 37 analyzed primer pairs produced monomorphic bands, while 19 of them (51.4%) were polymorphic. So, 60.6 % (57 primers) of 94 SSR primer pairs were out of the evaluation because of non-amplification in the PCR, and 19.2 % of them (18 primers) were monomorphic and 20.2 % of them (19 primers) were polymorphic.

32 of the 37 primer combinations produced alleles in single locus, 3 of them had amplification in two loci and 2 of them amplified in three loci. Sixteen out of 32 SSR primer pairs amplified in single locus were identified as monomorphic, while the rest of them were polymorphic. Three SSR primer pairs amplified two loci and they produced 4 monomorphic and 2 polymorphic loci. Two SSR primer pairs amplified three loci and they produced 2 monomorphic and 4 polymorphic ones. From 37 evaluated SSR primer combinations, 44 loci were produced and 22 (50.0 %) of them were monomorphic loci, while 22 (50.0%) of them produced polymorphic ones. Totally, 124 alleles were produced from 44 loci and there were an average of 2.8 alleles per locus.

## Discussion

The low rate of amplification success which 37 (39.4 %) of 94 SSR primer pairs produced DNA band can be attributed to transferability of the SSRs from *J. nigra* to *J. regia*. Similar results were also reported earlier (Woeste et al. 2002; Dirlewanger et al. 2002).

In SSR primer development studies in walnut as reported in scientific literature, the primers have been developed in *J. nigra* species as in this study. The highest number of primer pairs have been developed by Zhang et al. (2010), followed by this study and Woeste et al. (2002), respectively. The allele sizes varied between 100 and 362 base pairs in this study. The highest rate of average number of allele per primer was obtained by Victory et al. (2006), whereas the lowest rates were obtained by Zhang et al. (2010) and in this study (Table 2).

In conclusion, 94 SSR primer combinations were designed from genomic DNA library of *J. nigra* and tested in *J. regia* . Consequently, new SSR primers were developed in this study. They can be used in genetic characterization, genetic mapping and population genetic studies in walnut. Moreover, polymorphic SSR markers together with monomorphic ones should be tested in the other walnut species in the genus *Juglans*.

Figure 1. A concentration image of walnut DNA after agarose gel electrophoresis.

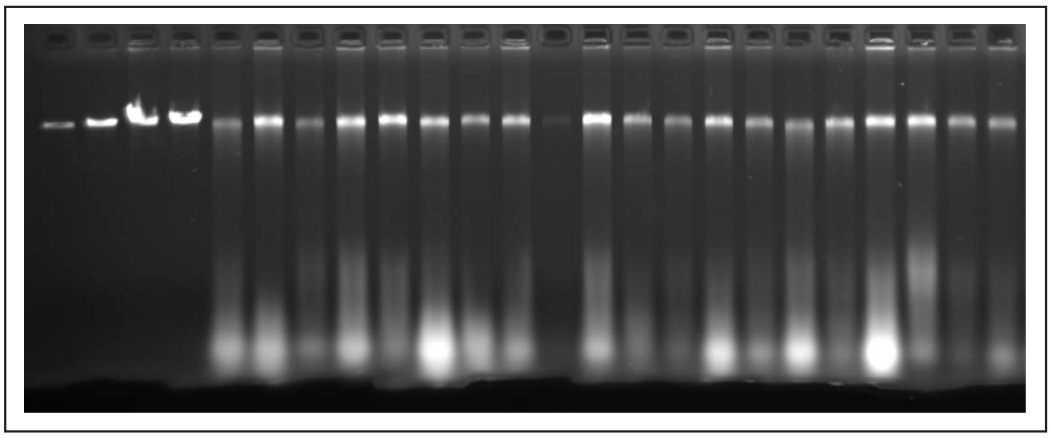
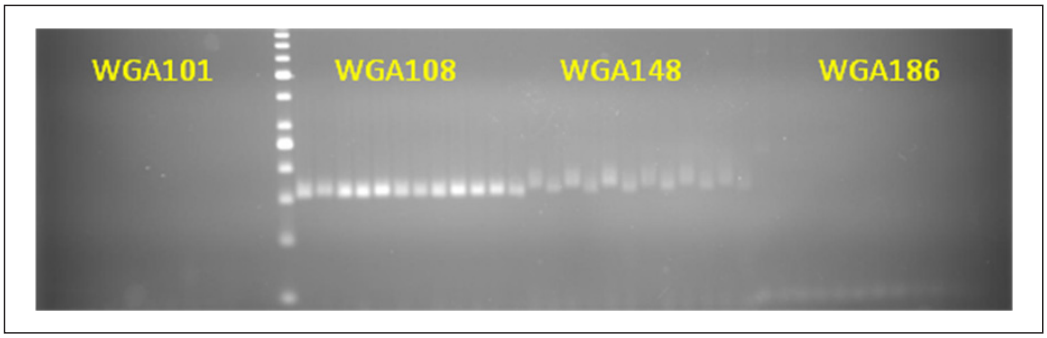


Figure 2. A gel image of three SSR primer pairs after gradient PCR.



