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Review Article

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Public Popcorn Breeding Studies of Turkey

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ABSTRACT

Popcorn (*Zea mays everta*) is one of the oldest forms of field maize and it was developed for higher popping volume from flint maize. Popcorn is a very popular appetizer in Turkey and its cultivation and consumption is increasing. It is widely cultivated in Aegean, Mediterranean, Southeast Anatolia and Marmara region of Turkey. Popcorn seed was supplied mainly by the public sector's varieties in the past. Currently both public and private sector's hybrids are produced by the growers. The primary concern in popcorn production is lack of enough high yielding and quality local popcorn varieties. According to the Variety Registration and Seed Certification Center of Turkey sources, there are only a few local popcorn hybrids in the production. Therefore, popcorn growers are often having difficulty finding sufficient seeds. A national big scale popcorn breeding project was initiated by Public, Private Sector and University partnership in order to develop and release high yielding popcorn hybrids in 2015. With the Project, it was targeted to develop local popcorn hybrids and inbreeds to meet high yield and quality variety need and increase national production. In order to develop new inbreds, genotypes derived from populations and pedigree breeding procedures was applied. During the studies, both field and greenhouses were used for generations. General combining ability tests have been done by topcrossing method and the trails were carried out in different 4 locations representing different regions of Turkey. On the other hand, to develop local new varieties in a near future, promising inbreds of public and private sector were crossed to each other in the light of genetic distances and breeding performances. Experimental hybrids will be evaluated in 4 locations in the 2nd and 3rd years of the Project. In the present paper, information on popcorn genetic resources such as developed populations, collections and inbred lines in Turkey are given, current and future studies of the breeding projects that carried out by the public sector are evaluated.

Keywords**:** genetic resources, population, inbred, hybrid, yield, quality

1. Popcorn Genetic Resources

Popcorn is a maize type that puffs up when heated. In order to maximize popping expansion selections were done in flint type maize in the past. Although yield is a very important trait in selecting popcorn germplasm, quality futures such as popping volume, flavor, mouth feel and also disease and insect resistance are very important selection criteria (Alexander, 1988).

In order to develop popcorn varieties for Turkey, different popcorn genetic resources beginning from 1980's to to date were used. Populations developed by Public National Research Institutes of Turkey are main sources for popcorn hybrid development projects.

Turkey has carried out intensive breeding studies on popcorn in the late 1990s. During that time, new popcorn breeding populations were developed. Ant-Pop-C6 popcorn source population was developed by Bati Akdeniz Agricultural Research Institute (BATEM) using half-sib recurrent selection in between 1996-2002. Ant-pop-C6 population consists of 256 family. The popping volume of the initial pop-

ulation, Ant-Pop-C3, was 21.6 cc/100g, while it was 27 cc/100g in the Ant-Pop-C6 population. Genetic progress has been made with the selections for popping volume (Koc *et al*., 2005).

On the other hand, ADAPOP-8 and ADAPOP-10 populations were developed by Maize Research Institute in 2008. Pop corn germplasm that has both white and yellow kernel around Turkey was collected and mass selection was applied to the collection in order to form ADAPOP-8 population. Recurrent Selection Method (S_1) was applied to the genetic material starting from 1992. The first cycle of the population was completed in 1996 and the studies started again in 1997 to obtain second cycle. Out of initial populations three populations (S_1C_1, S_1C_2, and S_1C_3) that were developed by S_1 recurrent selection method and one population (S_1C_3FS) which was developed by full-sib recurrent selection method were tested in Antalya (west Mediterranean) and Adapazari (Marmara region) ecological conditions (Figure 1). The study was carried out according to randomized complete block design using four replications. According to the results, the grain yield of the populations were changed between 5810 kg/ha and 7890 kg/ha in Adapazari location. The highest yield was obtained from S_1C_3 population in that location. Antalya site yields were lower than Adapazari location. The yields varied between 2770 kg/ha and 6330 kg/ha. The very similar yield trend in populations in both location was observed. It can be seen from the Figure1 that there were significant genetic improvement in the cycle three (C_3) in terms of grain yield (Figure 1). Also yield of full-sib population of C_3 was lower than S_1 recurrent population. This situation showed that S_1 recurrent selection method was more successful than full-sib recurrent selection method in the population improvement studies in popcorn. A similar approach for population improvement regarding to full-sib recurrent selection method was reported by Pandey and Gardner (1992) and Banziger *et al*., (2000). The researchers highlighted that full-sib method may be ineffective for the population improvement in terms of grain yield in maize. In addition. Hallauerand Miranda (1981) stated that the S_1receurrent selection method should be used between 2 and 4 cycles, after which the genetic structure of the population narrowed. Most probably, the current constriction is due to additive gene effects.

Another important popcorn source germplasm is ADAPOP-10. This population is composed of maize individuals with yellow grain kernel and mid-late maturity group. To date several popcorn inbred lines have been derived from the ADAPOP-10 source germplasm.

Public inbredlines from United States and Europe were used in the variety development projects. Generally the public inbredlines were crossed to adapted popcorn germplasm and lines derived from the F_2 populations. Especially, P206, P208, HP301, HP68-07, HP72-11 and P608 U.S public inbredlines were used in the pollinations.

Local popcorn populations or land races are valuable popcorn source germplasm for breeding popcorn in Turkey. Farmers used their own popcorn seed in the past since they could not find hybrid seed. This helped also to develop their own seed by selecting best germplasm for using in the future. Currently this germplasm were collected around the Turkey and characterized either by molecular markers or morphological data. Studies showed that there is a wide genetic distance between the popcorn germplasm (Figure 2) of Turkey (Ilarslan *et al*., 2001; Ozturk *et al*., 2017)

2. Popcorn Hybrid Breeding

Pedigree breeding approach was applied to the source germplasm to develop high yielding and quality popcorn single crosses. According to the method, inbred lines were derived from populations and general combining ability of the candidate lines were determined by using top crossing method in between S_2-S_5 selfing generations. Lines that have good combining ability were selected for further evaluations. During selections, yield, popping volume, popping rate and tenderness traits were the selection criteria. Candidate hybrids were assessed in different locations to select best hybrids. In a study Erdal *et al*., (2012) investigated the yield performances of 14 popcorn hybrids across four different regions of Turkey by using additive main effects and multiplicative interactions analysis (AMMI). The study showed that genotype by environment interaction is very important in these lection promising popcorn hybrids. After many studies on popcorn, the first improved local public popcorn hybrid (Ant Cin-98) was developed in Turkey in 1998. The hybrid was used by farmers for a long time and later NerminCin and KocCin were registered in 2002 and 2005, respectively (Anonymous, 2014). Information about these mentioned public popcorn hybrids are summarized in table 1.

3. Current Popcorn Breeding Studies

Popcorn production in Turkey is increasing. The most important problems encountered in the production of popcorn are lack of number of sufficient high quality and efficient popcorn varieties. According to the Variety Registration and Seed certification Center of Turkey sources. there are only a few pop-

corn hybrids including public varieties in the production (Anonymous 2014). In order to support popcorn growers, a public, private sector and university partnership was established in 2015. With the partnership, a national big scale popcorn breeding was initiated to develop and release high yielding popcorn hybrids.

The Project studies were illustrated in the Figure 3. Project works were planned in two main work packages. These are developping elite/advanced stage popcorn inbredlines and superior local popcorn hybrids. In the work package of developing elite/advanced popcorn-lines, 103 populations were used as starting material. 56 of these popcorn source germplasm belonged to BATEM and Maize Research Institute, 5 populations from gene banks around the World and 42 populations were provided from Turkey Eagean Agricultural Research Gene Bank. Selfing studies were done according to the Russel and Eberhart (1975) method. During the studies field and greenhouse have been used for generations. It was seen that green houses or shelters can be used very effectively for obtaining generations (Figure 4, 5, 6, 7). To date 314 S_4-S_5 level popcorn advanced inbred lines were developed and subjected to topcrossing method for general combining ability. After topcross yield tests across four locations in 2017 best lines will be selected for diallel crosses. The lines will be defined by morphological traits and thus will be ready for developing new hybrids.

On the other hand, to develop local new popcorn varieties in a near future, 66 promising popcorn inbred lines were used to generate hybrids in 2015 and 2016. These lines belonged to BATEM (34 lines), Black Sea Agricultural Research Institute (22 lines) and POLTAR (10 lines) which is a private sector seed company in Turkey. Hybrid parents were selected based on genetic distances that obtained by molecular markers and pedigree information given by breeders.

Experiments were conducted in four different geographical region of Turkey in 2016 and summer season of 2017 was used for final evaluations of the candidate hybrids. 59 candidate hybrids and 5 commercial checks (Antcin 98, NerminCin, SH9201, Baharcin and Elacin) including public varieties were tested in 2016. Hybrids were subjected to quality analysis after harvest.

4. Conclusions

With the efforts of National Turkish public research institutes, popcorn populations were developed and several inbred lines derived from the improved populations. Several inbred lines characterized by molecular and morphological markers. Public popcorn hybrids were developed and released for use in popcorn production. However, a strong relationship was needed to develop lines and hybrids of popcorn and therefore a national cooperation was established. The studies related popcorn hybrid breeding is going on and final results will be obtained in 2019. With the current public popcorn breeding studies popcorn genetic resources have been used effectively and it is believed that the study has the potential to provide a contribution to Turkish popcorn production.

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Table 1. Information about registrated public popcorn hybrids in Turkey.

Hybrid	Registration year	FAO Maturity Group	Plant Height (cm)	1000 GrainWeight (g)	Earlength (cm)	Popping Volume (cc g^{-1})	Yield (t/ha)
Ant Cin98	1998	550-600	210	135-150	18	26-28	5.5
NerminCin	2002	600	195	165	18	27	6
KocCin	2005	600	200	100	18	27	5- 6.5

Figure 1. Genetic improvement in the developed popcorn populations.

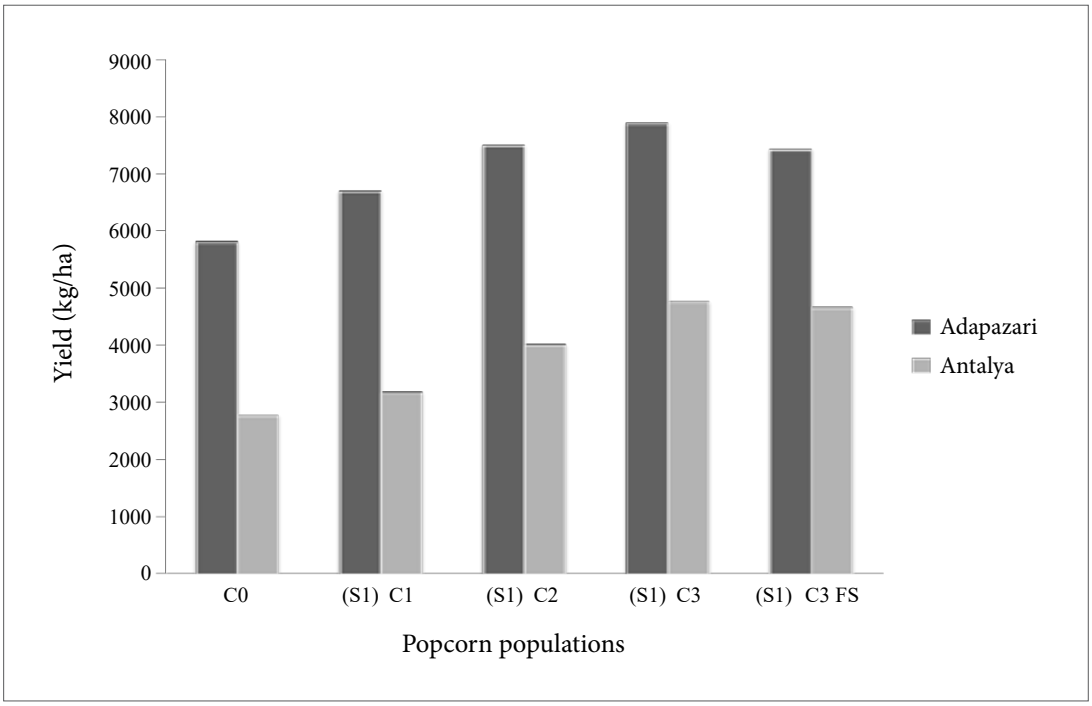

Figure 2. Different popcorn genetic resources of Turkey.

Figure 3. The figure shows the current popcorn breeding studies that carried out by public and private sector in Turkey.

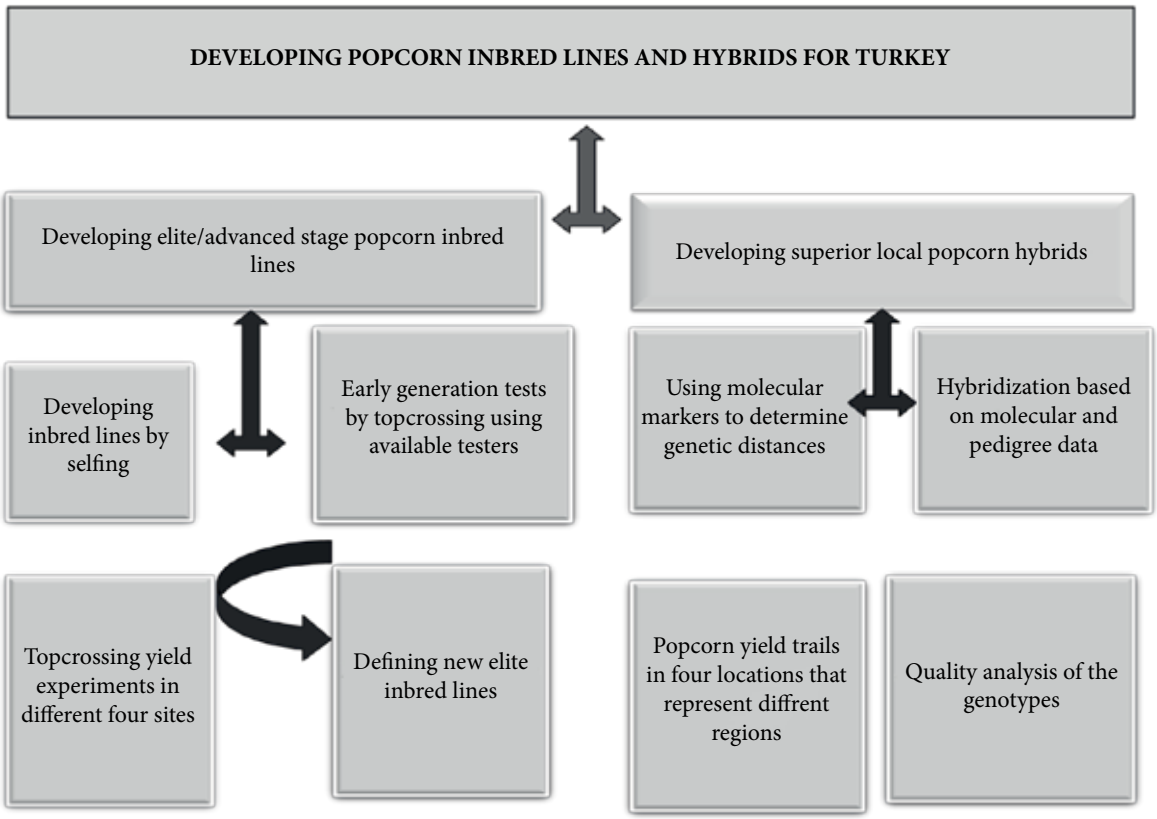

Figure 4. Popcorn seedlings during winter season.

Figure 5. Popcorn seedlings are ready for planting in greenhouse.

Figure 6. Popcorn plants during vegetation period in the greenhouse.

Figure 7. Selfing studies in popcorn in the greenhouse.

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Poaceae Weed Hosts of *Yellow dwarf viruses* (YDVs) in the Trakya Region of Turkey

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ABSTRACT

Trakya Region of Turkey has been one of the important cereal growing areas in Turkey. Previously sporadic and temporary infections of *Yellow dwarf viruses* (YDVs) have been reported in some parts of Turkey. YDV diseases on cereals however have been prevailing and causing yellowing, dwarfing, reddening and the reduction of grain yield on cultivated cereals since 1999 in the Trakya Region. YDV have been identified and their incidence and the rate of infections were investigated. *Barley yellow dwarf virus-PAV* (BYDV-PAV) was diagnosed as the most virulent and dominant one as *Cereal yellow dwarf virus-RPV* (CYDV-RPV) was also identified as another important virus in the area. In order to determine sources of YDVs and their over summering and overwintering hosts among the Poaceae weed species 326 symptomatic weed leaf samples and 82 intact weed plants were collected from road sides and hedge grows of cereal fields in 2010. In second year 357 weed leaf samples, 13 voluntary cereal leaves and 50 intact weed plants were also collected from same sites. Separately 7 aphid species were identified and 5 of them were used for vector transmission tests of YDVs from potted intact weeds to indicator barley (cv. Barbaros) seedlings. As a result of aphid transmissions from 15 weed species, 156 symptomatic barley leaf samples and from 6 weed species, 50 symptomatic barley samples were obtained in 2010 and 2011 respectively. So, totally 902 leaf samples were obtained from 42 weed, 3 voluntaries and 1 indicator barley species. DAS-ELISA and RT-PCR tests on 326 weed samples revealed the corresponding incidence rates were 54.60% for BYDV-PAV, 7.05% for CYDV-RPV, 5.52% for PAV+RPV, 14.41% for the other YDVs and being 81.59% total rate of virus incidence in weed samples in 2010. Test results on 370 leaf samples also revealed the incidences of BYDV-PAV as 14.86%, CYDV-RPV as 10.81%, PAV+RPV as 7.56% and the other YDVs as 48.91% totally being 82.16% rate of virus incidence from weed and voluntary cereal samples in 2011. Aphid transmitted barley samples revealed the similar incidences of viruses too. For molecular characterization the genomic region containing coat protein (CP) regions of BYDV-PAV and CYDV-RPV were amplified from selected weed species and samples by RT-PCR method. Specific DNA fragments in the sizes of 531 bp and 400 bp were amplified from 45 BYDV-PAV isolates from 24 weed species and 34 CYDV-RPV isolates from 15 weed species respectively. The selected DNA fragments of BYDV-PAV and CYDV-RPV were purified and sequenced for the determination of nucleotide sequences of CP genes of both virus isolates. Partial nucleotide sequences of 20 Turkish PAV weed isolates were determined and compared with other nine BYDV-PAV isolates in databases. Phylogenetic analysis of obtained and published nucleotide and amino acid sequences revealed the identity ranged from 86.67 - 99.80% and 70.05 - 99.40% respectively. Partial nucleotide sequences of 6 CYDV-RPV isolates were also compared with seven isolates of CYDV-RPV isolates in GenBank/EMBL. The nucleotide and amino acid sequences revealed the identity ranged from 80.44 - 95.86% and 62.50 - 93.33% identities respectively. To our knowledge, this is the first report of YDV's in Poacea weed hosts in Turkey.

