

A review of screening and conventional breeding under different seed priming conditions in sunflower (*Helianthus annuus* L.)

Waqas Amin, *Saif-ul-Malook, Sharmin Ashraf and Amir Bibi.

Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

*Corresponding author: saifulmalookpbg@gmail.com

Abstract: Pakistan is facing severe shortage of edible oil. Sunflower can be a good alternative for fulfilling oil requirement due to its ideal characteristics like high oil contents, protein contents and short life cycle. Seed priming is a technique which is used to decrease the harmful effects of different abiotic factors and to increase the seed germination and crop production. It is highly affected by biotic and abiotic stresses like drought, heat, clod, insect/pest attack, fungal, viral and bacterial diseases. There is great loss of yield and productivity of maize due to water stress. The present review will provide its readers an opportunity to understand the breeding procedure to develop drought tolerant varieties and hybrids. Heritability, specific combining ability, dominance effects, heterosis provides a chance to develop hybrid while additive, general combining ability and genetic advance provide chance to develop hybrid for higher grain.

[Waqas Amin, Saif-ul-Malook, Sharmin Ashraf and Amir Bibi. **A review of screening and conventional breeding under different seed priming conditions in sunflower (*Helianthus annuus* L.).** *Nat Sci* 2014;12(10):7-22]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 2

Key words: *Helianthus annuus*, drought, gene action, heritability, genetic advance, heterosis

1. Introduction

Sunflower (*Helianthus annuus* L.) is an oilseed crop having high oil contents (39-52%) and protein contents (42%). It belongs to family *Astraceae*, its oil is rich in linoleic acid and oleic acid (Arshad *et al.*, 2007; Arshad and Amjad, 2012) which are very important unsaturated fatty acids. It is highly adaptable to harsh environmental conditions such as in arid and semi-arid regions (Flagella *et al.*, 2002; Kazemeini *et al.*, 2009; Ali *et al.*, 2012; Ali *et al.*, 2011a; Ali *et al.*, 2011a; Ali *et al.*, 2014).

Being a dual season oilseed crop, sunflower can be used for fulfilling our local oilseed requirement. It can also be easily fitted to our cropping pattern due to its short life cycle of 90 to 110 days. Sunflower was cultivated on an area of 1750 thousand hectares while its estimated production was 378 thousand tons (Pakistan Economic Survey, 2012-13). In Pakistan, there are many constraints behind the low production of sunflower; among them less availability of local varieties and costly imported hybrids is one of the main problems of sunflower which have to be addressed through local production. Different abiotic factors such as soil salinity, high temperature and water deficiency etc. are the major constraints of seed germination which alter different metabolisms of carbohydrates and transportation of sucrose during seedling stage (Gupta *et al.*, 1993; Kaya *et al.*, 2003; Amin *et al.*, 2014).

Sunflower is susceptible to water shortage at germination stage. Seed germination percentage and various abiotic stresses have negative correlation. Osmotic stress of sunflower seed increases at

germination stage due to salinity and drought stress which affects the uptake of water and increase the germination time, ultimately decrease total plant population and harvest index (Ahmad *et al.*, 2009).

Seed priming helps in rapid and uniform germination and ultimately increases crop production (Basra *et al.*, 2005; Saif-ul-malook *et al.*, 2014c). It also decreases the harmful effects of various stress factors on growth of crop plants. Seed priming includes the techniques like hydro priming, hardening, osmo conditioning and osmo hardening (Chiu *et al.*, 2002; Windauer *et al.*, 2007). Seed priming is a technique by which seeds are partially hydrated to a point where germination process initiated early but radicle emergence does not occur. There are different types of chemicals like poly ethylene glycol solution, ascorbic acid and alpha tocopherol which are being used for sunflower seed priming (Basra *et al.*, 2005; Saif-ul-malook *et al.*, 2014b). In this study, CaCl₂ will be used as a suitable method in improving the germination percentage and salt tolerance of sunflower. Although there is lot of research work on seed priming but certain physiological fluctuations persuaded by CaCl₂ priming have been infrequently studied in plants (Cayuela *et al.*, 1996; Saif-ul-malook *et al.*, 2014a).

2. Seedling parameter

Shanthamalliah *et al.* (1976) observed that pre-soaking of sunflower seed in water for 12 hours or 24 hours followed by drying ensured higher germinability, early emergence, better growth of crop and gave increased yield. Dimov *et al.* (1977) observed improved field emergence and uniformity

of emergence, when tomato and capsicum seeds were pre-soaked for three hour in one per cent potassium nitrate. Hussain *et al.* (2006) conducted a research study to evaluate the influence of seed priming techniques on the seedling establishment, yield and quality of sunflower hybrids. Seeds were hydro primed, hardened for 24 hrs, matri-primed for 24 and 48 hrs, osmoprimed with 0.5% KNO₃ for 12 hrs and with 0.1% NaCl for 12 hrs. The results suggested that hydro priming and osmopriming with NaCl decreased overall time taken to 50% emergence and mean emergence time and increased final emergence, energy of emergence, plant population, achene yield and yield contributing factors and achene protein contents. But seed priming did not affect the time to start emergence, plant height and achene oil contents significantly. Hardening treatment resulted in similar to or even less significant performance than that of untreated seeds.

Ehsanullah, (2011). Saicylic acid and water (hydropriming; control) priming for enhancing germination and early seedling growth in hybrid sunflower were evaluated. Sunflower achenes were primed with 50, 100, 150 and 200 ppm solutions of SA, while controlled achenes were soaked in distilled water. After 24 hours of soaking achenes were air dried for 24 hours. Half the portion of achenes was repeated with the priming applications. Both SA and hydropriming did not affect the time to start emergence while the energy of emergence was decreased by freshly primed achenes with SA compared with hydropriming and it remained unaffected in case of dry primed achenes. The emergence index was decreased in fresh primed achenes with SA compared with hydropriming while it was enhanced in dry primed achenes with SA as compared with hydropriming. Final emergence percentage was not affected in case of dry primed achenes but it was decreased for the freshly primed achenes with SA at all concentrations compared with hydropriming. Mean emergence time was low in hydroprimed achenes compared with fresh primed achenes with SA, while it remained unaffected in case of dry primed achenes. Maximum root length was resulted in freshly achenes with SA at 150 ppm. Shoot length was increased in fresh primed achenes with SA and decreased in dry primed achenes. The dry weight of achenes was not affected by the use of salicylic acid for achenes. Fresh prime achenes performed better than dry primed achenes. It was found that fresh primed achenes performed better. During this study it was concluded that the hydroprimed achenes performed better compared with achene priming with SA at all concentrations.

. Draganic and Lekic (2012) observed that anti-oxidants enhance the germination and seedling vigor

of sunflower under stress conditions. It was also observed that cold test decreased germination percentage, shoot length but had no effect on root length. There were positive effects of accelerated ageing on germination of seed and root length but no effects on shoot length and percentage of abnormal seedling. Girolamo and Barbanti (2012) reported that osmopriming with polyethylene glycol (PEG) used as osmotic agent showed an average of 11% increase in percent germination and 36% shorter mean germination time (MGT) as compared to unprimed seed. It was observed that primed seed was less dependent on temperature. Priming effects were mainly influenced by osmotic potential, temperature, time and major biochemical processes. The major disadvantage of seed priming was reduction of seed shortage life, which can be increased by initial moisture dehydration. Kaya *et al.* (2012) revealed that significant changes had been observed in hydro priming for germination percentage, oleic and linoleic acid in sunflower seeds. They also suggested that hydro priming was useful technique for high germination percentage and for high oleic sunflower seeds among the control and KNO₃ priming. Moghanibashi *et al.* (2012) reported that seed priming especially hydro priming increased the germination percentage and seedling growth parameter under drought and salinity stress. They concluded that this technique might be effective priming technique for greater germination under stress condition.

. Eskandari (2013) evaluated the effects of seed priming on germination, seedling growth and made a comparison of priming response in different field crops. He reported that critical stages in plant life cycle were germination and seedling establishment. Seed priming could be used to improve the rate and uniformity of germination. Controlled imbibition of seed followed by dehydration was shown to improve germination and early seedling growth as compare to seedlings grown from untreated seed. He also studied several pre-hydration or priming treatments applied to increase the speed and synchrony of seed germination.

Sheidaie *et al.* (2013) conducted an experiment to study the effect of hydro priming on two sunflower hybrids (Azargol and Hysun-36). The experiment was planned at CRD with three replications. There were four treatments of hydro priming and two treatments of time duration. They maintained osmotic potential levels at (0, -0.3, -0.6, -0.9 MPa). They concluded that hydro priming for short duration (6 hours) had significant positive effects on seed germination at water stress condition as compare to longer duration (18 hours). So they suggested that

hydro priming for more than 6 hours had negative influence on germination indices.

3. Agronomic traits

Bajaj *et al.* (1997) conducted an experiment for observing the sunflower lines which showed a good SCA and GCA and the additive gene effects which was important in the inheritance for the characters like 1000-seed weight, 50% flowering, oil contents having all the characters under the control of non-additive genes. He studied 44 F₁ lines having 4 male sterile lines, 11 restorer lines and their parents. These lines were analyzed in two different environments to estimate the combining ability. From the lines female parents CMS-207B CMS-10B, CMS-234B and CMS-295B and male lines P35R, RHA-856, P 13R and P 179R2 had been evaluated for good combining ability for its yield and yield related traits. Crossed parents showed good SCA effects.

