LINE × TESTER ANALYSES USING SUNFLOWER (*HELIANTHUS ANNUUS* L.) INBRED LINES

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Dedicated to my parents,
For endless support & encouragement.

ABSTRACT

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in Pakistan. In the present study, plant material comprised of 8 cytoplasmic male sterile lines and 3 restorers was used to develop F_1 hybrids through line×tester mating design. The experiment was conducted in the research area of University College of Agriculture, University of Sargodha during 2014 by using Randomized Complete Block Design (RCBD) in triplicate. Genetic variability, general combining ability and specific combining ability were determined. The lines B-2, B-6 and B-21 expressed highly significant GCA for days to flowering, head diameter, plant height, internodal distance, stem girth, 100-achene weight and achene weight per head. Among testers, R-12 expressed highly significant GCA for days to flowering, plant height, head diameter, internodal distance, stem girth, number of leaves per plant, number of seeds per head, 100-achene weight and achene weight per head. The cross CMS-21×R-18 revealed high SCA for days to flowering, plant height, stem girth, achene yield per plant, number of seeds per head and oil contents. Achene yield and oil contents showed significantly positive association with days to flowering, head diameter and head shape. The cross CMS-2×R-12 exhibited significantly high heterosis for achene weight per head. The results of analysis of variance were determined among enteries for all the traits at significance level ($p \geq 0.01$).

CHAPTER I

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is indigenous to North America and was originated about 3000 B.C. Sunflower belongs to family Asteraceae having 67 brown species. These species include weedy types, rare types and some of them showing natural vegetation. There are only two species which are cultivated as a food source; *Helianthus annuus* L. is cultivated for oil characteristics and *Helianthus tuberosus* L. (also called Jerusalem artichoke) is cultivated for its tubers (Kistler and Shapiro, 2011).

It is an important oilseed crop all around the world including Pakistan. It ranks fourth among oilseed crops all over the world. It is under cultivation in about 70 countries and mainly cultivated in China, Russia, United States, Argentina, France, Romania, Spain, Hungary and Turkey (Rauf *et al*., 2012). In Pakistan sunflower as oil seed crop was introduced in early 1960s due to its high oil contents. Sunflower oil consists of number of fatty acids including stearic acid, palmitic acid, oleic acid and linoleic acid. Sunflower oil contains about 90% oleic acid and linoleic acid of the total fatty acids (Kinman and Earle, 1964).

In sunflower, inbred lines are produced through inbreeding and depend upon their heterosis performance. Selection has been done within and between inbred lines to get hybrid vigour (Skoric *et al.*, 2007). When two parents with dissimilar genotypes are crossed, first generation offspring is hybrid (Weiss, 2000). The hybrid seed that propagated ensure uniformity. The significance of hybrid cultivars in sunflower has improved in recent times due to its superior seed yield as compared to open cross-pollinated varieties in various countries of the world. Hybrid of sunflower is more established, extremely self-fertile, high yield and extra uniform at maturity (Kaya and Atakisi, 2004).

Line × tester analysis is one of the most important techniques to evaluate maximum number of inbreds for combining abilities (GCA and SCA) to understand the genetic basis of plant parameters (Miller and Frick, 1997). Combining ability was used to understand the nature of gene action that was concerned with the expression of quantitative characters and to estimate the performance of progenies. Both additive and non-additive gene actions were involved to determine yield and other traits (Mijic *et al*., 2008). Because heterosis takes place in hybrids that are developed by crossing different inbred lines that are genetically different from each other. Plant breeders can take advantage from these phenomena of heterosis during selection for the development of high yielding and resistant hybrids (Skoric, 1992).

Mahgoub (2011) proposed a model to determine the combining abilities (GCA & SCA) of each of the parent that is used as male or female in the hybrid seed development. The purpose of the study was to evaluate the general and specific combining ability effects after and before partitioning, to estimate the contribution of each of the parent when it is used in cross combination as male or female parent for development of hybrid seed, to determine the maternal effect in the form of general and specific combining abilities and the association between reciprocal and maternal effect. According to the Griffing model (Griffing 1956), estimated GCA is equal to the standard GCA for each of the parent either it used as female or male parent in cross combination while the average difference between female GCA effect and male GCA effect provided accurate maternal effects that proves involvement of favorable alleles (additive gene action). According to the proposed model, the average SCA effect of each cross is equal to its reciprocal effect, it proves that reciprocal effect provide précised result of interaction between nuclear and cytoplasmic genes. In view of above discussion, present study was planned to estimate the general and specific combining ability of inbred lines by using line × tester crossing technique and to evaluate the performance of developed hybrids along with standard / commercial hybrids.

Objectives of the study

Keeping in view the above discussion, the present study was designed with the following objectives:

- ❖ To evaluate sunflower inbred lines for general and specific combining ability.
- ❖ To develop high yielding hybrids with improved achene yield and oil quality by using line x tester method.

CHAPTER II

REVIEW OF LETERATURE

Suzer and Atakisi (1993) conducted the research to evaluate yield and its components in sunflower hybrids (semidwarf hybrids included DO-855, Sunbred-265, an average heighted hybrid Trakva-259 and tall hybrid Tordillo) with differed plant heights in Random Complete Block Design with four replications. They reported that the semidwarf hybrids had high seed yield with high plant population (71430 plants per hectare) than average height (47620 plants per hectare) and tall hybrids (35710 plants per hectare). Plant height and plant population both had significant effect on seed yield and its components. From average and tall hybrid, the average plant population was more suitable for seed and oil yield than tall hybrid plant population.

Gangappa *et al*. (1997) conducted an experiment to determine the direction and magnitude of combining abilities of 72 hybrids that developed from 6 CMS lines and 12 testers. The parental lines (RHA-99RTNBr, RFIA-299, CMS-BO2 and RHA-284) exhibited genes for earliness while the hybrids (CMS-852×RFIA-299RTNBr) showed best combination for days to 50 percent flowering. The parents (CMS-lines: CMS-8S2 and CMS-207, testers: RFA-214Br and RFIA-6D-5-3-6) were appeared to be transmit genes for high seed yield and therefore utilized in hybrid seed production for better yield. The hybrids (CMS-234×RHA-2284 and CMS-302×RFIA-273) showed best combinations for oil contents and seed yield. All the traits were found to be under control for additive and non-additive gene action with predominance.

Sassikumar *et al*. (1999) determined the combining ability in sunflower hybrids that developed 56 crosses from four cytoplasmic male sterile lines and fourteen testers through line× tester mating design. The combining abilities (General and Specific) for all parameters had been studied. They reported that the parents, CMSPET-2, BLCI-78 and CMSPF showed high general combining ability for many of the plant traits like head diameter, 100-seed weight, seed yield per plant and oil yield. The crosses included CMSF× RLC-215, CMSI× BLC-178 and CMSPET-2×RHA-RR-1 exhibited positive and significant specific combining ability for many of the traits especially yield contributing traits.

Rojas *et al*. (2000) studied line×tester analysis favored by Griffing (1956) statistical method for assessment of combining ability. They observed that multi-branched CMS lines and restorers were not used in sunflower production commercially. Due to economic value of exclusively F_1 hybrid, factorial mating design or North Caroline design II was the superlative method to measured combining ability in cross pollinated crop species.

Laureti and Gatto (2001) determined the combining abilities (General combining ability and Specific combining ability) of different 245 tests cross progenies obtained by crossing CMS lines and restorer lines and experiments were carried out in two different locations. They reported that the combining abilities (GCA&SCA) of the parents were significant for all the studied characters with randomly selected lines. When testers were selected on the basis of first year performance, the general combining ability of the new CMS lines and specific combining ability of the new testers were non-significant for seed yield but significant for all other characters. The general combining ability of testers were more than that of CMS lines indicated that selection of testers could be more valuable than selection of CMS lines.

Ortis *et al.* (2005) conducted an experimental trial to develop 80 F_1 hybrids by using 20 cytoplasmic male sterile lines and 4 restorers. Test crossed progenies were checked in three different environments. The traits like plant height, days to 50% flowering, 1000-kernal weight, seed yield and oil contents were greatly contributed by the parents (CMS-lines & testers), they reported predominant role of additive components while line × tester mating design showed highest contribution towards seed yield indicating the presence of non-additive gene effect.

Masood *et al.* (2005) studied the combining abilities (GCA, RE & SCA) for inherited agronomic characters by using 16 genotypes in diallel analysis. The result exhibited significant combining abilities (GCA, RE & SCA) for all the characters except days to maturity with non-significant reciprocal effect. The general combining effect was less than specific combining effect that exhibited more share of non-additive gene action. Both additive variance and non-additive variance were involved in description of all the studied traits. The three inbred lines (ARI, TF-11 and TF-4) exhibited good general combining ability for all the characters while ARI and GUL were best general combiners for seed yield. The hybrids (ARI, PESH, TF-4 and TF-335) gave better performance for all the studied characters. Reciprocal crosses gave better results for all the studied traits than direct crosses.

Haq *et al.* (2006) evaluated 17 F_1 hybrids. According to the results, the crossed F_1 hybrid (ORL-1× RL-110) exhibited maximum heterosis for seed yield/plant while the cross (ORl-43× RL-110) gave maximum seed yield/plant. The cross combination (OR2 × V-214) developed tall plants with maximum days (82) to flower initiation and flower completion also with maximum number of leaves and heat diameter. The cross (ORl-1× RL-110) gave maximum oil contents and the cross (ORl-43× RL-84) gave maximum 100-seed weight.

Habib *et al.* (2007) developed 84 F_1 hybrids by using 14 cytoplasmic male sterile lines and 6 restorers in a line × tester mating design and found significant difference among crosses for all the studied traits. The crosses, ORI-3 × RL-77 and ORI-3 × RL-84 exhibited high heterosis and heterobeltiosis for stem girth. The cross ORI-3 × RL-84 showed maximum increase over mid and high parent for 100-achene weight. The cross ORI-6 × RL-27 and ORI-47 × RL-69 showed maximum heterosis and heterobeltiosis for oil content. The crosses ORI-29 × RL-84 and ORI-3 × RL-77 revealed maximum heterosis and heterobeltiosis for head diameter. The cross, ORI-20 × RL-77 showed maximum significant heterosis and heterobeltiosis for plant height. The hybrid, ORI-3 × RL-77 exhibited maximum heterosis for oil content and number of seed per head.

Hladni *et al.* (2007) conducted an experiment to develop 21 F_1 hybrids by using 7 cytoplasmic male sterile lines and 3 testers in line × tester mating design. Stable and high yielded hybrids of sunflower depend upon interspecific hybridization and exhibited heterosis that occurred in F_1 generation. They found significant difference in mean values for all the studied characters (seed yield per plant, total number of seeds per head and 1000- seed weight). The results exhibited that heterosis for seed yield per plant (55.8 to 223.2%) was highly significant and positive as compared to parents (CMS-lines & restorers) average (98.4 to 274.1%) while less heterosis value was present for total number of seeds per head (69.6 to 203.7%) as compared to parents' average (47.6 to 183.3%). The heterosis for 1000- seed weight was 26.5 to 48.8% as compared to the parents average (-42.4 to 30.9%).

Mijic *et al*. (2008) proposed that the GCA and SCA both were important for oil yield, oil content and yield improvement. He found that GCA was greater in magnitude than SCA for above said traits.

Khan *et al.* (2008a) determined the magnitude of combining ability and approach of gene action by using 25 F_1 sunflower hybrids that developed by crossing 5 CMS lines and 5 testers in line × tester mating design at two different locations. The results revealed that combining abilities (GCA& SCA) were significant for all the studied traits like days to 50 % flowering, days to maturity, number of seeds per head, 1000-seed weight, seed yield and oil contents. The cytoplasmic male sterile lines (TS-4 & TS-335) and the testers (TR-5 and TR-6023) were good general combiners for all the studied traits. The specific combining ability effect was greater in magnitude than general combining ability that exhibited more expression of non-additive gene action. The GCA/SCA ratios revealed higher proportion of non-additive gene action among CMS lines (TS-18 & TS-335) and testers (TR-5 & TR-13) for most of the studied parameters. The F_1 hybrids in crossed combinations (TS-17×TR-6, TS-335×TR-3, TS-17× TR- 13 and TS-18×TR-5) exhibited high SCA effects for seed yield in one location while the remaining F_1 hybrids showed high SCA effects for seed yield second location.