Keywords**:** YDVs, BYDV-PAV, CYDV-RPV, weed host, cereal

Introduction

Trakya Region of Turkey has been one of the important cereal growing areas in Turkey. Almost one million ha of arable land covers 65% of the region has been allocated for field crops and cereal production. Annual average precipitation has been 590 mm, providing necessary moisture under dry farming for cereal production. Grain yield usually varies for the unsuitable weather conditions as well as the pest and diseases in the area. Beside important fungal diseases, sporadic and temporary infections of *Yellow dwarf viruses* (YDV) namely *Barley yellow dwarf virus-PAV* (BYDV-PAV) and *Cereal yellow dwarf virus-RPV* (CYDV-RPV) and their vectors on cereals were reported in some parts of Turkey (Bremer and Raatikainen 1975). YDV diseases on cereals however have been prevailing since 1999 in the Trakya Region (Ilbagi 2003). In addition to Trakya Region YDV diseases and *Wheat dwarf virus* (WDV) infections were also reported in 15 other cereal producing provinces of Turkey (Pocsai *et al.,* 2003; Ilbagi *et al.*, 2003). Those YDV diseases on winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), triticale (X *Triticosecale* Wittmack) and bird seed (*Phalaris canariensis* L.) caused yellowing, dwarfing, reddening and the reduction of grain yield and quality. Viruses of YD diseases have been identified and their incidence and the rate of infections were investigated (e.g., Ilbagi *et al.,* 2005; Ilbagi *et al.,* 2008). Up to now 8 YDV species were named and classified into Luteoviridae family (King *et al.,* 2011). Among them a luteovirus species *Barley yellow dwarf virus-PAV* (BYDV-PAV) was diagnosed the most virulent and dominant one as *Barley yellow dwarf virus-MAV* (BYDV-MAV) was found moderately virulent. *Cereal yellow dwarf virus-RPV* (Polerovirus, Luteoviridae) was also identified as another important virus in the area. The other YDVs of Luteoviridae family were weakly virulent and found in lower incidences. Control strategies and the prevention of YDV disease epidemics in field condition have been included in the assessments of host plants, environmental conditions, viruses and vector aphid species (D'Arcy and Burnett 1995). Beside cultivated cereal species D'Arcy (1995) complied and listed 96 annual, 2 biannual and 111 perennial Poaceae weed host in the world. Later on Poaceae weed host as a sources of YDV inoculums were reported in different countries by Garret and Dendy (2004) in the USA, Pokorny (2006) in Chech Republic, Bisnieks *et al.,* (2004) in Latvia and Sweden, Bakardjieva (2006) in Bulgaria. At the same time Ilbagi (2006) identified common reed (*Phragmites communis* Trin.) as over summering and overwintering host of BYDV-PAV, CYDV-RPV, *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) in the Trakya Region of Turkey. Güncan (2010) suggested effective weed control for their being sources of YDVs, as well as competition with cultivated cereals for plant nutrients and water. YDVs are phloem-limited and obligatorily transmitted viruses in a persistent manner by a number of aphid species. Halbert and Voegtlin (1995) reported and described the biology of 25 aphid species as the vector of YDVs however 10 of them are commonly found on cereal fields. In the case of aphid vectors infestation in Turkey Kinacı and Yakar (1984) reported the presence of *Rhopalosiphum padi* L. and *Rhopalosiphum maidis* Fitch. four provinces of Central Anatolian Region 4 aphid species were identified as the vectors of BYDV's by Çalı and Yurdakul (1996) as in Trakya Region Özder and Toros (1999) identified 7 aphid species in wheat fields in Tekirdağ Province. As long as the presence of direct interactions between viruses and vectors, aphids and host plants beside weed hosts it is utmost important to investigate aphid vectors too (Power and Gray 1995). YDVs and their aphid vector specificity has been considered as a rule not the exception since the work of Rochow and Muller (1971). Merely names of some aphids and the term of BYDV-strains changed into YDV species. In order to determine sources of YDV's and their over summering and overwintering hosts among the Poaceae weed species this study was initiated in 2009. For this purpose survey trips to 12 districts of Trakya Region have been done and Poaceae weed and weed leaf samples were collected. For the identification of YDV's, DAS-ELISA and RT-PCR tests were used and nucleotide sequence and phylogenetic analysis were implemented. By employing nucleotide sequences of 531 bp fragments of code protein gene of BYDV-PAV isolates and 400 bp fragments of code protein gene of CYDV-RPV isolates obtained from Poaceae weeds phylogenetic trees were constructed and compared them with Gene Bank accessions of both viruses.

Material and Methods

Survey studies and sampling: Extensive survey studies were implemented daily by travelling at least 72 Km up to 160 Km distances from Tekirdağ in the Trakya Region where 12 counties were visited in May and June 2010 and 2011 as exhibited in Figure 1.

Totally 829 symptomatic weed plants and weed leaf samples were collected from road side verges, hedge grows, banks of creeks and fallowed cereal fields. Herbariums of intact weeds were made for

their identifications. Weed leaf samples were packed into polyethylene bags and maintained in deep freeze working at -27°C until their usage for identification of YDV's. 82 symptomatic intact weed plants however collected and transplanted into sterile mixture of soil, sand and compost (1:1:1:) filled 3 L pots maintained into insect proof cages in 2010 and similarly 50 plants were transplanted in 2011.

Aphid collections and identifications: Weeds and voluntary cereal plants were examined for aphids. Whenever possible, aphids were identified at the sampling sites. Otherwise they were collected with their colonized plants by wrapping into papers and packed in polyethylene bags, brought to laboratory. Apterous aphid colonies free from their parasites, were collected into bottles containing 70% ethanol for identification later under Olympus SZ51 Stereo microscope. Rest of the aphids were transferred and cultured on potted healthy wheat (cv. Pehivan, and Attila 12) and barley (cv. Barbaros) plants grown in sterile greenhouse conditions. So 7 aphid species were identified,5 of them were cultured for aphid transmissions of YDV's and maintained in insect proof cages as suggested by Halbert and Voegtlin (1995)

Indicator plant and aphid transmission: Barley (cv. Barbaros) was selected as indicator plants of YDV's. Seeds were sown into 500 cc pots filled with sterilized mixture of soil, sand and compost (1:1:1) having 6 seeds in each pot. So 300 pots of indicator barley seedlings were grown in 2010 and repeatedly 300 pots of barley were grown in 2011. Aphid transmissions were made as suggested by Du *et al.,* (2007) by collecting apterous individuals into petri dishes by using camel hair brush and placing them on transplanted weeds for accusation of YDV's and let them feeding for 2 days. So, 1 plant was allocated for each aphid species from which 5 aphids per plant and totally 25 aphids from 5 species were used for transmission in each pot. After accusation period, aphids were transferred to indicator plants as 5 aphids per plant as one plant saved for control. After 5 days of inoculation period aphids were killed by spraying Marshall-25 insecticide and maintained them in insect proof greenhouse conditions at 20, 25°C for the exhibition of virus symptoms.

ELISA Procedures: Totally 901 leaf samples were tested with polyclonal antibodies (manufactured by AGDIA Inc.; Elkhart IN, USA) for the presence of BYDV-PAV, BYDV-MAV and CYDV-RPV viruses by employing Double Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA) as described by Clark and Adams (1977) and the procedure suggested by AGDIA Inc.

Nucleic acid isolation from YDV infected samples: Obtained 901 weed and aphid transmitted cereal leaf samples were subjected to isolation of the viral nucleic acid by employing the total nucleic acid extraction method described by Falke *et al.,* (2000).

cDNA synthesis: First strands cDNA molecules were obtained from total isolated RNA's of the code protein gene fragments belong to BYDV-PAV and CYDV-RPV by using Omniscript reverse transcriptase synthesize Kit (Fermentas; Vilnius, Lithuania). For each reaction 2 µl total RNA, 1 µl primer pairs (100 pmol/µ) were used and processed according to the manufacturer's instructions.

RT-PCR amplifications: Primer pairs for BYDV-PAV (5'-CCAGTGGTTRTGGTC-3' antisense) and (5'-GTCTACCTATTTGG-3' sense) as designed by Robertson *et al.,* (1991) were used for the amplification by RT-PCR. Amplified fragments were 531 bp long and corresponded to BYDV-PAV genome nucleotides between 2938 and 3469. The PCR reaction for BYDV-PAV consisted of 3 µl 10x PCR buffer, 2 µl $MgCl_2$ (25mM), 1 µl dNTP (10mM), 2 µl primer1, 2 µl primer2, 1 µl Taq DNA polymerase enzyme, 1 µl cDNA and 13 µl RNase free water. The amplification protocol for BYDV-PAV was as fallows; initial denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, 43°C for 1 min, 72°C for 1 min. and the final extension step at 72°C for 10 min in a Techne thermal cycler. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide (EtBr) and viewed under UV illumination in a gel documentation system (Vilber Lourmet; Marne La Vallee Cedex 1, France).

Similarly, primer pairs for CYDV-RPV (5'-ATGTTGTACCGCTTGATCCAC-3'antisense) and (5'-GCGAACCATTGCCATTG-3'sense) as designed by Deb and Anderson (2007) were used for the amplification by RT-PCR. Amplified fragments were 400 bp long and corresponded to CYDV-RPV genome nucleotides between 3275-3675. Those primer pairs for all viruses were obtained from IDT Inc. Coralville, Iowa, USA. The PCR reaction for CYDV-RPV consisted of 3µl 10x PCR buffer, 2 µl $MgCl_2$ (25mM), 1µl dNTP (10mM), 0.5µl primer 1, 0.5µl primer 2, 0.3µl Taq DNA polymerase enzyme, 2 µl cDNA, 15.7 µl RNase free water. The amplification protocol for CYDV-RPV was as follows; Initial denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 60°C for 45 sec, 72°C for 1 min and the final extension step at 72°C for 10 min in thermal cycler. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with EtBr and viewed under UV illumination in a gel documentation system (Vilber Lourmet; Marne La

Vallee Cedex 1, France). By employing proper primer pairs for the viruses of BYDV-MAV, BYDV-RMV and BYDV-SGV fragments consisting necessary compounds and following similar protocols PCR products were obtained and analyzed for their identifications too.

Sequencing of RT-PCR products: For sequence analysis, PCR products of BYDV-PAV and CYDV-RPV were purified from agarose gels by employing QIAquick gel extraction kits manufactured by MBI Fermentas; StLeon-Rot, Germany. Purified gels were sequenced in accordance with the manufacturer's protocol at Refgen Biotechnology Company, Ankara, Turkey. Obtained nucleotides sequences of both BYDV-PAV and CYDV-RPV isolates were aligned with Mega5 Program. The alignments were used as input data to construct phylogenetic trees with the Neighbor-Joining Distance method implemented in Mega 5.0 Program Tamura *et al.,* (2011) and compared with International Gene Bank accessions.

Results and Discussion

During the survey studies, 326 weed leaf samples from 14 annual, 3 biannual and 9 perennial totally 26 weed species were collected in 2010. Beside leaf samples 82 symptomatic intact weed plants from 15 species were also obtained and transplanted to pots for aphid transmission tests. In addition to 13 leaf samples from 3 voluntary cereal species, 357 weed leaf samples from 21 annual 1 biannual, 10 perennial species were collected. So total 370 leaf samples, 50 symptomatic intact weed plants were obtained and transplanted to pots for aphid transmission in 2011. By evaluating the distribution of weed species in 12 districts revealed that Hayrabolu was the most infested district with 21 weed species as Kırklareli Central District was found the least infested district with 7 weed species. In confirmation of our results most of those species were reported as the competitive weeds in cereal fields in Turkey (Güncan 2010). Collected and identified aphid species are listed in Table 1. *Metopolophium dirhodum* (Walker) was collected from 8 districts as infested on 8 weed species. *Rhopalosiphum padi* L. was in second place collected from 7 districts and found infested on 7 weed species. *Rhopalosiphum rufiabdominalis* (Sasaki) and *Sitobium fragariae* (Walker) were found in Hayrabolu as infested on *Bromus arvensis* and *Avena sterilis* respectively. These findings confirmed the observations of Kinaci and Yakar (1984), Cali and Yurdakul (1996) and the results of Ozder and Toros (1999) whom they identified same aphid species in cereal fields in Tekirdağ Province in the Trakya Region. As considered being predominant vectors of YDVs by Lister and Ranieri (1995) 5 aphid species; *Rapholosiphum padi* L., *Rapholosiphum maidis* Fitch., *Stobion avenae* Fabricus, *Schizaphis graminum* Rondeni and *Metopolophium dirhodum* Walker were employed for the aphid transmission tests.

Aphid transmission test results are shown in Table 2, in which 156 barley plants exhibited systemic symptoms and revealed the 79 out of 156 (50%) presence of YDV's in 2010 as 15 out of 50 (30%) of them had YDV's in 2011.

Among those weed species *Avena sterilis* was identified the best sources of YDVs as *A. barbata* and *Hordeum bulbosum* were found the least important sources of YDV's. *R. padi* was determined the most efficient vector which verified the Halbert and Voegtlin (1995)'s results. Test results of ELISA and RT-PCR implemented in 2010 are displayed in Table 3. The results revealed that; 178 of 326 weed samples (54.60%) had BYDV-PAV, 23 of 326 (7.05%) had CYDV-RPV, as 18 out of 326 (5.52%) had the mixture of BYDV-PAV+ CYDV-RPV and 45 of 326 (14.41%) of them found infected with other YDV's. So, totally 266 out of 326 (81.59%) samples from 22 poaceae weed species were identified as potential over summering and overwintering hosts of YDV's.

Four species of weeds; *Gastridium ventricosum*, *Lolium temulentum, Phleum bertolonii*, and *P. subulatum* had no virus at all. Obtained results revealed that BYDV-PAV was found as the dominant virus species on weed samples confirming the results of previous works of Ilbagi (2003), Ilbagi *et al.,* (2003), and Pocsai *et al.,* (2003) on cereal crops in 2010. The results of ELISA and RT-PCR tests implemented in 2011 were exhibited in Table 4.

It revealed the presence of BYDV-PAV at the rate of 54 of 369 (14.86%), CYDV-RPV as 40 of 369 (10.81%), mixture of BYDV-PAV+CYDV-RPV diagnosed as 28 of 369 (7.56%) and the other YDV's as 181 out of 369 (48.91%). Thus, totally 303 out of 369 (82.16%) incidence of viruses taken place on Poaceae weeds in Trakya Region of Turkey. Among the voluntary cultivated cereal only oat samples found infected with viruses. Among the weeds, *Aegilops cylindrical, A. geniculata* and *A. neclecta* were found free from viruses. All the outcomes in two years confirmed the results about the rates of YDV disease incidences on cereal crops reported by Ilbagi (2003), Ilbagi *et al.,* (2003) and Pocsai *et al.,* (2003). Two of virus free 7 weed species *Gastridium ventricosum* and *Aegilops cylindrica* however were listed as the susceptible hosts to Luteoviridae viruses by D'Arcy (1995). A perennial weed *Phalaris aquatica* was determined the most important source of YDV inoculum as *Bromus tomentellus, Avena fatua, Avena sterilis* and *Echinochloa crus-galli* followed it.

Our results in this study confirmed the findings of Garret and Dendy (2004)'s 4 wide spread grass species of being the sources of YDV's inoculum in the USA, Pokorny (2006)'s findings of *Echinochloa crus-galli, Seteria pumila* and *Phalaris canariensis* as the sources of BYDV-PAV in Chech Republic, Bakardjieva *et al.,* (2006)'s findings of *Elymus repens*, *Avena fatua* and *Sorghum halepense* as being sources of YDV diseases of cereals in Bulgaria. Obtained results in this study also confirmed findings of Bisnieks *et al.,* (2004)'s about the *Festuca elatior, Lolium perenne* and *Dactylis glomerata* as sources of BYDV-PAV and CYDV-RPV in the summer crops of cereals in Latvia and Sweden. Our findings in this study about Poaceae weed hosts and YDV infections on cereals in the Trakya Region confirmed the finding of Ilbagi (2006) about the widespread perennial weed *Phragmites communis* (*Phragmites australis*) being the over summering and overwintering host of BYDV-PAV, CYDV-RPV as well as MDMV and SCMV too.