Gangappa *et al.* (1997) conducted an experiment to evaluate combining ability of sunflower lines. Six testers and twelve restorer lines were used. Lines resulted for the earliness was RHA-284, RHA-99 RTNBr, RHA-299, and CMS-302. The F₁ lines CMS-852×RHA-99 RTNBr were selected for days to 50% flowering. Cultivars CMS-207 and CMS-852 were selected for the good transmitter of genes which were good for obtaining good seed yield. The hybrids CMS-234×RHA-284 and CMS-302×RHA-273 showed best combining ability for the good yield. All these characters were controlled by the additive and non-additive genes. Kandalkar (1997) conducted a research used nine lines and three cross pollinated lines of sunflower using line × tester analysis. From F₁ generation and parents it was resulted that the yield of the plant is controlled by both additive and non-additive genes. The lines showed good combining ability effects for the seed yield and none of the cultivar had good GCA effects. The cross combination showed good SCA effects are Su-IB6× Modern and Sun IBI × Surya. Lande *et al.* (1997) conducted a study to estimate heterosis and combining ability of eleven yield components in sunflower parents and crosses. The study showed non-additive gene action for all traits except days to 50% flowering. The hybrids 336A×855R, 21A×855R and 234A×IBK96 may be used to combine important traits for yield improvement.

Virupakshappa *et al.* (1997) conducted an experiment to evaluate the maximum number of male lines need to arrange the cultivars according their GCA. Research was conducted also to find out the relationship between the combining ability and performance of the lines of the sunflower. In the research 48 lines were crossed in which three were testers, an inbred line and cross pollinated line were involved. Lines were ranked according to their GCA

effects which were not significant. High GCA effects were observed in plant height, 50 % flowering, days to maturity and for oil contents. For yield and its components performance considered as a good criteria for the selection. Yenice and Arslan (1997) developed a synthetic variety of sunflower from 3 broomrape resistant selfed lines through open pollination. This variety was evaluated for heterosis of yield and yield components.

The heterosis observed for oil yield, seed yield, 1000-seed weight, husk percentage, oil percentage and stalk yield were 92.62%, 77.0%, 8.87%, 7.57%, 5.51% and 4.90% respectively under irrigated conditions. Under non irrigated condition heterosis observed for oil yield, seed yield, head diameter, plant height, oil percentage and stalk yield were 67.95%, 54.03%, 11.49%, 11.89%, 7.79% and 4.92% respectively.

Gill *et al.* (1998) evaluated the five varieties under four different condition. Lines were evaluated on the bases of better parent and varieties were checked under these four environmental conditions. It was resulted that hybrid CMS 86A × RHA 271 had selected for the earliness. F₁ hybrid CMS 234A × RHA 271 had selected for the lower magnitude for earliness. These showed the maximum heterosis for the yield of seed per plant and head diameter. These lines can be used as commercial for high heterosis. Hussain *et al.* (1998) determined the combining abilities of 6 inbred lines of sunflower for oil content, achene yield and other related traits. It was observed that, variances of SCA were more than GCA for all studied parameters and non-additive gene effects were dominant. Additive gene effects were showed for plant length and oil content. The best general combiner lines were GIMSUN-459 and GIMSUN-318 for yield whereas GIMSUN-459×GUNSUN-477 was best specific combiner for yield.

Limbore *et al.* (1998) evaluated 50 F₁ hybrids developed from 2 male sterile lines and 25 restorer lines in line × tester fashion. The high degree of heterosis for number of seeds per head and seed yield per plant was shown by hybrids 2A×IB-222 and 2A×IBC-13211 respectively. Ansaril *et al.* (1999) reported significant differences in seed yield and other traits among the parents and hybrids. The cross combinations CMS-232×RHA-229 and HOIA×RHP-44 were found to be the good combinations for seed yield so these hybrids may be used in further breeding program.

Castiglioni *et al.* (1999) studied to CMS lines and 7 S4 restoral lines of sunflower for general and specific combining ability. For seed yield and oil content the best general combiners were CMSHA 302, 89 V2345-3382 and 89V2345-3311. It was observed that non-additive gene action was dominant

for seed yield. For oil content and oil yield the cross combinations CMSHA30379NW22×89V2345-3382 were good whereas for seed yield the cross combination CMSHA 302 ×89V22396-5333 was good. Goksoy (1999) reported that mean value for plant height, head diameter, 1000 seed weight, seed per head and seed yield ranged from 154.5 to 169.6, 17.7 to 20.3, 54.4 to 63.4, 50.0 to 58.5 and 2155 to 2442 kg ha⁻¹ respectively. It was evaluated that synthetic varieties were superior to standard varieties

Naik *et al.* (1999) estimated the combining ability for twenty four hybrids of sunflower. The studied parameters were eleven related to achene yield and quality traits. For number of leaves per plant, days to fifty percent flowering, harvest index and yield per plant dominance gene action was found whereas per plant length, head diameter, hundred achene weight, husk content and oil content, over-dominance gene effects were observed. Nirmala *et al.* (1999) studied the heterotic effects for inbred lines of sunflower by using line × tester fashion. For eleven studied parameters, the performance of hybrids was superior as compared to parents and in inheritance of all studied parameters non additive gene action was found for achene yield and its related traits.

Sassikumar *et al.* (1999) estimated combining ability for achene yield and its related traits in *Helianthus annuus* L. under line × tester fashion comprising of 4 male sterile lines and fourteen restore lines. It was observed that due to the unavailability of hybrids, inadequate seed filling and shortage of self-fertile lines the production of oil seeds was less.

Ashok *et al.* (2000) developed forty hybrids of sunflower by crossing parents under Line × tester fashion. The studied parameters were plant length, stem girth, days to maturity, days to fifty percent flowering, hundred seed weight, oil content and yield/plant. In the genetics of all studied traits, the dominance of additive gene action was observed excluding oil content and hundred achene weights. The best general combiners for parents were RHA 299, RHA 273, 338 A and RHA 855 on the basis of GCA and their performance. The good specific combiners were cross combinations of 338 A × RHA 855 and 338A × RHA 296 on the basis of heterotic effects and their SCA values.

Goksoy *et al.* (2000) created 24 hybrids of sunflower artificially by crossing four restorer and six cytoplasmic male sterile lines, to approximate the heterotic effects and combining ability for achene yield and its related traits. For no. of seeds per head and achene yield variances of GCA and SCA were significant. For achene weight per head, hundred achene weight and head diameter, the variance due to SCA was highly significant whereas variance due to

GCA was significant only for plant height. For all traits excluding plant length, dominance of non-additive gene action was observed. The parents of less GCA × less GCA or more GCA × less GCA had maximum heterotic effects for most of hybrids. Hussain *et al.* (2000) determined the significant variation in CMS lines, restorer lines and test crosses for oil content. Minimum level of variation was observed in restorer lines and maximum variation in test crosses for oil content. The better parent heterotic effects were showed in maximum number of test crosses for seed yield and SCA effects contributed a major role in the performance of better quality test crosses. It was also determined that early flowering, small headed, shorter and rapid growing crosses had heavy seeds.

Jovan *et al.* (2000) estimated the combining ability, type of gene action, heterosis and genetic variance of eight inbred lines of sunflower and fifteen F₁ hybrids. In ten cross combinations, positive heterosis was found in the genetics of head diameter whereas in five remaining hybrids dominance of better parent was observed. Non additive gene effects were observed in the inheritance of studied parameters. For head diameter, significant and positive values of GCA were found in inbred lines RHA-178 and HA-BCPL whereas significant and negative values of SCA were observed in inbred lines RHA-N-K and HA-74. Nehru *et al.* (2000) evaluated three restorer lines (HA 234B, No. 61 and KBSH-1) and forty-eight inbred sunflower lines and their 144 F₁ hybrids; 81 hybrids showed positive heterosis over better parent for seed yield and 42 hybrids showed positive heterosis for oil content. The hybrid BLC-5R-2-7-3×HA 234B had maximum oil yield. The line HA 234B has good potential for seed yield and oil content so it should be used for future breeding programmes.

Siddique and Baig (2000) crossed four CMS lines and ten restorer lines of sunflower to create forty sunflower hybrids during 1995-1996 in three seasons viz. Rabi (E1), Kharif (E2) and summer (E3) in India. It was observed that CMS 338 A had highest achene yield in all seasons. All the crosses showed significant positive heterobeltiosis. The cross combinations CMS 234 A × RHA 273, CMS 207A × RHA 273, CMS 207A × RHA 298, CMS 338 A × RHA 298 showed significant heterotic effects over the standard control LDMRSH 3.

Shekar *et al.* (2000) studied combining ability of important yield components in sunflower parents and hybrids for days to 50% flowering, plant height, days to maturity, seed yield per plant, head size, 100 seed weight and oil content and reported additive gene action governing there traits. The best general combining ability for seed yield per plant, head

diameter, 100 seed weight, oil content was shown by testers RHA-83R6 and RHA-345. The best hybrid combinations were CMS-338×RHA-297, CMS-338×RHA-274, CMS-234 ×RHA-83R6 and CMS-851×RR-1 for days to 50% flowering, days to maturity, oil content and seed yield per plant respectively. Skoric *et al.* (2000) evaluated twenty Rf-lines in which 5 A-tester lines, their hundred cross combinations were used. Maximum number of important agronomically parameters of these genotypes were studied. Seed oil content (%), achene yield (Kg/ha) and plant height was also reported. In all the traits significant heterotic effects were observed of various intensity levels. Regarding to the general combining ability (GCA) significant differences were observed among A-tester lines and Rf-lines. Some lines showed positive and negative highly significant general combining ability. For the inheritance of parameters both additive and non-additive genes were responsible. The maximum contribution to the expression of the parameters was due to A-testers.