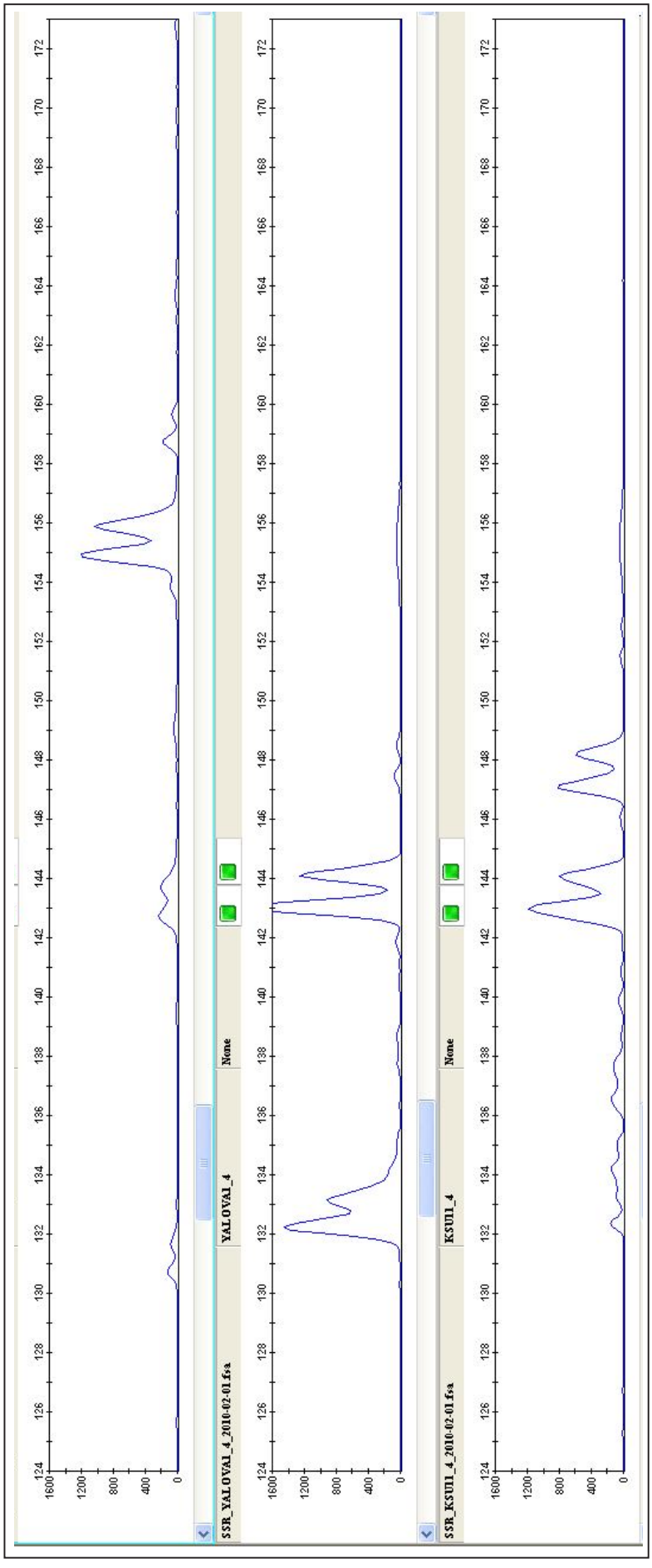



Figure 3. An electropherogram of WGA123 locus in *Juglans* cultivars obtained from capillary electrophoresis.

Table 1. Annealing temperatures, number of locus, allele sizes and allele numbers of the SSR primer combinations.