Being the most important YDV on cereals in Turkey BYDV-PAV deserved the investigation about its molecular features. So partial CP gene sequences of 20 Turkish PAV isolates obtained from weed species were aligned and compared with the published sequences of 9 isolates of PAV available in the GenBank⁄EMBL databases. Multiple sequence alignments and pair wise sequence comparisons were performed BioEdit Software. The results of phylogenetic analysis demonstrated that the PAV isolates divided into two major groups as shown in Figure 2. In the first group, 14 weed isolates of PAV clustered with the other PAV isolates from China, Iran, Sweden, New Zealand, Pakistan and USA available in the Genbank databases by forming two subgroups. The other 6 isolates from 6 weed species of *A. stolonifera, L. rigidum, V. ciliate, A. sterilis, B. hordeaceous,* and *B. scoparius* were clustered among themselves in second group and they also had two subgroups. Sequences analysis among all the PAV isolates included the nucleotide identities was 86.67 - 99.80%. The lowest level of identity was 86.67% between Priekuli1 isolate from Sweden (Acc.No. AJ563415.1) and TR-AGR isolate of *A. stolonifera*, while the highest level identity was 99.80% between Yolo274 isolate from USA (Acc. No. DQ631850.1) with TR-VULM isolate of *V. myosurus*, TR-PHR1 isolate of *P. austrialis*, TR-CYNO isolate of *C. echinatus* by confirming Rastgou *et al.,* (2005), Pakdel *et al.,* (2010)'s results. The Cluster I included that the highest nucleotide identities were 94.63-99.80% isolates between 06KM25 isolate Chine (Acc. No. EU332333.1) and Yolo274 isolate USA (Acc. No. DQ631850.1) with TR-PHR1, TR-HMUR, TR-VULM, TR-PHR1 of weed isolates while Cluster II included that the highest nucleotide identities were 86.67 - 90.05% between Priekuli1 with TR-AGR isolate and Yolo274 with TR-VULC isolate respectively. Nevertheless, Cluster II included that the comparisons among themselves of the PAV isolates in this study revealed that the nucleotide identities were 87.67-100.00%. The lowest level of identity was 87.67% between TRAQUA1 isolate of *P. aquatica* and TR-LP-ER isolate of *L. perenne.* The highest level of identity was 100% between TR-VULM isolate of *V. myosurus*, TR-PHR1 isolate of *P. austrialis* and between TR-VULM isolate of *V. myosurus*, TR-CYNO isolate of *C. echinatus*. PAV isolates grouped according to their hosts, not grouped according to their geographical distribution or their genetic diversity as described by Bisniek *et al.,* (2004), Mastari *et al.,* (1998). Amino acid multiple sequence alignment revealed the lowest level of identity was 70.05% between Priekuli1 isolate from Sweden (Acc.No. AJ563413.1) and TR-AQUA1 isolate of *P. aquatica,* while the highest level of identity was 99.40% between Yolo274 isolate from USA and TR-VULM isolate of *V. myosurus*, and TR-PHR1 isolate of *P. austrialis*.

The identified nucleotide sequences of 6 Turkish RPV weed isolates were also aligned and compared to sequences of 7 isolates of CYDV-RPV available in GenBank⁄EMBL. Multiple sequence alignments and pair wise sequence comparisons were performed using BioEdit Software. The results of the phylogenetic analysis demonstrated that the RPV isolates were divided in two major groups as shown in Figure 3. The sequences of the RPV isolates that were analyzed uncovered that the nucleotide identities were 80.44 - 95.86%. The lowest level of identity was 80.44% between RPV-TR2 (Acc. No. KR005847) and RPV 05P4b02 isolate (Acc.No. DQ988088.1) while the highest level of identity was 95.86% between RPV-TR2 and RPV 44P4b04 isolate (Acc.No. DQ988108.1). The studied 6 weed isolates from this study were clustered in the first group with 3 RPV isolates from the USA (Acc.No. DQ988108.1, Acc.No. EF521839.1, Acc.No. DQ988105.1). The lowest level of nucleotide identity was 91.46% for RPV-TR2 and RPV 046 (Acc.No. EF521839.1). The highest level of identity was 95.86% between RPV-TR2 and RPV 44P4b04. The other 5 Turkish isolates, except RPV-TR2, formed second subgroups among themselves. The lowest nucleotide identity of the latter isolates was 80.99% between RPV-TR3 (Acc.No. KT923454), RPV 05P4b02 (Acc.No. DQ988088.1) and RPV 010 (Acc.No. EF521830.1). while the highest identity level was 95.04% between RPV-TR6 (Acc.No.

KT923457), RPV 44P4b04 and RPV 046. Bisniek *et al.,* (2004) and Mastari *et al.,* (1998) reported that host plant species play an important role in genetic diversity of BYDVs, which is in accordance with our findings. In parallel to the variations of the nucleotide sequences among the RPV isolates, the same variations are also visible in the amino acids sequences that indicates a lowest identity level of 62.50% between RPV-TR2 and RPV 44P4b04.

The molecular, serologic, as well as the transmission tests conducted in this study revealed that Poaceae weeds species might be reservoirs of *Yellow dwarf viruses* (YDVs). This investigation, because it identifies the potential sources of BYDV-PAV, CYDV-RPV, BYDV-MAV, BYDV-SGV, BYDV-RMV inoculum, provided the means for an effective control of viral infections in Trakya, for example by controlling the Poaceae weed hosts. To our knowledge, this is the first report of YDV's in Poacea weed hosts in Turkey.

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Table 1. Aphid vectors of *yellow dwarf viruses* (YDVs) of cereals and their infested weed species in Trakya Region of Turkey.

Name of Aphid species	District Name	Names of weeds aphids were obtained
Rhopalosiphum padi L.	Edirne Central	*Avena sterilis* L.
	Ipsala	*Phragmites australis* (Cav) Trin. Exsteudel
	Uzunköprü	*Echinochloa crus-galli* (L.) P. Beauv.
	Lüleburgaz	*Avena fatua* L.
	Pınarhisar	*Avena sterilis* L.
	Tekirdağ Central	*Phragmites australis* (Cav) Trin. ExSteudel
	Çorlu	*Avena sterilis* L.
Rhopalosiphum maidis L.	Tekirdağ Central	*Bromus sterilis*
	Çorlu	*Phragmites australis* (Cav) Trin. ExSteudel
Rhopalosiphum rufiabdominalis (Sasaki)	Hayrabolu	*Bromus arvensis* L.
Sitobion avenae (Fab.)	Ipsala	*Hordeum murinum* L.
	Pınarhisar	*Bromus tectorum* L.
	Saray	*Avena fatua* L.
Sitobion fragariae (Walker)	Hayrabolu	*Avena sterilis* L.
Metopolophium dirhodum (Walker)	Ipsala	*Avena sterilis* L.
	Lalapaşa	*Avena sterilis* L.
	Kırklareli Central	*Hordeum bulbosum* L.
	Lüleburgaz	*Avena fatua* L.
	Pınarhisar	*Hordeum murinum* L
	Tekirdağ Central	*Phalaris aquatic* L.
	Çorlu	*Bromus hordeaceus* L.
	Malkara	*Avena sterilis* L.
Schizaphis graminum (Ron)	Tekirdağ Central	*Avena barbata* Pott ex Link

Table 2. Aphid transmission test results of YDVs to cv. Barbaros Barley by using 5 aphid species and verified by DAS-ELISA and RT-PCR tests in 2010 and 2011.

Year	Number of weed species	Number of plants as virus sources	Number of barley plants YDV's transmitted to				Total number of infected barley plants	Rate of infection
			PAV	RPV	PAV+RPV	Other		
2010	15	82	55	1	5	12	79	50%
2011	6	50	6	4	3	2	15	30%

Table 3. *Yellow dwarf virus* (YDV) disease incidences within naturally infected Poaceae weed species determined by using DAS-ELISA and RT-PCR tests in 2010 in the Trakya Region of Turkey.

Name of weed species	Number of samples	Number of weed samples *yellow dwarf virus* (YDVs) or their mixtures detected				Total number of samples YDV's detected
		BYDV-PAV	BYDV-PAV	PAV+RPV	Other YDV's (MAV-RMV+SGV)	
Aegilops triuncialis	1	1	-	-	-	1
Agrostis stolonifera	1	1	-	-	-	1
Alopecurus aequalis	3	3	-	-	-	3
Avena fatua	20	12	-	3	5	20
Avena sterilis	50	37	1	3	10	51
Bromus arvensis	42	21	4	4	2	31
Bromus hordeceaus	5	4	1	-	-	5
Bromus sterilis	39	21	2	2	7	32
Bromus tectorum	14	10	-	-	1	11
Bromus tomentellus	18	12	1	1	4	18
Cynosorus echinatus	2	2	-	-	-	2
Descampsia caespitosa	3	3	3	-	-	6
Echniochloa crus-galli	10	4	-	1	4	9
Gastridium ventricosum	1	-	-	-	-	-
Hordeum bulbosum	3	1	-	-	-	1
Hordeum murinum	2	1	-	-	-	1
Lolium perenne	11	5	2	-	-	7
Lolium rigidum	16	8	2	1	5	16
Lolium temulentum	4	-	-	-	-	-
Phalaris aquatica	10	8	-	2	-	10
Phleum bertolonii	1	-	-	-	-	-
Phleum subulatum	1	-	-	-	-	-
Phragmites australis	53	19	2	1	7	29
Poe trivialis	4	2	2	-	-	4
Sorghum halepense	9	-	3	-	2	5
Vulpia ciliata	3	3	-	-	-	3
Total 26 species	326	178	23	18	47	266
		54.60%	7.05%	5.52%	14.41%	81.59%

Table 4. *Yellow dwarf virus* (YDV) disease incidences determined by employing DAS-ELISA and RT-PCR tests within the naturally infected weed species and voluntary cereal plants in 2011 in Trakya Region of Turkey.

Names of weed species and voluntary cereals	Number of samples	Identified number of *yellow dwarf viruses* and their mixtures				Total number of viruses identified
		BYDV-PAV	CYDV-RPV	PAV+RPV	Other YDV's (MAV-RMV+SGV)	
Aegilops cylindrica	1	-	-	-	-	-
Aeligops geniculata	1	-	-	-	-	-
Aegilops neclecta	3	-	-	-	-	-
Aegilops triuncialis	2	-	1	-	1	2
Alopecurus myosuroides	7	3	1	-	3	7
Alopecurus rendlei	1	-	-	-	1	1
Apera spica venti	4	1	1	1	2	5
Arrhenatherum elatius	2	-	-	-	2	2
Avena barbata	8	2	2	1	4	9
Avena fatua	1	-	-	-	-	-
Avena sativa (voluntary)	10	2	2	-	11	15
Avena sterilis	42	9	3	3	22	37
Bromus hordeceaus	12	1	2	-	4	7
Bromus rigidus	4	1	-	1	2	4
Bromus scoparius	4	1	-	-	1	2
Bromus sterilis	31	8	6	2	11	27
Bromus tectorum	11	-	-	-	9	9
Cynodon dactylon	3	-	-	-	1	1
Dactylis glomerata	2	-	-	-	2	2
Dasyprum villosum	3	1	-	-	-	1
Echinochloa crus-galli	1	-	-	-	1	1
Elymus repens	13	-	-	-	1	1
Hordeum bulbosum	8	-	-	-	6	6
Hordeum murinum	34	3	4	5	16	28
Lolium perenne	8	1	1	-	3	5
Lolium rigidum	42	6	4	4	20	34
Phalaris aquatica	16	8	1	5	9	23
Phleum exaratum	25	3	3	3	10	19
Phragmites australis	29	1	1	1	23	26
Poa trivialis	19	2	8	1	4	15
Secale cereal (voluntary)	1	-	-	-	-	-
Sorghum halepense	18	1	-	1	12	14
Triticum aestivum (voluntary)	2	-	-	-	-	-
Vulpia ciliate	1	-	-	-	-	-
Vulpia myuros	1	1	-	-	-	1
Total 35 species	370	55	40	28	181	304
		14.86%	10.81%	7.56%	48.91%	82.16%

Figure 1. Twelve districts in the Trakya Region of Turkey where YDVs investigated Totally 829 symptomatic weed plants and weed leaf samples were collected from road.

Figure 2. Constructed Phylogenetic tree of 20 Turkish BYDV-PAV isolates with 9 PAV isolates in database.

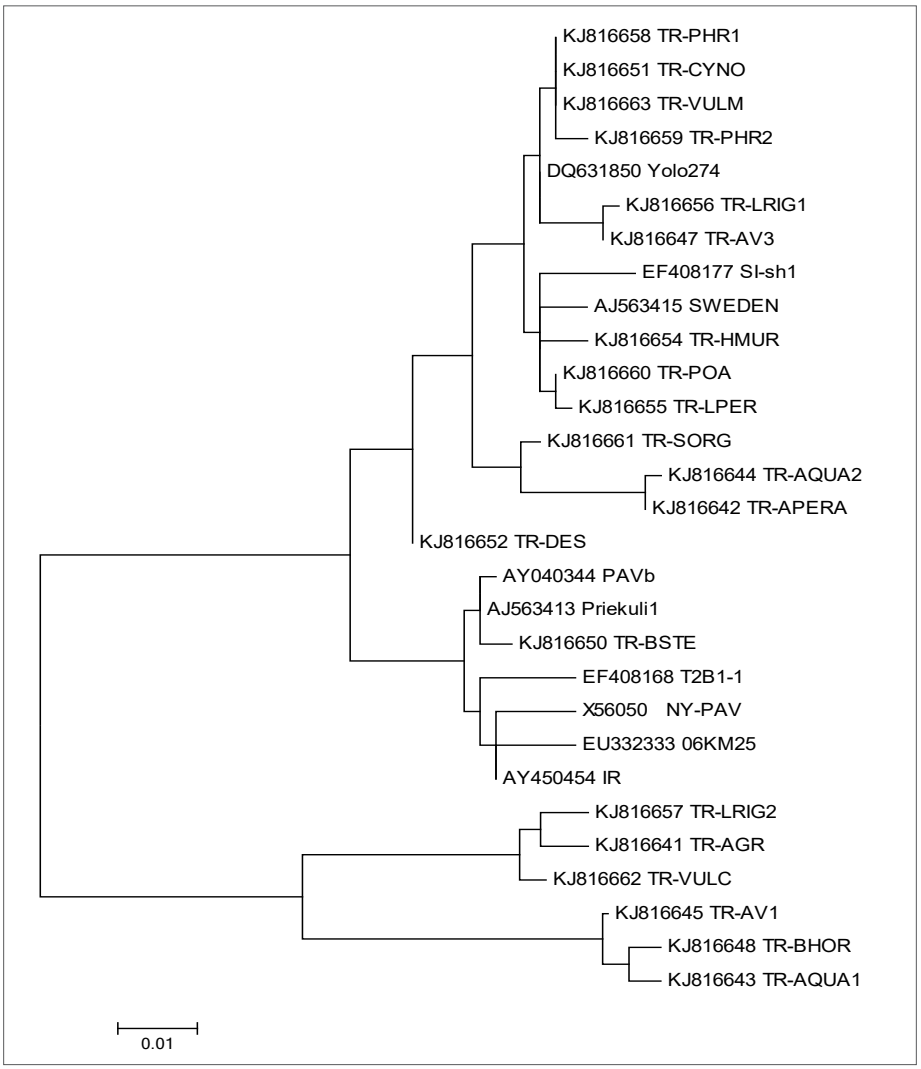
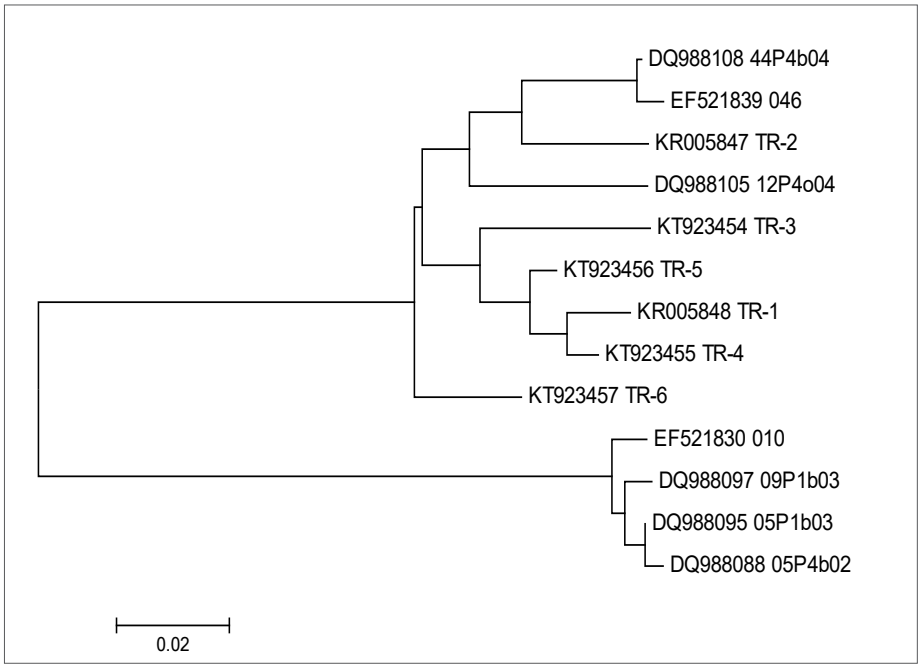


Figure 3. Constructed Phylogenetic tree of 6 Turkish CYDV-RPV isolates with 7 RPV isolates in database.



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Combining Ability Analysis and Heterotic Effects for Cotton Fiber Quality Traits

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ABSTRACT

Combining ability and gene action can help breeders to select suitable parents and appropriate breeding strategy. In the present study combining ability analysis and heterotic effects for cotton fiber quality traits were studied in a set of diallel crosses involving six cotton genotypes. The aim of this study was to estimate general combining ability of parents, special combining ability of F_1 diallel crosses, heterotic effects of F_1 diallel crosses in in the breeding programme to develop high quality cotton varieties. Randomized complete block design was used to test 15 F_1 diallel crosses, and 6 parents. Analysis revealed significant general combining ability effects for all the investigated traits and additive gene effects were important in the inheritance of the traits. Analysis also revealed significant SCA effects for only fiber fineness and spinning consistency index traits and additive and non-additive gene effects were important in the inheritance of the traits. General combining ability was found significant for all investigated traits, revealing the important role of additive gene effects. Specific combining ability was found significant for the Mic and SCI, revealing that non-additive gene effects, as dominant or epistatic are important, but not for Len, Str and SFI.

Keywords**:** cotton, fiber quality, gene action, diallel analysis

Introduction

Raw cotton and cotton products play an important role in the economy of advanced cotton growing countries. Improvement of fiber quality and cotton fiber quality parameters play a vital role in the cotton price in textile sector.Breeding programs of cotton generally aim to increase fiber quality parameters. The success of cotton breeding programme is based on choice of superior genotypes for hybridization and selection for favorable genes and gene complexes in combination. Since fiber quality traits are quantitatively inherited, a simple genetic model having several genetic parameters needs a lot of work to solve complex relationship of successful breeding (Bolek *et al*., 2010). Over the years fiber traits were significantly improved by the plant breeders by bringing new allelic recombination and subsequent selection of valuable trans-segregants (Ali *et al*., 2010).