Khan (2001) reported correlation for traits, heritability and yield performance of 20 different genotypes of sunflower. All the morphological characters were positively correlated with seed yield. Days to 50% flowering, days to maturity, oil content and 100 achene weight were observed for heritability. Path analysis indicated that number of seeds per head, first flower, head diameter, and 100 achene weight had positive direct effects on seed yield while days to 50% flowering had negative effect on yield. Results showed that these traits played significant role in enhancing the seed yield but comparison should be made with all other characters because of their indirect effect on seed yield.

Laureti and Gatto (2001) estimated the GCA and SCA values of sunflower parents to select superior genotypes to utilize in breeding program and to justify the performance of hybrids. For all the studied traits significant values of GCA and SCA effects were observed for restorer and cytoplasmic male sterile lines. Jayalakshmi *et al.* (2001) developed 200 inbred lines of sunflower from crosses between lines ACC 1260, ACC 456, Morden, PSCP 1, 207 B and 234 B to evaluate for achene yield and oil content during 1999, in Andhra Pradesh, India. For oil content and seed oil, the superior lines were RGP 257, RGP 515-3, RGP 81-1, RGP 307, RGP 18-4 and RGP 240. After analysis of combining abilities, these lines can be utilized in breeding program for heterosis.

Tahir *et al.* (2002) performed correlation and path analysis for morphological characters in sunflower. They reported that percentage of filled achene per plant and head diameter was significantly

correlated with seed yield. Increase in seed yield mainly depended upon the % filled achenes, head diameter and 1000 seed weight. These traits were considered the important characters for seed yield improvement. Goksoy *et al.* (2002) carried on an experiment to estimate the genetics of studied parameters. The significant and positive heterotic effects were observed for all studied parameters. For achene yield the best general combiners were two to three parental lines whereas for achene yield and plant length, the promising hybrids were eight.

4. Heterosis

Phad *et al.* (2002) evaluated forty hybrids of sunflower with their parents for combining ability and heterotic effects. The negative range of heterobeltiosis was observed in plant length, no. of days to fifty percent flowering and maturity whereas head diameter, no. of filled sunflower seeds, hundred achene yield and yield per plant showed positive heterobeltiosis. GCA and SCA values were estimated for different plant characters. Gatto *et al.* (2002) suggested that significant general combining ability was found among the lines. In the inheritance of studied characters, non-additive gene action was most important. The ratio of SCA/GCA was greater than one. Among test crosses, 47/6-6 was best combiner and good tester that showed maximum variance. For most of the crosses SCA values were higher. The superior cross combinations had high and low GCA values of parents or two low GCA values.

Dagustu and Goksoy (2002) determined heritability, heterobeltiosis and combining abilities of six characters in sunflower. They crossed five restorer lines and five CMS lines of sunflower under Line × tester design to select the superior parents and good hybrids with maximum GCA and SCA gene effects. For achene yield and other related components, the maximum GCA values were showed by parents RHA-7 and CMS-1. Significant and positive SCA values were determined by cross combinations of 3 × 10 and 5 × 9. For all studied characters, positive and significant heterotic effects were estimated. Haq *et al.* (2002) estimated the combining abilities for seventeen hybrids of sunflower. For achene yield per plant maximum heterobeltiosis was observed in cross combination ORI-1 × RI-110 whereas maximum achene yield/plant was found in cross ORI-43 × RL-10. The hybrid ORI-2 × V-214 had tall plants and more no. of days for flower initiation (82 days) and completion of flower (86.3 days). The maximum hundred achene weight was found in cross ORI-43 × RL-84 whereas more oil contents were found in cross ORI-1 × RL-110.

Sharma *et al.* (2003) studied three cytoplasmic male sterile lines and twenty inbred lines in

line×tester scheme for the estimation of combining ability. The study showed the importance of additive gene effects in the inheritance of seed yield per plant, head diameter and oil content but plant height, 1000 seed weight, days to 50% flowering showed non additive gene effects. The lines CMS 10B, CMS 44B and testers IL-M-197, IL-IP 234, IL-IP-238 were estimated best general combiner. The crosses high × average, high × high combiners showed the highest specific combining ability effects for seed yield per plant and oil content.

Ahmad *et al.* (2004) studied heterotic effects and inbreeding depression in the crosses of sunflower. The planted source was parent inbred lines, their F_1 and F_2 populations under randomized complete block design (RCBD) using three replications. The recorded data was yield and important agronomic parameters. In all the parameters significant differences were found among the parental lines, their F_1 and F_2 populations. In F_1 population, highly significant heterotic effects were observed for leaf area and yield ranging from 46.3 to 163.9% and 102 to 309% respectively. In F_1 hybrids minimum heterotic effects were found in leaves per plant ranging from -0.9 to 39.7%.

Kaya and Atakisi (2004) performed an experiment to estimate combining ability of 25 sunflower varieties during the year 2000 and 2001 at three locations. Average flowering in sunflower hybrids varied from 66.6 to 72.8 days, physiological maturity 97.9 to 104.4 days, hectoliter weight 354.2 to 407.5 g, 1000- seed weight from 32.5 to 42.5g, plant height from 98.3 to 134.3 cm and head diameter from 12.5 to 14.2 cm. Based on all combining ability analyses performed in this research, the cross 2453-A × R-1001 was determined as the best hybrid in the experiment. Among the parental lines, HA89-A and 2644-R were chosen as first female and male testers, respectively; BAH8-A and 2280-R were chosen as second testers for testing combining ability of lines to be used in future breeding programs in sunflower improvement.

Khan *et al.* (2004) reported that yield contributing traits affected by heterosis. The parents showed highly significant genetic differences for seed weight per head, 1000 seed weight, head weight per plant, harvest index while hybrids showed significant genetic differences for all traits except head weight per plant. The hybrids TS-18×TR-120 and TS-17×TR-120 showed high level of heterosis for seed weight per head and head weight per plant respectively. The maximum heterosis for 1000 seed weight and harvest index was shown by the hybrid TS-4×TR-11. These parents and their hybrids suggested for further breeding programs due to their high heterotic effects for important characters.

Yalcin and Ibrahim (2004) estimated combining ability of hybrids of sunflower for six important morphological traits. The cross 2453-A × R-1001 was considered as the excellent hybrid on the basis of combining ability. 2644-R and HA89-A were selected as first male and female testers respectively among the parental lines whereas second testers were BAHB-A and 2280-R to estimate combining ability in sunflower. Devi *et al.* (2005) studied heterosis and combining ability for ten sunflower inbred lines. The lines 8A, 9A and 10A and testers 21R found to be best general combiners for yield components. The highest specific combining ability effects and heterosis were shown by the crosses 10A×21R, 8A×22R and 9AK×21R.

Gvozdenovic *et al.* (2005) measured the plant length and head diameter of sunflower which are related to yield. They crossed 3 tester lines (A lines) and 6 restorer lines (Rf lines) to synthesize eighteen hybrids using Line × tester fashion. For plant length and head diameter, significant differences were observed in testers, restorer lines and their F_1 generation. Maximum mean value for plant length was observed in tester HA-48A and F_1 hybrid HA-48A × RHA-SELEUS whereas minimum value was found in RHA-BRE-1 and F_1 hybrid L-19A × RHA-BRE-1. The maximum average value was observed in line L-19A and F_1 cross combination HA-48A × RHA-TR-20 for head diameter. The cross combination L-19A × RHA-BRE-1 was excellent specific combiner for plant height whereas HA-26A × RHA-SELEUS was good combiner for head diameter.

Hladni *et al.* (2005) synthesized firm and good yielding hybrids of sunflower by interspecific hybridization. The hybrids were evaluated on the basis of heterotic effects for plant length, head diameter and achene yield under line × tester design. By crossing each female line of sunflower with each tester, F_1 generation was obtained. Significant differences were found among F_1 generation and their parents for all studied traits. For achene yield per plant, the values of heterosis were significant and positive as compared to the better parent (54.8-223.2%) and mean of parental lines (98.4-274.1%). Minimum heterosis was estimated in case of plant length as compared to better parent (-3.9-51.6%) and mean of parental lines (19.0-66.0%). Jan *et al.* (2005) studied heterosis in sunflower for achene yield (kg ha⁻¹) and oil content (g kg⁻¹). The study showed that the heterosis for both traits affected by direct and reciprocal crosses. The study showed 1.29 % heterosis for achene yield, 31.19 % for oil content in direct crosses and 3.73 % heterosis for achene yield, 5.71 % for oil content in reciprocal crosses.

Maria *et al.* (2005) observed that at least one plant with short height, short internodal length and thick was found in fifteen genetic materials out of thirty genetic materials. The genetic material was consisted of three OPP and twelve inbred lines. This short height syndrome was homogenously found by all plants in the case of inbred lines and only one to four plants in the case of OPP. In the descendants syndrome was fixed and plants were self. The molecular study revealed that ten out of fifteen short height genotypes, at the HaDellel gene sequence had DDR codon modification. Ortis *et al.* (2005) estimated general and specific combining abilities among 20 cytoplasmic male sterile inbred lines and 4 testers. The largest proportional contribution of CMS inbred lines and testers, for seed oil content, plant height and 1000-kernel weight indicated the predominant role of additive components for these traits. On the other hand, line \times tester interaction exhibited the greatest contribution to gain yield suggesting the presence of non-additive genetic effect.