| No | Primer Name | Annealing Temperature | Fluorescent Dye | Allele Size Range (bp) | No. of Alleles | Number of Locus | Type of marker |
|---|---|---|---|---|---|---|---|
| **1** | WGA101 | 58 | PET | 120 | 1 | 1 | Monomorphic |
| **2** | WGA104 | 60 | PET | 121-139 | 6 | 1 | Polymorphic |
| **3** | WGA108 | 58 | VIC | 145-157 | 2 | 1 | Monomorphic |
| **4** | WGA110 | 54 | VIC | 103 | 1 | 1 | Monomorphic |
| **5** | WGA111 | 60 | NED | 250 | 1 | 1 | Monomorphic |
| **6** | WGA112 | 56 | VIC | 106 | 1 | 1 | Monomorphic |
| **7** | WGA116 | 50 | FAM | 362 | 1 | 1 | Monomorphic |
| **8** | WGA123 | 52 | FAM | 105-271 | 13 | 3 | Polymorphic |
| **9** | WGA125 | 52 | NED | 173-253 | 2 | 2 | Monomorphic |
| **10** | WGA126 | 52 | NED | 100-350 | 5 | 3 | Polymorphic |
| **11** | WGA127 | 52 | PET | 238-281 | 6 | 1 | Polymorphic |
| **12** | WGA131 | 56 | FAM | 179-298 | 5 | 2 | Polymorphic |
| **13** | WGA133 | 50 | PET | 121-130 | 3 | 1 | Polymorphic |
| **14** | WGA134 | 52 | NED | 362 | 1 | 1 | Monomorphic |
| **15** | WGA135 | 58 | NED | 355 | 1 | 1 | Monomorphic |
| **16** | WGA136 | 54 | FAM | 218-268 | 9 | 1 | Polymorphic |
| **17** | WGA137 | 54 | PET | 227 | 1 | 1 | Monomorphic |
| **18** | WGA139 | 56 | NED | 171-186 | 4 | 1 | Polymorphic |
| **19** | WGA140 | 58 | FAM | 230 | 1 | 1 | Monomorphic |
| **20** | WGA142 | 54 | VIC | 243-271 | 5 | 1 | Polymorphic |
| **21** | WGA145 | 56 | VIC | 153-161 | 3 | 1 | Polymorphic |
| **22** | WGA148 | 52 | VIC | 151-171 | 3 | 1 | Polymorphic |
| **23** | WGA150 | 60 | FAM | 199-209 | 4 | 1 | Polymorphic |
| **24** | WGA153 | 58 | FAM | 113 | 1 | 1 | Monomorphic |
| **25** | WGA160 | 54 | NED | 245 | 1 | 1 | Monomorphic |
| **26** | WGA167 | 52 | FAM | 241-253 | 4 | 1 | Polymorphic |
| **27** | WGA168 | 54 | NED | 222-259 | 2 | 1 | Monomorphic |
| **28** | WGA169 | 52 | PET | 175-188 | 6 | 1 | Polymorphic |
| **29** | WGA171 | 58 | VIC | 131-147 | 5 | 1 | Polymorphic |
| **30** | WGA182 | 60 | PET | 181-254 | 3 | 2 | Monomorphic |
| **31** | WGA185 | 54 | VIC | 262-269 | 2 | 1 | Polymorphic |
| **32** | WGA190 | 58 | PET | 135-143 | 3 | 1 | Polymorphic |
| **33** | WGA193 | 56 | FAM | 228-267 | 8 | 1 | Polymorphic |
| **34** | WGA195 | 54 | NED | 172-198 | 6 | 1 | Polymorphic |
| **35** | WGA196 | 52 | NED | 260 | 1 | 1 | Monomorphic |
| **36** | WGA198 | 52 | PET | 137-178 | 2 | 1 | Monomorphic |
| **37** | WGA200 | 54 | PET | 181 | 1 | 1 | Monomorphic |

Table 2: Comparison of data in this study with the SSR primer development studies in the literature.

| No. | Species | Reference | Number of primer pair | Allele Size range (bp) | Average number of alleles |
|---|---|---|---|---|---|
| **1** | *J. nigra* | Woeste et al. (2002) | 30 | 150-242 | 7.3 |
| **2** | *J. nigra* | Dangl et al. (2005) | 12 | 143-275 | 5.2 |
| **3** | *J. nigra* | Foroni et al. (2005;2007) | 4 | 120-266 | 6.0 |
| **4** | *J. nigra* | Victory et al. (2006) | 4 | 162-236 | 23.8 |
| **5** | *J. nigra* | Robichaud et al. (2006) | 1 | 208-250 | - |
| **6** | *J. nigra* | Ross-Davis and Woeste (2008a) | 5 | 161-164 | 12.2 |
| **7** | *J. cinerae* | Hoban et al. (2008) | 13 | 103-358 | 13.6 |
| **8** | *J. regia* | Zhang et al. (2010) | 41 | - | 3.0 |
| **9** | *J. nigra* | In this study | 37 | 100-362 | 3.4 |

**References**


Aleta N, Olarte C, Truco MJ and Arus P (1990) Identification of walnut cultivars by isozyme analysis. Acta Hort 284: 91-96.

Arulsekar S, Parfitt DE and McGranahan GH (1985) Isozyme gene markers in *Juglans* species inheritance of Gpi and Aat in *J. regia* and *J. hindsii*. J of Heredity 76: 103-106.

Aruselkar S, McGranahan GH and Parfitt DE (1986) Inheritance of Phosphoglucomutase and Esterase isoyzmes in Persian walnut. Jl of Heredity 77:220-221.