Combining ability analysis, to compare the performance of F_1 combinations is used in breeding programs (Griffing, 1956) and allow estimation of different genetic parameters (Verhalen and Murray, 1967). Additive-dominance model can direct plant breeder about the validation of data and design as well as usage of data. Heterosis is useful in determining the most appropriate parents for specific traits (Khan *et al*., 2010).

Cotton fiber quality is expressible by a multitude of measurements (Hake *et al*., 1996). Fiber length, fiber fineness, fiber strength, short fiber index and the spinning consistency index are the most important fiber quality traits. Because of the high potential for cotton fiber quality *G. barbadence* L. still are used in cotton breeding programs. Thus new cotton varieties with high fiber quality can be obtained. The variation in a fiber trait through plant breeding approaches requires knowledge of the cultivar's genetics

(Aguado *et al*., 2010). Heterosis is one of the significant techniques in cotton breeding programs (Khan *et al*., 2010, Bhadate *et al*., 1980, Basbag *et al.*, 2007). Little work has been reported on the heterosis of fiber quality traits in cotton breeding (Ashokkumar *et al*., 2013). Estimation of heterotic effects is necessary to identify the new suitable cross combinations.

The aim of this study was to determine the general combining ability of the parents, the specific combining ability of F_1 diallel crosses and the heterotic effects of the hybrids in the breeding programme to develop high quality cotton varieties.

Materials and Methods

The parent genotypes belonged to G. *hirsutum* L. (Sayar-314, Stoneville-453, Nazilli-84S, and Fantom) and G. *barbadense* L. (Giza-45 and Delcerro) species. During first year of experiment, six cotton genotypes were crossed in a half diallel mating design in the experimental fields of Dicle University of Agriculture Faculty in 2011. In second year, all parents (6) and F_1 crosses (15) were planted in the randomized complete block design with three replicates at the same experimental area in 2012. In all experiments, plot length was 12 m, spacing between and within rows was 70x20 cm. Standard cultural practices were applied as suggested by Diyarbakır ecological region. Fertilizers were 160kg ha^{-1} N and 120 kg ha^{-1} P_20_5 and irrigations were total 9 times in about 8-10 days intervals as furrow irrigation during growing seasons. Data were recorded on fiber obtained from randomly selected boll in each of the three replicates as the fiber length (Len.) (mm) (2.5% Span Length), fiber fineness (Mic.)(micronaire), fiber strength (Str) ($gtex^{-1}$), short fiber index (SFI) and the spinning consistency index (SCI). Investigated fiber quality traits were determined by HVI (High Volume Instruments). The data were analyzed using analysis of variance method by using Dial-98 (Ukai, 2006) and SAS (SAS Institute, Cary, NC). Traits found significant were further analyzed by Griffing's (1956) method-II and model-I. Heterosis (Ht) (%) and heterobeltiosis (Hb) (%) were calculated according to formulas of Hallauer and Miranda (1981).

Results and Discussion

Mean squares of δ^2GCA, δ^2SCA, δ^2GCA/δ^2SCA and GCA for investigated traits in cotton parents are presented in Table1. Mean squares of general combining ability (GCA) were found significant for all investigated traits, revealing the important role of additive gene effects (Table 1). Specific combining ability (SCA) was found significant for the Mic and SCI, revealing that non-additive gene effects, as dominant or epistatic are important, but not for the Len, Str and SFI (Table 2).

Variance of GCA effects were higher than variance of SCA effects ((δ^2GCA)/(δ^2GCA)>1) for the all investigated traits which indicated that additive gene action is prevailing with non-additive gene actions for the expression of these traits (Table 1). The results are in agreement with earlier reported the findings. (Bolek *et al*., 2010; Lukonge *et al*., 2008; Aguiar *et al*., 2007; Cheatham *et al*., 2003; Leidi 2003; Tariq *et al*., 1992; Green and Gulp, 1990;Kanoktip, 1987).

SCA was found highly significant for the Mic. and SCI, revealing an important role of non-additive gene effects (Table1). (Green and Culp 1990; Bhardwaj and Kapoor; 1998;Cheatham *et al*., 2003). Additive genetic effects with enough genetic variability were also noticed for the traits permitting for the effective selection (Lukonge *et al*., 2008). The reason for this difference may be due to different genetic structure of genotypes utilised and the different ecological condition (Bolek *et al*., 2010).

Positive GCA effects for the Len and SCI were ascertained in Giza-45 and Delcerro, whereas a negative GCA effects were ascertained in Sayar-314, Stoneville-453, Nazilli-84S and Fantom. Positive GCA effects for the Str were obtained from Stoneville-453, Fantom and Delcerro, while on the contrary negative GCA effects were obtained from Sayar-314, Nazilli-84S and Giza-45. Negative GCA effects for the Mic. were detected in Giza-45, Delcerro, and Nazilli-84S, while positive GCA effects were detected in Sayar-314, Stoneville-453, and Fantom. Negative GCA effects for the SFI were ascertained in Giza-45, Delcerro, and Fantom inasmuch as positive GCA effects were ascertained in Sayar-314, Stoneville-453, and Nazilli-84S. In order to do genetic crosses within the parents, Giza-45 and Delcerro were selected for the Len, Mic., SFI, SCI.

These genotypes would be more promising to develop higher fiber quality progenies. The GCA effects of parents indicated that the Giza-45 genotype was greatest general combiner for the Len, Mic., and SFI followed by Delcerro which was the best combiner for the Str and SCI.

Significant GCA effects for all the traits determined suggest at least one parent superior to the others, regarding mean performance in hybrid combinations. Giza-45 and Delcerro had affirmative and significant GCA effects on the Len, Mic., and SFI values, respectively, indicating some dominance genes for the Str, Mic., and SFI in the Giza-45 and Delcerro

genotypes, which are consistent with the results (Aguiar *et al*., 2007).

Determinated SCA effects, Ht (%) and Hb (%) for the investigated traits in cotton F_1 diallel crosses. (Table 2).

The main aim in cotton breeding is obtain a lower value for the Mic. and SFI, while it is referred a high value for the Len, Str and SCI. In this context, higher negative SCA, negative Ht, and negative Hb values are desirable for the Mic. and SFI in cotton breeding studies.

Among the crosses, the determined SCA effects varied from -0.65 to 0.72 for the Len, -0.10 to 0.03 for the Mic., -1.65 to 0.85 for the Str, -0.44 to 0.78 for the SFI, -1.30 to 2.34 for the SCI. Stoneville-453xGiza-45, Sayar-314xDelcerro, Sayar-314xGiza-45, Stoneville-453xFantom, Nazilli-84SxDelcerro and FantomxDelcerro for the Len were determined to be greater specific combinations. Stoneville-453xDelcerro, Sayar-314xFantom, Sayar-314xDelcerro, Stoneville-453xFantom, Nazilli-84SxDelcerro for Str were found to be the best specific combinations. Significant and positive SCA effects for the SCI were observed in the three of the fifteen cross combinations; Sayar-314xDelcerro, DelcerroxGiza-45, Nazilli-84SxGiza-45. On other hand, Sayar-314xGiza-45, Nazilli-84SxFantom, Stoneville-453x Giza-45, Nazilli-84SxDelcerro for the SFI were found to be the greater specific combinations. Negative and significant SCA effects were determined for FantomxGiza-45, Sayar-314xGiza-45, and Stoneville-453xGiza-45 diallel crosses for the Mic.

Among the crosses the determinate Ht values varied from -1.77% to 3.81% for the Len, -3.43% to 10.58% for the Mic., -1.33% to 7.85% for the Str, -10.48% to 24.86% for the SFI, -12.82% to 7.06% for the SCI. In addition among the crosses the estimated Hb values varied from -7.87% to -0.03% for the Len, -17.98% to 5.19% for the Mic., -5.06% to 3.80% for the Str, -26.51% to 19.24% for the SFI, -18.22% to 4.63% for the SCI. Similar results were reported by Rauf *et al*., (2005). High and positive Hb (%) values were determined for the crosses Sayar-314xGiza-45, Sayar-314xDelcerro, Stoneville-453xGiza-45 and Nazilli-84SxGiza-45 for the Len, Sayar-314xDelcerro, Stoneville-453xFantom, Stoneville-453xDelcerro, Nazilli-84SxDelcerro for the Str, FantomxGiza-45, Stoneville-453xGiza-45, Sayar-314xGiza-45 for the SCI, while high and negative Ht values were determined for the crosses DelcerroxGiza-45, FantomxGiza-45, Nazilli-84SxGiza-45, Stoneville-453xGiza-45, Sayar-314xGiza-45 and Stoneville-453xDelcerro for the Mic., Sayar-314xGiza-45 Stoneville-453xGiza-45 and Nazilli-84SxGiza-45 for the SFI. The lower Hb values were determined for the crosses Sayar-314xGiza-45 (-17.98%) for the Mic, and Sayar-314xGiza 75 for the SFI (-26.51%) (Table. 2). Performance of parents and crosses could vary widely with genetic background and growing conditions (Ashokkumar *et al*., 2013; Bolek *et al*., 2010; Ehsan *et al*., 2008; Copur, 2006).

Conclusion

As a result of this study it may be concluded that additive genetic effects were noticed for the Len, Str, SFI. On the other hand, both additive and non-additive genetic effects were defined for the Mic. and SCI. Hence, selection in early generations may be more preferred for the Len, Str, SFI, while selection in the late generations may be more preferable for the Mic. and SCI. Suitable parents were determined to improve fiber quality traits of cotton. Namely Giza-45 for the Len, Mic., and SFI; Delcerro for the Str and SCI were selected as the thriving parents. These results implyed that Stoneville-453xGiza-45, Sayar-314xDelcerro, Sayar-314xGiza-45, Stoneville-453xFantom, Nazilli-84SxDelcerro and FantomxDelcerro for the Len; FantomxGiza-45, Sayar-314xGiza-45, Stoneville-453xGiza-45 for the Mic, Stoneville-453xDelcerro, Sayar-314xFantom, Sayar-314xDelcerro, Stoneville-453xFantom, Nazilli-84SxDelcerro for the Str; Sayar-314xDelcerro, DelcerroxGiza-45, Nazilli-84SxGiza-45 for the SCI may be proposed as the utmost promising cross combinations.

Table 1. Values of δ2GCA, δ2SCA, δ2GCA/δ2SCA and GCA for investigated traits in cotton parents (Griffing, 1956).

Parameters	Genotypes		Len	Mic.	Str	SFI	SCI
GCA	1	Sayar-314	-1,28**	0,62**	-0,281	0,58**	-8,39**
	2	Stoneville-453	-0,82**	0,0004	0,054	0,23*	-0,03
	3	Nazilli-84S	-0,81**	-0,15**	-1,302**	0,4**	-5,54**
	4	Fantom	-0,75**	0,26**	1,171**	-0,03	-2,66**
	5	Delcerro	1,20**	-0,30**	**1,841****	-0,35**	**10,00****
	6	Giza-45	**2,44****	**-0,43****	-1,484**	**-0,85****	6,62**
δ^2 GCA			52,42**	3,65**	41,97**	6,68**	1210,13**
δ^2 SCA			0,64	0,07**	1,8	0,54	112,96**
(δ^2GCA)/(δ^2GCA)			81,16	51,51	23,32	12,18	10,71

σ^2GCA: variance of general combiningability, σ^2SCA: variance of specificcombiningability * and **: significant at P ≤ 0.05 and P ≤ 0.01 respectively; Len: Fiber Length (mm), Mic: Fiber Fineness (micronaire), Str: Fiber Strength (g/tex), SFI: Short Fiber Index, SCI: Spinning Consistency Index

Table 2. Determinated SCA effects, Ht (%) and Hb (%) for the investigated traits in cotton F_1 diallel crosses.

Genotypes			LEN			MIC			STR			SFI			SCI	
			SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)
1	x	2	-0.12	0.17	-2.25	0.01	2.84	-8.43	-0.028	0.89	-0.16	0.78	**24.86**	13.25	-7.07**	-12,82	**-18,22**
1	x	3	-0.18	0.45	-1.56	0.16**	5.84	-8.43	-0.304	-1.29	-1.68	-0.05	2.07	1.72	-9.31**	-12,72	-15,76
1	x	4	-0.11	0.29	-2.28	0.25**	8.43	1.12	0.388	-0.84	-4.79	0.16	9.30	-1.89	-0.73	-5,65	-7,27
1	x	5	0.60	3.11	-4.78	-0.01	0.77	-14.42	0.684	4.16	-0.76	0.19	9.31	-7.06	**5.29****	1,45	-5,84
1	x	6	0.63	**3.81**	-6.60	-0.07**	-1.57	**-17.98**	0.044	-0.62	-2.15	**-0.44**	**-10.48**	**-26.51**	-4.73**	5,47	1,36
2	x	3	-0.27	-0.78	-1.21	-0.04	0.62	-2.64	-0.607	**-1.33**	-2.74	0.57	17.90	7.28	-4.77*	**-12,48**	-15,03
2	x	4	0.10	0.12	**-0.03**	0.34**	**10.58**	**5.19**	0.419	5.32	2.16	0.45	20.64	19.24	-5.43**	-5,67	-10,04
2	x	5	-0.65	**-1.77**	-7.17	-0.07	-1.77	-6.95	**0.848**	**7.85**	**3.80**	0.03	11.14	3.38	-5.64**	-10,88	-11,90
2	x	6	**0.72**	3.20	-5.05	-0.06*	-2.46	-9.59	-0.025	1.04	-1.53	-0.43	-7.09	-16.91	-4.31**	3,19	0,63
3	x	4	0.008	0.25	-0.33	-0.003	2.58	-5.41	-0.557	0.34	-4.01	-0.29	-2.41	-12.13	-1.30	-11,11	-12,74
3	x	5	0.30	1.68	-4.30	0.03	0.39	-1.79	0.372	3.93	-1.34	-0.14	-0.61	-15.25	0.47	-6,11	-9,85
3	x	6	0.12	2.94	-5.67	-0.06	-2.95	-7.18	-1.642	0.33	-0.83	0.09	-6.53	-23.05	4.97*	2,39	1,93
4	x	5	0.27	1.25	-4.18	-0.10	-1.32	-10.82	0.132	1.76	0.94	0.26	11.85	5.19	2.34	-6,34	-11,65
4	x	6	-0.36	0.00	**-7.87**	**-0.15****	-3.42	-14.50	0.025	0.91	-4.53	0.01	-1.80	-11.26	-5.06*	**7,06**	**4,63**
5	x	6	0.01	1.49	-1.35	-0.01	**-3.43**	-5.63	-0.229	1.12	**-5.06**	0.06	-1.54	-5.65	0.96*	2,50	-1,16
S. Error			**0.37**	**1.55**	**2.46**	**0.13**	**4.19**	**5.90**	**0.59**	**2.55**	**2.47**	**0.33**	**10.42**	**12.72**	**4.24**	**6.91**	**7.02**
Means			**0.07**	**1.08**	**-3.64**	**0.01**	**1.01**	**-7.16**	**-0.03**	**1.57**	**-1.51**	**0.08**	**5.11**	**-4.64**	**-2.29**	**-4.11**	**-7.39**
Max.			**0.72**	**3.81**	**-0.03**	**0.34**	**10.58**	**5.19**	**0.85**	**7.85**	**3.80**	**0.78**	**24.86**	**19.24**	**5.29**	**7.06**	**4.63**
Min.			**-0.65**	**-1.77**	**-7.87**	**-0.15**	**-3.43**	**-17.98**	**-1.64**	**-1.33**	**-5.06**	**-0.44**	**-10.48**	**-26.51**	**-9.31**	**-12.82**	**-18.22**

Where: SCA: variance of specific combining ability, Ht: Heterosis (%), Hb: Heterobeltiosis (%), * and **: significant at $P \leq 0.05$ and $P \leq 0.01$ respectively; LEN: Fiber Length (mm), MIC: Fiber Fineness (micronaire), STR: Fiber Strength (g/tex), SFI: Short Fiber Index, SCI: Spinning Consistency Index 1: Sayar-314, 2: Stoneville-453, 3: Nazilli-84S, 4: Fantom, 5: Delcerro, 6: Giza-45

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Diversity of Rhizobia Associated With *Lablab purpureus* Isolated from Algeria by PCR Amplification of the 16S rDNA PCR / RFLP

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ABSTRACT

The objective of this study was determination of the taxonomic position of these isolates and the evaluation of the level of approximation or divergence between these strains and the reference strains belonging to different genus of rhizobia. Amplification of the ribosomal 16S rDNA gene (PCR / RFLP of 16S rDNA) was digested with four different restriction enzymes: *Msp* I, *Hinf* I, *Hha* I and *Taq* I. The results of different electrophoretic profiles of fragments obtained shown the selection of the most discriminating enzymes *Msp* I and *Hinf* I. The length polymorphism of the restriction fragments (RFLP) analysis of PCR amplified 16S rDNA was compared with those of reference strains. Numerical analysis of molecular characteristics showed that 20 strains studied were divided into three distinct groups; we noted that three isolates only *Lablab purpureus* have a high level of similarity with the reference strain "*Bradyrhizobium",* while 17 isolates did not exhibit precise taxonomic status and therefore their exact phylogenetic classification is to be determined. The nearly complete sequence of the 16S rRNA gene from a representative strain of each REP-PCR pattern showed that the strains were closely related to the members of the family *Bradyrhizobium.*

Keywords**:** *Lablab purpureus*, PCR/RFLP, numerical analysis, genetic diversity, repetitive extragenic palindromic (REP).