Yalcin (2005) studied twenty five hybrids of sunflower for four important morphological traits i.e. achene yield, oil content, oil yield and hull ratio. On the basis of analysis of specific combining ability, the best superior hybrid was 2453-A \times 2644-R in the experiment. The male lines 2644-R and R-1001 and female lines 0704-A and 2453-A had maximum achene yield and oil production. Maximum oil percentage was found in female lines BAH-8-A and HA89-A and restorer lines 2280-R and 2644-R. The female parental line 2453-A and male parental line 2644-R were the good general and specific combiners.

Habib *et al.* (2006) performed an experiment of 84 crosses and observed highly significant differences among parents, genotypes and parents vs crosses for heterosis. The maximum heterotic effects and heterobeltiosis were found in days taken to first flowering by the crosses ORI-6 \times RL-77 and ORI-6 \times RL-69 respectively. For flowering period maximum increase over mid and better parent was observed by the cross ORI-20 \times RL-77 whereas highest decrease over mid and better parent was found by the crosses ORI-20 \times RL-27 and ORI-6 \times RL-46 respectively for days taken to maturity. For plant length maximum positive heterotic effects and heterobeltiosis was observed by the cross ORI-20 \times RL-77. Khokhar *et al.* (2006) conducted an experiment on 10 genotypes of sunflower viz. A- 43, A- 75, A- 88, A- 132, G-33, G- 68, FH- 243, HBRS- 1, Bemisal- 205 and Bemisal- 4710 to determine the variability for different characters. Head size, number of whorls per head, 100 seed weight and stem diameter had positive correlations with seed weight per head. It was

concluded that improvement in these characters might also improve the seed yield.

Joksimovic *et al.* (2006) estimated combining ability for number of florets and fertilization %age in three testers, five inbreds of sunflower and their fifteen F₁ hybrids under Line \times tester fashion. In the studied characters non additive gene effects were observed as the major components of genetic variance. RHA 178 and HA 74 were good general combiner for fertilization percentage.

Haq *et al.* (2006) estimated GCA and SCA of seventeen sunflower hybrids. They observed that maximum heterosis (284.37%) was found in cross ORI-1 \times RL-110 for achene yield per plant. The maximum achene yield per plant was observed in cross ORI-43 \times RL-10 whereas taller plants were produced by cross ORI-2 \times V-214, they took maximum days (86.3) to flower completion and flower initiation (82 days). It was also found that ORI-2 \times V-214 had maximum no. of leaves per plant (24.67) and head diameter (18.20 cm). More oil contents (48.53%) were found in ORI-1 \times RL-110 whereas cross ORI-43 \times RL-84 had maximum hundred seed weight (6.04 g). Hladni *et al.* (2006) observed significant differences between A line, R line and F₁ hybrids for plant height, head diameter, seed yield per plant, 1000-seed weight and oil content. The highest positive and negative general combining ability value for oil content were shown by lines NS-GS-4 and NS-GS-5 respectively. The highest positive specific combining ability value for oil content was showed by hybrid NS-GS-6 \times RHA-R-PL-2/1.

Kaya M. D 2006. The treated seeds (control, KNO₃ and hydropriming) of sunflower (*Helianthus annuus* L.) cultivar Sanbro were evaluated at germination and seedling growth for tolerance to salt (NaCl) and drought conditions induced by PEG-6000 at the same water potentials of 0.0, -0.3, -0.6, -0.9 and -1.2MPa. Electrical conductivity (EC) values of the NaCl solutions were 0.0, 6.5, 12.7, 18.4 and 23.5 dSm⁻¹, respectively. The objective of the study was to determine factors responsible for germination and early seedling growth due to salt toxicity or osmotic effect and to optimize the best priming treatment for these stress conditions. Results revealed that germination delayed in both solutions, having variable germination with different priming treatments. Germination, root and shoot length were higher but mean germination time and abnormal germination percentage were lower in NaCl than PEG at the same water potential. Seeds were able to germinate at all concentrations of NaCl but no seed germination was observed at -1.2MPa of PEG treatments. NaCl had less inhibitor effect on seedling growth than the germination. It was concluded that

inhibition of germination at the same water potential of NaCl and PEG resulted from osmotic effect rather than salt toxicity. Hydropriming increased germination and seedling growth under salt and drought stresses.

Habib *et al.* (2007) crossed six testers and fourteen CMS lines to develop eighty four sunflower hybrids in a Line \times tester design. The parents and their crosses were genetically different. For days to flower initiation, the maximum heterosis was observed by cross ORI-6 \times RL-69 and maximum heterobeltiosis by cross ORI-6 \times RL-77. For flowering period, the maximum increase over mid and high parent heterosis was observed by cross ORI-20 \times RL-77, whereas the maximum positive heterosis and heterobeltiosis was showed by cross ORI-20 \times RL-77 for plant height. For seed yield, the maximum positive mid and better parent heterosis was observed by ORI-3 \times RL-77.

Arshad *et al.* (2007) studied 20 different sunflower hybrids for various parameters under field conditions to estimate the genetic parameters, correlations and path coefficients. Mean performance and analysis of variance for yield and its components indicated significant differences among all the genotypes for all characters. All hybrids had highly significant results for all traits. Days to maturity showed positive correlation with head diameter but negative association with seed yield. However, seed yield had highly positive genotypic correlation with oil contents but non-significant with 100 seed weight. Skoric *et al.* (2007) studied the gene action of agronomic traits in F₁ and F₂ generations. For study of yield and yield components in sunflower GCA and SCA was determined. To increase effects of heterosis for achene yield and oil content, it is essential to increase the variability of germplasm before breeding, in this way effective results can be achieved at inbreeding stage and process of evaluation of GCA and SCA values can be quick using various biotechnology methods and molecular markers to get breeding objectives.

Thombre *et al.* (2007) crossed four CMS lines and 9 testers under line \times tester design. The studied parameters were nine. For achene yield per plant, the best general combiners were CMS-207 B and CMS-234B. The highest SCA effects and achene yield was observed by cross CMS 234A \times LTRR-1-822.

Binodh *et al.* (2008) crossed three cytoplasmic male sterile lines and ten inbreds of sunflower to create thirty hybrids for eight traits during 2005-2006. For all the studied traits significant differences were found in parents and their hybrids. Dominant gene action was found in all traits. The relationship between GCA and SCA effects showed that main type of gene action was non additive viz., dominant \times

dominant and additive \times dominant gene action for most hybrids that can be utilized for heritability in sunflower.

Ghassemi 2008 evaluated the effects of hydro and osmo - priming (PEG: Polyethylene glycol 6000 at -0.8MPa) on seed germination and field emergence of lentil. Analysis of variance for laboratory data showed that hydropriming significantly improved germination rate and root weights, compared to other seed treatments. However germination percentage for seeds primed with water and PEG were statistically similar, but higher than those for unprimed seeds. Over all, hydropriming treatment was comparatively superior in laboratory tests. Invigoration of lentil seeds by hydropriming resulted in higher seedling emergence in the field, compared to control and seed priming with PEG. Seedling emergence rate was also enhanced by priming seed with water. Thus, hydropriming could be used as a simple method for improving seed germination and seedling emergence of lentil in the field. Mijic *et al.* (2008) conducted an experiment and determine GCA and SCA for grain yield, oil yield and oil content of sunflower inbred lines. The study showed the relationship between inbred lines, two-line and three-line hybrids. The highest general combining ability effects for oil yield, grain yield and oil content showed by OS-1 line. The largest SCA effects for grain yield, oil yield and oil contents were shown by OS-1A \times OS-6B. OS-1 was used to transfer its positive effects for oil yield and oil content in three way cross hybrids and it was used for further breeding programmes.

Machikowa and Saetang (2008) reported correlations among agronomic and yield related traits in sunflower. In the experiment twelve candidate synthetic varieties and four checks were tested. The results indicated that plant height, head diameter, percent seed set and 100 seed weight were strongly correlated with seed yield. Days to flowering and oil contents were negatively correlated with each other. Correlation and path coefficient analysis indicated that by selection of head diameter and plant height sunflower yield could be increased.

Sawargaonkar and Ghodke (2008) exploited the crosses with SCA effects according to their performance, GCA of their parents and heritability over their standard check hybrids KBSH-1 and LSFH-35. Good GCA effects and highest average performance traits were found between low \times low, low \times high or high \times high general combiners. The cross combinations 6D-1R \times DMLT-1Y, NDR-1 \times LR-451 and 6D-1R \times DMLT-1Y were good for head diameter, hundred seed weight and oil content.