Khan *et al.* (2008b) evaluated 16 F_1 hybrids by using 8 inbred lines from 2003-04 at ARI Tarnab to determine mid parent heterosis and high parent heterosis for oil contents and seed yield. The results revealed that parents and crosses showed high genetic difference for seed yield, moisture factor, harvest index and oil content. F_1 hybrids exhibited 5.6 to 185.02 % and -9.06 to 181.73% for seed yield. Hybrid TS-18 × 291RGI exhibited maximum mid and high parent heterosis for seed yield. TS-335 × 291RGI high positive mid and high parent heterosis for harvest index. TS-228 × 291RGI gave optimum mid and high parent heterosis for moisture factor while TS-535 × 291RGI showed maximum mid and high parent heterosis for oil contents.

Tavade *et al*. (2009) conducted an experiment in diallel mating design to developed 45 F_1 hybrids after crossed ten restorers. These hybrids were checked for different quantitative traits. Among these restorers ID-3/147 R was considered good general combiner for seed yield/plant and oil contents. The hybrid that developed by crossing BC-3-IR x ID-3/147 R gave maximum seed yield including oil contents. Therefore, it revealed that ID-3/147 R had potential to transfer desired characters in the progeny.

Dudhe *et al.* (2009) evaluated heterotic potential of sunflower restorer lines by using half diallel with 45 hybrids and their parents. Out of 45 crossed F_1 hybrids, 40 crosses exhibited high significant heterosis over parents. Among parents, 270R, 272R, 273R, 278R and 859R exhibited superior in hybrids combination. Smililarly, Karasu *et al.* (2010) conducted an experiment during 2005-2007 to study the combining abilities (GCA&SCA) of 6 F_1 sunflower hybrids that developed through crossing among 3 cytoplasmic male sterile lines and 2 testers. The ratio of GCA & SCA variances were less than 1 for head diameter and plant height during both years, for 1000-seeds weight and number of seeds per head during 2007 and seed yield during 2006. During 2006, number of seeds per head and 1000-seeds weight; for seed yield and plant height during 2007 had significant additive gene effect while the ratio of GCA and SCA for these traits were more than 1. The non-additive gene effects were more helpful than other polygenic actions. The parent lines CMS-10 and RHA-10 exhibited superior combiners with high positive general combining ability effect in yield and its attributes. The crosses developed from these 2 superior parents might be formed good hybrids in terms of seed yield. The entire F_1 hybrids exhibited significant and positive heterosis for seed yield.

Mohanasundaram *et al*. (2010) developed 48 hybrids by using 24 cytoplasmic male sterile lines and 2 testers in line x tester crossing fashion to determined the combining ability for seed yield. Non-additive genetic variance played dominant part in the inheritance of all studied traits (plant height, head diameter, 100 seed weight, seed yield per plant, days to maturity and 50% flowering). The two lines (27, 436) and a tester (234A) regarded to be as dominant parents as they performed significant GCA for seed yield per plant and their hybrids considered to be superior hybrids as they performed significant SCA for seed yield per plant.

Tan (2010) determined the combining abilities of newly developed 20 F_1 hybrids by crossing 5 cytoplasmic male sterile lines and 4 restorers in line × tester mating design. The studied characters were days to flowering, days to maturity, plant height, stem diameter, head diameter, 1000-seed weight, seed yield, oil content, fatty acids (oleic acid, linoleic acid, stearic acid and palmitic acid. The combining abilities (GCA & SCA) variances were extremely significant for most of the studied parameters. The ratio of GCA and SCA variances represented the presence of non-additive gene action for the studied traits except head diameter, stem diameter, plant height, stearic and palmitic acid content but additive and non-additive gene actions were observed for 1000-seed weight, stem diameter, hull percentage, seed yield and oil content at curve position. The results exhibited highly significant GCA for all the studied traits while insignificant SCA for majority of the parameters. Based on GCA effect, the cytoplasmic male sterile lines (CMS-0043, CMS-0046, CMS-0583, CMS-0195 and CMS-0704) and testers (Rf-0951, Rf-0708, Rf-0951 & Rf-1097) exhibited good general combiners for most of the studied parameters that may be further used in development of high yielded hybrid seeds.

Chandra *et al.* (2011) developed 42 F_1 hybrids by using 7 cytoplasmic male sterile lines and 6 testers in line × tester design. The resultant hybrids with their parents were checked at three different locations. The analysis of variance for combining ability exhibited that specific combining ability was higher in magnitude than general combining ability for all the traits except oil contents indicated that predominance of non-additive gene action for all the traits while additive gene action for oil contents.

Nasreen *et al.* (2011) conducted an experiment to develop high yielded sunflower 36 F_1 sunflower hybrids by using 6 CMS lines and 6 testers. The traits (head diameter, leaf area, 1000-seed weight, harvest index and yield hā$^{1)}$ were studied for yield and its components. The result of analysis of variance exhibited low to high range of genetic variability between hybrids for all the studied parameters. The hybrids were evaluated on the basis of genetic difference among the both parental lines. The results revealed that the hybrid (CMS-H55-2-2-1× C206R) gave high heterotic value for yield hā^{1}. The hybrids (CMS-HAR-1× RHA-854 & CMS-64 × C-206R) with optimum genetic variability for yield and its components were further utilized in heterosis breeding program.

Andakhor *et al*. (2012) developed 48 F_1 hybrids by crossed among 8 cytoplasmic make sterile lines and 6 restorer lines along with 2 commerical hybrids were planted in simple lattice design in two replicates. According to the results, analysis of variances exhibited significance difference between genotypes for all the traits including plant duration, plant height, head diameter, 1000-seed weight, seed yield and oil contents. Line × tester fashion showed significant effects of parents, hybrids and their interaction for all the studied traits. The lines (AF80-488/1/2/1 and AF80-488/2/1/1) showed good combining results for seed yield and 1000-seed weight while the restorer lines (RF81-25 and RF81-30) exhibited positive GCA for seed yield and 1000-seed weight. The F_1 hybrids (AF80-460/2/1/1× RF81-25 and AF8-6937× RF81-30) exhibited significant positive specific combining ability for seed yield and oil contents. The estimation of low narrow heritibility for all studied characters that showed the importance of non-additive gene action.

Kang *et al*. (2013) conducted an experiment in line × tester mating design by using 9 cytoplasmic sterile lines and 4 testers to develop 36 hybrids. Among parental lines, 2 lines (G-93 & G-79) and 2 testers (A-85 & A-5) of them showed highly significant GCA. Crossed among them revealed highly significant SCA for head diameter, 100-achene weight per plant, oil contents, 50% flowering and days to maturity.

Khan *et al*. (2013) conducted an experiment at two different locations to determine heritability and genotype by environment (GE) interaction for 25 hybrids developed by crossing 5 cytoplasmic male sterile lines and 5 testers and studied traits including oil yield, plant height, head diameter, leaf area and stem girth. The analysis of variance at both locations regarded high significant difference within the genotypes of all characters. A significant interaction (Genotype by location) was observed for all studied characters except plant height and stem girth. On the other side, it revealed high heritability for oil yield and head diameter followed by leaf area, seed yield and stem girth. Plant height was observed to be low heritable trait and greatly influenced by environment. After testing genotypes, it assumed that heritability increased over a wide range of locations.

Andakhor *et al*. (2013) conducted an experiment to determine the combining ability and heritability of yield and its components of 48 F_1 hybrids (8 CMS lines & 6 restorers) and two commercial hybrids by using line × tester mating design. The analysis of variance results exhibited significant variation among parents and their crosses for all parameters (seed thickness, seed width, seed length, seed yield and oil content). All the studied parameters showed significant effects with yield except seed thickness. The resultant hybrid (AF-6937 × RF81-30 exhibited significant positively SCA effects for seed width seed yield and oil content while the hybrids (AF8-6937 × RF81-30 and AF80-460/2/1/1 × RF81-25 × RF81-25) showed significant SCA effects for seed yield and oil content. Estimated low narrow sense heritability for all parameters except seed thickness indicated the significance of non-additive gene action.

Saleem-ud-din *et al*. (2014) developed an experiment to study line x tester mating design to determine genetic difference due to yield and its related components in 16 F_1 hybrids that developed by crossing 4 cytoplasmic male sterile lines and 4 testers. The parents (CMS line CMS HA-99 & restorer RHP-76) revealed high GCA effect with maximum additive gene action and considered as good general combiner and also had potential to improve studied characters. Among sixteen hybrids, CMS line CMS HA-99 × restorer RHP-76 was considered to be excellent specific combiner for seed yield per head. It was also noted that maximum results may be obtained if this hybrid included in selection and hybridization for the above studied traits.

Memon *et al.* (2014) determined phonological, seed yield and oil traits in sunflower. They were conducted an experiment to develop 18 F_1 hybrids by using 6 cytoplasmic male sterile lines (PAC-0505, HO-1, Hysun-33, T-4-0319, CMS-03 and Peshawar=93) and 3 restorer lines (SF-187, PAC-64-A and PAC-0306) in line × tester fashion. Significant difference was observed among parents and their crosses for phonological, seed yield and oil traits. Line × tester analysis determined the significance of dominant variance while importance of mean squares of the parents estimated additive variance. Data was taken to estimate physiological traits, yield traits and heritability of earliness. They reported that degree of dominant genes was greater than unity of genes so, the main role of dominant genes was very important. Therefore, estimated heritability was normally low to moderate due to dominant variance. The results exhibited that if chance of recombination was given to desirable genes during selection for traits. Correlation studies suggested that head diameter, 1000-achene weight and number of leaves per plant showed strong relation between with seed and oil yield so; these traits may be used to improve seed and oil yield in sunflower breeding.

Razzaq *et al*, (2014) conducted an experiment to determine the genetic variability and relationship of agronomic traits (quantitative traits including days to 50% flowering, days to 50%maturity, plant height, leaf area, head diameter, number of leaves per plant, percentage of filled achenes, 1000-achene weight and achene weight per head, qualitative traits including leaf shape, head shape, leaf habit, achene size, head angle at maturity, achene colour and achene strips) of ten accessions. The research was subjected to determine variance analysis, path way coefficient analysis and correlation analysis. The accessions (A-79, HBRS-1, G-8 & G-33) showed significant results for many traits (leaf area, number of leaves per plant, head diameter, 100-achene weight, percentage of filled achene and achene weight per head). The phenotypic relationship was insignificant of all the studied parameters with achene weight per head while genotypic relationship of achene weight was positively significant with head diameter, 100-achene weight, number of leaves per plant and leaf area. Head diameter, achene weight per head, leaf area and 100-achene weight per head was indirectly highly positively affected by days to 50% maturity while leaf area, achene weight per head and days to 50% maturity was directly highly affected by 100-achene weight. The positive results of head diameter, leaf area and 100-achene weight may be suggested to be used in development of high yielding sunflower hybrid breeding program.

Hladni *et al.* (2014) developed 39 F_1 hybrids by using 13 new cytoplasmic male sterile lines and 3 testers. They reported that the parents and their crosses were significantly different in their mean values for plant height and head diameter. The parent with more plant height was dominant over parent with head diameter while parent with more head diameter was dominant over parent with average plant height. The results exhibited significantly high positive general combining ability for plant height and head diameter in CMS line (NS-G-7) and in tester (RHA-N-49). In the newly developed hybrids, significantly high positive specific combining ability was present in NS-G-8× RUS-RF-OL-168 for head diameter and NS-G-1× RHA-N-49 for plant height. The cutoplasmic male sterile line (A-line) were more contributed towards plant height and head diameter than restorers. By GCA/SCA ratio in the F_1 hybrids which was smaller than unit, it was confirmed that non-additive gene action played dominant role in the inheritance of both traits (plant height and head diameter).

CHAPTER III

MATERIALS AND METHODS

The research trials were conducted in the experimental area at University College of Agriculture, University of Sargodha, Sargodha, during the crop growing season 2014. The experimental material comprised of ten CMS lines and three testers planted in the field and crossed in line × tester fashion, each female line was crossed with each of three testers to develop F_1 crosses. CMS lines were covered with kraft paper bags to avoid pollen contamination. Pollen grains from tester lines were collected and dusted on the CMS lines to develop F_1 hybrids. Continued cycle of pollination was carried out. Sunflower heads were harvested, dried and seeds were threshed from capitula separately. The seeds from all selected parents and F_1 hybrids were stored in kraft paper bags and placed in cool condition to protect from sunlight.