Dangl G, Woeste K, Aradhya M, Koehmstedt A, Simon C, Potter D, Leslie CA and McGranahan G (2005) Characterization of 14 microsatellite markers for genetic analysis and cultivar identification of walnut. J Amer Soc Hort Sci 130: 348-354.

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P and Laigret F (2002) Development of microsatellite markers in peach (*Prunus persica* (L) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127-138.

Doyle JJ and Doyle JL (1987) A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11-15.

FAOSTAT (2014) http://faostatfaoorg/site/567/defaultaspx Accessed 14 April 2014.

Fjellstrom RG, Parfitt DE and McGranahan GH (1994) Genetic relationships and characterization of Persian walnut (*J. regia* L.) cultivars using restriction fragment lenght polymorphisms ( RFLPs ). J Amer Soc Hort Sci 119:833-839.

Fornari B, Malvolti ME, Taurchini D and Fineschi S (2001) Isozyme and organellar DNA analysis of genetic diversity in natural/naturalised European and Asiatic walnut (*Juglans regia* L.) populations. Acta Hort 544: 167-178.

Foroni I, Rao R, Woeste K and Gallitelli M (2005) Characterisation of *Juglans regia L.* with SSR markers and evaluation of genetic relationships among cultivars and the 'Sorrento' landrace. J of Hort Sci & Biotech 80:49-53.

Foroni I, Woeste K and Monti LM (2007) Identification of 'Sorrento' walnut using simple sequence repeats (SSRs). Genet Resour Crop Evol 54:1081-1094.

Germain E, Hanguier I and Monet R (1993) Identification of eight *Juglans* spp and their interspecific hybrids by isoenzimatic electrophoresis. Acta Hort, 311, 73-85.

Hoban S, Anderson R, Mccleary T, Schlarbaum S and Romero-Severson J (2008) Thirteen nuclear microsatellite loci for butternut (*Juglans cinerea* L.). Mol Ecol Resour 8:643-646.

Kafkas S (2006) Phylogenetic analysis of the genus *Pistacia* by AFLP markers. Plant Syst and Evol 262: 113-124.

Malvolti ME, Paciucci M, Cannata F and Fineschi S (1993) Genetic variation in Italian populations of *Juglans regia* L. Acta Hort 311: 86-94.

Malvolti ME, Spada M, Beritognolo I and Cannata F (1997) Differentiantion of walnut hybrids (*Juglans nigra* L x *Juglans regia* L.) through RAPD markers. Acta Hort 462:43-52.

Malvolti ME, Fornari B, Maccaglia E and Cannata F (2001) Genetic linkage mapping in an

intraspesific cross of walnut (*Juglans regia* L.) using molecular markers. Acta Hort 544:179-185.

Mannıng WE (1978) The classification within the *Juglandaceae.* Ann Mo Bot Gard 65:1058-1087.

Nicese FP, Hormaza JI and Mcgranahan GH (1997) Characterization of walnut (*Juglans regia* L.) cultivars using RAPD. III Int Walnut Congress Acta Hort 442: 51-63.

Pollegioni P, Woeste K, Major A, Scarascia Mugnozza G and Malvolti ME (2008) Characterization of *Juglans nigra* (L), *Juglans regia* (L) and *Juglans* x *intermedia* (Carr) by SSR markers: a case study in Italy. Silvae Genetica, 58:1-2.

Potter D, Gao F, Aiello G, Leslie C and Mcgranahan GH (2002) Intersimple sequence repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. J Amer Soc Hort Sci 127: 75-81.

Powell W, Machray GC and Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends in Plant Sci 1:215-221.

Robichaud RL, Glaubitz JC, Rhodes Jr OE and Woeste K (2006) A robust set of black walnut microsatellites for parentage and clonal identification. New Forests 32:179-196.

Ross-Davis A and Woeste KE (2008a) Microsatellite markers for *Juglans cinerea* L. and their utility in other Juglandaceae species. Conserv Genet 9: 465-469.

Ross-Davis A, Huang Z, Mckenna J, Ostry M and Woeste K (2008b) Morphological and molecular methods to identify butternut (*Juglans cinerea*) and butternut hybrids: relevance to butternut conservation. Tree Physiology 28:1127-1133.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotech 18: 233-234.

Solar A, Smole J and Stampar F (1994) Identification of walnut cultivars by polen isozymes. Acta Hort 311: 95-100.

Şen SM (1986) Ceviz Yetiştiriciliği. Eser Matbaası, Samsun, 229 S.

Victory ER, Glaubitz JC, Rhodes-JR OE and Woeste KE (2006) Genetic homogenety in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. American Journal of Botany 93:118-126.