Volotovich *et al.* (2008) conducted an experiment and evaluated 28 hybrids by using

line×tester in which crosses occurred between seven male sterile lines and four testers. The parents and their crosses evaluated for combining ability of important yield characters. Two hybrids Donskoy 22 and signal showed highest value for oil yield and suggested to use in the hybrid breeding of sunflower for Byelorussian soil climate condition. Wahid *et al.* (2008) evaluated the basis of growth vigor enhancement by sunflower seed priming with hydrogen peroxide, thiourea salicylic acid, gibberelic acid, etc. Sunflower seed priming decreased the solute leakage, increased the soluble sugar pool in achene and reduced the days to 50% germination and mean germination time. They concluded that seed priming were positively correlated with shoot length, dry weight of shoot and root. Illahi *et al.* (2009) evaluated twelve genotypes of sunflower for genotypic and phenotypic correlations among morphological traits. Achene weight per head had positive genotypic and phenotypic correlations with plant height, internodal length and head diameter. The trait 100 achene weight had positive significant genotypic and phenotypic correlations with stem diameter and head diameter. Stem diameter, head diameter and 100 achene weights could be used as indirect selection traits for the improvement of achene yield.

Jan *et al.* (2009) conducted a study to determine the magnitude of heterosis for head size, achene yield/plant, achene number per plant, 100 achene weight, achene yield (kg ha⁻¹) and oil content in sunflower. The study showed the magnitude of heterosis maximum for head size 32.88% and minimum for achene yield ha⁻¹ 1.29%. The traits achene yield ha⁻¹, achene number per plant and oil content exhibits significantly visible maternal effects but this effect was not visible for achene yield per plant, head size and 100 achene weights. Kausar *et al.* (2009) studied that sunflower seeds at high temperature and high relative humidity lose their vigor during storage. Seed priming with KH₂PO₄ ($\Psi=-1.25$ MPa) in four hybrids (Hysun 33, Hysun 38, Hysun 44 & F-330) was directed to invigorate the performance of low-vigor seeds. Priming was effective in decreasing the time for 50% germination and mean germination time (MGT) and increase in germination percentage in low-vigor seeds of all hybrids. Priming of normal/low-vigor seeds developed the vigor of seedling in term of radicle length, plumule length and their root/shoot fresh weight. Priming significantly improve the banding pattern and intensity of protein of low-vigor seeds. Neelima and Parameshwarappa (2009) conducted a research to study 30 hybrids produced by using six restorers and five cytoplasmic male sterile lines in line×tester scheme to evaluate GCA and SCA for

agronomic traits. The parents DCMS 51A and CMS 17A evaluated good general combiners for seed yield, days to maturity, head diameter, plant height and oil content. The tester RHA6D-1 showed maximum general combining ability for oil content. The hybrids CMS 4546A×RHA-271, CMS 17A×R-298, CMS 4546A×RHA-272 and CMS 103A×RHA-272 showed significant heterobiltiosis for oil content.

Khan *et al.* (2009) conducted line× tester analysis in sunflower. The variation due to general and specific combining ability indicated additive gene action for first flowering, days to hundred percent flowering and plant height while predominance of non additive gene action was observed for days to maturity. For morphological and physiological characters in sunflower both additive and non additive gene interactions were important. Through simple selection method in F₁ generation these characters might be advanced. Non additive gene interaction is desired in heterosis breeding or in hybrid seed production.

Tavade *et al.* (2009) evaluated forty five crosses of sunflower for eleven quantitative traits. It was observed that ID-3/147 R was good general combiner for achene yield per plant and oil contents, whereas cross combination of 298R × ID-3/147 was excellent for achene yield per plant. For achene yield and its related traits, the best cross was BC-3-IR × ID-3/147. It was also found that parental line ID-3/147 R was common in most superior crosses which can be utilized to transfer desirable traits. Aslam *et al.* (2010) developed sixteen hybrids by crossing four restorer lines TR-6023, TR-9, 291RGI, R-25 and four CMS lines TS-18, TS-17, TS-335 and TS-228 in randomized complete block design at Peshawar. The sunflower parents and their F₁ hybrids were genetically different for linoleic acid (C18:2), behenic acid (C20:0) and oleic acid (C18:1). For C18:1, the cross TS-18 × R-25 had highest positive mid and high parent heterosis whereas TS-18 × TR-6023 had highest positive mid and high parent heterosis for C18:2. Maximum negative mid parent heterosis was shown by crosses TS-17 × TR-9 and TS-18 × 291 RGI for C20:0. It was concluded that to improve the oil quality of the parents, mid and high parent heterosis has to be determined and can be used in further breeding programmes. Bajehbaj (2010) studied the effect of KNO₃ priming on germination and seedling traits of four sunflower cultivars sown under saline conditions. Seeds were primed with KNO₃ (-1MPa) for 24 hours in continuous 30°C with various levels of osmotic pressures induced by NaCl (5, 10, 15, 20 and 25 dSm⁻¹). Germination percentage, radical length, seedling height and total leaves per plant of primed seeds were higher than unprimed seeds. It was observed that salinity

tolerance in primed plantlets was due to high potential of these plants for osmosis regulation. Moeinzadeh *et al.* (2010) conducted an experiment to check the effectiveness of various priming agents for enhanced germination. They also studied the biopriming effect of UTPf76 and UTPf86 bacterial strains in seedling growth and germination percentage. As a conclusion biopriming with *Pseudomonas fluorescence* UTPf76 and UTPf86 had provided very well adherence of bacteria to the seed before planting and thus was suggested as a proper treatment for enrichment of seed indices and improvement of seedling growth.

Karasu *et al.* (2010) synthesized 6 artificial hybrids using 3 CMS and 2 restorer lines in Sunflower during 2005-2007. The ratio of GCA and SCA variance was lower than 1 for plant height and head diameter in 2006 and 2007, for seed yield in 2006, for number of seeds per head and thousand seed weight in 2007. The non-additive effects were more important than the other effects in all the characters. The parental lines RHA 10 and CMS 10 had high positive GCA effect for yield and yield components and were good combiners. The crosses CMS 01×RHA 10, CMS 23×RHA 10, CMS 10×RHA 10, CMS 10×RHA 03 were considered as promising hybrids for seed yield.

Mohanasundaram *et al.* (2010) studied twenty four lines and two testers in line×tester method to determine the combining ability for seed yield in sunflower. Non-additive genetic variance played a vital role in the inheritance of maximum plant traits such as days to maturity, days to 50% flowering, head diameter, 100 seed weight and seed yield/plant. The tester 234A and line 436 showed highly significant general combining ability effects for seed yield per plant. The cross combinations 234A×235, 234A×436 and 234A×440 were found to be best hybrids for seed yield per plant. Tan (2010) studied heterosis and general and specific combining ability of inbred line and their hybrids. The research showed highly significant GCA effects for all traits but non-significant SCA effects for most of the traits. The inbred lines 0583CMS, 0046CMS, 0704CMS, 0195CMS, 0043CMS, 0845RF, 1097RF and 0951RF showed maximum GCA effects and considered as best general combiner for maximum traits.

Yasin and Singh (2010) assessed 24 different genotypes of sunflower for eight characters and performed correlations and path coefficient analysis. The yield per plant showed highly significant and positive correlations with number of seeds per head, head length in diameter and 1000-seed weight at both genotypic and phenotypic levels. Path coefficient analysis revealed that number of seeds per head, 1000-seed weight and head length in diameter had

the highest and positive direct effects on yield per plant. Achenes per head, head diameter and 100 seed weight were basic parameters for the improvement of yield in sunflower.

Ghaffari *et al.* (2011) studied genetic variances, general and specific combining abilities for different agronomic traits in sunflower. F₁ hybrids (24) were developed by the use of six lines and four testers. Significant differences were found between hybrids for all the traits except head diameter. Variances among cytoplasmic male sterile lines were greater than restorer lines for all traits. Both additive and dominant effects were observed for 1000 seed weight and number of seeds per head. Dominance effect was observed for seed yield while additive effects were observed for plant height and oil content. The hybrid R23×CMS78 was found to be the best hybrid for early maturity and the highest oil content.

Machikowa *et al.* (2011) synthesized 21 F₁ hybrids and checked general combining ability and specific combining ability of seven inbred lines for thousand seed weight, plant height, head diameter, achene yield and oil contents to select superior parents. It was found that mean squares of GCA were more significant for head diameter as compared to oil content and yield, while results of mean squares of SCA were highly significant for plant height and thousand seed weight. The variance of GCA was high than the SCA variance for oil content, head diameter and yield and non-additive gene action was less important than additive gene action for these traits. The two inbred lines 2A and 5A had highest GCA effects for oil content and yield and were considered to be used as parents for hybrid production.

Nasreen *et al.* (2011) crossed six cytoplasmic male sterile lines and six fertile restorer lines to synthesize thirty six hybrids of sunflower under line×tester fashion to achieve maximum production. The studied parameters were harvest index, thousand achene weight, leaf area, moisture factor, head diameter and yield per hectare. The genetic variability was found in all studied traits. For achene yield and its related components, the excellent cross combinations were CMS-HAR-1 × RHA-854 and CMS-64 × C-206R. Ahmad *et al.* (2012) developed fifty crosses of sunflower using five testers and ten sunflower lines through line × tester analysis to evaluate for general and specific combining ability. The variation was found in days to flowering, plant height, leaf area, day to maturity, intermodal length, number of leaves per plant, stem girth, head diameter, hundred seed weight, percentage of filled achenes and achene yield per plant. In all plant traits non additive gene action was found which is useful for heterosis breeding. Crosses A-41×A-35, A-41×HBPS-1, A-165×A-26 and A-1×G-12 had

positive and significant SCA effects for hundred seed weight, achene yield per plant and percentage of filled achenes.