3.1. Evaluation of hybrids

The seeds of 11 parents (8 CMS lines & 3 restorers) and 30 hybrids (CMS-2×R-5, CMS-2×R-12, CMS-2×R-18, CMS-3×R-5, CMS-3×R-12, CMS-3×R-18, CMS-5×R-5, CMS-5×R-12, CMS-5×R-18, CMS-6×R-5, CMS-6×R-12, CMS-6×R-18, CMS-7×R-5, CMS-7×R-12, CMS-7×R-18, CMS-9×R-5, CMS-9×R-12, CMS-9×R-18, CMS-16×R-5, CMS-16×R-12, CMS-16×R-18, CMS, CMS-17×R-5, CMS-17×R-12, CMS-17×R-18, CMS-20×R-5, CMS-20×R-12, CMS-20×R-18, CMS-21×R-5, CMS-21×R-12 and CMS-21×R-18) along with two commercial hybrids Hysun-33 and S-278 were sown in the field during February (2015) in randomized complete block design (RCBD) with three replications keeping plant to plant and row to row distances 23 and 76cm, respectively. Normal cultural practices were carried out from germination to maturity of the crop. The seeds of two CMS-lines (CMS-17 and CMS-20) did not germinate during hybrid evaluation so we discarded the seeds of their F_1 hybrids and evaluated the remaining 24 F_1 hybrids and compared them with two commercial hybrids. At maturity data of different morphological traits were collected from 5 randomly selected plants from each line within each replication.

3.2. Traits studied

3.2.1. Days to flower initiation

Number of days from sowing to the opening of disc when yellow florets just appeared were counted and recorded for 5 randomly selected plants of each line within replication.

3.2.2. Days to flower completion

Number of days from planting to physiological maturity when flowers/heads ended to open completely were counted and recorded.

3.2.3. Plant height (cm)

Plant height was measured with the help of meter rod from ground level to top of the head. Average was computed after taking plant height of 5 randomly selected plants from each replication.

3.2.4. Number of leaves per plant

The leaves originated from the main stem were calculated at maturity for 5 plants in each replication and average was recorded.

3.2.5. Stem girth (cm)

Stem girth was measured at maturity from the center of main stem with the help of measuring tape for 5 plants from each replication and average was calculated.

3.2.6. Leaf area (cm^2)

Leaf area was measured by leaf area meter for 5 fresh weighted leaves from each replication and average was calculated.

3.2.7. Internodal distance (cm)

Internodal distance of sixth node of 5 randomly selected plants from each replication was measured with help of measuring tape and average was calculated.

3.2.8. Head diameter (cm)

Head diameter was measured at maturity with the help of measuring tape for 5 plants from each replication and average was recorded.

3.2.9. Distance from head to soil surface (cm)

Distance from head to soil surface was measured at maturity by using measuring rod of 5 plants from each replication and average was calculated.

3.2.10. Number of seeds per head

Numbers of seeds were calculated by seed counter from 5 randomly selected heads and average was recorded.

3.2.11. 100-achene weight (g)

100 seeds from 5 plant heads of each line within each replication were randomly sampled and weighed in grams on an electrical balance.

3.2.12. Achene weight per plant (g)

Heads of 5 five plants from each line within each replication was threshed manually and weighed with the help of an electrical balance. Achene weight per plant also refers as achene weight per head.

3.2.13. Oil content (%)

Soxhlet apparatus was used to estimate the oil contents of developed 24 hybrids along with 2 commercial hybrids. Firstly, samples were oven dried for 2-3 hours and weighed than crushed and placed into soxhlet apparatus along with hexane and kept for 5 hours. The oil contents percent was calculated by using following formula:

$$\text{Oil content (\%)} = [\text{Weighed oil} / \text{Weighed oven dried seeds}] \times 100$$

3.3. Phenotypically important traits

3.3.1. Degree of head orientation

Head position was noted visually at time of physiological maturity of 5 randomly selected plants from each replication.

3.3.2. Degree of head shape

Head shape was noted visually at the time of physiological maturity of 5 randomly selected plants from each replication.

3.3.3. Shape of convocular brackets

Shape of convocular brackets was noted visually at time of physiological maturity of 5 randomly selected plants from each replication.

3.4. Statistical analysis

All the traits under study were subjected to analysis of variance (Steel *et al.*, 1997). Data was further analyzed for general combining ability and specific combining ability, line × tester analysis was done as narrated by Kempthorne *et al.* (1957).

Estimation of GCA effects;

Lines gi = {(xj../tr) – (x…/ltr)}

Testers gt = {(x.j./lr) – (x…/ltr)}

Where,

l = number of lines

t = number of testers

r = number of replications

x i.. = Total number of F_1 resulting from crossing ith lines with all the testers.

x...= Total number of all the crosses.

Estimation of SCA effects;

si= {(xij.)/r) – (xi../tr) – (x.j./lr) + x…/ltr

Where,

xij = Total of F_1 resulting from crossing ith lines with jth tester.

xi = Total of all the crosses of ith line with all testers.

xj = Total of all the crosses of jth tester with all lines.

Personion correlations were computed through excel function. Significance of each correlation was determined at 0.05 and 0.01 probability level. Commercial heterosis and commercial heterobeltiosis were estimated as narrated by Comstock *et al*. (1952); Hayman, (1957). Estimation of heterosis and commercial heterosis;

$$\text{Commercial Heterosis} = (F_1\text{-}AC/\ AC) \times 100$$

$$\text{Commercial Heterobeltiosis} = (F_1\text{-}BC/\ BC) \times 100$$

Where;

AC = average of all commercial hybrids value.

BC = best commercial hybrid.

Significance of heterosis and heterobeltiosis was done as referred by Wynne *et al*., (1970). Significance of heterosis and heterobeltiosis

$$\text{t-test for mid parent} = (F_1\text{-}MP)/\sqrt{3/8 \times \sigma^2_E}$$

$$\text{t-test for high parent} = (F_1\text{-}HP)/\sqrt{3/8 \times \sigma^2_E}$$

Where;

F_1= average of each F_1 hybrid.

MP= mean of all commercial hybrids.

HP = mean of best commercial hybrid.

σ^2_E = mean square error (MSE).

CHAPTER IV

RESULTS

4.1. Line × tester analysis of studied traits in sunflower (*Helianthus annuus* L.)

4.1.1. Intermodal distance (cm)

Analysis of variance for internodal distance showed high significant ($P \leq 0.05$) differences among sunflower genotypes. Genotypes were further divided into parents and crosses and their analysis also revealed significant variation among parents, crosses and parents vs. crosses (Appendix 1). Line x Tester analysis showed non-significant ($P \geq 0.05$) variation among testers and lines which indicated the absence of additive gene action while significant variation due to line x tester interaction showed that internodal distance was predominantly controlled by dominance type of gene action. Dominance or over dominance gene action is the basis of heterosis therefore; this trait can be exploited through hybrid breeding in sunflower (*Helianthus annuus* L.).

4.1.2. Plant height (cm)

Analysis of variance showed highly significant ($P \geq 0.05$) difference among sunflower genotypes (Appendix II). Genotypes were further divided into parents and crosses and their analysis exhibited highly significant variation among parents, crosses and parents vs. crosses. Line × tester analysis (Appendix II) revealed insignificant ($P \leq 0.05$) variation among lines and testers while highly significant variation was revealed by line × tester interaction which showed that plant height was controlled by dominant gene action. Dominance or over-dominance is the main source of heterosis. Hence, plant height would be exploited through hybrid breeding in sunflower (*Helianthus annuus* L.).

4.1.3. Stem girth (cm)

Analysis of variance exhibited highly significant variation among sunflower genotypes (Appendix III). Genotypes were further divided into parents and crosses and their analysis also indicated significant difference among parents, crosses and parents vs. crosses. Line × tester analysis (Appendix III) showed non-significant variation in testers with presence of additive gene action while lines and line × tester showed significant variation which meant that stem girth was predominantly controlled by dominant gene action and dominance may be stronger as compared to additive gene action. Heterosis is due to dominance or over-dominance. As a result, this trait could be exploited through hybrid breeding in Sunflower (*Helianthus annuus* L).

4.1.4. Number of leaves per plant

The results of analysis of variance among studied sunflower genotypes exhibited highly significant ($P \leq 0.05$) variation. Genotypes were further splited into parents and F_1 crosses. Their analysis also revealed significant differences among parents, crosses and parent vs. crosses (Appendix IV). Line×tester analysis (Appendix IV) exhibited insignificant ($P \geq 0.05$) variation among testers and lines while highly significant variation due to line × tester interaction which showed that trait was controlled by dominant genes and heterosis is due to dominant genes. Therefore; number of leaves per plant could be used through hybrid breeding in Sunflower (*Helianthus annuus* L.)

4.1.5. Days to flowering

Analysis of variance showed significant ($P \leq 0.05$) differences among sunflower genotypes. Genotypes were further divided into parents and crosses (Appendix V). Their analysis also represents significant variation among parents and crosses while non-significant ($P \geq 0.05$) variation exhibited by parents vs. crosses. Line×tester analysis (Appendix V) showed non- significant variation in testers and line. On the other hand, line × tester interaction showed significant variation which meant that inheritance of this trait was controlled by dominant gene action and dominance or over-dominance is the source of heterosis and it should be exploited for hybrid breeding in Sunflower (*Helianthus annuus* L.)

4.1.6. Head diameter (cm)

Analysis of variance showed significant ($P \leq 0.05$) variation among studied sunflower genotypes. Genotypes were further splited into parents and crosses (Appendix VI). Their analysis also exhibited significant variation among parents, crosses and parents vs. crosses. Line×tester analysis (Appendix VI) revealed significant variation in lines while testers and line × tester interaction showed non-significant ($P \geq 0.05$) variation, which indicated that inheritance of head diameter was controlled by additive gene action and there is no advantage of additive gene action in hybrid breeding in Sunflower (*Helianthus annuus* L.).

4.1.7. Number of seeds per head

Analysis of variance showed significant ($P \leq 0.05$) variation among sunflower genotypes. Genotypes were further divided into parents and crosses. This exhibited significant variation among crosses and parents vs. crosses while parents showed non-significant variation. Line×tester analysis (Appendix VII) showed non-significant ($P \geq 0.05$) variation among lines and testers while significant variation was due to line × tester interaction. It meant that inheritance of this trait was predominantly controlled by dominant genes. Dominance or over-dominance is the basis of heterosis. Therefore; trait such as number of seeds per head could be exploited through hybrid breeding in sunflower (*Helianthus annuus* L.).

4.1.8. 100-Achene weight (g)

Analysis of variance showed significant ($P \leq 0.05$) variation among genotypes. Genotypes were divided into parents and crosses. Their analysis also revealed significant variation among parents, crosses and parents vs. crosses which indicated the presence of dominant type of gene action.

Line×tester analysis (Appendix VIII) displayed non-significant ($P \geq 0.05$) variation among male and females while highly significant variation was due to line × tester interaction which showed that the trait was controlled by dominant gene action and it was the reason of heterosis. Hence; the trait, 100-achene weight could be exploited through hybrid breeding in sunflower (*Helianthus annuus* L.)

4.1.9. Achene weight per head (g)

Analysis of variance of achene weight per head showed significant ($P \leq 0.05$) variation among sunflower genotypes. They were further classified into parents and crosses (Appendix IX). Their analysis also revealed significant variation among crosses and parents vs. crosses while parents showed non-significant ($P \geq 0.05$) variation. Line×tester analysis (Appendix IX) showed non-significant variation among lines and testers while line×tester interaction revealed significant variation which means that inheritance of achene weight per head was controlled by dominance gene action and it was the main source of heterosis. Thus, this trait could be exploited in sunflower (*Helianthus annuus* L.) hybrid breeding program.

4.2. General combining ability (GCA) for yield and yield component in sunflower (*Helianthus annuus* L.)

4.2.1. Intermodal distance

General combining ability (GCA) for yield and yield related components in sunflower (*Helianthus annuus* L.) of 8 CMS lines and 3 testers was estimated (Table 4.1). These inbred lines showed different general combining ability for intermodal distance. In a single plant, if there is less internodal distance than there will be more leaves and it is good for transpiration, photosynthesis as well as positive results towards yield. So, among lines, B-2, B-3, B-6 and B-21 were not good general combiners while B-5, B-7, B-9 and B-16 showed good general combining ability for intermodal length. Among testers (R-5, R-12 and R-18), only R-5 was not found good general combiner while R-

12 and R-18 were good general combiners for internodal length. The lines and tester with good GCA may be used further for development of F_1 hybrid seed.

4.2.2. Plant height (cm)

General combining ability of inbred lines (8 CMS and 3 restorers) for yield and its components was estimated (Table 4.1). Inbred lines showed different combining abilities for plant height. Among lines B-3 and B-6 were very good general combiners while B-5 and B-21 showed good general combining ability. The lines B-2, B-7, B-9 and B-16 were not good general combiners. Among testers, R-12 showed good general combining ability while R-5 and R-18 were not good general combiners.

4.2.3. Stem girth (cm)

General combining ability of 11 inbred lines (8 CMS and 3 testers) for stem girth was estimated (Table 4.1). The lines included B-2, B-3, B-6, and B-21were found good general combiners while lines B-5, B-7, B-9 and B-16 were not good general combiners. Among testers, R-5 and R-12 were good general combiners while R-18 was not a good general combiner for stem girth.