Vyas D, Sharma SK and Sharma DR (2003) Genetic structure of walnut genotype using leaf isozymes as variability measure. Sci Hort 97:141-152.

Wang H, Pei D, Gu RS and Wang BQ (2008) Genetic diversity and structure of walnut populations in Central and Southwestern China revealed by microsatellite markers. J Amer Soc Hort Sci 133: 197-203.

Woeste K, Mcgranahan GH and Bernatzky R (1996) Randomly amplified polymorphic dna loci from a walnut backcross [(*Juglans hindsii* x *Juglans regia)* x *Juglans regia*]. J Amer Soc Hort Sci 121:358- 361.

Woeste K, Burns R, Rhodes O and Michler C (2002) Thirty polymorphic nuclear microsatellite loci from black walnut. J.Hered 93:58-60.

Zaloğlu S (2008) Antepfıstığında mikrosatellit primerlerin geliştirilmesi ve diğer *Pistacia* türlerinde kullanılma durumlarının belirlenmesi. Master Thesis, Cukurova University, Adana.

Zhang R, Zhu A, Wang X, Yu J, Zhang H, Gao J, Cheng Y and, Deng X (2010) Development of *Juglans regia* SSR Markers by Data Mining of the EST Database. Plant Mol Biol Rep 28:646–653.

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# Improved vegetable varieties for Central Asia and the caucasus developed from AVRDC - The World Vegetable Center Germplasm

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**ABSTRACT**

After the breakup of the Soviet Union in 1991, all countries in Central Asia and the Caucasus experienced difficulties in obtaining vegetable germplasm for breeding programs. The genebank at AVRDC – The World Vegetable Center conserves a diversity of vegetable germplasm. From 2005-2012, the Center introduced 1370 genebank accessions and 26 improved lines of vegetable species representing 9 families to countries in Central Asia and the Caucasus through the Regional Network for Vegetable System Research and Development. This network fostered faster variety development and dissemination by encouraging partner research institutes to study vegetable crops in regional variety trials under various agroecosystems. Currently a total of 38 new varieties of 12 species are under State Variety Trials. Through collaboration, 35 new varieties of 8 vegetable crops including tomato, sweet and hot pepper, eggplant, vegetable soybean, mungbean, yard-long bean and cabbage have been released and registered in state registries. From 35 new varieties, 14 have been developed by conventional selection methods using germplasm received from the AVRDC genebank. All released varieties demonstrate economically valuable traits: early maturity, high yield, resistance to diseases and pests, high nutrient content and other marketable features. Seeds of released varieties are multiplied by research institutes for distribution to farmers. For the first time, new varieties of non-traditional species such as vegetable soybean, mungbean, yard-long bean and Chinese leafy cabbage have expanded the diversity of vegetables grown in the region, and have become popular for cultivation and consumption. Increasing vegetable production will help diversify diets, increase farmers' income, and enhance the well-being of families throughout the region.

***Keywords:*** Central Asia and the *caucasus*, vegetable germplasm, regional variety trials, yield..

## Introduction

Vegetables are important for food security and livelihoods of people in Central Asia and the Caucasus. Human populations are steadily increasing in the region, and vegetable production is increasing as well: from 13,114,077 t in 2006 to 20,032,668 t in 2012 – an increase of almost 53% in only 6 years. However, this increase came about because the total vegetable sowing area expanded from 682,592 ha to 770,881 ha. Average yield (32.2 t/ha) for vegetables remains below potential yield (FAOSTAT, 2013).

Vegetable diversity in the region comprises about 40 vegetable species including traditional and non-traditional species. The most popular vegetables are cabbages and other brassicas, tomato, watermelon, cucumber, onion, carrot and these occupy most of the crop area in the region. Approximately 15% of the region's total vegetable production occurs during winter from November to March (Ali *et al*., 2006). This includes production of tomato, cucumber and greens in heated greenhouses and vegetables harvested in autumn to sell in winter and spring.

The region needs more productive varieties with improved resistance to pests and diseases, and tolerance to heat, drought, and saline soils. Underutilized traditional and non-traditional

vegetable crops have yet to be fully exploited (Mavlyanova 2013**b**).