Yadav (2011). Seed performance improved by seed priming and overcomes the negative effects associated with stress exposure. Seeds of capsicum (*Capsicum annuum* L.) were primed with warm water, CuSO₄, potassium nitrate, and polyethylene glycol, PEG-6000 along with hydropriming and unsoaked control to study the effect of seed priming. The seed priming significantly increased the rate as well as percentage of seed germination. Further, survival response of the seedlings obtained from primed seeds was better than the control on subsequent exposure to salt or cold (4°C) stress for 10 days. All the plants obtained from potassium nitrate-primed seeds survived the salt-stress exposure, whereas those obtained from PEG-primed seeds tolerated both the cold and salt stresses. The control seedlings did not survive exposure to either stress. In addition, the growth performance of the plants obtained from primed seeds was better than control, suggesting chemical seed priming was a cost-effective and eco-friendly approach for developing cold or salt-stress tolerance in capsicum.

5. Combining ability

Andarkhor *et al.* (2012) reported that 48 sunflower hybrids developed by the use of eight lines and six testers in line×tester method. The parents and hybrids were checked and significant differences obtained plant height, head diameter, grain yield, 1000 seed weight, oil content and oil yield. A significant positive general combining ability effect for grain yield and 1000 seed weight showed by testers RF 81-25 and RF 81-30 respectively. A significant negative general combining ability effect for plant height showed by RF-131/1. A significant positive specific combining ability effects for oil content and grain yield showed by crosses AF8-6937×RF81-30 and AF80-460/2/1/1×RF81-25 respectively. Muhammad *et al.* (2012) developed 50 crosses using ten sunflower lines and five testers through line × tester design and evaluated them for general and specific combining abilities. Genetic variation was observed in plant length, leaf area, days taken to flowering, days taken to maturity, number of leaves per plant, stem diameter, head diameter, hundred seed weight, %age of filled achenes and achene yield per plant. For days taken to maturity, days taken to flowering, leaf area, achene yield per plant and internodal length, the sunflower lines A-39, A-27, A-1 had significant general combining ability effects.

Siddiqi *et al.* (2012) studied parental sunflower lines viz, TS-4, TS-7, TS-1, TS-11, TR-120, TR-2, TR-6023, SMTR-17 and their crosses for

morphological and oil traits. Among the parental lines TR-6023 had more achene yielded (1.2 ton per hectare). Maximum achene yield (2.8 ton per hectare) was found in TS-7× TR-6023 and minimum achene yield (1.08 ton per hectare) in TS-11×SMTR-17 among the crosses. The parental line TR-120 had maximum oil content (42%) and TR-6023 had minimum oil content (35%). Ali *et al.* (2013) laid out an experiment to investigate the growth and yield performance of different sunflower hybrids (Hysun-33, Hysun-38, S-278, SF-187, Pioneer-64A93FH-314 and FH-337). All the hybrids showed significant differences in all the parameters under study except number of days to emergence and number of plants. Hysun-38, Hysun-33 were taken more days to flower completion and crop maturity while Hybrids FH-337 and S-278 were taken minimum number of days to complete flowering and to maturity of crop. S-278 indicate highest head diameter which was statistically different than rest of the hybrids. Maximum number of achenes per head and 1000-achene weight was indicated by the hybrids S-278 and Hysun-33 while hybrid FH-337 showed minimum number of achenes per head and lowest 1000-achene weight. Various hybrids showed significant differences for achene yield. S-278 gave maximum achene yield (2735kg ha⁻¹) followed by Hysun-33(2542 kg ha⁻¹) whereas Hybrid FH-337 showed lowest achene yield (1650kg ha⁻¹). So S-278 and Hysun-33 gave high yield and were best adapted to climatic conditions of Sargodha. Hassan *et al.* (2013) evaluated sunflower genotypes for yield related traits in randomized complete block design with three replications. Correlation among various yield related traits and direct and indirect effects of various traits on achene weight per head were estimated. Path analysis revealed direct and indirect effects of plant height, head diameter, 100-achene weight and oil contents were positive while direct effects of stem diameter, internodal length, number of fertile whorls and protein contents were negative on achene weight per head. So selection based on these traits will be more successful in improving yield. Himidi and Anosheh (2013) concluded that seed germination rate and seedling percentage establishment under abiotic stress condition might be improved by seed priming. They evaluated effect of PEG, urea and KNO₃ priming on sunflower germination and other yield related traits. They conducted two experiments; in first experiment urea was applied with PEG while in second experiment KNO₃ was used with PEG having four levels (0.00, -0.05, -0.10 and -0.15 bar). Urea and KNO₃ had no positive effect on germination percentage but increased the radicle and shoot length. PEG decreased the germination percentage but increased the seedling growth.

Kang *et al.* (2013) crossed four testers and nine lines through Line × tester design and developed thirty six hybrids. They checked their general and specific combining abilities and genetic variability among genotypes. The highly significant GCA effects were shown by lines G-79 and G-93 for days to maturity, head diameter, no. of days to flowering, no. of days to maturity, percentage of filled sunflower seeds, hundred achene weight, oil contents and per plant achene yield. The tester A-85 showed significant GCA effects for days to maturity, days to flowering, per plant achene yield, weight of hundred sunflower seeds and oil content. The highest SCA effects were shown by cross G-65×A-85 for head diameter, weight of hundred sunflower seeds, days to maturity, per plant achene yield oil contents and days to 50% flowering.

Corresponding author:

Saif-ul-Malook

Department of Plant Breeding and Genetics

University of Agriculture, Faisalabad

E-mail: saifulmalookpbg@gmail.com

References

- Ahmad, M.W., M.S. Ahmad and H.N. Tahir. 2009. Combining ability analysis for achene yield and related traits in sunflower (*Helianthus annuus* L.). Chilean J. Agric. Res. 72(1): 21-26.
- Ahmad, S., M.S. Khan, M.S. Sawati, G.S. Shah and I.H. Khalil. 2004. A study on heterosis and inbreeding depression in sunflower (*Helianthus annuus* L.). Songklanakarin J. Sci. Technol. 27(1): 1-8.
- Ali, A., M. Aziz, S. W. Hassan, M. A. S. Ahmad, M. Mubeen and M. Yasin. 2013. Growth and yield performance of various spring planted sunflower (*Helianthus annuus* L.) hybrids under semi-arid conditions of Sargodha. Pakistan. Sci. Int. (Lahore) 25(2): 341-344.
- Ali, Q., M. Ahsan, M.H.N. Tahir, S.M.A. Basra. 2012. Genetic evaluation of maize (*Zea mays* L.) accessions for growth related seedling traits. IJAVMS, 6: 164-172.
- Ali, Q., M. Ahsan, M.H.N. Tahir, S.M.A. Basra. 2014. Gene action and correlation studies for various grain and its contributing traits in maize (*Zea mays* L.). Bothalia, 44: 80-91.
- Ali, Q., M. Ahsan, I. Khaliq, M. Elahi, M. Shahbaz, W. Ahmed and M. Naees, 2011a. Estimation of genetic association of yield and quality traits in chickpea (*Cicer arietinum* L.). Int. Res. J. Plant Sci., Vol. 2: 166-169.
- Amin, W., Saif-ul-malook, A. Mumtaz, S. ashraf, H. M. ahmad, K. Hafeez¹, M. Sajjad and A. Bibi. 2014. Combining ability analysis and effect of seed priming on seedling traits in Sunflower (*Helianthus annuus*). Report and Opinion, 6: 19-30.
- Ansari, A.H., M.A. Naz, S.A. Taran and A.A. Kakar. Heterosis and heterobeltiosis studies on *Helianthus annuus* (Linn). Pah. J. Agri. Sci. 360: 10-13.
- Arshad, M. and M. Amjad. 2012. Medicinal use of sunflower oil and present status of sunflower in Pakistan. Sci. Tech. Dev. 31: 99-106.
- Arshad, M., M.K. Ilyas and M.A Khan. 2007. Genetic divergence and path coefficient analysis for seed yield traits in sunflower (*Helianthus annuus* l.) hybrids. Pak. J. Bot. 39: 2009-2015.
- Ashok, S., N.M. Sherrif and S.L. Narayanan. 2000. Character association and path coefficient analysis in sunflower (*Helianthus annuus* L.). Crop Res. 20: 453-456.
- Ashraf, M., and M.R. Foolad. 2005. Pre-sowing seed treatment shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv Agron 88: 223-271
- Aslam, S., S.M. Khan, M. Saleem, A.S. Qureshi, A. Khan, M. Islam and S.M. Khan. 2010. Heterosis for the improvement of oil quality in sunflower (*Helianthus annuus*L.). Pak. J. Bot. 42(2): 1003-1008.
- Bajaj, R.K., Ahuja, K. and Chahal, G.S. 1997. Combining ability studies in sunflower (*Helianthus annuus*). Crop Improv. 24(1): 50-54.
- Basra, S.M.A., M. Farooq, R. Tabassum and N. Ahmad. 2005. Physiological and biochemical aspects of pre-sowing seed treatment in fine rice (*Oryza sativa* L.) Seed Sci. Technol. 33: 623-628.
- Binodh, A.K., N. Manivannan and V. Varman. 2008. Combining ability analysis for yield and its contributing characters in sunflower (*Helianthus annuus* L.). Madras, Agric. J. 95(7-12): 295-300.
- Castiglioni, V.B.R., M.F. Deoliveira and C.A.A. Arias. 1999. Combining ability analysis among inbred lines of Sunflower. Pesquisa Agropecuaria Brasileira 34(6): 981-988.
- Cayuela, E., Perez-Alfocea F., Caro M. and M.C. Bolarin. 1996. Priming of seeds with NaCl induces physiological changes in tomato plants grown under salt stress. Physiol. Plant. 96: 231-236.
- Chiu, K.Y., C.L. Chen and J.M. Sung. 2002. Effect of priming temperature on storability of primed *sh-2* sweet corn seed. Crop Sci. 42: 1996-2003.