4.2.4. Number of leaves per plant

General combining ability of 11 inbred lines (8 CMS and 3 testers) for number of leaves per

Table 4.1: General combining ability (GCA) of sunflower (*Helianthus annuus* L.) inbred lines for yield and its components

Parents	IND	PH	SG	NL	DTF	HD	SPH	100-AW	AWH
B-2	0.75	-1.89	0.17	2.82	-0.04	1.47	43.03	1.18	119.77
B-3	0.95	7.30	0.31	2.60	0.29	1.64	-22.47	-0.11	-15.57
B-5	-0.82	3.33	-0.42	-2.18	3.18	-2.46	-46.89	-0.90	-29.02
B-6	0.71	11.03	0.14	-0.74	-0.26	-0.92	-93.73	0.12	-9.46
B-7	-0.06	-2.89	-0.09	-1.18	-2.26	-1.14	-133.44	0.89	-20.40
B-9	-0.95	-7.76	-0.09	-1.63	-0.04	-2.17	26.53	-0.75	-19.74
B-16	-0.77	-12.0	-0.12	-1.07	-0.82	0.53	111.75	-0.80	-21.52
B-21	0.18	2.88	0.10	1.38	-0.04	3.04	115.22	0.38	-4.07
R-5	0.21	-3.95	0.02	-0.56	1.36	-0.24	-84.88	0.31	-24.95
R-12	-0.18	8.81	0.05	1.40	2.19	0.98	93.78	0.22	43.10
R-18	-0.03	-4.86	-0.07	-0.85	-3.56	-0.74	-8.90	-0.53	-18.15

**Internodal distance (IND), Plant height (PH), Stem girth (SG), Number of leaves (NL), Days to flowering (DTF), Head diameter (HD), Seeds per head (SPH), 100-Achene weight (100-AW) and Achene weight per head (AWH).

plant was calculated (Table 4.1). The lines, B-2, B-3 and B-21 showed good GCA and may be used for further development of F_1 hybrids while B-5, B-6, B-7, B-9 and B-16 were not good general combiners. Among testers, R-12 exhibited good general combing ability while R-5 and R-18 were not good general combiners for number of leaves per plant.

4.2.5. Days to flowering

General combining ability for days to flowering was estimated for 11 inbred lines (8 CMS and 3 testers) (Table 4.1). Among lines, B-3 and B-5 were not good general combiners as they started late flowering as compared to remaining lines while B-2, B-6, B-7, B-9, B-16 and B-21 showed good general combining ability and may be used for further development of F_1 hybrids. Among testers; R-5 and R-12 were not good general combiners while R-18 showed good general combining ability for days to flowering.

4.2.6. Head diameter (cm)

General combining ability of 8 CMS and 3 restorers was calculated (Table 4.1) for head diameter. Among lines, B-2, B-3, B-16 and B-21 exhibited good general combining ability while B-5, B-6, B-7 and B-9 were not good general combiners. Among restorers, R-12 showed good general combining ability while R-5 and R-18 were not good general combiners. Inbred lines with good general combining ability may be used for further development of F_1 hybrids.

4.2.7. Number of seeds per head

General combining ability of 11 inbred lines (8 CMS and 3 testers) was calculated (Table 4.1) for number of seeds per head. Among lines, B-16 and B-21 showed excellent general combining ability, B-2 and B-9 exhibited good general combining ability while B-3, B-5, B-6 and B-7 were not good general combiners. Among testers, R-12 was a good general combiner while R-5 and R-18 were not good general combiners.

4.2.8. 100-Achene weight (g)

General combining ability for 100-achene weight (Table 4.1) of 11 parental lines (8 CMS and 3 testers) was estimated. Among lines, B-2, B-6, B-7 and B-21 showed good general combining ability while B-3, B-5, B-9 and B-16 were not good general combiners. Among testers, R-5 and R-12 exhibited good general combining ability while R-18 was not a good general combiner for 100-achenes weight.

4.2.9. Achene weight per head (g)

General combining ability for seed weight per head of 11 inbred lines (8 CMS and 3 testers) was calculated (Table 4.1). Among lines, B-2 was an excellent general combiner while the remaining lines B-3, B-5, B-6, B-7, B-9, B-16 and B-21 were not good general combiners. Among testers, R-12 showed good general combining ability while R-5 and R-18 were not good general combiners for achene weight per head.

4.3. Specific combining ability (GCA) for yield and yield components in sunflower (*Helianthus annuus* L.).

4.3.1. Internodal length

Specific combining ability (SCA) for yield and yield contributing component in 24 sunflower (*Helianthus annuus* L.) F_1 hybrids were estimated (Table 4.2) which was developed by using 8 lines and 3 testers. Among F_1 hybrids, different hybrids show specific combining ability in different range for internodal length. The crosses, CMS-2×R-5, CMS-2×R-12, CMS-3×R-18, CMS-5×R-5, CMS-5×R-18, CMS-6×R-5, CMS-7×R-12, CMS-9×R-5, CMS-16×R-18, CMS-21×R-12 and CMS-21×R-18 were not good specific combiners for internodal length, while CMS-2×R-18, CMS-3×R-5, CMS-3×R-12, CMS-5×R-12, CMS-6×R-12, CMS-6×R-18, CMS-7×R-5, CMS-7×R-18, CMS-9×R-12, CMS-9×R-18, CMS-16×R-5, CMS-16×R-12 and CMS-21×R-5 showed good specific combining ability for internodal length. The crosses with good specific combining ability should be exploited for commercial means.

4.3.2. Plant height (cm)

Specific combining ability of 24 F_1 hybrids (8 CMS × 3 testers) was determined and different crosses showed different range of specific combining abilities for plant height (Table 4.2). The cross CMS-9×R-5 exhibited excellent specific combining ability while CMS-2×R-5, CMS-3×R-18, CMS-5×R-18, CMS-6×R-12, CMS-7×R-12, CMS-7 × R-18 and CMS-21×R-12 showed good specific combining abilities. The crosses CMS-2×R-12, CMS-3×R-12, CMS-5×R-12, CMS-16×R-5 and CMS-21×R-5 revealed average specific combining abilities. The crosses, CMS-2×R-18, CMS-3×R-5, CMS-5×R-5, CMS-6×R-5, CMS-6×R-18, CMS-7×R-5, CMS-9×R-12, CMS-9×R-18, CMS-16×R-18 and CMS-21×R-18 did not show good specific combining ability for plant height. The crosses having excellent and good specific combining abilities should be exploited for commercial hybrid seed development.

4.3.3. Stem girth (cm)

Specific combining ability of 24 F_1 hybrids for stem girth was observed (Table 4.2). Among crosses; CMS-2×R-5, CMS-2×R-12, CMS-3×R-5, CMS-3×R-18, CMS-5×R-18, CMS-6×R-12, CMS-7×R-12. CMS-9×R-5, CMS-9×R-12, CMS-16×R-12 and CMS-21×R-12 were good specific combiners while CMS-2×R-18, CMS-3×R-12, CMS-5×R-5, CMS-5×R-12, CMS-6×R-5, CMS-6×R-18, CMS-7×R-5, CMS-7×R-18, CMS-9×R-18, CMS-16×R-5, CMS-16×R-18, CMS-21×R-5 and CMS-21×R-12 did not show good SCA for stem girth. The F_1 hybrids with good stem girth should be used for further commercial means.

4.3.4. Number of leaves per plant

Specific combining ability of 24 F_1 hybrids was estimated for number of leaves per plant (Table 4.2). Among F_1 crosses, CMS-2×R-5, CMS-2×R-12, CMS-3×R-18, CMS-5×R-5, CMS-5×R-18, CMS-6×R-12, CMS-7×R-12, CMS-9×R-5, CMS-9×R-18, CMS-16×R-12, CMS-21×R-5 and CMS-21×R-12 showed good specific combining ability while CMS-2×R-18, CMS-3×R-5, CMS-3×R-12, CMS-5×R-12, CMS-6×R-5, CMS-6×R-18, CMS-7×R-5, CMS-7×R-18, CMS-9×R-12, CMS-16×R-5, CMS-16×R-18 and CMS-21×R-18 were not good specific combiners for number of leaves per plant.

4.3.5. Days to flowering

Specific combining ability of 24 F_1 hybrids was calculated for days to start flowering (Table 4.2). Among F_1 hybrids, CMS-2×R-18, CMS-3×R-5, CMS-5×R-12, CMS-5×R-18, CMS-6×R-12, CMS-6×R-18, CMS-7×R-18, CMS-9×R-5, CMS-9×R-12, CMS-16×R-5, CMS-16×R-12, CMS-21×R-5 and CMS-21×R-12 did not show good specific combining ability due to late flowering as compared to other F_1 hybrids while CMS-2×R-5, CMS-2×R-12, CMS-3×R-12, CMS-3×R-18, CMS-5×R-5, CMS-6×R-5, CMS-7×R-5, CMS-7×R-12, CMS-9×R-18, CMS-16×R-18 and CMS-21×R-18 were good specific combiners for days to start flowering and they should be exploited for further commercial means.

4.3.6. Head diameter (cm)

Specific combining ability of 24 F_1 hybrids was calculated for head diameter (Table 4.2). Among crosses CMS-2×R-5, CMS-2×R-18, CMS-3×R-12, CMS-3×R-18, CMS-5×R-18, CMS-6×R-12, CMS-7×R-18, CMS-9×R-5, CMS-16×R-5, CMS-16×R-12 and CMS-21×R-12 exhibited good specific combining ability and could be exploited for further commercial means while CMS-2×R-12, CMS-3×R-5, CMS-5×R-5, CMS-5×R-12, CMS-6×R-5, CMS-6

Table 4.2. Specific combining ability (SCA) of F_1 crosses for yield and yield components in sunflower (*Helianthus annuus* L.)

Cross combinations	IND	PH	SG	NL	DTF	HD	SPH	100-AW	AWH
CMS-2×R-5	0.51	13.17	0.09	0.56	-3.58	1.62	277.36	0.09	-101.90
CMS-2×R-12	1.82	4.28	0.08	2.60	-4.08	-2.17	-224.12	1.44	198.42
CMS-2×R-18	-2.33	-17.45	-0.17	-3.15	7.67	0.55	-53.24	-1.53	-96.52
CMS-3×R-5	-0.39	-10.30	0.16	-1.56	6.08	-0.50	-86.64	-0.24	4.51
CMS-3×R-12	-0.71	1.19	-0.25	-0.18	-0.42	0.50	120.75	0.17	-19.43
CMS-3×R-18	1.10	9.11	0.09	1.74	-5.67	0.00	-34.11	0.07	14.82
CMS-5×R-5	0.50	-9.83	-0.05	0.89	-5.47	-0.86	111.41	-0.13	27.56
CMS-5×R-12	-0.62	3.00	-0.15	-3.07	3.69	-1.24	-157.34	-0.07	-48.82
CMS-5×R-18	0.12	6.84	0.20	2.18	1.78	2.10	45.93	0.20	21.27
CMS-6×R-5	0.72	-5.04	-0.04	-0.22	-7.03	-1.26	-152.81	0.15	2.50
CMS-6×R-12	-0.64	5.71	0.14	0.82	4.81	3.22	309.09	0.10	1.29
CMS-6×R-18	-0.08	-0.67	-0.10	-0.60	2.22	-1.96	-156.28	-0.24	-3.79
CMS-7×R-5	-0.51	-16.62	-0.01	-0.78	-0.03	-0.10	-131.29	0.88	13.95
CMS-7×R-12	0.53	6.73	0.19	0.93	-3.86	-0.56	-117.45	-0.23	-28.10
CMS-7×R-18	-0.02	9.89	-0.18	-0.15	3.89	0.66	248.74	-0.65	14.15
CMS-9×R-5	0.72	24.33	0.13	2.33	0.75	1.27	221.24	0.18	34.28
CMS-9×R-12	-0.48	-9.75	0.04	-3.29	1.25	-0.28	79.25	-0.34	-19.77
CMS-9×R-18	-0.24	-14.58	-0.18	0.96	-2.00	-0.99	-300.49	0.17	-14.51

CMS-16×R-5	-1.04	0.42	-0.21	-1.56	5.86	1.24	-62.57	-0.41	12.39
CMS-16×R-12	-0.30	5.92	0.40	5.07	3.44	1.49	169.38	0.82	33.60
CMS-16×R-18	1.34	-6.34	-0.18	-3.51	-9.31	-2.73	-106.81	-0.41	-45.99
CMS-21×R-5	-0.50	3.86	-0.08	0.33	3.42	-1.40	-176.71	-0.52	6.61
CMS-21×R-12	0.12	10.28	-0.20	1.63	6.67	2.48	25.82	-0.37	5.32
CMS-21×R-18	0.38	-14.14	0.28	-1.96	-10.08	-1.07	150.89	0.90	-11.93

**Internodal distance (IND), Plant height (PH), Stem girth (SG), Number of leaves (NL), Days to flowering (DTF), Head diameter (HD), Seeds per head (SPH), 100-Achene weight (100-AW), Achene weight per head (AWH).