After the breakup of the Soviet Union in 1991, breeding programs in all countries in the region were weakened by the lack of germplasm. AVRDC – The World Vegetable Center's collaboration with the National Agricultural Research and Extension Systems (NARES) of Central Asia and the Caucasus through the Regional Network on Vegetable System Research & Development (CACVEG) became one of the most important sources for vegetable germplasm and a platform for faster variety development and dissemination (Mavlyanova, 2013**a**). This collaboration opened access for researchers who were evaluating vegetable crops in regional varietal trials (Aytbayev *et al*., 2012; Mavlyanova *et al*., 2010). New fresh market and processing tomato varieties created business opportunities, and cherry tomato was introduced for the first time (Martirosyan, 2012; Osmanalieva, 2013; Dzhantasov *et al*., 2013). Evaluation of sweet and hot pepper collections enriched local pepper diversity with new unique varieties (Azimov and Mavlyanova, 2010; Sariksyan and Sagsyan, 2012; Lin *et al*., 2013). The introduction of non-traditional species encouraged research in new directions, and increased vegetable diversity in the region (Mavlyanova, 2013**c**; Kim, 2013; Kiseleva and Baytureeva, 2013).

## Materials and methods

From 2005-2012, the Center introduced 1370 accessions and 26 improved lines of vegetable species representing 9 families (*Alliaceae, Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Poaceae and Solanaceae*) to countries in Central Asia and the Caucasus. This germplasm was evaluated in partner research institutes in regional varietal trials under various agroecosystems in Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan. AVRDC's crop production and field guides were used in research institutes. Investigations were carried out in accordance with standard procedures with four replications. Vegetable germplasm was evaluated for biological, morphological and agronomic characters, and commercially valuable traits. Conventional selection methods were used to develop new vegetable varieties.

## Results

Through collaboration, 35 new varieties of 8 vegetable crops including tomato, sweet and hot pepper, eggplant, vegetable soybean, mungbean, yard-long bean and cabbage have been released and registered in state registries. Among them, vegetable soybean, yard-long bean and leafy cabbage are non-traditional species introduced to the region for the first time. Genebank accessions as well as breeding lines are good sources for development of new varieties. For example, from 35 new varieties, 14 (40%) were developed by conventional selection methods using germplasm received from the AVRDC genebank. The newly released varieties demonstrate economically valuable traits: early maturity, higher yield than local varieties, disease resistance, high nutrient content and other marketable features. Local names given to new varieties mainly refer to specific characteristics or appearance. Currently, a total of 38 new varieties of 12 species are under state variety trials in eight countries across the region.

***Tomato*:** Some farmers and countries in the region still rely on low-yielding tomato varieties that are 70-90 years old with limited disease resistance. New cherry tomato varieties developed from AVRDC genebank accessions such as Armine (VI006852) and Narek (VI006972) and from improved lines such as Zeytun (CH154), Janna (CLN2413D), Rubina (CLN1558B) in Armenia, and Solnechnaya jemchujina (CLN2070C) and Zolotaya businka (CLN 2071D) in Kazakhstan can open new markets for growers. The new varieties are early, medium and late maturing; indeterminate type; resistant to fusarium, bacterial wilt and tomato mosaic virus; and have plum, roundish, or ellipse-shaped fruit that is yellow, orange or red in color, with fruit weight of 10-25 g and yield potential of 50-70 t/ha.

Fresh market tomato varieties Saadreo (CLN2026D) in Georgia and Alsu (CLN2545A) in Azerbaijan have fruit weight of 95 g and yield potential of up to 70 t/ha. All released varieties have high nutrient content, good transportability and very good processing qualities.

***Hot pepper*:** Although hot pepper is a popular crop in the region, only a few varieties are available. AVRDC germplasm has opened growers' access to new varieties. New early maturing (100 days) variety Punj (VI013538) based on a AVRDC genebank accession was developed in Armenia with small fruits (3 g) but high yield (14.7 t/ha), elongated fruit shape, and red fruit color at the mature stage. New mid-maturity varieties with long conical fruits and red color at ripening such as Zspanak (VI014204) and Kon (VI037591) have been developed in Armenia. Erekshe (VI059345), developed in Kazakhstan, has

large fruits (28-49 g) and yield of 28 t/ha. AVRDC improved lines of mid-maturing hot pepper include Gita (PP0337-7546) with small fruits (5-7 g) and yield of 28 t/ha released in Armenia; Piquant (PP0107-7058) with large fruits (14 g) and yield of 14.5 t/ha released in Kazakhstan; and Uchkun (PP0337-7069) and Tillarang (PP9955-15) released in Uzbekistan, with large fruits (30 g) and yield of 28 t/ha.