Introduction

Many legumes plants with grains, forage as *Lablab purpureus* and pasture legumes form symbiotic associations with a group of bacteria, generally called as rhizobia (Harrier *et al*., 1995, Yue li *et al*., 2011). With the advancement of bacterial phylogenetics based on the sequences of the small conserved subunit of 16S ribosomal RNA (Day *et al*., 1965, Diouf *et al*., 2010), the taxonomy of rhizobia is rapidly changing. However, it is not only the taxonomy of rhizobia which is changing from time to time. The selection of appropriate rhizobial microsymbionts is becoming a complex procedure due to the fact that several legumes species can be nodulated by single rhizobia (Bringer *et al*., 1992). Yet, the symbiotic association between the legumes and their microbial symbionts play a significant role in agriculture worldwide by reducing ca. 100 million metric tons of atmospheric nitrogen saving US$ 8 billion/year on fertilizer N (Burnie *et al*., 2006, Nera *et al*., 2009).

The association between rhizobia and the members of the family Leguminosae accounts for 80% of biologically fixed nitrogen and contributes 25-30% of the 'protein intake in the world (Vance *et al*., 1997). To date, more than 98 species have been described for legume-associated symbiotic nitrogen-fixing bacteria within the genus *Rhizobium, Mesorhizobium, Ensifer, Bradyrhizobium, Burkholderia, Phyllobacterium, Microvirga, Azorhizobium, Ochrobacterium, Methylobacterium, Devosia,* and *Shinella* in the *Alphaproteobacteria* group, as well as *Burkholderia* and

Cupriavidus in the *Betaproteobacteria* group (http://www.bacterio.cict.fr). Rhizobia were characterized from wild and tree legumes, and several novel taxa have been proposed on the basis of these studies (Wolde- Meskel *et al*., 2005, Yan *et al*., 2007, Shetta *et al*., 2011). The isolation and characterization of new Rhizobium isolates from different legumes species is an interesting field of work that helps to understand the diversity and evolution of rhizobia.

Considering the potential value of *Lablab purpureus* for sustainable agriculture, agroforestry, and the lack of studies on the diversity of rhizobia associated with these plants, we aimed to collect and characterize rhizobia associated with this plant in Algeria.

The aim of the present study was to assess the diversity of *Lablab* micro-symbiotes and molecular characterization of rhizobia associated with this legume by using PCR/RFLP of 16S rDNA in Algeria.

Materials and methods

Authentication of isolates

All the rhizobia isolates were evaluated as pure cultures that can serve as nodules on their respective host plants. The seeds of the leguminous plants were previously germinated in petri-dishes after scarification with conc. H_2SO_4. The pre-germinated seeds have been planted in growth pouches containing N-free nutrient solution (Somasegaran *et al*., 1994). Seven days after planting, the growth pouches were inoculated with 1 ml broth YEM culture of each isolate with each treatment replicated four times. Uninoculated pouches have served as control. The pouches were placed in racks and kept in the green house. Plants were harvested 12 weeks after planting and their roots assessed for the presence of nodules. The results obtained after two months of culture have revealed that the 20 isolates (100% of the isolates) are able to nodulate their host plants.

Bacterial strains and culture medium

The colonies obtained on the solid YEM medium in each of the 20 pure isolates culture were collected and cultivated on TY medium (tryptone-yeast extract) (Bringer *et al*., 1992) diluted in half.

Extraction of genomic DNA

DNA preparation: Total genomic DNAs from all strains were isolated using standard phenol-chloroform-isoamyl extraction and ethanol precipitation in the presence of sodium acetate (0.3 mol/L). The pellets were washed with 70% ethanol, dried and re-dissolved in 150 µL of TE buffer.

The concentration and purity of DNA have been estimated spectrophotometrically at 260 nm and 280 nm, respectively. From the bacteria grown on TY medium for two days at 28 °C, a multi-well-formed colony were picked and suspended in 25 µl of sterile double distilled water.

PCR amplification PCR amplification of the 16S rDNA PCR/RFLP

The amplification reactions were performed using a protocol optimization initially described by Bruijn *et al*., (1992). Amplification reactions were performed in a total volume of 25 µl and contain the following: 1× reaction buffer (10 mM Tris-HCl, 50 mM KCl) with 1.5 mM $MgCl_2$, 2.5 units *Taq* polymerase, 200 µM of each dNTP (dATP, dCTP, dGTP and dTTP), 5 pmol of each forward and reverse primer and 100 ng of genomic DNA. The temperature profile was as follows: Initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 3 min. The amplified products were kept at a temperature of 4°C. All amplifications were carried out in Thermocycler. The PCR product was run on a 1% agarose gel stained with ethidium bromide.

Digestion of the amplification products with restriction enzymes

The universal primers FGPS 6 (5' GGA GAG TTA GAT CTT GGC ATT G 3 ') and FGPS 1509 (5'AAG GAG GGG CAG ATC CGC CA CAC 3') developed by Norman *et al*. (1996).

PCR products were separately digested with each of the following restriction endonucleases *Msp* I, *Hinf* I, *Hha* I and *Taq* I. The restriction fragment length polymorphism (RFLP) patterns were resolved by gel electrophoresis on 1.8% agarose for 4h at 120 mV.

Statistical Analysis

The results of the different profile of restriction have been treated by the UPGMA method with the Statistica software. The similarities between the various strains tested were evaluated by comparing the profile of restriction taken in pair's.

PCR amplifications

Repetitive extragenic palindromic (REP)-polymerase chain reactions (PCR) were performed using primers REPIR-I and REP2-I, according to Bruijn *et al*. 1992. PCR amplifications of 16S rRNA gene fragments were carried out using the two opposing primers 41f and 1488r as previously

reported (Wang *et al*., 2003). Amplification products were purified using the Qiagen PCR product purification system and subjected to cycle sequencing using the same primers as for PCR amplification, with ABI Prism dye chemistry. The products were analyzed with a 3130 × l automatic sequencer at the sequencing facilities of Estación Experimental del Zaidin, CSIC, Granada, Spain. The obtained sequences were compared to those in the GenBank database using the BLAST program (Ando *et al*., 1999) and with the sequences held in the EzTaxon-e server (Niemann *et al*., 1997). The sequences were aligned using Clustal W software (Bontemps *et al*., 2015). The distances were calculated according to Kimura's two-parameter model (Gyaneshwar *et al*., 2011). Phylogenetic trees were inferred based on the maximum likelihood (ML) method (Mohammed *et al*., 1997), using MEGA 5.0 software.

Results

PCR amplification of the 16S rDNA PCR/RFLP

RFLP Aanalysis of PCR amplification of 16S rDNA PCR/RFLP genes of almost all the 20 rhizobia isolates of *Lablab purpureus* has produced a single band 1500 bp representing the 16S rDNA PCR /RFLP gene amplified in all the *Lablab purpureus* rhizobial strains.

All the restriction enzymes tested produced polymorphic patterns. The most discriminative were those obtained with MSP I (Figure 1).

Numerical Analysis of phylogenetic groups established by the UPGMA

The results of the different restriction patterns were treated by UPGMA. The dendrogram derived from this analysis is shown in (Figure 2, Table 1). At a level of 83% similarity yields three clusters:

The first cluster (A) the strains of Rhizobium genus to a level of 75% similarity, the second cluster (B) includes *Mesorhizobium* strains to a level of 69% similarity; and the third cluster (C) groups of *Bradyrhizobium s*trains to a level of similarity 89%.

Comparing our isolates with reference strains, we noted that only three isolates of *Lablab purpureus* have a high level of similarity with the reference strain *"Bradyrhizobium"*. These isolates 2007, DLB (DLB 2008 and 2009) that form one and the same lineage with the reference strain *Bradyrhizobium.*

The most interesting results derived from the analysis by PCR/RFLP of the rDNA 16S is that isolates *Lablab purpureus* studied are totally distinct from *Bradyrhizobium s*trains.

In addition, 17 isolates did not present a specific taxonomic status, therefore their exact phylogenetic classification is to be determined.

Discussion

In this study, we performed molecular characterization by PCR RFLP 16S of 20 symbiotic bacteria isolated roots of *Lablab purpureus*. The amplification of the 16S rRNA gene of almost all the rhizobia isolates used in this study resulted in a single band 1.5 kb in size. This band size corresponds to the expected size reported earlier by Weisburg *et al*., (1991).

Polymorphism of lengthof the restriction fragments (RFLP) analysis of PCR amplified 16S rDNA were compared with those of reference strains. Numerical analysis of the molecular characteristics showed that 20 strains studied fall into three distinct groups, we noted that three isolates only of *Lablab purpureus* have a high level of similarity with the reference strain *"Bradyrhizobium",* while 17 isolates did not exhibit precise taxonomic status and therefore their exact phylogenetic classification is to be determined.

REP-PCR fingerprinting was used to group the strains. This technique has been extensively used to cluster bacteria at the subspecies or strain level (Jensent *et al*., 1968, Walkley *et al*., 1934) and is known to be a powerful tool for studies on microbial ecology and evolution (Ishii *et al*., 2009).

The combined restriction of the 16S rRNA genes of the rhizobia isolates with four endonucleases distinguished clearly different combinations of patterns or fingerprints at 80% similarity level which represents three distinct 16S rRNA genotypes among the isolates. This finding indicates great variations among the isolates and suggests that the soils harbour populations of highly diverse strains that nodulates the legume. This finding is in agreement with the results obtained in other parts of the world (Bremmer *et al.* 1967, Yue Downer *et al*., 2017).

These results, however, agree with those previously published, in which Yue *et al*., (2011) have shown in a study on Five strains isolated from root nodules of *Lablab purpureus* and *Arachis hypogaea* grown in the Anhui and Sichuan provinces of China were classified as members of the genus *Bradyrhizobium*. These strains had identical 16S rRNA gene sequences which shared 99.48%, 99.48% and 99.22% similarity with the most closely related strains of *Bradyrhizobium jicamae*, respectively. Parallel to our results in three distinct groups, we noted that three isolates only of *Lablab purpureus* have a high level of similarity with the reference strain *"Bradyrhizobium*".

Conclusion

We have focused our investigation on the genetic study using PCR/RFLP of 16S rDNA gene from 20 strains resulted in three groups, the first group includes the genus *Rhizobium* strains to a level of 75% similarity, the second combines the *Mesorhizobium* strains a level of similarity of 69% and the third groups of *Bradyrhizobium* strains to a level of similarity of 89%. Statistical Analysis of phylogenetic groups established by the UPGMA statistical software shows that among the twenty strains studied, 17 strains of the species described in the literature could be new species; this needs to be confirmed first by the complete sequencing rDNA16S.

Acknowledgments

The authors thank all those who have given us constructive ideas to shape this manuscript.

Table 1. Phylogenetic classification of bacterial strains.

Strains	REP-PCR pattern	Closest related genus	Family
DLB5011	1	*Rhizobium*	Rhizobiaceae
DLB5020	2	*Rhizobium*	Rhizobiaceae
DLB4012	3	*Rhizobium*	Rhizobiaceae
DLB4016	4	*Rhizobium*	Rhizobiaceae
DLB5017	5	*Rhizobium*	Rhizobiaceae
DLB5018	6	*Rhizobium*	Rhizobiaceae
DLM1111	7	*Mesorhizobium*	Rhizobiaceae
DLM1114	8	*Mesorhizobium*	Rhizobiaceae
DLM1120	9	*Mesorhizobium*	Rhizobiaceae
DLB4020	10	*Mesorhizobium*	Rhizobiaceae
DLM1121	11	*Bradyrhizobium*	Rhizobiaceae
DLM1123	12	*Bradyrhizobium*	Rhizobiaceae
DLM1122	13	*Bradyrhizobium*	Rhizobiaceae
DLb2006	14	*Bradyrhizobium*	Rhizobiaceae
DLb2005	15	*Bradyrhizobium*	Rhizobiaceae
DLb2004	16	*Bradyrhizobium*	Rhizobiaceae
DLb2007	17	*Bradyrhizobium*	Rhizobiaceae
DLb2008	18	*Bradyrhizobium*	Rhizobiaceae
DLb2009	19	*Bradyrhizobium*	Rhizobiaceae
DLB5015	20	*Bradyrhizobium*	Rhizobiaceae

Figure 1. Types of restriction patterns of PCR-amplified 16S rDNA digested with *Msp*I obtained with strains used in this study. Molecular size marker: M2, 20 bp ladder.

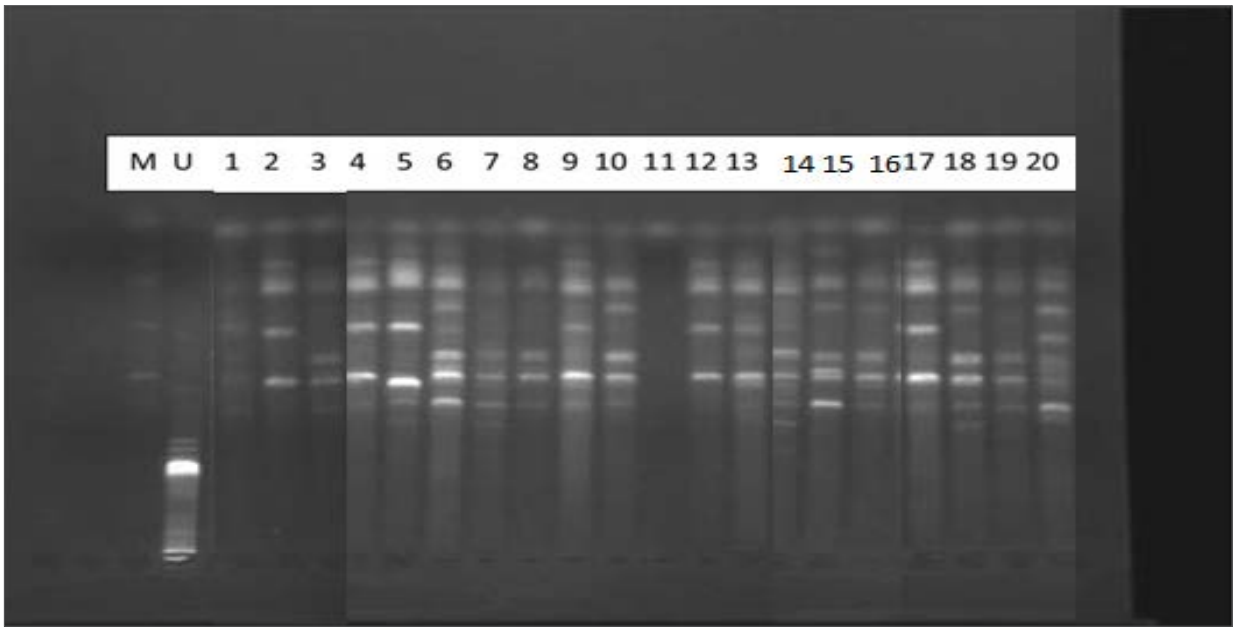
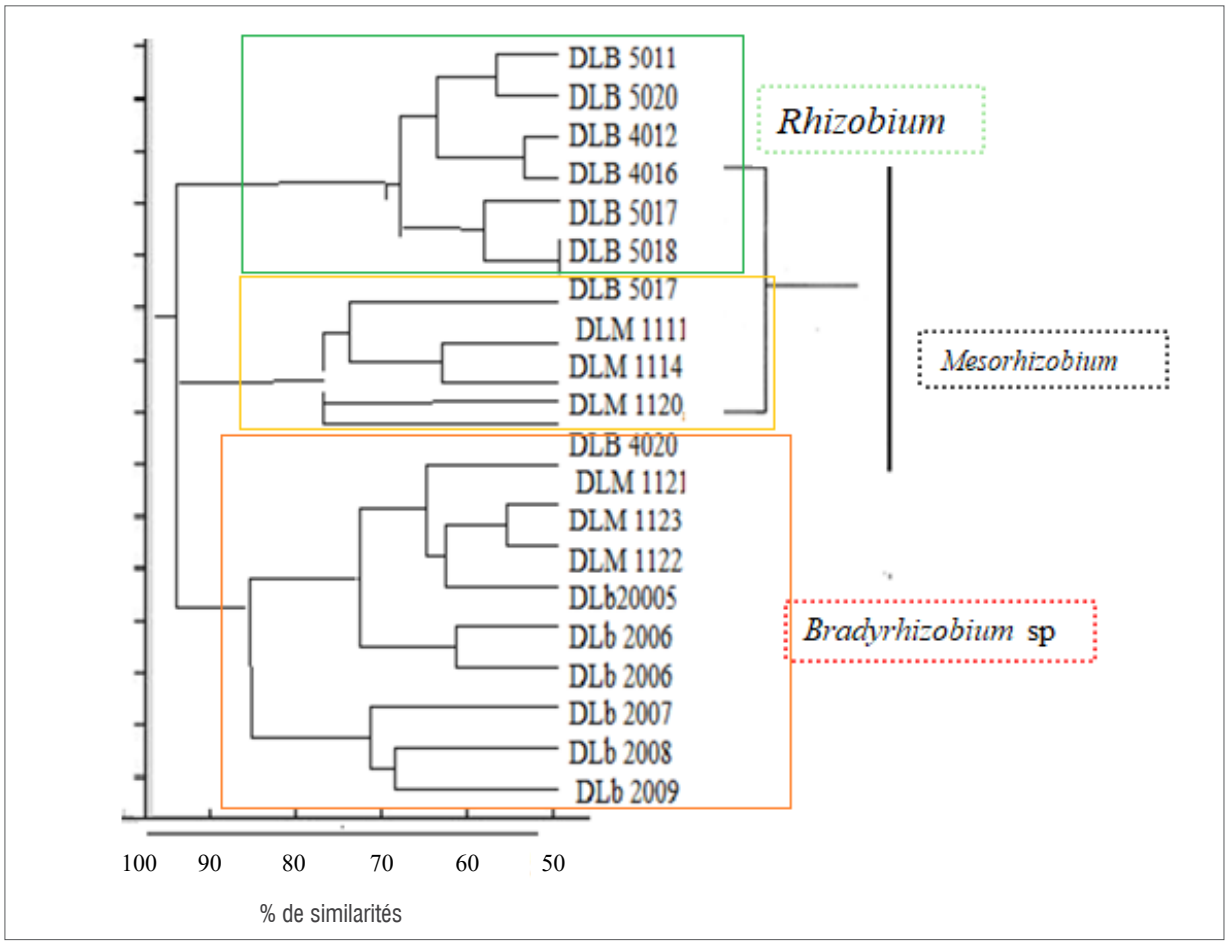


Figure 2. Dendrogram constructed by UPGMA indicating the phylogenetic position strains isolated from the legume plant *Lablab purpureus* compared to the reference strains used on the basis of the PCR / RFLP of 16S rDNA.