× R-18, CMS-7×R-5, CMS-7×R-12, CMS-9×R-12, CMS-9×R-18, CMS-16×R-18, CMS-21×R-5 and CMS-21×R-18 were not good specific combiners for head diameter.

4.3.7. Number of seeds per head

Specific combining ability of the 24 F_1 hybrids was determined for number of seeds per head (Table 4.2). Among F_1 crosses CMS-2×R-5, CMS-3×R-12, CMS-5×R-5, CMS-5×R-18, CMS-6×R-12, CMS-7×R-18, CMS-9×R-5, CMS-9×R-12, CMS-16×R-12, CMS-21×R-12 and CMS-21×R-18 were good specific combiners for number of seeds per head and could be used for further commercial means while CMS-2×R-12, CMS-2×R-18, CMS-3×R-5, CMS-3×R-18, CMS-5×R-12, CMS-6×R-5, CMS-6×R-18, CMS-7×R-5, CMS-7×R-12, CMS-9×R-18, CMS-16×R-5, CMS-16×R-18 and CMS-21×R-5 were not good specific combiners for number of seeds per head.

4.3.8. 100-Achene weight (g)

Specific combining ability of the 24 F_1 hybrids was calculated (Table 4.2) for 100-achene weight. Among crosses CMS-2×R-5, CMS-2×R-12, CMS-3×R-12, CMS-3×R-18, CMS-5×R-18, CMS-6×R-5, CMS-6×R-12, CMS-7×R-5, CMS-9×R-5, CMS-9×R-18, CMS-16×R-12 and CMS-21×R-18 were good specific combiners for 100-achene weight and should be exploited for further commercial means while CMS-2×R-18, CMS-3×R-5, CMS-5×R-5, CMS-5×R-12, CMS-6×R-18, CMS-7×R-12, CMS-7×R-18, CMS-9×R-12, CMS-16×R-5, CMS-16×R-18, CMS-21×R-5 and CMS-21×R-12 were not good specific combiners for 100- achenes weight.

4.3.9. Achene weight per head (g)

Specific combining ability of F_1 hybrids for achene weight per head was calculated (Table 4.2). Among crosses CMS-2×R-5, CMS-2×R-12, CMS-3×R-5, CMS-3×R-18, CMS-5×R-5, CMS-5×R-18, CMS-6×R-5, CMS-6×R-12, CMS-7×R-5, CMS-7×R-18, CMS-9×R-5, CMS-16×R-5, CMS-16×R-12, CMS-21×R-5 and CMS-21×R-12 were good specific combiners and should be used for commercial means while CMS-2×R-18, CMS-3×R-12, CMS-5×R-12, CMS-6×R-18, CMS-7×R-12, CMS-9×R-12, CMS-9×R-18, CMS-16×R-18 and CMS-21×R-18 were not good specific combiners for achene weight per head.

4.4. Correlation coefficients among yield and its components

4.1.1. Plant height (cm)

Plant height is an important trait and showed significant positive correlation with head diameter, number of leaves, stem girth, internodal distance, number of seeds per head, achene weight per head, 100-achene weight and oil contents while significant negatively correlated with days to flowering (Table 4.3).

4.4.2. Achene weight per head (g)

Seed weight per head is dependent trait and it is contributed by number of independent traits. Therefore correlation analysis was done to study the relationship between traits (Table 4.3). Correlation analysis showed that seed weight for head was positively associated with 100-achene weight showing that genotypes with large seed size have greater seed weight. 100-achene weight is an interesting trait in sunflower and seed size determines sunflower end use. For instance large seeded varieties have place in confectionary and bird food market. Larger seed tends to lower oil contents. In our study we used inbred lines of different achene size and correlation analysis showed that hybrids with large seed size tend to have greater yield. Seed weight per head was also positively correlated with head diameter, internodal distance and number of leaves per plant. Among the traits number of leaves per plant at anthesis seems good indicator of yield in sunflower.

4.4.3. Days to complete flowering

Days to complete flowering (DTCF) was significantly and positively interrelated with head diameter and internodal distance (Table 4.3), as days to complete flowering increased head diameter and internodal distance was also increased. DTCF was non-significantly positive correlated with seed weight per head, 100-achene weight, number of infected leaves and number of achene per head, as DTCF had no effect on these traits either DTCF increased or decreased. DTCF was significant negatively associated with days to flower initiation as days to flower initiation increased days to flower completion decreased. DTCF was non-significantly negative correlated with head shape and number of infected leaves, as it had no effect on these traits but negative association.

4.4.4. Internodal distance (cm)

Internodal distance is very important trait and it is directly related with number of leaves (Table 4.3). There will be less number of leaves if there is more internodal distance. Leaves are very important for transpiration and photosynthesis in plants so there should be less internodal distance with more number of leaves. In this study, internodal distance was significant and positively associated with number of infected leaves. Insect attack occurred at time of flowering, thus, there were more infected leaves with large internodal distance. Internodal distance had no effect on head diameter. It was non-significant and negative associated with number of achene per head and head shape as increased in internodal distance cause the decrease in these traits but non-significantly. Internodal distance was insignificant and positively associated with number of holes per infected leaf. Internodal distance was significant positively associated with achene yield per plant and 100-achene weight per plant.

4.4.5. Head diameter (cm)

Head diameter was significant and negatively associated with days to flower initiation, as increased in days to flower initiation cause the decrease in head diameter and vise versa (Table 4.3). Head diameter showed non-significant negative correlation with head shape. Head diameter exhibited significantly positive relationship with number of leaves per plant. Head diameter was non-significant and positively associated with number of holes per infected leaf it had no effect on infected leaves but linked positively.

4.4.6. Stem girth and number of leaves

Stem girth and number of leaves both exhibited positive and significant correlation (Table 4.3) with plant height, head diameter, internodal distance, achene weight per head, number of seeds per head and 100-achene weight but showed negative correlation with oil contents. Sunflower hybrids that take more time for vegetative growth gives low oil percentage and have strong stem and more number of leaves as compared to other hybrids that take more time during reproductive growth.

Table 4.3. Correlation coefficients among various yield and yield related components

Traits	***PH***	***HD***	***SG***	***IND***	***NL***	***NSPH***	***100-AW***	***AWPH***	***DTFC***
HD	0.31								
SG	0.30	0.53							
IND	0.43	0.16	0.57						
NL	0.44	0.60	0.68	0.60					
NSPH	0.31	0.60	0.25	-0.10	0.29				
100-AW	0.20	0.28	0.56	0.58	0.52	-0.02			
AWPH	0.21	0.18	0.36	0.44	0.61	0.11	0.61		
DTFC	-0.05	-0.12	0.16	0.55	0.14	-0.08	0.28	0.07	
OC	-0.11	-0.08	-0.28	0.04	-0.24	0.01	-0.17	-0.26	0.51

Where ** when P ≤ 0.01 and P ≤ 0.05

**Plant height (PH), Head diameter (HD), Stem girth (SG), Internodal distance (IND), Number of leaves (NL), Number of seeds per head (NSPH) , 100-Achene weight (100-AW), Achene weight per head(AWPH), Days to flower completion (DTFC) and Oil contents (OC).

4.4.7. Oil Content (%)

In Pakistan, the purpose of breeding sunflower hybrids is to get high oil contents. High oil contents are directly related to achene weight per head and number of seeds per head. According to the research of study, oil contents were significant positively correlated with head diameter while exhibited non-significant and negatively correlation with days to flower completion and achene weight per head. Oil contents were non-significantly correlated with 100-achene weight and internodal distance (Table 4.3).

4.5. Significance of heterosis

Average commercial heterosis and heterobeltiosis for seed yield per plant was estimated in 24 F_1 crosses (Table 4.4). Estimates showed that 17 F_1 crosses exhibited positive heterosis while 10 F_1 crosses showed positive heterobeltiosis indicating potential for commercialization. Heterosis range was 2 to 592.4 % that showed positive heterosis. However, only 10 F_1 crosses showed positive heterobeltiosis. Among these 10 crosses, cross CMS-2×R-12 showed highest heterosis and heterobeltiosis. Depending upon the oil or protein content this cross could be commercialized for its specific use. Among 24 F_1 hybrids, seven crosses showed negative heterosis that ranged from -5.6 to -37.6 % and forteen crosses showed negative heterobeltiosis from -2.3 to 47.7% and all of these crosses exhibited non-significant negative heterosis from mid to high parent among crosses. Among F_1 hybrids, CMS-2×R-12 showed positive and highly significant heterosis from mid and high parent. After this cross, CMS-6×R-12 (95.4 %) followed by CMS-21×R-18(83.5%) exhibited high heterosis and significant positive heterobeltiosis from mid and high parent among the remaining significant positive F_1 hybrids. The cross CMS-2×R-12 gave maximum yield (454.60 g) among all the F_1 hybrids. The purpose of this study was also to develop hybrid with high seed yield and oil contents with ultimate objectives to commercialization of specific hybrids for specific use.

Table 4.4. Significance of heterosis of sunflower F_1 hybrids for yield and its components

Genotypes	Achene weight per head (g)			Significance of hterosis	
	Average	Heterosis	Heterobeltiosis	MP	High parent
CMS-2×R-5	86.33	31.5	10.2	0.546	0.212
CMS-2×R-12	454.60	592.4	480.5	10.298	9.962
CMS-2×R-18	98.50	50.0	25.8	0.869	0.534
CMS-3×R-5	57.50	-12.4	-26.6	-0.216	-0.551
CMS-3×R-12	101.50	54.5	29.6	0.948	0.613
CMS-3×R-18	74.50	13.4	-4.9	0.234	-0.101
CMS-5×R-5	67.00	2.0	-14.5	0.035	-0.299
CMS-5×R-12	58.67	-10.7	-25.1	-0.185	-0.520
CMS-5×R-18	67.50	2.8	-13.8	0.048	-0.287
CMS-6×R-5	61.50	-6.3	-21.5	-0.110	-0.445
CMS-6×R-12	128.33	95.4	63.8	1.658	1.323
CMS-6×R-18	62.00	-5.6	-20.8	-0.097	-0.432
CMS-7×R-5	62.00	-5.6	-20.8	-0.097	-0.432
CMS-7×R-12	88.00	34.0	12.3	0.591	0.256
CMS-7×R-18	69.00	5.1	-11.9	0.088	-0.247
CMS-9×R-5	83.00	26.4	6.0	0.458	0.124
CMS-9×R-12	97.00	47.7	23.8	0.829	0.494
CMS-9×R-18	41.00	-37.6	-47.7	-0.652	-0.989
CMS-16×R-5	59.33	-9.6	-24.3	-0.167	-0.503
CMS-16×R-12	87.33	33.0	11.5	0.573	0.238
CMS-16×R-18	69.00	5.1	-11.9	0.088	-0.247
CMS-21×R-5	71.00	8.1	-9.4	0.141	-0.194
CMS-21×R-12	76.50	16.5	-2.3	0.287	-0.048
CMS-21×R-18	120.50	83.5	53.8	1.451	1.116

4.6. Comparison of mean values of Sunflower F_1 hybrids.

4.6.1. Plant height (cm)

Mean values of plant height are shown in Table 4.5. Mean values of all developed F_1 hybrids indicated that single cross hybrids showed plant height range of 158- 211 cm. The cross CMS-9×R-18 showed the lowest plant height while cross combination CMS-6×R-12 showed the largest value for plant height. However, most of crosses showed near to same plant height. The crosses combinations such as CMS-2×R-5, CMS-2×R-12, CMS-3×R-12, CMS-3×R-12, CMS-3×R-18, CMS-5×R-12, CMS-5×R-18, CMS-5×R-18, CMS-6×R-18, CMS-7×R-12, CMS-9×R-5 and CMS-21×R-12 showed statistically similar values for plant height and could regarded as cross combination with the high value for plant height. Higher plant height tend to induce lodging, therefore, dwarf plant height is normally favored in the crosses. However, our study showed that lower plant height was correlated with lower yield.