***Sweet pepper*:** Sweet pepper germplasm from the AVRDC genebank was used for development of new variety Kaz-Tai (VI046956) in Kazakhstan; it has red fruit color at biological ripening, conical shaped large fruits (125 g) and yield of 22 t/ha. AVRDC improved lines of sweet pepper were used to develop mid- and late-maturing varieties with intense orange color at biological ripening in Armenia: Natali (PP0137-7025) has a cylindrical shape and Emili (PP0137-7041) has a cube shape; both have large fruit size (150-160 g) and yield up to 57 t/ha. Bayan Sulu (PP0037-7645) in Kazakhstan (fruit weight: 125 g; yield: 30 t/ha) and Sabo (PP0437-7031) in Uzbekistan (fruit weight: 80 g; yield: 25 t/ha) are other promising sweet pepper for release. Among red-orange colored fruits, Shodlik (PP0636-6056), developed in Uzbekistan, has a fruit weight of 95 g and yields 26 t/ha. Sweet pepper variety Mili (PBC271) developed in Armenia is late ripening (142 days) with red colored large fruits (160 g) and potential yield of 50 t/ha. The sweet pepper variety Kozy-Korpesh (PP0237-7011) released in Kazakhstan is mid-maturing (120 days) and yields 22.0 t/ha. It has elongated fruits (80 g) with dark green color at technical ripening changing to dark red at biological ripening—a quality that has consumer appeal. New sweet pepper lines adapted to hot local climate conditions are being released in Kyrgyzstan, Tajikistan and Turkmenistan.

***Eggplant*:** Genebank germplasm was used to successfully breed Feruz (VI042320), the first new eggplant variety developed in Uzbekistan. Feruz has large, elliptical-shaped fruits (180 g) and yields 32 t/ha.

***Vegetable soybean*:** Soybean is a valuable crop; the green seeds and grain are used to cook a variety of dishes, oil can be extracted from the grain, and the grain and oilcake can be used as livestock and poultry feed. AVRDC introduced vegetable soybean to Central Asia and the Caucasus for the first time, and new varieties have been developed from AVRDC germplasm and breeding lines. Ilhom (VI053823; Misono Green) and Universal (VI032661) have been released in Uzbekistan; Sabostne 1 (VI045038; Jasuto-75) and Mtsvane parkiani (VI044024; AGS292) have been registered in Georgia. Their reduced photoperiod sensitivity and early maturity (95 days) fits in various crop systems, and they are high yielding, producing 9 t/ha of green pods and 3.5 t/ha of seeds, with high protein (42%) and oil (21%) content. New varieties developed from AVRDC improved lines are Inju (AGS-437), a mid-maturity (100 days) variety for Kazakhstan, and Sulton (AGS423), a late maturing (125 days) variety released in Uzbekistan. Sulton has high protein (42.5%) and oil (22%) content. Its green 1000 seed weight is 690 g and its ripe 1000 seed weight reaches up to 250 g; the green pods yield 18-20 t/ha and grain yield is 4-6 t/ha. Research has confirmed the capability of soybean and other vegetable legume crops to increase soil fertility (Mavlyanova, 2013d).

***Mungbean*:** Late maturing varieties grown in Central Asia are subject to lodging and produce crumbled pods. Durdona (VI002984; NM94), an early maturing (70 days) mungbean variety, was developed from AVRDC germplasm. Early maturing AVRDC improved line VC6492-59 has been released as Zhasyl Dan in Kazakhstan and Marjon in Uzbekistan. Mungbean variety Zilola (VC1178) has been released in Uzbekistan. These early maturing varieties are high yielding (2.1-2.8 t/ha) with upright stems; they resist lodging and perform well under heat stress and as a repeat crop. Mid-maturity variety Turon (VC6153B-20G) is characterized by similar traits and has a higher yield (3.2 t/ha) than Zilola, Marjon, and Zhasyl Dan. All these varieties have large marketable seeds, and are appropriate for spring and summer sowing.

***Yard-long bean*:** Oltin soch was developed from an AVRDC genebank accession by multiplying a selection of flowering plants under hot summer conditions. It is a compact bush type. This early maturing variety (80 days) yields green pods (5.3 t/ha) and is well adapted as a repeat crop in Uzbekistan.

***Chinese leafy cabbage*:** New species such as Chinese leafy cabbage (pak choi) have been introduced in Uzbekistan. Early maturing (43 days) variety Sharq guzali was developed by using the polycross method among accessions, with the selection of plants focused on early leaf formation, intensive growth, and tolerance for high planting density. This new variety grows well in greenhouses and tunnels in early spring, as well as in open fields in spring and autumn; it yields up to 20 t/ha.

## Conclusion

AVRDC genebank accessions and improved lines have made a significant contribution to the development of new varieties adapted to various agroecosystems in Central Asia and the Caucasus. Seed of released varieties are multiplied by research institutes and distributed to farmers. New varieties of non-traditional species such as vegetable soybean, yard-long bean and Chinese leafy cabbage have expanded the diversity of vegetables grown in the region, and have become popular for cultivation and consumption. Increasing vegetable production will help to diversify diets, increase farmers' income, and enhance the well-being of families throughout the region.