20. Dagustu, N. and A.T. Goksoy. 2002. Combining ability and hybrid performances in sunflower (*H. annuus* L.). *Turk. J. Field Crops* 7(1): 6-14.
21. Devi, K.R., A.K. Ranganatha and M.Ganesh. 2005. Combining ability and heterosis for seed yield and its attributes in sunflower. *Agric. Sci. Digest* 25(1): 11-14.
22. Dharmalingam, C. and N.R. Basu., 1978. Control of seed deterioration in cotton (*Gossypium hirsutum* L.) *Current Sci.*, 47: 484-487.
23. Dimov, I., L. Ivanov and K. Miliev. 1977, A method for enhancing seed germination, rate and uniformity of emergence. *Gradinarstro*, 58 (4): 13-16.
24. Draganic, I. and S. Lekic. 2012. Seed priming with antioxidants improves sunflower seed germination and seedling growth under unfavorable germination conditions. *Turk. J. Agri. For.* 36: 421-428.
25. Ehsanullah, K. Jabran, M. Ismail, M. Hussain, M. Zafar and U. Zaman. 2011. Hydroprimed sunflower achenes perform better than salicylic acid primed achenes. *J. Agri. Technol.*, 7: 1561-1569
26. Eskandari, H. 2013. Effects of priming technique on seed germination properties, emergence and field performance of crops: a review. *Int. J. Agron. Plant Prod.* 4: 454-458.
27. Flagella, Z., T. Rotunno, E. Tarantino, R. Caterina, A. Caro, R.D. Caterina, A.D. Caterina and A.D. Caro. 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. *Eur. J. Agron.* 17: 221-230.
28. Gangappa, E., K.M. Channakrishnajiah, M.S. Harini and S. Ramesh. 1997. Studies on combining ability in sunflower. *Helia* 20(27): 73-84.
29. Ghassemi G. k., A. A. Alilool, M. Valizadeh and M. Moghaddam. 2008. Effects of Hydro and Osmo-Priming on Seed Germination and Field Emergence of Lentil (*Lens culinaris* Medik.) *Not. Bot. Hort. Agrobot. Cluj.*, 36: 29-33.
30. Gill, H.S., S.R. Khurana, T.P. Yadava and R.K. Sheoran. 1998. Expression of heterosis for different characters in sunflower over environments. *Haryana Agric. Uni. J. Res.* 28(2-3): 95-100.
31. Girolamo, G.D. and L. Barbanti. 2012. Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian J. Agron.* 7: 178-188.
32. Goksoy, A.T., A. Turkec and Z. Turan. 2002. Determination of some agronomic characteristics and hybrid vigour for new improved synthetic varieties in sunflower (*Helianthus annuus* L.). *Helia* 20(44): 101-110.
33. Goksoy, A.T., A. Turkec and Z.M. Turan. 2000. Heterosis and combining ability in sunflower (*Helianthus annuus* L.). *Indian J. Agric. Sci.* 70(8): 525-529.
34. Govt. of Pakistan. 2004. Ministry of Finance, Economic Advisor's Wing Islamabad.
35. Govt. of Pakistan. 2009-10. Ministry of Finance, Economic Advisor's Wing Islamabad.
36. Govt. of Pakistan. 2012-13. Ministry of Finance, Economic Advisor's Wing Islamabad.
37. Gvozdenovic, S., J. Joksimovic, D. Skoric. 2005. Gene effect and combining abilities for plant height and head diameter in sunflower. *Genetika* 37(1): 57-64.
38. Habib, H., S. S. Mehdi, A. Rashid, S. Iqbal and M. A. Anjum. 2006. Heterosis studies in sunflower (*Helianthus annuus* L.) crosses for agronomic traits and oil yield under Faisalabad conditions. *Pak. J. Agric. Sci.* 43(3-4): 131-135.
39. Habib, H., S.S. Mehdi, A. Rashid, M.Zafar and M.A. Anjum. 2007. Heterosis and heterobeltosis studies for flowering traits, plant height and seed yield in sunflower (*Helianthus annuus* L.). *Int. J. Agric. Biol.* 9(2): 355-358.
40. Haq, A., A. Rashid, M.A. Butt, M.A. Akhter, M. Aslam and A. Saeed. 2006. Evaluation of sunflower (*Helianthus annuus* L.) hybrids for yield and yield components in central Punjab. *J. Agric. Res.* 44(4): 277-284.
41. Hassan, S.M., M.S. Iqbal, G. Rabbani, Naeem-din, G. Shabir, M. Riaz and I.R. Noorka. 2013. Correlation and path analysis for yield and yield components in sunflower (*Helianthus annuus* L.). *African J. Biotech.* 12(16): 1968-1971.
42. Himidi, R. and H.P. Anosheh. 2013. Comparison effect of different seed priming methods on sunflower germination and seedling growth. *Int. J. Agron. Plant Prod.* 4: 1247-1250.
43. Hladni, N., D. Skoric and M.K. Balalic. 2005. Line \times tester analysis of morphophysiological traits and their correlations with seed yield and oil content in sunflower (*Helianthus annuus* L.). *Genetika* 40(2): 135-144.
44. Hladni, N., D. Skoric., M. Kraljevic- Balalic, Z. Sakac and D. Jovanovic. 2006. Combining ability for oil content and its correlation with other yield components in sunflower (*Helianthus annuus* L.). *Helia* 30(47): 191-198.
45. Hussain, M., M. Farooq, S.M.A. Basra and N. Ahmad. 2006. Influence of seed priming

- techniques on the seedling establishment, yield and quality of hybrid sunflower. *Int. J. Agri. Biol.* 8: 1560–8530.
46. Hussain, S.Z., R.J. Anandam and A.S. Rao. 2000. Effect of different fungicides and homeopathic drugs on seed borne fungi of sunflower (*Helianthus annuus* L.). *Ind. J. Plant Prot.* 28(2): 148- 151.
 47. Illahi, F., M.H.N. Tahir and H.A. Sadaqat. 2009. Correlation and path coefficient analysis for achene yield and yield components in sunflower. *Pak. J. Sci.* 46(1): 20-24.
 48. Jan, M., F. Raziudin and G. Hassan. 2005. Combining ability analysis in sunflower (*Helianthus annuus* L.). *Pak. J. Biol. Sci.* 8(5):710-713.
 49. Joksimovic, J., A. Jovanka, R. Marinkovic and D. Jovanovic. 2006. Genetic control of oleic and linoleic acid contents in sunflower. *Helia* 29(44): 33-40.
 50. Kandalkar, V. S., 1997, Phenotypic stability analysis in open pollinated varieties of sunflower (*Helianthus annuus* L.) in North West and South East Madhya Pradesh during winter season. *Indian J. Agric. Sci.* 67: 606-607.
 51. Kang, S.A., F.A. Khan, M.Z. Ahsan, W.S. Chatha and F. Saeed. 2013. Estimation of combining ability for development of hybrid genotypes in *Helianthus annuus* L. *J. Biol.Agric. Health* 3(1): 68-75.
 52. Karasu, A., M. Oz, M. Sincik, A. Tanju and Z.M. Turan. 2010. Combining ability and heterosis for yield and yield components in sunflower (*Helianthus annuus* L.). *Not. Bot. Hort. Agrobot.* 38(3): 259-264.
 53. Kausar, M., T. Mahmood, S.M.A. Basra and M. Arshad. 2009. Invigoration of Low Vigor Sunflower Hybrids by Seed Priming. *Int. J. Agri. Biol.* 11: 521–528.
 54. Kaya, M. D., G. Okc, M. Atak , Yakup C, İkıl , C and O. Zer K. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.) *Europ. J. Agron.*, 24: 291–295
 55. Kaya, M.D., G. Kaya and S. Bayramin. 2012. Comparison of seed priming efficiency on germination of high linoleic and high oleic acid contents in sunflower (*Helianthus annuus* L.) Seeds. *Int. J. Agri. Biosystems Sci. Eng.* 69:147-148.
 56. Kaya, Y. 2005.. Hybrid vigor in sunflower (*Helianthus annuus* L.). *Helia* 28(43):77-86.
 57. Kaya, Y. and A. Ibrahim. 2004. Combining ability analysis of some yield characters of sunflower, *Helianthus annuus* L. *Helia* 27(41):75-84.
 58. Kaya, Y. and I.K. Atakisi. 2004. Combining ability analysis of some yield characters of sunflower (*Helianthus annuus* L.). *Helia* 27(41): 75-84.
 59. Kazemeini, S.A., M. Edalat and S. Avat. 2009. Interaction effects of deficit irrigation and row spacing on sunflower (*Helianthus annuus* L.) growth, seed yield and oil yield. *African J. Agri. Res.* 4: 1165-1170.
 60. Khan, A. 2001. Yield performance, heritability and interrelationship in some quantitative traits in sunflower. *Helia*, 24: 35–40.
 61. Khan, A.A., J.D. Maguire, G.S. Abawi and S. Illas. 1992. Matricconditioning of vegetable seed to improve stand establishment in early field planting. *J. Amer. Soc. Hort. Sci.*, 117: 41-47.
 62. Khan, S.A., H. Ahmad, A. Khan, M. Saeed and S. Masaud. 2009. Using line × tester analysis for earliness and plant height in sunflower (*Helianthus annuus* L.). *Recent Res. Sci. Tech.* 1(5): 202-206.
 63. Khan. S.M., I.H. Khalil, and M.S. Sawati. 2004. Heterosis for yield components in sunflower (*Helianthus annuus* L.) *Asian J. plant Sci.* 3(2): 207-210.
 64. Khokhar , M. I., H. A. Sadaqat and M. H.N. Tahir. 2006. Association and effects of yield related traits on achene yield in sunflower. *Int. J. Agric. Biol.* 8: 450-451.
 65. Laureti, D. and A.D. Gatto. 2001. General and specific combining ability in sunflower (*Helianthus annuus* L.). *Helia* 24(34): 1-16.
 66. Limbore, A.R., D.G. Weginwar, S.S. Lande, B.D. Gite and K.M. Ghodke. 1998. Heterosis in Sunflower (*Helianthus annuus* L.). *Ann. Plant Physiol.* 12: 38-42.
 67. Machikowa, T. and C. Saetang. 2008. Correlation and path coefficient analysis on seed yield in sunflower. *Suranaree J. Sci. Technol.* 15(3): 243-248.
 68. Machikowa, T., C. Saetang and K. Fungpeng. 2011. General and specific combining ability for quantitative characters in sunflower. *J. Agric. Sci.* 3(1): 91-95.
 69. Maria, G.L., K.R. Sridhar and N.S. Raviraja. 2005 Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. *J. Agric. Technol.* 1: 67-80.
 70. Mijic, A., I. Liovic, Z. Zdunic, S. Maric, A. Marjanovic, Jeromela and M. Jankulovska. 2008. Quantitative analysis of oil field and its components in sunflower (*Helianthus annuus* L.). *Romanian, Agric. Res.* : 41-16.
 71. Moeinzadeh A., F. S.Zadeh, M. A.zadeh and F. H. Tajabadi . 2010. Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas*