4.6.2. Head diameter (cm)

Mean values for head diameter are shown in a Table 4.5. Mean values indicated that single cross F_1 hybrids exhibited range of 19-28 cm head diameter. The crosses CMS-6×R-18 and CMS-9×R-18 showed lowest head diameter while cross CMS-21×R-12 showed highest value for head diameter. The cross combinations CMS-2×R-5,CMS-2×R-18, CMS-3×R-5, CMS-3×R-12, CMS-3×R-18, CMS-6×R-12, CMS-21×R-18, CMS-21×R-5, CMS-16×R-12, CMS-16×R-5 showed statistically similar values for head diameter. More head diameter tends to increase achene yield and achene yield is the main purpose in hybrid breeding program. Hence, our study also showed that less head diameter was correlated with lower achene yield.

4.6.3. Internodal distance (cm)

Mean values of internodal distance indicated that single cross F_1 hybrids exhibited range of 6-10 cm (Table 4.5). The crosses, CMS-2×R-18, CMS-5×R-12, CMS-9×R-12, CMS-9×R-18 and CMS-16×R-5 showed lowest values for internodal distance while crosses; CMS-2×R-12 and CMS-3×R-18 showed highest values for internodal distance. CMS-5×R-18, CMS-7×R-5 and CMS-16×R-12 showed statistically similar values for internodal distance and could consider as F_1 hybrids with less internodal distance. Hence, less internodal distance tends to increase number of leaves per plant which leads toward more transpiration and photosynthesis.

4.6.4. Days to flower initiation

Mean values for days to flowering indicated that single cross hybrids exhibited range of 65-83 (Table 4.5). The cross, CMS-3×R-18 showed lowest value for days to flowering while the cross, CMS-5×R-12 showed highest value for days to flowering. The cross combinations, CMS-9×R-18, CMS-16×R-18 and CMS-21×R-18 showed statistically similar values for days to flowering and regarded as lower values for days to flowering. However, F_1 hybrids with early flowering tend to increase yield and cause decrease in plant height that is good for hybrid seed production.

4.6.5. Leaf area (cm^2)

Mean values for leaf area are shown in Table 4.5. Mean values indicated that single cross F_1 hybrids showed range of 183-477 cm^2. The cross, CMS-2×R-18 showed highest value for leaf area while the cross, CMS-9×R-18 showed lowest value for leaf area. The cross combinations; CMS-3×R-5, CMS-5×R-5, CMS-9×R-5, CMS-9×R-12 and CMS-21×R-18 showed statistically similar and high values for leaf area. Hence, F_1 hybrid with more leaf area tends to increase transpiration and is also good for plant photosynthetically.

4.6.6. Number of leaves per plant

Mean values for number of leaves per plant indicated that single cross F_1 hybrids showed range of 19-30 (Table 4.5). The cross CMS-2×R-12 showed highest value for number of leaves per plant while the crosses CMS-5×R-12 and CMS-9×R-12 showed lowest value number of leaves. The cross combinations; CMS-2×R-5CMS-3×R-12 and CMS-16×R-5 showed statistically similar and high values for number of leaves. Hence, F_1 hybrids with more number of leaves tend to increase transpiration with reduce internodal distance and is also good for plant photosynthetically.

4.6.7. Stem girth (cm)

Mean values for Stem girth is shown in Table 4.5. Mean values indicated that single cross F_1 hybrids showed range of 1.6-2.6 cm. The crosses, CMS-3×R-5 and CMS-21×R-18 showed highest value for stem girth while the cross CMS-5×R-12 showed lowest value stem girth. The cross combinations; CMS-2×R-5, CMS-2×R-12, CMS-3×R-18 and CMS-3×R-18 showed statistically similar and high values for stem girth. Hence, F_1 hybrids with more stem girth tend to increase number of achene per head, head diameter and achene weight that's why more stem girth is the most important trait in sunflower hybrid seed production.

4.6.8. 100-achene weight (g)

Mean values for 100-achene weight is shown in Table 4.5Mean values indicated that single cross F_1 hybrids showed range of 5-9 g. The cross CMS-2×R-12 showed highest value for 100-achene weight while the cross CMS-5×R-18 showed lowest value for 100-achene

Table 4.5. Means, Range and Lsd values of sunflower F_1 hybrids for seed yield and its components

Genotypes	PH**	HD	IND (cm)	DTFI	LA (cm^2)	NL	SG	100-AW	SYH	OC (%)
CMS-2×R-5	193±3.21	26±0.92	9±0.415	72±0.575	281±40	26±0.578	2.4±0.029	8±0.65	86±39.62	36.2±1.01
CMS-2×R-12	196±7.00	23±1.6	10±0.6	72±0	358±98	30±0.578	2.4±0.065	9±0.25	455±339.3	33.5±1.54
CMS-2×R-18	161±23.52	24±5	6±0	78±5	477±46	22±6.506	2.1±0.65	6±0.90	99±45.5	28±1.66
CMS-3×R-5	178±5.25	24±0.875	8±9.625	82±0.57	365±137	23±0.578	2.6±0.075	6±0.13	58±19.67	35.2±1.02
CMS-3×R-12	203±11.17	26±2.167	8±1.08	76±0	285±143	27±2.516	2.2±0.225	7±0	102±3.5	27.7±1.52
CMS-3×R-18	197±3.25	24±0.125	10±0.375	65±0	223±17	26±1.527	2.4±0.09	6±0.15	75±3.5	36.2±2.01
CMS-5×R-5	175±3.75	19±0.167	8±0.5	73±0	385±147	21±0	1.7±0.265	6±0.20	67±2	40.9±1.62
CMS-5×R-12	200±5.51	20±0	6±0	83±0	193±16	19±0	1.6±0.1	6±0.55	59±5.68	36.4±2.06
CMS-5×R-18	191±2.75	22±0.875	7±1.125	75±0.57	208±34	22±1	1.8±0.125	5±0.68	68±7.5	40.4±2.06
CMS-6×R-5	187±5.25	20±0.5	9±0.25	68±0	200±13	21±0.578	2.2±0.125	7±0.29	62±0.5	38.9±1.58
CMS-6×R-12	211±1.75	26±2.5	8±0.5	81±1.52	327±56	24±1.527	2.4±0.01	7±1.27	128±14.15	37.9±1.61
CMS-6×R-18	191±6.10	19±0.1	8±0.2	72±0.57	262±64	21±0.578	2.1±0.065	6±0.16	62±0	35.8±1.50
CMS-7×R-5	162±24.65	21±3.75	7±1.25	73±3	306±93	20±3.511	2±0.475	9±0.65	62±20	35.9±1.57
CMS-7×R-12	198±0.35	22±2.5	8±0.1	70±0	223±3.21	24±2	2.3±0.163	7±1.23	88±15	35.4±1.07
CMS-7×R-18	187±4.51	22±2.5	8±1.5	72±4	311±82	21±0.578	1.8±0.225	6±0.08	69±24.24	36±1.59
CMS-9×R-5	198±5.17	22±1.08	8±1.08	76±0	359±94	23±1	2.2±0.075	6±0.28	83±8	36.3±2.03

CMS-9×R-12	177±26.50	21±5.25	6±0	77±1.15	363±212	19±3.51	2.1±0.575	6±1.37	97±57.19	29.3±2.05
CMS-9×R-18	158±1.00	19±1.57	6±0.625	68±0.57	183±13	21±0.578	1.8±0.14	5±0.97	41±9	41.3±2.01
CMS-16×R-5	170±10.50	24±0.25	6±0.5	80±1.52	234±15	26±0.578	1.8±0.10	6±0.04	59±21.22	42.5±1.58
CMS-16×R-12	174±9.25	24±1	7±0.5	73±0	244±51	20±2	2.3±0.475	6±1.18	87±26.63	39.2±1.90
CMS-16×R-18	176±0.67	22±0.5	8±1.33	66±0	278±11	20±0.578	1.9±0.135	6±0.26	69±5	42.9±1.54
CMS-21×R-5	158±7.50	24±3.875	8±0.5	79±4.5	293±166	24±0	2.2±0.15	7±0.24	21±36	34.5±2.50
CMS-21×R-12	194±16.50	28±1	8±0.125	77±1	233±21	25±4	2±0.50	6±0.98	77±29.5	43±1.64
CMS-21×R-18	183±5.75	26±2.89	8±0.5	66±0	388±67	24±0.578	2.6±0.05	8±0.18	121±1.5	43.6±1.54
Range	158-211	19-28	6-10	65-83	183-477	19-30	1.6-2.6	5-9	21-455	27.7-43.6
LSD at $t_{0.05}$	20.18	3.667	0.965	2.973	125.051	3.1011	0.411	0.9642	10.23	1.35

**: Plant height (PH), Head diameter (HD), Internodal distance (INTD), Days to flower initiation (DTFI), Number of leaves per plant (NL), Stem girth (SG), 100-Achene weight (100-AW), Seed yield per head (SYH) and Oil contents (OC%).

weight. The cross combinations; CMS-2×R-5, CMS-2×R-5, CMS-3×R-12, CMS-6×R-5, CMS-6×R-12, CMS-7×R-12 and CMS-21×R-18 showed statistically similar and high values for 100-achene weight. Hence, F_1 hybrids with more 100-achene weight tend to increase oil contents. So, it is the most important trait in sunflower hybrid seed production.

4.6.9. Achene weight per head (g)

Mean values for achene weight per head indicated that single cross F_1 hybrids showed range of 21-455 g (Table 4.5). The cross, CMS-2×R-12 showed highest value for Achene weight per head while the cross CMS-21×R-5 showed lowest value for Achene weight per head. There is very difference in mean value of crosses with highest and lowest achene weight per head. The cross combinations; CMS-6×R-12, CMS-21×R-18 and CMS-3×R-12 showed statistically similar and high values for Achene weight per head. Hence, F_1 hybrids with more Achene weight per head tend to increase number of achenes per head and high yield is the main purpose of breeding. So, it is the most important trait in sunflower hybrid breeding program.

4.6.10. Oil content (%)

Mean values for oil content percentage is shown in Table 4.5. Mean values indicated that single cross F_1 hybrids showed range of 27.7-43.6%. The cross CMS-21×R-18 showed highest value for oil content percentage 43.6% while the cross CMS-3×R-12 27.7% showed lowest value for oil content percentage. The cross combinations; CMS-2×R-5, CMS-5×R-5, CMS-5×R-12, CMS-5×R-18, CMS-3×R-12, CMS-6×R-5, CMS-6×R-12, CMS-7×R-18, CMS-9×R-5, CMS-9×R-18, CMS-16×R-5, CMS-16×R-12, CMS-16×R-18 and CMS-21×R-12 showed statistically similar and high values for oil content percentage. Hence, F_1 hybrids with small seed size and more 100-achene weight tend to increase oil contents. So, it is one of the most important trait in sunflower hybrid seed production. The hybrids with good oil content percentage could be exploited for commercial hybrid production.

4.7. Comparison of mean values of Parents with LSD value at 0.05.

4.7.1. Plant height (cm)

Mean values for plant height of parents are shown in Table 4.6. Mean values indicated that inbred lines showed range of 80-159 cm. The inbred line B-16 showed the lowest plant height while inbred line B-9 showed the largest value for plant height. However, most of inbred lines showed similar plant height. For instance, B-9 showed the highest plant height while inbred lines such as B-5, B-6, B-7 and B-21 showed statistically similar values of plant height and could consider as good inbred lines with the high value for plant height.

4.7.2. Head diameter (cm)

Mean values for head diameter of parents are shown in Table 4.6. Mean values indicated that inbred lines showed range of 9-15 cm head diameter. The inbred line B-3 and B-16 showed the lowest head diameter while inbred line B-5 and B-6 showed the largest value for head diameter. However, most of inbred lines showed similar head diameter. For instance, B-3 and B-16 showed the highest head diameter while inbred lines such as B-2, B-7, B-9 and B-21 showed statistically similar values for head diameter and could regarded as good inbred lines with the high value for head diameter.

4.7.3. Internodal distance (cm)

Mean values for internodal distance in inbred lines are shown in Table 4.6. Mean values indicated that inbred lines showed range of 3-6 cm. The inbred line B-2 and B-16 showed the lowest internodal distance while inbred line B-6 and B-9 showed the largest value for internodal distance. The inbred lines such as B-3, B-5, B-7 and B-21 showed statistically similar values for internodal distance and and could regarded as F_1 hybrids with less internodal distance.

4.7.4. Days to flower initiation

Mean values for days to flowering of inbred lines are shown in Table 4.6. Mean values indicated that inbred lines showed range of 68-81. The inbred line B-2, B-3 and B-21 showed the lowest value for days to flowering while inbred line B-16 showed the largest value for days to flowering. The inbred lines such as B-5, B-6, B-7 and B-9 showed statistically similar values for days to flowering.