## References


Ali, M., Mavlyanova, R., Wu, M., Farooq, U., Lin, L. and Kuo, C.G. (2006) Setting research and development priorities for market-oriented vegetable production systems in Central Asia and the Caucasus. In: Proceedings of the Workshop "Increasing market-oriented vegetable production in Central Asia and the Caucasus through collaborative research and development" held in 25-27 April 2005. Tashkent. Uzbekistan. pp. 105-137.

Aytbayev, T., Amirov, B. and Dzhantasov, S. (2012) Regional varietal trial of vegetable crops' accessions from the World Vegetable Center in Republic of Kazakhstan. In: Proceedings of III International Vavilov's Conference "Vavilov's ideas in the modern world" held in 6-9 November 2013, St-Petersburg, Russia, pp. 128-129 (in Russian).

Azimov, B.B. and Mavlyanova, R.F. (2010) Study of hot pepper collection in Tashkent region of Uzbekistan. In: Proceedings of Republic Conference "Gene fund, breeding and seed production of agricultural crops and modern technologies" held in 18 August 2010, Tashkent, Uzbekistan, pp. 87-90 (in Russian).

Dzhantasov, S., Nusupova, A. and Mirmanova, E. (2013) Promising cherry tomato varieties. In: Proceedings of International Conference "Gene fund and plant breeding" Siberian Research Institute of Plant Industry and Breeding" held in 8-12 April 2013, Novosibirsk, Russia (in Russian).

FAOSTAT 2013 http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor Updated: 07 February 2014.

Kim, V. (2013) Vegetable soybean variety 'Universal' is new crop in Uzbekistan. In: Proceedings of International Conference "Innovative technologies for secure and sustainable development of the agrarian sector" held in 3-4 October 2013, Tbilisi, Georgia, pp. 113-120 (in Russian).

Kiseleva, N. and Baytureeva, A. (2013) Vegetable soybean in Kazakhstan. In: Proceedings of International Conference "Promising cultivation technologies of oil and grain legume crops and soil fertility regulation," KNAU, Almaty, Kazakhstan, pp. 160-162 (in Russian).

Lin, S., Chou, Y., Shieh, H., Ebert, A., Kumar, S., Mavlyanova, R., Rouamba, A., Tenkouano, A., Afari-Sefa, V. and Gniffke, P.A. (2013) Pepper (*Capsicum* spp.) Germplasm dissemination by AVRDC-The World Vegetable Center: an overview and introspection. J. Chronica Horticulturae **53**(3) 21-27.

Martirosyan, G.S. (2012) Evaluation of tomato and pepper accessions for revealing of value initial material for breeding. In: Proceedings of III International Conference "Modern tendencies in breeding and seed production of vegetable crops: tradition and perspectives" held in 8-9 August 2012, VNIISSOK, Moscow, Russia, pp. 355-359 (in Russian).

Mavlyanova, R., Mamedov, F. and Mamedova, K. (2010) Collaboration of Azerbaijan Research Institute of Vegetable Growing with AVRDC-The World Vegetable Center. J Agrarian science of Azerbaijan Baku (**5**):23-24 (in Russian).

Mavlyanova, R.[1] (2013a) Enhancing livelihoods in Central Asia and the Caucasus through the increased production and consumption of nutritious vegetables, In: The Basis of Human Civilization. Food, Agriculture and Humanity. Present Scenario, Nath P. (edit), Vol. 1, India, pp.75-95.

Mavlyanova, R.[2] (2013b) Strategic approaches for research and promotion of underutilized vegetable crops for food security in Central Asia and the Caucasus. J Acta Horticulturae **2** (979): 541-547.

Mavlyanova, R.[3] (2013c) AVRDC-The World Vegetable Center's germplasm using for new varieties development of non-traditional crops in Central Asia and the Caucasus. In: Proceedings of the International Conference on "Plant introduction: achievements and prospects" held on 23-25 May 2013, Tashkent, Uzbekistan, pp. 71-75 (in Russian).

Mavlyanova, R.[4] Dzumaniyazova GS, Gafurova LA, Murodova SS, Pirnazarov DR. (2013d) AVRDC vegetable legumes: new varieties for soil fertility improvement. Soil-Water Journal. Special issue

for AGRICASIA-2013. 1st Central Asia Congress **2** (1): 645-653.

Osmanalieva, K. (2013) New tomato accessions of the World Vegetable Center that meet the modern requirements of production and industrial processing in Kyrgyzstan. In: Proceedings of International Conference "Innovative technologies for secure and sustainable development of the agrarian sector" held on 3–4 October 2013, Tbilisi, Georgia, pp. 147 -150 (in Russian).

Sarikyan, K.M. and Sagsyan, G.J. (2012) Results of study of sweet pepper new cultivars and hybrids introduced from the World Vegetable Centers' collections (AVRDC) in Ararat lowland of Armenia. J Agroscience **1-2**:27-30 (in Armenian).

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