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Varietal Screening in Chickpea Against Gram Pod Borer, *Helicoverpa armigera* (Hub.) in Field Conditions Using Biochemical Parameters

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ABSTRACT

A field experiment was conducted to screen the 15 chickpea genotypes against gram pod borer by using biochemical parameters at Research Farm of Pulses Section, Deptt. of G&PB, CCS Haryana Agricultural University, Hisar, Haryana (India). The pooled analysis of two winter season 2013-14 & 2014-15 revealed thatthe maximum and minimum mean larval population of *Helicoverpa armigera* was 17.99 and 28.50 l/mrlon GNG 1581 and HK-2 genotype, respectively. The yield, per cent pod damageand pest susceptibility rating (PSR) ranged from 136.94 to 326.94 kg/ha, 33.26 to 83.30% and 3 to 7, respectively. Among the 15 chickpea genotypes maximum yield (326.94 kg/ha) minimum pod damage (33.26%) and PSR (3) was recorded on GNG 1581 while, in HK-2 minimum yield (136.94 kg/ha),maximum pod damage (83.30%) and PSR (7) was observed. The content of different biochemical in chickpea plants*viz.,* Malic acid, Total phenol, Total soluble sugars, Cellulose, Hemicellulose and Lignin were evaluated for screening and these listed biochemicalranged from 137.51 to 265.65 mg/g, 35.62 to 60.06 mg/g, 2.28 to 4.12 mg/g, 16.05 to 27.15 mg/g, 16.30 to 22.65 mg/g and 6.80 to 12.10 mg/g, respectively. The significant and negative correlation coefficient was recorded between malic acid and mean larval population of *H. armigera* (r= -0.69**) while, all other parameters were correlated non-significantly. Thus malic can be used as selection criteria for identifying *H. armigera* tolerant genotypes in chickpea.

Keywords**:** biochemical, chickpea, *Helicoverpa*, pod borer, screening

Introduction

Chickpea (*Cicer arietinum* Linn.), also known as Bengal gram is one of the most important pulse crops of India and is considered as "king of' pulses" (Bhatt and Patel, 2001). Chickpea is an important source of carbohydrates, dietary fiber and protein, and the protein quality is considered to be better than other pulses (Jukanti *et al.*, 2012). Nearly sixty insect pest species feed on chickpea worldwide, of which gram pod borer, *Helicoverpa armigera* (Hubner) is the major insect pests in the Indian subcontinent.Gram pod borer, *H. armigera* is a polyphagous,multivoltine and cosmopolitan pest and is reported to feed and breed on 182 species of host plants belonging to 47 families in India (Sithanantham, 1987; Pawar, 1998). The yield loss in chickpea due to the pod borer has been estimated to be 10 to 60% under normal weather conditions and may elevate to 50 to 100% in favourable weather conditions (Vaishmpayam and Veda. 1980). Biochemical traits such as malic acid, phenolic compounds, cellulose, hemicelluloses, lignin, free amino acids, etc. of crops have been identified that could be responsible for resistance to insect pests (Yoshida *et al.,* 1995; Grija *et al.*, 2008). Identification and detailed knowledge of insect pest resistance traits of chickpea are of immense importance for developing resistant varieties.In present paper results on varietal screening conducted under field conditions in collection of fifteens genotypes has been reported.

Materials and Methods

The present experiment was conducted at Research Farm of Pulses Section, Deptt. of Genetics &Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana (India) during the two winter season 2013-14 and 2014-15. Fifteen genotypes of chickpea were screened against the gram pod borer under field conditions by using various biochemical parameters *viz.,* malic acid, total phenols, total soluble sugars, cellulose, hemicellulose and lignin. The experiment was laid in randomised block design with 3 replications with plot size of 5 rows of 4 m length. The observationson larvae of *H. armigera* were recorded from 3 selected places per meter row length in each plot after initiation of pest at 15 days interval.At the time of maturity all the pods of 10 randomly selected plants were plucked and number of healthy and damaged pods were counted and per cent pod damage was calculated. Pest Susceptibility rating was also calculated for the genotypes

$$\text{Pod damage (\%)} = \frac{\text{Number of damaged pods}}{\text{Total number of pods}} \text{x100}$$

$$\text{PSR} = \frac{\text{C - T}}{\text{C}} \text{x100}$$

C= Check genotype
T= Treatment genotype
Scale: 1 to 9
*1 – Resistance, **9 – Highly susceptible

Yield of each plot was recorded at harvest. The pods collected from 20 plants for recording per cent pod damage was also added to record the total yield/ plot. It was converted as kg/ha and analysed statistically. Correlation coefficient were computed between biochemical parameters and mean larval population.

Results and Discussion

The larval population of H. armigera

The pooled mean larval population (Table 1) of two years 2013-14 and 2014-15, the minimum mean larval population of *H. armigera* was recorded on GNG 1581 (17.99 l/mrl) and it was statistically on par with GNG 1488 (20.26 l/mrl) and H 07-121 (20.41 l/mrl). Maximum mean larval population was recorded on HK-2 (28.52 l/mrl) and it was statistically on par with ICC 3137 (26.92 l/mrl), C-235 (27.13 m/mrl) and HC-1 (28.06 l/mrl). None of the genotypes showed resistance against *H. armigera*. These results are in conformity with Shankar *et al.,* (2014) who reported that, larval population of *H. armigera* on chickpea genotype ICC 3137 (38.6 l/5plants) and ICCL 86111 (16.6 l/5plants) at flowering stage among the 10 selected genotypes. The results are not in agreement with Lateef (1985), Chhabra *et al.,* (1990), Singh and Yadav, (1999). Chickpea germplasm accessions with resistance to *H. armigera* have been identified by several workers. The genotypic responses have been found to be quite variable across seasons and locations (Sharma *et al*., 2003).

Biochemical traits of chickpea genotypes

Malic acid plays a major role against incidence of major insect pests in chickpea. Malic acid content in selected chickpea genotypes ranged from (Table 2) 137.51 to 265.65 mg/g. Maximum amount of malic acid was recorded on GNG 1488 (265.65 mg/g) and minimum on C-235 (137.51 mg/g). Negative and significant correlation (Table 3) was recorded between malic acid with larval population of *H. armigera* (r= -0.6901**), during pooled mean of 2013-14 and 2014-15, respectively.

Varieties with highest amount of malic acid had the highest resistance to *H. armigera* (Rembold, 1981; Rembold *et al*., 1990). Malic acid acts as deterrents to the *H. armigera* larva and pod borer resistant lines have more amount of malic acid than the susceptible lines (Bhagwat *et al*., 1995). Oxalic acid inhibits the growth of *H. armigera* larvae when incorporated in artificial diet, while malic acid shows no growth inhibition (Yoshida *et al*., 1995). Low acidity in the leaf extracts is associated with susceptibility to *H. armigera* however, resistance expressed by PDE 2-3, PDE 7-3 and ICC 506 of chickpea have been attributed to factors other than acidity while that of PDE 7-2 is due to high acidity (Patnaik and Senapati, 1995).

Pod damage and yield

Thepooled mean of two years 2013-14 and 2014-15 showed that minimum per cent pod damage was recorded on variety GNG 1581 (33.26%) and it was statistically on par with GNG 1488 (33.50%) and H 07-158 (40.66%), H 07-121 (38.42%) and H 03-56 (40.51%). Maximum per cent pod damage was recorded on HK-2 (83.30%) and it was statistically on par with HC-1 (75.11%) and C-235 (74.16%).

The pooled mean of years 2013-14 and 2014-15 showed that yield of the selected genotypes ranged from 102.50 to 371.67 kg/ha. Maximum yield was realized from H 03-56 (371.67 kg/ha) and it was statistically on par with H 07-121 (304.17 kg/ha), GNG 1581 (326.94 kg/ha) and HC-5 (335.56 kg/ ha) whereas minimum yield was from ICCL 86111 (102.50 kg/ha) and it was statistically on par with HC-1 (111.39 kg/ha) and C-235 (127.50 kg/ha).

These results are in agreement with Shankar *et al.,* (2014) who evaluated the chickpea genotypes for resistance to *H. armigera* under filed condition. Data revealed that grain yield was significantly greater in ICCV 10 (1732.0 kg/ha) than in susceptible check, ICC 3137 (73.3 kg/ha) and significantly maximum pod damage among the selected genotypes namely ICCL 86111, ICC 10393, ICC 12475, RIL 20, RIL 25, ICCV 10, EC 583264, KAK 2 and EC 583264. Nadeem *et al.,* (2011) studied ten advanced Kabuli genotypes against pod borer and reported that pod damage ranged from 8.2 to 15.8% whereas yield from 197 to 1259 g/plot.

Pest susceptibility rating

The pooled mean of two years 2013-14 and 2014-15, revealed that GNG 1581 and GNG 1488 were recorded with pest susceptibility rating 3, which was considered as increasing resistance. Moderately resistant genotypes *viz.,* H 07-158, HC-5, ICC 3137, H 07-121, H 03-45, H 01-27, H 03-56, GNG 1958 and ICCL 86111 were recorded with PSR of 4 and 5. Maximum PSR (7) was found in the HK-2 and considered as increasing susceptibility against the population of *H. armigera*. A method of grading the test materials by using a 1 to 9 rating scale based on pod damage was suggested by Lateef and Reed (1995). Hossain (2009) recorded pod damage range from 2.80 to 13.47/plant in 20 different chickpea genotypes and found that genotype with maximum pod damage was most susceptible.

Conclusion

Our studies revealed that chickpea genotypes differ in their response to insect pest (*Helicoverpa armigera*) attack as exhibited by differences in larval population and corresponding damage to chickpea pods and hence seeded. Studied 15 genotypes exhibited that GNG 1581 was fairly tolerant to *H. armigera* as it showed least larval population and yield damage. The biochemical analysis supplemented our field observations. The malic acid content was significantly negatively correlated with larval population and the malic acid was high in tolerant genotypes as well. Therefore our studies fairly conclude that large number of genotypes of chickpea should be screened under field conditions both for larval population, damage to pods and reduction in yield so that a set of genotypes with different genetic backgrounds could be identified for further breeding programme in chickpea for its tolerance to dreadful pest *H. armigera.*

Table 1. Larval population of *H. armigera*, pod damage, yield and PSR on different genotypes of chickpea (pooled data).

No.	Genotypes	8th	10th	12th	14th	16th	Average	Pod damage (%)	Yield (kg/ha)	PSR
1	GNG 1581	0.00 (1.00)	0.22 (1.10)	1.12 (1.45)	6.61 (2.76)	82.02 (9.11)	17.99 (4.36)	33.26 (35.19)	326.94	3
2	GNG 1958	0.00 (1.00)	0.61 (1.27)	1.89 (1.70)	9.28 (3.21)	107.79 (10.43)	23.91 (4.99)	60.70 (51.18)	193.61	5
3	GNG 1488	0.00 (1.00)	0.50 (1.22)	1.45 (1.56)	8.00 (3.00)	91.35 (9.61)	20.26 (4.61)	36.19 (36.88)	260.00	3
4	CSJ-140	0.00 (1.00)	0.84 (1.35)	2.39 (1.84)	10.06 (3.32)	112.52 (10.65)	25.16 (5.11)	69.95 (56.75)	176.11	6
5	HK-2	0.00 (1.00)	1.61 (1.62)	3.06 (2.01)	12.73 (3.70)	125.98 (11.27)	28.52 (5.43)	83.30 (66.78)	136.94	7
6	H 07-158	0.00 (1.00)	0.73 (1.31)	2.00 (1.73)	9.45 (3.23)	105.37 (10.31)	23.51 (4.95)	43.88 (41.45)	265.28	4
7	HC-5	0.00 (1.00)	0.78 (1.33)	2.00 (1.73)	9.45 (3.23)	110.68 (10.57)	24.58 (5.06)	51.55 (45.87)	335.56	4
8	ICC 3137	0.00 (1.00)	0.89 (1.37)	2.44 (1.85)	10.11 (3.33)	121.17 (11.05)	26.92 (5.28)	49.58 (44.74)	139.72	4
9	ICCL 86111	0.00 (1.00)	0.62 (1.27)	2.06 (1.75)	8.95 (3.15)	103.96 (10.24)	23.12 (4.91)	67.08 (55.01)	102.50	5
10	H 07-121	0.00 (1.00)	0.39 (1.18)	1.28 (1.51)	7.34 (2.89)	93.07 (9.70)	20.41 (4.63)	42.27 (40.45)	304.17	4
11	H 03-45	0.00 (1.00)	0.73 (1.31)	1.78 (1.67)	9.39 (3.22)	112.57 (10.66)	24.89 (5.09)	44.07 (41.58)	220.00	4
12	H 01-27	0.00 (1.00)	0.67 (1.29)	1.84 (1.68)	9.34 (3.21)	106.57 (10.37)	23.68 (4.97)	49.25 (44.55)	242.50	4
13	H 03-56	0.00 (1.00)	0.56 (1.25)	1.67 (1.63)	8.11 (3.02)	98.60 (9.98)	21.79 (4.77)	46.00 (42.68)	371.67	4
14	HC-1	0.00 (1.00)	1.06 (1.43)	2.78 (1.94)	10.50 (3.39)	125.20 (11.23)	28.06 (5.39)	75.11 (60.55)	111.39	6
15	C-235	0.00 (1.00)	0.89 (1.37)	2.67 (1.92)	10.11 (3.33)	121.96 (11.09)	27.13 (5.30)	74.16 (59.47)	127.50	6
CD at 5%		(N.S.)	(0.10)	(0.10)	(0.28)	(0.52)	(0.26)	(7.90)	98.82	
SEm(±)		-	(0.03)	(0.03)	(0.10)	(0.20)	(0.08)	(2.71)	33.94	

Data presented in parentheses are square root transformed value

Table 2. Bio-chemical constituents in different genotypes of chickpea.

No.	Genotypes	Malic Acid	Total Phenol	Total Soluble Sugars	Cellulose	Hemicellulose	Lignin
1	GNG 1581	260.26	45.70	3.21	19.10	21.90	9.05
2	GNG 1958	240.62	46.87	3.38	22.40	21.50	7.80
3	GNG 1488	265.65	53.34	2.42	27.15	21.10	10.20
4	CSJ-140	200.77	60.06	4.12	19.20	21.20	9.60
5	HK-2	213.04	37.55	2.62	20.35	21.15	9.95
6	H 07-158	207.41	45.11	3.14	15.85	20.05	8.50
7	HC-5	195.11	35.87	2.99	19.30	20.25	9.00
8	ICC 3137	230.54	37.63	3.82	20.20	20.80	9.25
9	ICCL 86111	210.81	27.97	2.83	21.40	22.40	9.95
10	H 07-121	242.30	49.31	2.53	21.60	22.65	9.10
11	H 03-45	218.32	41.08	2.28	16.05	21.80	9.15
12	H 01-27	212.45	47.80	3.91	21.10	19.33	8.65
13	H 03-56	236.24	40.40	3.21	19.10	14.50	12.10
14	HC-1	199.68	38.22	4.07	21.35	19.45	6.80
15	C-235	137.51	35.62	4.12	19.95	16.90	6.90

Table 3. Correlation coefficients among biochemical traits of chickpea genotypes with mean larval population of *H. armigera* (pooled data).

No.	Biochemical traits	*H. armigera*
		Pooled
1	Malic acid	-0.69**
2	Total phenols	-0.37^{ns}
3	Total soluble sugars	0.43^{ns}
4	Cellulose	-0.18^{ns}
5	Hemicellulose	-0.18^{ns}
6	Lignin	-0.39^{ns}

** significant at P≤0.01
ns non-significant

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Use of Heat Susceptibility Index and Heat Response Index as a Measure of Heat Tolerance in Wheat and Triticale

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ABSTRACT

Wheat, a cool temperature-loving crop is encountering serious problem of high temperature particularly at the grain filling stage. The present investigation was carried out to compare wheat and triticale genotypes for heat tolerance using seven genotypes from each group. This experiment was conducted in randomized block design with three replications under two dates of showing *i.e.* 25th November and 25th December. Morphological traits like days to flowering, days to maturity, spike length, number of effective tillers per plant, grain yield per plant, biological yield per plant, harvest index and thousand grain-weight were studied and used for the calculation of heat susceptibility index (HSI). In wheat group wherein, genotypes Raj 3765, WH 1080 and WH 1142 showed minimum HSI; while in triticale group, almost all genotypes had minimum values of HSI for different traits, representing high temperature tolerance of these genotypes. Based on heat response index (HRI) also same results were revealed. Overall genotypes Raj 3765, WH 1142, TL 3001, TL 3002, TL 3005 and TL 2942 figured most suitable for late sown conditions. Further these results were also supported by correlation analysis in which HSI and HRI were negatively correlated with each other.

Keywords**:** wheat, triticale, heat stress, HSI, HRI

Introduction

Wheat (*Triticum aestivum* L em. Thell), the'king of cereals' is a member of *Poaceae* family. It is an important staple food all over the world. By the year 2020, the demand of wheat is expected to grow at 1.6 percent per annum, which can be fulfilled if productivity of wheat is increased upto 3.5 tons per hectare (Ortiz *et al*., 2008). However, the present scenario of global warming and climate change may pose problems in wheat production as wheat is seriously affected by elevated temperature. The global temperature is increasing at a rate of 0.13°C per decade since 1950 and still there is expectation that it will take pace of 0.2°C per decade in the next few decades (IPCC, 2007). As wheat is a winter season crop, it requires a long period of low temperature to give maximum yield. The mean temperature of 15 to 18°C is considered as optimum during vegetative growth of wheat (Chowdhury and Wardlaw, 1978). Wheat plant need a long period of low temperature at vegetative and grain filling stage and heat stress at any of these stages may result in decreased productivity of wheat. A mean temperature greater than 17.5°C in the coolest month is defined as heat stress for wheat plant (Fischer and Byerlee, 1991). For every one degree rise in ambient temperature there is a reduction of 3 to 4 percent wheat yield (Mishra *et al.,* 2002).