4.7.5. Leaf area (cm^2)

Mean values for leaf area are shown in Table 4.6. Mean values indicated that inbred lines showed range of 95-182 cm^2. The inbred line, B-7 showed highest value for leaf area while the B-2 showed lowest value for leaf area. However, most of the inbred lines exhibited similar values for leaf area. The inbred lines (B-5, B-6 and B-9) showed statistically similar and high values for leaf area.

4.7.6. Number of leaves per plant

Mean values for number of leaves of inbred lines are shown in Table 4.6. Mean values indicated inbred lines showed range of 11-20. The inbred line B-6 showed highest value for number of leaves while the inbred line B-16 showed lowest value number of leaves. However, most of the inbred lines exhibited similar values for number of leaves. The inbred lines; B-5 and B-9 showed statistically similar and high values for number of leaves.

4.7.7. Stem girth (cm)

Mean values for Stem girth of parental lines is shown in Table 4.6. Mean values indicated that inbred lines showed range of 1-1.6 cm. The inbred lines, B-5 and B-6 showed highest

Table 4.6. Means, Range and Lsd values of sunflower various inbred lines for seed yield and its components

Parents	PH (cm)	HD (cm)	IND(cm)	DTFI	LA(cm^2)	NL	SG (cm^2)	100-AW	SYPH
B-2	104±1.527	12±0	3±0	68±0	99±32	13±0	1.3±0	4±0.10	7±0.5
B-3	113±9.50	9±0.25	5±0	68±0	80±25	15±0.57	1±0.09	3±0.38	5±2.17
B-5	124±5.50	15±1.5	5±0.57	79±5.29	150±34	18±1.52	1.6±0.25	6±0.7	9±1.75
B-6	142±0	15±0	6±0	79±0	128±17	20±0	1.6±2.72	6±0.36	18±3
B-7	135±0	14±0	5±0	76±0	182±32	14±0	1.5±0	5±0.32	18±2
B-9	159±0.57	14±6.11	6±0.57	77±1.52	170±20	17±2.30	1.5±0.47	6±0.37	30±12.58
B-16	80±0	9±0	3±0	81±0	109±63	11±0	1±0	4±0.35	5±0.14
B-21	113±2.64	14±1	4±0	68±0	105±20	13±0	1.2±0	3±0.11	6±1.18
Range	80-159	9-15	3-6	68-81	99-182	11-20	1-1.6	3-6	5-30
LSD at t=0.05	20.18	3.667	0.965	2.973	125.05	3.1011	0.411	0.964	10.23

**: Plant height (PH), Head diameter (HD), Internodal distance (INTD), Days to flower initiation (DTFI), Number of leaves per plant (NL), Stem girth (SG), 100-Achene weight (100-AW) and Seed yield per head (SYH).

value for stem girth while the inbred line B-3 and B-16 showed lowest value stem girth. The inbred lines; B-5, B-6, B-7and B-9 showed statistically similar and high values for stem girth.

4.7.8. 100-achene weight (g)

Mean values for 100-achene weight of parents is shown in Table 4.6. Mean values indicated that inbred lines showed range of 3-6 g. The inbred line, B-3 and B-21 showed lowest value for 100-achene weight while inbred lines B-5, B-6 and B-9 showed highest value for 100-achene weight. The inbred lines; B-2, B-7 and B-16 showed statistically similar and high values for 100-achene weight.

4.7.9. Achene weight per head (g)

Mean values for parental achene weight per head is shown in Table 4.6. Mean values indicated that inbred lines showed range of 5-30 g. The inbred line B-9 showed highest value for achene weight per head while the inbred lines B-3 and B-16 showed lowest value for achene weight per head. There is very difference in mean value of crosses with highest and lowest achene weight per head. However, most of the inbred lines exhibited similar values for achene weight per head. The inbred lines; B-2 and B-7 showed statistically similar and high values for Achene weight per head.

4.7.10. oil content (%)

Mean values for oil content percentage is shown in table 5. Mean values indicated that single cross F_1 hybrids showed range of 27.7-43.6%. The cross CMS-21×R-18 showed highest value for oil content percentage 43.6% while the cross CMS-3×R-12 27.7% showed lowest value for oil content percentage. The cross combinations; CMS-2×R-5, CMS-5×R-5, CMS-5×R-12, CMS-5×R-18, CMS-3×R-12, CMS-6×R-5, CMS-6×R-12, CMS-7×R-18, CMS-9×R-5, CMS-9×R-18, CMS-16×R-5, CMS-16×R-12, CMS-16×R-18 and CMS-21×R-12 showed statistically similar and high values for oil content percentage. Hence, F_1 hybrids with small seed size and more 100-achene weight tend to increase oil contents. So, it is one of the most important trait in sunflower hybrid seed production. The hybrids with good oil content percentage could be exploited for commercial hybrid production.

DISCUSSION

5.1. Analysis of variance for yield and its components

The major objective of sunflower breeding is to develop hybrids with improved genetic potential for yield and yield related components. Line × tester analysis for yield and its components as shown in (Appendixs 1-X) revealed highly significant difference among parents, crosses and parents vs. crosses for plant height, internodal distance, stem girth, number of leaves, 100-achene weight, days to flowering and head diameter. While parents showed insignificant results for number of achene per head and achene weight per head. Line × tester analysis exhibited highly significant difference for all the above studied traits except head diameter; it indicated the importance of dominant variances (Memon *et al*., 2014). Significant difference had also been reported by many early researchers among sunflower genotypes our results get support from the findings of Habib *et al*., 2007, Mohanasundaram *et al*., 2010, Kang *et al*., 2013 and Saleem-ud-din *et al*., 2014.

5.2. General combining ability for yield and its components

The parental lines were checked for their general combining ability by developing crosses in all possible combinations. In this experiment eight female lines and three male lines were crossed in all possible combinations to develop F_1 hybrids (Table 4.1). Among eight female lines, B-2 performed overall good for yield and its components, B-6 and B-7 found good for 100-achene weight, achene weight per head and oil contents, B-9 and B-16 showed positive significant results for number of achenes per head while B-21 was positively associated with achenes per head and 100-achene weight. Among three male lines, R-12 gave overall good performance for yield and its components while R-5 showed positive significant result for achene weight per head. Some parental lines showed negatively significant general combining ability effect. Advantage of studying a particular trait should be given to the inbred line that is the best combiner for a particular trait regardless of where the value is positive or negative; it depends upon the direction of selection for specific trait is going on. The results of our study are in agreement with Sassikumar et al., 1999, Hladni *et al*., 2007, Khan *et al*., 2008 and Andarkhor *et al*., 2012 who also reported such types findings.

5.3. Specific combining ability for yield and its components

There was significant difference between different cross combinations that developed by using parental lines. All newly developed twenty four F_1 hybrids showed satisfactory performance under specific environmental condition. Out of 24 F_1 crosses, 13 F_1 crosses showed significant negative specific combining ability for plant height indicating non-additive gene action for decreasing of this trait. (Andarkhor *et al*., 2012). The parental lines that showed good general combining ability were then evaluated to specific combining ability under different cross combinations. The crosses CMS-2×R-5, CMS-5×R-18, CMS-6×R-12, CMS-9×R-5 and CMS-16×R-12 exhibited over all very good performance for yield and yield related components, these hybrids could be exploited to commercialization through hybrid breeding program (Table 4.2). The cross combinations, CMS-2×R-12, CMS-3×R-18, CMS-6×R-5 and CMS-7×R-5 showed positive significant results for 100-achene weight and oil contents. The F_1 hybrids, CMS-5×R-5, CMS-7×R-18 and CMS-21×R-12 exhibited significantly positive results for number of seeds per head and achene weight per head. The F_1 hybrids CMS-3×R-12 and CMS-21×R-18 showed positive significant results for achenes per head and 100-achene weight per head. The F_1 crosses CMS-3×R-5, CMS-16×R-5 and CMS-21×R-5 exhibited positive results for achene weight per head but negatively associated with 100-achene weight and number of achenes per head. The F_1 cross CMS-9×R-12 gave positive result for number of achenes per head but showed negative results for achene weight per head and 100-achene weight. The F_1 cross CMS-9×R-18 exhibited positive 100-achene weight but gave negative results for number of achenes per head and achene weight per head. The results are in accordance with the findings of Kang *et al*., 2013 and Hladni *et al*., 2014 that reported to some extent the same kind of results. Over all, single cross F_1 hybrids, CMS-2×R-18, CMS-5×R-12, CMS-6×R-18 and CMS-7×R-12 showed non-significantly negative results for yields and its components, their characteristics may be improved through development of improved inbred lines.

5.4. Correlation coefficients for yield and its components

Achene yield is the main trait in sunflower hybrid breeding. In our research, oil contents showed significant positive association with head diameter while achene yield was positively associated with seeds per head, number of leaves per plant, 100-achene, head diameter, plant height and stem girth (Table 4.3). Hladni *et al.*, 2008 and Razzaq *et al.*, 2014 also reported the similar type of findings. Small seeded hybrids associated with high oil contents while large seed size is interrelated with achene yield, most of the hybrids under study were small seeded and give good oil yield as compared to large seeded hybrids.

5.5. Commercial heterosis, heterobeltiosis and their significance for achene yield per head

High achene weight is a desirable plant parameter in sunflower (*Helianthus annuus* L) breeding. Therefore, positive result for achene yield per head is useful. Seventeen out of 24 F_1 crosses showed positive heterosis for achene yield but nine F_1 crosses revealed significant values (Table 4.4). Also 9 F_1 hybrids showed significant positive values over mid parent heterosis. Significance of heterosis showed positive heterosis over mid parent for 17 crosses while significance of heterosis exhibited positive heterosis over high parent for 10 crosses. Habib et al., 2007, Khan *et al*., 2008b, Dudhe *et al*., 2009 also observed high better parent heterosis for achene yield and differences happened may be due to different genotypes and environmental conditions which are in agreement with the present study.

6.1. Screening of F_1 hybrids based on phenotypically important plant traits

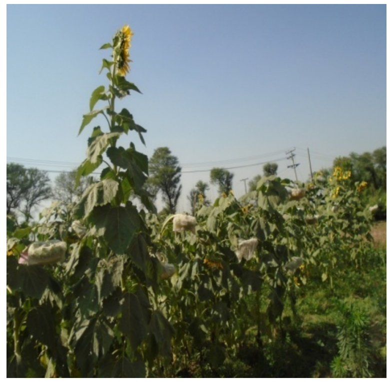
6.1.1. Infected leaves

When sunflower hybrids start flowering they were infected by army worm (*Spodoptera frugiperda*) that caused a little damage to some hybrid heads and severe damage to leaves, the surface of leaves became dry and small holes formed in the leaves (Fig. 4.1.). In this study, the insect damage varied from hybrid to hybrid. The leaves of some hybrids were more infected. Pubescence prevents from severe damage and become resistant to insects. The leaves of F_1 hybrids including CMS-2×R-12 and CMS-3×R-12 were more infected than other developed hybrids and commercial hybrids were least infected.

Figure 4.1. An overview of infected leaves of sunflower

6.1.2. Head orientation at maturity

There are three kinds of head orientation at maturity. Upward position, Straight position and downward position (Fig 4.2). The sunflower heads with upward position were most infected by birds, it occurred very less in those hybrids which had straight head position as compared to heads with upward position. The most beneficial and resistant to bird damage position was downward position and all hybrids that grown had downward head position at maturity were least infected by birds.


a) Downward position **b) Straight position of head** **c) Upward position of head**

Figure 4.2. An overview of head orientation at maturity in sunflower

6.1.3. Degree of head shape

There are three types of head position flat, Convex and concave (Fig. 4.3). Convex shape heads gave more yield than flat and concave, Flat gave more yield than concave. The most of the F_1 hybrid heads were of convex shape and gave more yield. Heads with flat and concave



a) Concave shape head

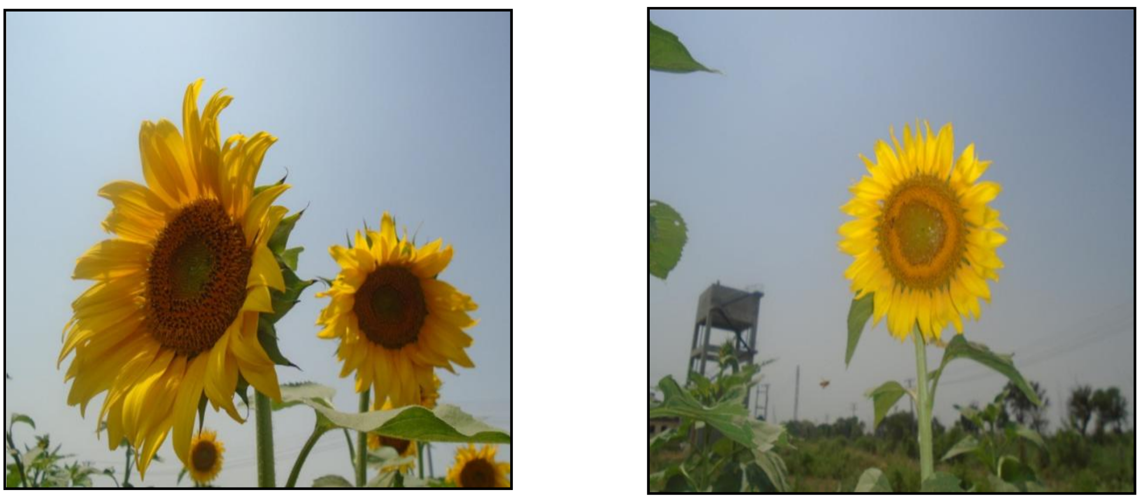
b) Convex Shape head **c) Flat shape head**

Figure 4.3: An overview degree of head shape

shape were in very less number. Head shape is correlated with head position; heads with convex shape were bended downward while heads with flat shape were in straight position or in upward position.