- fluorescens for improvement of seed invigoration and seedling growth. A.J.C. Sci., 4: 564-570
72. Moghanibashi, M., H. Karimmojeni, P. Nikneshan and D. Behrozi. 2012. Effect of hydropriming on seed germination indices of sunflower (*Helianthus annuus* L.) under salt and drought conditions. Plant Knowledge J. 1: 10-15.
 73. Naik. V.R., S.R. Hiremath and J.K. Girira. 1999. Gene action in sunflower. Karnataka J. Agric. Sci. 12(1-4): 43-47.
 74. Nasreen, S., Z. Fatima., M. Ishaque., A.S. Mohmand., M. Khan., R. Khan and M.F. Chudhary. 2011. Heritability analysis for seed yield and yield components in Sunflower (*Helianthus annuus* L.). Pak. J. Bot. 43(2): 1295-1306.
 75. Neelima, S. and K.G. Parameshwarappa. 2009, Heterosis and combining ability for seed yield, oil content and other quantitative traits in sunflower, *Helianthus annuus* L. J. Oilseeds Res. 26(2): 94-97.
 76. Nehru, S.D., A. Manjunath, and D. Basavarajaiah. 2000. Extent of heterosis for seed yield and oil content in sunflower. Karnataka J. Agric. Sci. 13(3): 718-720.
 77. Nirmala, V.S., A. Gopalan and D. Sassikumar. 1999. Correlation and path coefficient analysis in sunflower. Agric. J. Madras 86: 269-72.
 78. Ortis, L., G. Nestares, E. Frutos and N. Machado. 2005. Combining ability analysis for agronomic traits in sunflower (*Helianthus annuus* L.). Helia, 28(43): 125-134.
 79. Pill, W.G. and W.E. Finch-Savage. 1988. Effects of combining priming and plant growth regulator treatments on the synchronisation of carrot seed germination. Ann. Appl. Biol. 114: 383-389.
 80. Punia, M.S. and H.S. Gill. 1994. Correlations and path coefficients analysis for seed yield traits in sunflower (*Helianthus annuus* L.). Helia 17: 7-12.
 81. Saif-ul-malook, M. Ahsan, Q. Ali and A. Mumtaz. 2014a. Genetic variability of maize genotypes under water stress and normal conditions. Researcher, 6: 31 – 37.
 82. Saif-ul-malook, M. Ahsan, Q. Ali, A. Mumtaz. 2014b. Inheritance of yield related traits in maize under normal and drought condition. Nature and Science, 12: 36 – 49.
 83. Saif-ul-malook, Q. Ali, A. Shakeel, M. Sajjad and I. Bashir. 2014c. Genetic variability and correlation among various morphological traits in students of UAF, Punjab Pakistan. 2014. International Journal of Advances in Case Reports, 1:1-4.
 84. Sassikumar, D., A. Goplan and T. Thirumurugan. 1999. Combining ability analysis in Sunflower (*Helianthus annuus* L.). Tropical Agric Res. 11: 134-142.
 85. Sawargaonkar, S.L. and M.K. Ghodke. 2008. Heterosis in relation to combining ability in restorer lines of sunflower. Helia 31(48): 95-100.
 86. Shanthamallaiiah, N.R., G. Pushpha, N. Venugopal and N.M. Patil. 1976. Effect of pre-soaking of sunflower seeds in water on the growth and yield of sunflower. Mysore J. Agri. Sci. 10: 34-57.
 87. Sharma, K., N. Dutta, A.K. Pattanaik, and Q.Z. Hassan. 2003. Replacement value of undecorticated sunflower meal as a supplement for milk production by crossbred cows and buffaloes in Northern plains of India. Animal Health and Production, 35: 131-145.
 88. Sheidaie, S., M. Divsalar, B. Oskouei, H. Sadeghi and E. Rezvan. 2013. Seed hydro-priming application to alleviation of water stress during germination in sunflower hybrids (*Helianthus annuus* L.). Intl. J. Agri. Crop Sci. 5: 155-159.
 89. Shekar, G.C., H. Jayaramgowda, S.D. Nehru, B.H. Halaswamy and S. Ashok. 2000. Combining ability of early maturing *cms* lines and restorers in sunflower. Mysore J. Agric. Sci. 34(4): 289-293.
 90. Siddiqui, H.M., S. Ali, J. Bakht, A. Khan, S.A. Khan and N. Khan. 2012. Evaluation of sunflower lines and their crossing combinations for morphological characters, yield and oil contents. Pak. J. Bot. 44(2): 687-690.
 91. Škorić, D., S. Jocić, and I. Molnar. 2000. General (GCA) and Specific (SCA) combining abilities in sunflower. In: Proc. of the 15th International Sunflower Conference. Toulouse, France June 12-15. E: 23-29.
 92. Skoric, D., S. Jocić, N. Hladni and G.P. Vannozzi. 2007. An analysis of heterotic potential for agronomically important traits in sunflower (*Helianthus annuus* L.). Helia 30(46): 55-73.
 93. Tahir, M.H. N., H.A. Sadaqat and S. Bashir. 2002. Correlation and path coefficient analysis of morphological traits in Sunflower (*Helianthus annuus* L.) population. Int. J. Agri. Biol. 4(3): 341-343.
 94. Tavade, S. N., S. S. Lande and S. P. Patil. 2009. Combining ability studies in some restorer lines of sunflower (*Helianthus annuus* L.). Karnataka J. Agric. Sci. 22: 32-35.

95. Taylor, A.G., P.S. Allen, M.A. Bennett, K.J. Bradford, J.S. Burris and M.K. Misra. 1998. Seed enhancements. *Seed Sci Res.* 8: 254–256.
96. Virupakshappa, K., S.D. Nehru, J. Gowda and S. Hedge. 1997. Selection of testers for combining ability analysis and relationship between per se performance and GCA in sunflower (*H. annuus* L.). *Helia* 20(26): 79-88.
97. Volotovich, A.A., T.A. Silkova, N.S. Fomchenko, O.V. Prokhorenko, K.O.G. Davyden. 2008. Combining ability and heterosis effects in sunflower of Russian origin. *Helia* 31:111-118.
98. Wahid, A., A. Noreen, S.M.A. Basra, S. Gelani, and M. Farooq. 2008. Priming-induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth. *Bot. Studies* 49: 343-350.
99. Wahid, A., M. Parveen, S. Gelani, and S.M.A. Basra. 2007. Pretreatment of seeds with H₂O₂ improves salt tolerance of wheat seedling by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.* 164: 283-294.
100. Windauer, L., A. Altuna and R.B. Arnold. 2007. Hydrotimic analysis of *Lesquerella fendleri* seed germination responses to priming treatments. *Indus Crop Prod.* 25: 70-74.
101. Yadav P. V., M. Kumari, and Z. Ahmed. Chemical Seed Priming as a Simple Technique to Impart Cold and Salt Stress Tolerance in Capsicum. *J. C. Improv.*, 25:497–503, 2011
102. Yasin, A.B. and S. Singh. 2010. Correlation and Path coefficient analysis in sunflower. *J. Plant Breed. Sci.* 2: 129-133. Ali, Q., and M. Ahsan, 2011b. Estimation of Variability and correlation analysis for quantitative traits in chickpea (*Cicer arietinum* L.). *IJAVMS*, 5: 194-200.

9/3/2014