The high temperature stress occurring at grain filling stage, commonly known as terminal heat stress is most severe for wheat production (Wahid *et. al*, 2007). About 40 percent of wheat producing areas face this problem worldwide (Reynolds *et al*., 1994). This situation may be more severe in South Asian countries where rise in temperature is projected by as much as 3-4°C by the end of this century. In India, nearly 60

percent of wheat area is planted late due to the late harvesting of *kharif* crops. Under such circumstances where on one side demand of wheat production is increasing to feed the huge population of the world and on other side elevated temperature due to global warming is creating problem in sustaining wheat productivity, there is a quick need to identify genotypes which can perform well under temperature stress conditions. Triticale (*X Triticosecale*), the first man made cereal, can be an alternate option under such situation because it is a cross of wheat and rye (*Secale cereale*) (Wilson, 1876). Rye is more tolerant to abiotic stresses as compared to wheat. So it is possible that triticale genotypes with high yield and tolerance to abiotic stresses can be identified. In present investigation these both species are compared for their heat tolerance based on heat susceptibility index (HSI) and heat response index (HRI).

Material and Method

The present experiment was conducted during *Rabi* season of 2015-16 in research area of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The research material was sown in randomized block design under two dates of sowing *i.e.* 25th November (Normal sowing) and 25th December (late sowing). The research material consisted of seven genotypes; each from bread wheat and triticale group. Genotypes included were WH 1080, WH 1105, WH 1142, PBW 550, HD 3086, DBW 88 and Raj 3765 from bread wheat group and TL 3001, TL 3002, TL 3003, TL 3004, TL 3005, TL 2942 and TL 2969 from triticale group. The experimental material was sown in paired rows of 2.5 m length, with row to row spacing 20 cm and plant to plant spacing 10 cm. All the recommended package and practices were followed.

Various morphological and phenological observations were on traits like days to flowering, days to maturity, spike length, number of effective tillers per plant, grain yield per plant,biological yield per plant, harvest index and thousand grain weights were collected from randomly selected five plants per genotype per replication. Heat Susceptibility Index was calculated according to the formula given by Fisher and Maurer (1978)

$$HSI = (1-YD/YP)/D$$

Where,

YD = mean of the genotypes in stress environment

YP = mean of the genotypes under non-stress environment

D = 1-(mean YD of all genotypes/ mean YP of all genotypes).

The Heat Response Index of individual genotype was computed using the formula given by Bidinger *et al.*, (1987) as

$$HRI = (Ya- Yest)/SES$$

Where,

Yest and Ya are the yields estimated by regression analysis and actual yields, respectively, and SES is the standard error of the dependent trait *i.e.* grain yield.

Results

Under both normal and late sown conditions, significant genotypic variability was present for all traits as shown by analysis of variance (Table 1). This variation can be exploited for selection of heat tolerant genotypes. Based on these traits, heat susceptibility index was calculated (Table 2). The genotypes with high positive HSI values are susceptible to higher temperature and vice versa (Fisher and Maurer, 1978). In wheat group, genotypes Raj 3765, WH 1080 and WH 1142 showed minimum HSI for a number of traits. While in triticale group, almost all genotypes have minimum values of HSI for different traits. HSI values for the three important traits *i.e.* grain yield per plant, biological yield per plant and harvest index can be utilized for selection of tolerant genotypes. HSI value for grain yield per plant was minimum for TL 3005 (0.39) followed by TL 3002 (0.47), TL 2942 (0.56), Raj 3765 (0.65), TL (0.73) and WH 1142 (0.89). For the trait biological yield per plant minimum HSI was shown by TL 3005 (-0.70) followed by TL 3002 (-0.19). Similarly, for harvest index HSI was minimum for genotype Raj 3765 (0.65). All these genotypes are more tolerant to high temperature than other genotypes.

Other than Heat Susceptibility Index, Heat Response Index (HRI) was also calculated to confirm the above results. HRI is more useful criteria of selection as it categorise the genotype based on the mechanism of heat tolerance *i.e.* escape, resistance or tolerance (Munjal and Dhanda, 2016). The HRI values of different genotypes is shown in table 3. Significant positive values of HRI denote heat tolerance, while negative values denote heat susceptibility. Maximum heat response index (HRI) in *T. aestivum* was noticed in Raj 3765 (1.05) followed by WH 1142 (0.94);while in triticale group maximum HRI was found in TL 3005 (0.86) followed by TL 2942 (0.83), TL 3002(0.77), TL 3001 (0.11) and TL 3004 (0.02). A negative value of HRI represents a genotype with susceptibility to high temperature. It is clear from both Heat Susceptibility Index and Heat Response Index that triticale genotypes are more tolerant to high temperature than wheat genotypes.

Similar study was also conducted by Bhardwaj *et. al* (2017) who classified wheat genotypes in four groups *i.e.* highly heat tolerant, tolerant, moderately tolerant and susceptible based on HSI values. Munjal and Dhanda (2016) used HSI and HRI to screen wheat genotypes tolerant to drought condition. There result revealed that significant variability exist in wheat genotypes for various yield attributing traits and Heat Response Index is very useful criteria for selection of genotypes tolerant to abiotic stress condition.

Further correlation coefficient analysis was carried out between percentage reduction of yield and heat Susceptibility Index and Heat Response Index (Table 4). It is clear that reduction in yield under late sown condition is significantly positively correlated with HSI (1.000) whereas it is negatively correlated with HRI (-0.870). There is a negative correlation between HSI and HRI (-0.867). These results reveals that yield reduction will be minimum if HSI is less and HRI is more for a genotype.

Conclusion

The global warming is a major challenge for crop production. Every year temperature is rising. Also within year fluctuations in temperature is more in recent years. Under such circumstances, only resistance genotypes is a solution for crop production. With this objective the present investigation was carried out using wheat and triticale genotypes. Heat Susceptibility Index and Heat Response Index were used to select genotypes tolerant to high temperature. Based on this study we can conclude that wheat genotype Raj 3765 and WH 1142 are highly tolerant to increased temperature as compared to other wheat genotypes. The second important result of this study was that triticale has genes for abiotic stress tolerance as most of the genotypes have shown very low HSI values for all the traits. Triticale has proved to be a good gene pool of abiotic stress tolerant genes. We can use these genes in wheat breeding programmes related to high temperature stress.

Table 1. Mean sum of squares for different traits under normal and late sown conditions.

Source of Variation		DF	MSS									
			DH	DM	T/P	PH	SL	GY	BY	HI	G/S	TW
Normal sown	Replication	2										
	Treatment	13	25.926*	48.888*	7.011*	343.742*	4.385*	114.919*	339.227*	65.743*	276.931*	24.969*
	Error	26	1.283	3.401	0.988	9.632	0.599	4.74	7.818	5.991	9.787	2.091
Late sown	Replication	2	2.21	8.21	8.16	1.96	0.24	19.06	6.52	0.67	2.14	23.83
	Treatment	27	49.56*	112.66*	86.74*	7.63*	6.68*	270.33*	106.46*	388.74*	227.30*	40.24*
	Error	54	1.49	1.7	2.41	0.783	0.284	6.87	10.55	3.03	20.1	13.42

* Significant at P≤0.05

Table 2: Heat Susceptibility Index (HSI) of different genotypes.

	Days to he ading	Days to maturity	Tillers per plant	Spike length	Yield per plant	Biological yield per plant	Harvest index	Grains per plant	1000-grain weight
WH 1080	0.88*	0.99*	0.99*	0.85*	1.23	0.93*	1.60	0.42*	1.18
WH1105	0.23*	1.05	0.72*	1.38	1.25	0.97*	1.61	0.41*	2.56
WH 1142	0.96*	1.25	0.53*	-0.28*	0.89*	0.67*	1.10	1.14	2.19
PBW 550	1.09	1.27	0.92*	2.01	1.21	1.73	0.65*	1.37	1.85
HD 3086	1.34	1.01	0.72*	1.59	1.23	1.23	1.31	0.93*	1.98
DBW 88	0.97*	1.25	0.95*	0.69*	1.15	1.22	1.13	1.07	1.67
RAJ 3765	0.92*	0.84*	0.72*	0.44*	0.63*	1.01	0.15*	0.80*	1.72
TL 3001	1.07	0.80*	1.00	0.93*	0.96*	1.14	0.78*	1.03	0.79*
TL 3002	0.93	1.01	0.89*	0.76*	0.47*	-0.19*	1.08	0.12*	-1.22*
TL 3003	1.17	0.99*	1.56	1.93	1.19	1.53	0.90*	1.83	-0.77*
TL 3004	2.01	0.95*	1.29	0.75*	0.73*	0.51*	0.95*	1.01	-2.13*
TL 3005	1.07	0.98*	1.26	0.79*	0.39*	-0.70*	1.32	0.39*	0.64*
TL 2942	0.38*	0.64*	1.17	0.77*	0.56*	0.67*	0.43*	1.35	1.84
TL 2962	0.85*	1.02	1.35	1.05	1.53	2.45	0.58*	1.60	0.71*

* *Genotype showing low susceptibility to increasing temperature.*

Table 3. Heat Response Index (HRI) of wheat and triticale genotypes.

T. aestivum	HRI	Triticale	HRI
WH1080	-0.12	**TL 3001**	0.11*
WH1105	-0.18	**TL 3002**	0.77*
WH1142	0.94*	**TL 3003**	-0.33
PBW550	-0.19	**TL 3004**	0.02
HD 3086	-0.27	**TL 3005**	0.86*
DBW 88	0.03	**TL 2942**	0.83*
RAJ3765	1.05*	**TL 2969**	-0.44
CD	0.8	**-**	0.8

* Significant at P≤0.05

Table 4: Correlation Coefficients between reduction in grain yield HSI and HRI in wheat.

	Reduction (%) in yield	HSI	HRI
Reduction (%) in yield	1.000	-	-
HSI	1.000*	1.000	-
HRI	-0.870*	-0.867*	1.000

* Significant at P≤0.05

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Genetic Variability and Character Association in Advance Inbred Lines of Pearl Millet Under Optimal and Drought Condition

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ABSTRACT

This study was conducted to investigate genetic variability among 50 advance inbred lines of pearl millet. The genotypes were evaluated for 15 growth traits, yield components, and grain yield. All the quantitative traits varied significantly among the tested genotypes. A wide range was observed for all parameters of genetic variability for all the traits. A higher PCV for various characters than its corresponding GCV suggested the role of considerable component of environment in the expression of all these characters. High to moderate heritability and genetic advance as per cent of mean for dry fodder yield per plant, grain yield per plant indicated that selection will be effective for further improvements. Comparative mean performance for various traits of Bawal location (drought) and Hisar location (normal) revealed that the characters expression at Bawal was much reduced causing significant reduction in grain yield. The correlation coefficients revealed that estimated genotypic correlations for most of the characters were greater than their corresponding phenotypic ones. Results revealed that number of tillers per plant at Hisar location and dry fodder yield at Bawal location can be used as indirect selection criteria to increase the seed yield.

Keywords**:** drought response index, heritability, genetic advance, morpho-physiological traits

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the staple food grain with a high nutritional value and is also used as a feed, fodder, construction material and even its potential as a source of bio-fuel. As compared to the early 1980s, pearl millet area in India declined by 26% during 2014-15, but production increased by 19% owing to 48% increase in productivity. It is cultivated in the most sandy, infertile soils and drought prone environments where no other cereal crop can survive even under these conditions, pearl millet yields 500-800 kg/ha of grain. Pearl millet hybrids maturing in 80-85 days, when cultivated as an irrigated summer season crop in parts of Rajasthan, Uttar Pradesh, Gujarat and Maharashtra states of India, have been reported to give as high as 5000-7000 kg/ha of grain yield (Anonymous 2016).

Pearl millet is principally a cross pollinated crop where heterozygocity *perse* has to be maintain to realize elite hybrids or improved population. That in termed can be achieved through incorporating diverse inbred lines in crossing programme (three lines system) for hybrids and/or development of open pollinated improved population. This necessitates evaluation of good number of inbred lines under different growth environment so that promising inbred line could be selected for further improvement. Moreover information on genetic variability parameters like GCV, PCV, heritability and genetic advance in given set of population and correlation co-efficient will among various traits could help deciding the selection intensity

and direction of selection for further improvement. Keeping these aspects in view the presence study was conducted to evaluate a set of 50 genotypes of pearl millet under two growth environments representing rainfed drought condition at Bawal and normally irrigated condition at Hisar location.

Material and Methods

The present investigation was conducted at the Research Area of Pearl millet Section at Hisar and Regional Research Station (RRS) at Bawal, CCS Haryana Agricultural University, Hisar representing typical semi-arid conditions during Kharif 2013.The material comprised 50 pearl millet inbred lines. The experiment was conducted in RBD with 3 replications. Three irrigations were applied at Hisar location whereas rainfed crop was grown at Bawal with plot size of two row of 4.0 meter length. Plant to plant distance within a row (10 cm) and row to row distance (50 cm) were maintained at both the locations. Analysis of Variance was computed for all the traits as described by Gomez and Gomez (1984). Correlation coefficients among characters were determined by using the variance and covariance components as suggested by Al-joubri *et al*., (1958). Estimates of appropriate components were substituted for the parameters to predict expected genetic gain as suggested by Johnson *et al*., (1955).

Results and Discussion

Drought stress is one of the major constraints for the crop productivity, which is affecting 1/3rd of arable land world-wide and will probably increase in the on-going climate changes. Therefore, for sustaining the productivity in future drought tolerance is important.

In both the locations ANOVA revealed (data not given for brevity) highly significant genotypic differences among the genotypes for leaf rolling, flag leaf area (cm^2), flag leaf angle, number of tillers per plant , stem thickness (mm), plant height (cm), grain yield (g), dry fodder yield (g), days to 50% flowering , panicle length (mm), grain yield per panicle (g) , grain volume, total panicle number per plant, grain mass (1000 grain wt.) and grain number per panicle were significant in both the environments. Over all mean of genotypes was less in Bawal as compared to that in Hisar location for all the characters.

Heritability in broad sense, genetic advance in terms of per cent of mean also showed a wide range in both the environments (Table 1). High estimates of coefficient of variation along with high to moderate heritability and genetic advance as per cent over mean for grain per panicle, grain yield per panicle and dry fodder yield are indicative of additive genetic variance for these characters. Grain yield and plant height and tillers per plant had moderate heritability with moderate genetic advance at both locations *viz*., Hisar and Bawal which indicated non-additive genetic variance. High heritability, low genetic advance and low variability were observed for panicle length *i.e.* [heritability 57.56, PCV 6.66, GCV 5.06 (Hisar), heritability 80.37, PCV 6.77, GCV 6.06 (Bawal), respectively] for days to 50% flowering which indicates prevalence of non-additive genetic variance. Selection in later generation might be more effective for such traits. Singh and Singh (2016) estimated high heritability for plant height and panicle length. All characters i.e. number of tillers per plant, plant height, panicle length, panicle diameter, number of leaves per plant, test weight, days to 50% flowering, and grain yield showed high heritability with high genetic advance that indicated the predominance of additive type of gene action for these characters.

Correlation co-efficient

Grain yield per plant expressed a positive and significant correlation with flag leaf area (0.1708*), number of tillers per plant (0.1760*), plant height (0.4226**), dry fodder yield (0.3834**), panicle length (0.2687**), grain yield per panicle (0.8620**), total panicle number per plant (0.1851*), grain number per panicle (0.6458**) while it expressed a negative correlation with days to 50% flowering (-0.2026*) at Hisar location (Table 2.2). Grain yield per plant expressed a positive correlation with flag leaf area (0.2080*), flag leaf angle (0.1712*), number of tillers per plant (0.1667*), plant height (0.5983**), dry fodder yield (0.1932*), panicle length (0.3352**), grain yield per panicle (0.7920**), total panicle number per plant (0.2472**), grain mass (0.2890**), grain number per panicle (0.4557**), while it expressed a negative correlation with days to 50% flowering (-0.3092**), at Bawal location (Table 2.1).

Number of tillers per plant (0.176*, 0.166*), dry fodder yield (0.383**, 0.193*) grain yield per panicle (0.862**, 0.792**) and grain number per panicle (0.645**, 0.457**) had positive significant correlation with grain yield at Hisar and Bawal locations respectively which indicated major yield attributing traits were played significant role. Kumar *et al*., (2014) evaluated a set of 26 pearl millet hybrids at Regional Research Station, Bawal, CCSHAU under rainfed conditions to estimate the genetic parameters, correlation and path coefficient analysis for yield and its component traits.

Significant differences were observed among the hybrids for all the characters studied. Genetic variability and character association among the twenty advanced hybrids, twelve R line and twelve B lines of pearl millet was studied by Dapke *et al*., (2014) for ten quantitative traits. Considerable amount of variation was observed for all the genotypes in their mean performances with respect to the characters studied that indicated presence of sufficient variability and scope for further selection and breeding superior and desirable genotypes. Ezeaku *et al*., (2015) studied 24 parental lines of pearl millet A/B pairs along with seed parent across five locations for eight characters to determine yield and yield component relationships, heritability estimates as well as genetic advance. Correlation coefficient analysis showed that stand count, plant height and head weight are significantly and positively correlated with grain yield while days to 50% flowering was significant but negatively correlated with grain yield.

Conclusion

The present study conclusively revealed that the performance of various inbred lines for various traits including grain yield and dry fodder yield were significantly different over locations as well as within a location. Different traits revealed different magnitude of mean range, GCV, PCV, co-efficient of variation, heritability and genetic advance. This indicated adequate genetic variation in the present material for various traits. The selection gains each trait will be determined by heritability and genetic advance and the selection intensity applied during selection phase. Accordingly traits with high variability and high genetic advance are more amenable for selection where as traits with high heritability and moderate to low genetic advance would need high selection intensity for tangible selection gain. The correlation co-efficient revealed that traits *viz*., stem thickness, plant height, panicle length, grain yield per panicle and panicles per plant were positively associated with grain yield and dry fodder yield. Hence selection for these yield components would result into grain yield improvement. The yield component at Bawal and Hisar location partially varied and these traits should be considered while basing selection at optimal (irrigated) and drought prone (rainfed) location.