6.1.4. Direction of convocular brackets

There are two types of convocular brackets. Open and naked (Fig. 4.4). Heads with naked convocular brackets were more resistant to bird damage as compared to open brackets. In newly developed F_1 hybrids, most of the hybrids had naked convocular brackets and became resistant to bird damage.



a) Naked convocular brackets **b) Open convocular brackets**

Figure. 4.4. An overview of direction of convocular brackets in sunflower

SUMMARY

The germplasm of sunflower including cytoplasmic male sterile lines, maintainers, restorers and single cross F_1 hybrids were evaluated using line×tester mating technique in experimental area of University College of Agriculture, Sargodha. The research was conducted in randomized complete block design (RCBD) having three replications. Single cross F_1 hybrids were developed by crossing eight cytoplasmic male sterile lines (CMS-lines) and three restorers (R-lines). The crosses were developed in all possible combinations (24 crosses) during crop season 2014. All the F_1 hybrids along with their parents were sown for evaluation during crop season 2015. The single cross F_1 hybrids and their parents were grown up to maturity to measure various morphological and yield contributing traits viz., days to flower initiation, days to flower completion, plant height, number of leaves per plant, stem girth, leaf area, internodal distance, distance from head to soil surface, head diameter, number of seeds per plant, 100-achene weight, achene weight per head and oil content percentage. Some other phenotypically important traits that contributed toward seed yield and its component were also measured viz., degree of head orientation, degree of head shape and shape of convocular brackets. The traits that showed significant variation among genotypes (parents and crosses) were further analyzed through correlation, commercial heterosis, heterobeltiosis and mean value for yield and its components.

Overall results revealed that 10 crosses out of 24 exhibited positive significance of heterosis towards yield and yield components while 17 crosses out of 24 showed only positive heterosis. Out of 24 crosses, eight F_1 hybrids (CMS-2×R-5, CMS-2×R-12, CMS-3×R-18, CMS-5×R-18, CMS-6×R-12, CMS-7×R-18, CMS-9×R-5 and CMS-16×R-12) showed significantly positive results towards plant height, head diameter, stem girth, number of leaves, internodal distance, days to flowering, numer of seeds per head, 100-achene weight and achene weight per head. These crosses with significant results

for yield and its components would be exploited to commercialization through sunflower (*Helianthus annuus* L.) hybrid breeding program.

The objective of this research was to develop high yielded hybrids with improved morphological characters that could be used for further commercialization. According to general combing ability, out of eight lines, 3 lines (B-2, B-6 and B-21) and testers R-12 performed overall good under studied traits like plant height, internodal distance, stem girth, number of leaves per plant, days to flowering, head diameter, number of seeds per head, 100-achene weight, achene weight per head and oil contents. These lines would be used to develop hybrid seed on commercial scale. Other lines (B-3, B-5, B-7, B-9 and B-16) and tester (R-5 and R-18) may be improved through further inbreeding.

On the basis of specific combining ability and heterosis out of 24 F_1 crosses, 8 crosses (CMS-2×R-5, CMS-2×R-12, CMS-3×R-18, CMS-5×R-18, CMS-6×R-12, CMS-16×R-12 and CMS-21×R-18) performed better than other developed hybrids in view of achene yield, number of seeds per head, oil contents, head diameter, stem girth, days to flowering, internodal distance, number of leaves per plant and plant height. These F_1 hybrids could further be used on commercial scale.

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Appendix i

Line × tester analysis for internodal distance (cm) in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	0.42	0.21	0.60	0.55
Genotypes	33	330.91	10.03	28.62	0.00
Parents	11	28.03	2.55	7.27	0.00
Crosses	23	89.62	3.90	11.12	0.00
Parents vs. Crosses	1	213.26	213.26	608.57	0.00
Lines	7	37.67	5.38	1.50	0.24
Tester	2	1.84	0.92	0.26	0.78
Line x Tester	14	50.11	3.58	10.21	0.00
Error	66	23.13	0.35		
Total	101	354.45			

** Highly significance at 0.01 probability

Appendix ii

Line × Tester analysis for plant height (cm) in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	57.45	28.73	0.19	0.83
Genotypes	33	107425.35	3255.31	21.23	0.00
Parents	11	18123.20	1647.56	10.75	0.00
Crosses	23	13571.78	590.08	3.85	0.00
Parents vs. Crosses	1	75730.37	75730.37	493.99	0.00
Lines	7	3695.39	527.91	1.05	0.44
Tester	2	2803.93	1401.96	2.78	0.10
Line x Tester	14	7072.46	505.18	3.30	0.00
Error	66	10118.07	153.30		
Total	101	117600.88			

** Highly significance at 0.01 probability

Appendix iii

Line × Tester analysis for stem girth (cm^2) in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	0.01	0.01	0.09	0.91
Genotypes	33	22.73	0.69	10.83	0.00
Parents	11	2.07	0.19	2.96	0.00
Crosses	23	5.63	0.24	3.85	0.00
Parents vs. Crosses	1	15.02	15.02	236.17	0.00
Lines	7	3.22	0.46	2.88	0.04
Tester	2	0.18	0.09	0.58	0.57
Line x Tester	14	2.23	0.16	2.51	0.01
Error	66	4.20	0.06		
Total	101	26.94			

** Highly significance at 0.01 probability

Appendix iv

Line × Tester analysis for number of leaves in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	0.49	0.25	0.07	0.93
Genotypes	33	1977.19	59.91	16.56	0.00
Parents	11	214.13	19.47	5.38	0.00
Crosses	23	524.55	22.81	6.30	0.00
Parents vs. Crosses	1	1238.40	1238.40	342.21	0.00
Lines	7	243.54	34.69	2.33	0.08
Tester	2	71.86	35.93	2.40	0.13
Line x Tester	14	209.25	14.95	4.13	0.00
Error	66	238.84	3.62		
Total	101	2216.52			

** Highly significance at 0.01 probability

Appendix V

Line × Tester analysis for days for flowering in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	3.78	1.89	0.57	0.57
Genotypes	33	2588.04	78.43	23.58	0.00
Parents	11	775.37	70.49	21.19	0.00
Crosses	23	1812.65	78.81	23.69	0.00
Parents vs. Crosses	1	0.02	0.02	0.01	0.94
Lines	7	144.55	20.66	0.24	0.97
Tester	2	463.44	231.72	2.69	0.10
Line x Tester	14	1204.56	86.04	25.86	0.00
Error	66	219.55	3.33		
Total	101	2811.37			

** Highly significance at 0.01 probability

Appendix vi

Line × Tester analysis for head diameter (cm) in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	2.07	1.04	0.20	0.82
Genotypes	33	2545.46	77.14	15.24	0.00
Parents	11	132.09	12.01	2.37	0.02
Crosses	23	401.86	17.47	3.45	0.00
Parents vs. Crosses	1	2011.51	2011.51	397.47	0.00
Lines	7	245.53	35.08	4.13	0.01
Tester	2	37.41	18.70	2.20	0.15
Line x Tester	14	118.92	8.49	1.68	0.08
Error	66	334.01	5.06		
Total	101	2881.55			

** Highly significance at 0.01 probability

Appendix vii

Line × Tester analysis for number of seeds per head in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	71078.12	35539.06	1.55	0.22
Genotypes	33	15014237.38	454976.89	19.79	0.00
Parents	11	386000.89	35090.99	1.53	0.14
Crosses	23	2956583.98	128547.13	5.59	0.00
Parents vs. Crosses	1	11671652.52	11671652.52	507.57	0.00
Lines	7	518539.14	74077.02	0.51	0.82
Tester	2	385879.39	192939.69	1.32	0.30
Line x Tester	14	2052165.46	146583.25	6.37	0.00
Error	66	1517679.38	22995.14		
Total	101	16602994.88			

** Highly significance at 0.01 probability

Appendix viii

Line × Tester analysis for 100-achene weight in sunflower (Helianthus annuus L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	0.99	0.50	1.42	0.25
Genotypes	33	212.33	6.43	18.39	0.00
Parents	11	30.01	2.73	7.80	0.00
Crosses	23	81.46	3.54	10.12	0.00
Parents vs. Crosses	1	100.86	100.86	288.23	0.00
Lines	7	39.30	5.61	2.46	0.07
Tester	2	10.25	5.13	2.25	0.14
Line x Tester	14	31.90	2.28	6.51	0.00
Error	66	23.09	0.35		
Total	101	236.41			

** Highly significance at 0.01 probability

Appendix ix

Line × Tester analysis for achene weight per head in sunflower (*Helianthus annuus* L.)

SOV	DF	SS	MS	F-Cal	Probability
Replications	2	4012.45	2006.23	0.53	0.59
Genotypes	33	584223.99	17703.76	4.55**	0.00
Parents	11	1921.94	174.62	0.05	1.00
Crosses	23	437745.58	19032.42	5.00	0.00
Parents vs. Crosses	1	144556.47	144556.47	37.97	0.00
Lines	7	151244.27	21606.32	1.38	0.29
Tester	2	67427.48	33713.74	2.15	0.15
Line x Tester	14	219073.82	15648.13	4.11**	0.00
Error	66	251253.65	3806.87		
Total	101	839490.09			

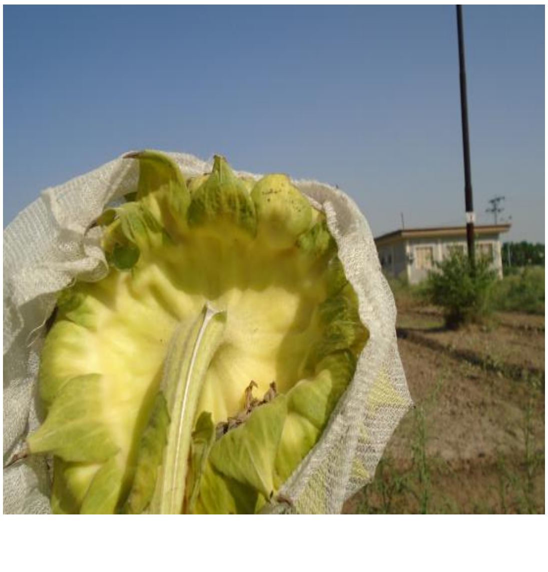
** Highly significance at 0.01 probability

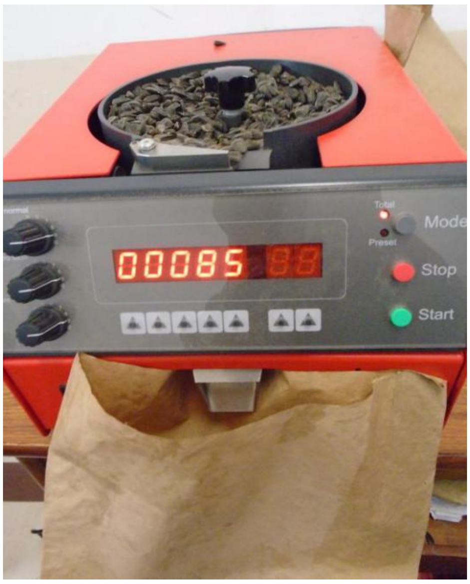
Appendix x

Analysis of variance for oil content (%)

SOV	SS	DF	MS	F	P-value	F cal
Crosses	1364.48	23	59.33	87.71	1.33E-30	1.766805
Replication	109.12	2	54.56	80.67	9.12E-16	3.199582
Error	31.11	46	0.68			
Total	1504.62	71				

** Highly significance at 0.01 probability




00085
Mode
Stop
Start


CMS-20 x R-5
0.073 kg
ON OFF
PRINT
MODE CAL
SET M
TARE


CMS-21xR-8


CMS-21 x R-12