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Table 1. Mean, range, co-efficient of variation (phenotypic and genotypic), heritability (broad sense), and genetic advance (as % of mean) for various characters at Hisar and Bawal locations.

No.	Characters		Mean ± SE	Range	Co-efficient of variations (%)		Heritability (broad sense)	Genetic advance as % of mean
					PCV	GCV		
1	Leaf Rolling	HR	2.71±2.33	1.00-4.5	29.23	25.06	73.54	1.20
		BL	4.38±0.32	2.16-6.50	31.39	28.60	83.05	2.35
2	Leaf Area (cm²)	HR	65.13±4.34	22.24-149.32	41.92	40.26	92.24	51.86
		BL	83.64±8.48	34.48-148.24	35.03	30.21	74.33	44.88
3	Flag Leaf Angle	HR	41.20±2.18	14.66-76.00	35.00	33.75	93.0	27.63
		BL	30.81±1.80	13.233-57.31	29.29	27.44	87.78	16.32
4	Tillers/ Plant	HR	3.60±0.33	1.83-6.33	29.34	24.79	69.54	27.63
		BL	2.94±0.18	1.00-5.20	33.17	31.30	89.01	1.78
5	Stem Thickness (mm)	HR	13.46±0.83	10.91-16.89	12.51	6.15	24.2	0.84
		BL	12.69±0.98	9.10-17.34	16.74	9.87	34.76	1.52
6	Plant Height (cm)	HR	154.86±8.70	90.66-212.833	20.99	18.55	78.07	52.29
		BL	136.76±6.86	71.80-208.46	25.89	24.36	88.5	64.56
7	Grain Yield (g)	HR	51.81±3.18	8.03-118.05	58.38	57.38	96.6	60.19
		BL	10.96±0.66	1.57-45.71	69.52	68.70	97.64	15.33
8	Dry Fodder Yield (g)	HR	588.16±10.27	125.00-1676.66	60.36	60.29	99.74	729.56
		BL	93.61±4.62	36.66-430.00	66.61	66.05	98.32	126.31
9	Days to 50% Flowering	HR	53.51±1.32	46.33-59.33	6.66	5.06	57.56	4.23
		BL	47.95±0.82	41.33-55.66	6.77	6.06	80.37	5.37
10	Panicle Length (mm)	HR	190.74±8.17	134.30-308.80	20.02	18.56	85.97	67.65
		BL	188.92±8.66	123.66-313.33	21.35	19.78	85.87	71.36
11	Grain Yield Per Panicle (g)	HR	14.78±1.26	1.51-38.10	59.97	58.07	93.79	17.13
		BL	4.23±0.25	1.01-13.37	64.18	63.32	97.35	5.45
12	Grain Volume	HR	9.48±0.87	4.40-16.01	33.33	29.15	76.46	4.98
		BL	8.59±0.73	3.82-15.13	32.83	29.21	79.17	4.60
13	Panicles/ Plant	HR	3.69±0.35	1.93-6.33	30.52	25.34	68.96	1.60
		BL	2.80±.25	1.00-5.2	37.44	34.02	82.56	1.78
14	Grain Mass (1000 Grain Wt.)	HR	6.36±0.60	2.30-10.67	35.38	31.27	78.11	3.62
		BL	5.66±0.51	1.81-10.08	35.10	31.36	79.84	3.26
15	Grains/ Panicle	HR	2659.72±156.50	202.33-8405.66	66.31	65.50	97.59	3545.59
		BL	828.72±87.23	231.00-2776.33	70.97	68.54	93.27	1130.14
16	Drought Response Index (DRI)	HR	1.30±0.016	0.21-3.98	75.68	75.65	99.92	2.02
		BL	1.30±0.017	0.20-3.98	75.69	75.65	99.91	2.02
17	Drought Susceptible Index (DSI)	HR	0.93±.0075	0.20-1.22	28.52	28.49	99.75	0.54
		BL	0.93±0.0096	.20-1.22	28.53	28.48	99.6	0.54
18	Canopy Temperature (°c)	HR	32.68±0.95	31.34-34.34	4.73	1.95	17.11	0.54
		BL	30.91±0.17	29.70-31.70	1.53	1.17	59.24	57.83
19	Relative Water Content (%)	HR	86.07±1.78	76.14-92.82	5.26	3.82	52.75	4.92
		BL	83.51±2.96	67.83-89.68	7.57	4.34	32.91	4.28
20	Osmotic Potential (MPA)	HR	3.66±0.15	1.75-5.00	25.77	24.66	91.57	1.78
		BL	3.55±0.33	1.72-4.82	25.33	16.35	58.35	1.08
21	Chlorophyll Fluorescence	HR	0.69±0.010	0.58-0.76	5.79	5.14	78.98	0.06
		BL	0.67±0.008	0.56-0.74	5.82	5.36	84.98	0.06

Table 2.1. Phenotypic Correlation Co-efficients between different Morho-physiological characters in pearl millet genotypes at Hisar location.

Character	Leaf Rolling	Flag Leaf Area (cm²)	Flag Leaf Angle	Tillers/ Plant	Stem Thickness (mm)	Plant Height (cm)	Dry Fodder Yield (g)	Days to 50% Flowering	Panicle Length (mm)	Grain Yield Per Panicle (g)	Panicles/ Plant	Grain Mass (1000 Grain Wt.)	Grains/ Panicle	Drought Susceptible Index	Canopy Temperature (°c)	Relative Water Content (%)	Osmotic Potential (MPA)	Chlorophyll Fluorescence
Leaf Rolling	**1.000**	0.1429	0.0891	-0.1132	0.1640*	-0.0563	-0.1049	-0.0129	0.0842	0.0112	-0.111	0.0828	0.012	0.0657	-0.1146	0.0585	-0.1556	0.0293
Flag Leaf Area (cm²)	0.1429	**1.000**	0.1221	-0.1473	0.1741*	0.4781**	0.6044**	-0.0064	0.2227**	0.2740**	-0.1834*	-0.0358	0.1947*	0.0035	0.035	-0.0538	-0.1343	-0.3726**
Flag Leaf Angle	0.0891	0.1221	**1.000**	-0.0204	-0.0787	0.2332**	0.0766	-0.1852*	0.1518	-0.0741	0.0083	0.0975	-0.127	0.0189	-0.0415	-0.087	0.0633	-0.0717
Tillers/ Plant	-0.1132	-0.1473	-0.0204	**1.000**	-0.1016	-0.0372	0.0586	-0.0945	-0.1102	-0.2383**	0.9521**	0.017	-0.1840*	0.0378	-0.0204	-0.0747	0.0831	0.0624
Stem Thickness (mm)	0.1640*	0.1741*	-0.0787	-0.1016	**1.000**	0.0764	0.1023	0.139	0.1732*	0.0093	-0.111	-0.0134	0.0121	-0.0024	-0.1416	0.0198	-0.041	-0.0085
Plant Height (cm)	-0.0563	0.4781**	0.2332**	-0.0372	0.0764	**1.000**	0.5257**	-0.0662	0.5302**	0.3892**	-0.0093	-0.1862*	0.3354**	0.1306	-0.0014	-0.1931*	0.013	-0.3022**
Dry Fodder Yield (g)	-0.1049	0.6044**	0.0766	0.0586	0.1023	0.5257**	**1.000**	0.0011	0.1508	0.4036**	0.0308	-0.0234	0.2752**	-0.02	0.0219	-0.0767	-0.1248	-0.2751**
Days to 50% Flowering	-0.0129	-0.0064	-0.1852*	-0.0945	0.139	-0.0662	0.0011	**1.000**	0.0516	-0.1566	-0.1633*	-0.1723*	0.0716	0.1694*	0.2118**	0.0494	0.0269	0.1062
Panicle Length (mm)	0.0842	0.2227**	0.1518	-0.1102	0.1732*	0.5302**	0.1508	0.0516	**1.000**	0.2839**	-0.1019	0.034	0.2418**	0.0166	0.0859	-0.1131	-0.0304	-0.1677*
Grain Yield Per Panicle (g)	0.0112	0.2740**	-0.0741	-0.2383**	0.0093	0.3892**	0.4036**	-0.1566	0.2839**	**1.000**	-0.2641**	0.0475	0.7377**	0.4682**	0.0514	-0.0705	-0.1368	-0.1811*
Panicles/ Plant	-0.111	-0.1834*	0.0083	0.9521**	-0.111	-0.0093	0.0308	-0.1633*	-0.1019	-0.2641**	**1.000**	0.0169	-0.1949	0.0408	-0.0631	-0.1485	0.0205	0.0297
Grain Mass (1000 Grain Wt.)	0.0828	-0.0358	0.0975	0.017	-0.0134	-0.1862*	-0.0234	-0.1723*	0.034	0.0475	0.0169	**1.000**	-0.4580**	-0.1832*	0.0418	0.0508	-0.0443	-0.1356
Grains/ Panicle	0.012	0.1947*	-0.127	-0.1840*	0.0121	0.3354**	0.2752**	0.0716	0.2418**	0.7377**	-0.1949*	-0.4580**	**1.000**	0.5042**	0.0028	-0.0873	-0.1015	-0.0205
Drought Susceptible Index	0.0657	0.0035	0.0189	0.0378	-0.0024	0.1306	-0.02	0.1694*	0.0166	0.4682**	0.0408	-0.1832*	0.5042**	**1.000**	0.1286	-0.2318**	-0.0308	0.0479
Canopy Temperature(°c)	-0.1146	0.035	-0.0415	-0.0204	-0.1416	-0.0014	0.0219	0.2118**	0.0859	0.0514	-0.0631	0.0418	0.0028	0.1286	**1.000**	-0.1630*	0.2312**	-0.2472**
Relative Water Content (%)	0.0585	-0.0538	-0.087	-0.0747	0.0198	-0.1931*	-0.0767	0.0494	-0.1131	-0.0705	-0.1485	0.0508	-0.0873	-0.2318**	-0.1630*	**1.000**	-0.0423	0.091
Osmotic Potential (MPA)	-0.1556	-0.1343	0.0633	0.0831	-0.041	0.013	-0.1248	0.0269	-0.0304	-0.1368	0.0205	-0.0443	-0.1015	-0.0308	0.2312**	-0.0423	**1.000**	0.1749*
Chlorophyll Fluorescence	0.0293	-0.3726	-0.0717	0.0624	-0.0085	-0.3022**	-0.2751**	0.1062	-0.1677*	-0.1811*	0.0297	-0.1356	-0.0205	0.0479	-0.2472**	0.091	0.1749*	**1.000**
Grain Yield (g)	**-0.0586**	**0.1708***	**-0.0144**	**0.1760***	**-0.0476**	**0.4226****	**0.3834****	**-0.2026****	**0.2687****	**0.8620****	**0.1851***	**0.0511**	**0.6458****	**0.5244****	**0.0297**	**-0.1716**	**-0.1292**	**-0.152**

Table 2.2. Phenotypic Correlation Co-efficients between different Morho-physiological characters in pearl millet genotypes at Bawal location.

Character	Leaf Rolling	Flag Leaf Area (cm²)	Flag Leaf Angle	Tillers/ Plant	Stem Thickness (mm)	Plant Height (cm)	Dry Fodder Yield (g)	Days to 50% Flowering	Panicle Length (mm)	Grain Yield Per Panicle (g)	Panicles/ Plant	Grain Mass (1000 Grain Wt.)	Grains/ Panicle	Drought Susceptible Index	Canopy Temperature (°c)	Relative Water Content (%)	Osmotic Potential (MPA)	Chlorophyll Fluorescence
Leaf Rolling	**1.000**	-0.0042	-0.0916	-0.0902	-0.0010	0.1970*	-0.0689	-0.0332	0.0149	0.1630*	-0.0890	0.1817*	0.0101	0.2228**	0.1499	-0.1450	0.3391**	-0.0388
Flag Leaf Area (cm²)	-0.0042	**1.000**	0.2253**	0.1011	-0.0113	0.1939*	0.3444**	0.0024	0.1583	0.1180	0.0721	0.0789	0.0729	0.0928	-0.0013	-0.0765	-0.0612	-0.1227
Flag Leaf Angle	-0.0916	0.2253**	**1.000**	0.0508	0.0891	0.3550**	0.0413	-0.1192	0.1774*	0.1259	0.1526	0.0631	0.0590	-0.1488	-0.0865	-0.0672	0.2273**	-0.2584**
Tillers/ Plant	-0.0902	0.1011	0.0508	**1.000**	-0.1999*	-0.0295	0.2211**	-0.2287**	-0.0308	-0.3237**	0.9159**	-0.0663	-0.2982**	-0.0037	-0.0856	0.1111	0.0218	0.2736**
Stem Thickness (mm)	-0.0010	-0.0113	0.0891	-0.1999*	**1.000**	0.1631*	-0.0616	-0.0439	0.1813*	0.1138	-0.1186	0.0443	0.1254	0.0298	-0.0018	0.0079	0.0058	-0.2567**
Plant Height (cm)	0.1970*	0.1939*	0.3550**	-0.0295	0.1631*	**1.000**	0.2131**	-0.2650**	0.5333**	0.5956**	0.1030	0.1216	0.3908**	-0.1613*	-0.0284	-0.0488	0.2416**	-0.3415**
Dry Fodder Yield (g)	-0.0689	0.3444**	0.0413	0.2211**	-0.0616	0.2131**	**1.000**	-0.2283**	0.2715**	0.0564	0.2046*	-0.0544	0.0463	0.0764	-0.1328	0.0924	-0.0696	-0.0513
Days to 50% Flowering	-0.0332	0.0024	-0.1192	-0.2287**	-0.0439	-0.2650**	-0.2283	**1.000**	-0.1088	-0.1512	-0.3293**	-0.1412	0.0057	-0.0564	0.1808*	0.0122	-0.2101**	-0.0280
Panicle Length (mm)	0.0149	0.1583	0.1774*	-0.0308	0.1813*	0.5333**	0.2715**	-0.1088	**1.000**	0.3944**	-0.0232	0.0144	0.3333**	-0.2178**	-0.0287	0.0336	0.1699*	-0.1532
Grain Yield Per Panicle (g)	0.1630*	0.1180	0.1259	-0.3237**	0.1138	0.5956**	0.0564	-0.1512	0.3944**	**1.000**	-0.2559**	0.2536**	0.7201**	-0.4954**	0.0467	0.0465	0.2033*	-0.2752**
Panicles/ Plant	-0.0890	0.0721	0.1526	0.9159**	-0.1186	0.1030	0.2046*	-0.3293**	-0.0232	-0.2559**	**1.000**	-0.0659	-0.2657**	0.0004	-0.1174	0.0566	0.0424	0.1650*
Grain Mass (1000 Grain Wt.)	0.1817*	0.0789	0.0631	-0.0663	0.0443	0.1216	-0.0544	-0.1412	0.0144	0.2536**	-0.0659	**1.000**	-0.3602**	-0.1579	0.0470	-0.0750	0.0021	-0.2756**
Grains/ Panicle	0.0101	0.0729	0.0590	-0.2982**	0.1254	0.3908**	0.0463	0.0057	0.3333**	0.7201**	-0.2657**	-0.3602**	**1.000**	-0.3281**	-0.0022	0.0757	0.2023*	-0.0796
Drought Susceptible Index	0.2228**	0.0928	-0.1488	-0.0037	0.0298	-0.1613	0.0764	-0.0564	-0.2178**	-0.4954**	0.0004	-0.1579	-0.3281**	**1.000**	-0.0213	-0.0941	-0.0403	0.0539
Canopy Temperature(°c)	0.1499	-0.0013	-0.0865	-0.0856	-0.0018	-0.0284	-0.1328	0.1808*	-0.0287	0.0467	-0.1174	0.0470	-0.0022	-0.0213	**1.000**	-0.2326**	0.1039	0.0318
Relative Water Content (%)	-0.1450	-0.0765	-0.0672	0.1111	0.0079	-0.0488	0.0924	0.0122	0.0336	0.0465	0.0566	-0.0750	0.0757	-0.0941	-0.2326**	**1.000**	-0.1699*	0.0355
Osmotic Potential (MPA)	0.3391**	-0.0612	0.2273**	0.0218	0.0058	0.2416**	-0.0696	-0.2101**	0.1699*	0.2033*	0.0424	0.0021	0.2023*	-0.0403	0.1039	-0.1699*	**1.0000**	0.0323
Chlorophyll Fluorescence	-0.0388	-0.1227	-0.2584**	0.2736**	-0.2567**	-0.3415**	-0.0513	-0.0280	-0.1532	-0.2752**	0.1650*	-0.2756**	-0.0796	0.0539	0.0318	0.0355	0.0323	**1.000**
Grain Yield (g)	**0.1382**	**0.2080***	**0.1712***	**0.1667***	**0.0019**	**0.5983****	**0.1932***	**-0.3092****	**0.3352****	**0.7920****	**0.2472****	**0.2890****	**0.4557****	**-0.3990****	**-0.1032**	**0.0810**	**0.1447**	**-0.2107****

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Journal article:

Toker C, (1998). Adaptation of kabuli chickpeas (*Cicerarietinum* L.) to the low and high lands in the West Mediterranean region of Turkey. Turk J Field Crop 3:10-15.

Toker C and Canci H, (2003). Selection of chickpea (*Cicerarietinum* L.) genotypes for resistance to ascochyta blight [Ascochytarabiei (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO, (2006). Estimation of outcrossing rate in chickpea (*Cicerarietinum* L.) sown in autumn.Euphytica 151: 201-205.

Article by Digital Object Identifier (DOI) number:

Yasar M, Ceylan FO, Ikten C and Toker C, (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicerarietinum* L.). Euphyticadoi:10.1007/s001090000086

Book:

Toker C, (2014). YemeklikBaklagiller. BISAB, Ankara.

Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM, (2007). Abiotic stresses. In: Chickpea Breeding and Management,YadavSS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:

FAOSTAT J ,(2013). http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 15 May 2013.

Dissertation (Thesis):

Yasar M, (2012). Penetrance and expressivity of double podding characteristic in chickpea (*Cicerarietinum* L.).Dissertation, Akdeniz University, Antalya.

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Abbreviations

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