Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.912
*Biol. Clin. Sci. Res. J., Volume, **2024**: 912*
Original Research Article

DIALLEL CROSSES AND SDS-PAGE PROFILING FOR UNDERSTANDING GENETIC COMPATIBILITY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

***LAIQUE A[1], MUSTAFA HSB[2], TANVEER M[3], ATHAR MH[2], HASSAN M[2], SARWAR S[2], SHAHZAD S[2], ASGHAR S[2], QAMAR R[2], KHAN QAT[2], ALTAF M[2], *IQBAL MA[1]**

[1]*Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan*
[2]*Oilseeds Research Institute, Ayub Agricultural Research Institute Faisalabad, Pakistan*
[3]*Department of Agronomy, University of Agriculture Faisalabad, Pakistan*
**Corresponding authors email address:* arubalaique085@gmail.com, ahsanuaf2003@uaf.edu.pk

(Received, 12th March 2024, Revised 7th June 2024, Published 17th June 2024)

Abstract *Sunflower (Helianthus annuus L.) is an economically significant oilseed crop cultivated globally, including Pakistan, due to its versatile applications in the food, feed, and oil industries. Various sunflower accessions were crossbred using a diallel technique. Following the harvest of the first season crop, these seeds were cultivated in a Randomized Complete Block Design (RCBD) with three replications in the spring of 2022–2023 for further evaluation. Data was collected on plant height, head diameter, leaf area, number of leaves per plant, internodal distance, stem diameter, head diameter, oil content, and protein content. SDS-PAGE was utilized to analyze the data using the 4×4 Griffing's technique for full diallel and to assess genetic diversity among superior crossings. The crosses F-44×F-58, F-48×F-58, F-44×F-58, and F-48×F-54 were identified as the best specific combiners due to their high SCA effect. Additionally, F-44×F-48, F-48×F-58, F-54×F-58, and F-44×F-54 demonstrated high RCA effect. Moreover, F-44×F-48, F-44×F-54, F-44×F-58, and F-58×F-44 exhibited significantly better or mid-parent heterosis. Gene action analysis revealed that plant height and internodal length were governed by additive gene action, while other traits showed dominant gene action. For advanced investigations, proteomic analysis of the genotypes was conducted, revealing variability among the genotypes for multiple traits. The standard SDS-PAGE protocol was followed, using a 15% resolving gel and a 10% stacking gel. Protein analysis PCR was conducted at a constant voltage of 80 volts for two hours, with F-48 showing the most optimal amplification. F-48 and P-58 emerged as the superior performers and best combiners.*

Keywords*:* *General combining ability; Specific combining ability; Reciprocal Combining Ability; Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis; sunflower*

Introduction

Nations with a reliable food supply can advance quickly in the current era of intense global economic competition. Oil, along with proteins and carbohydrates, is a crucial component of human nutrition. It provides fat-soluble vitamins like A, D, and E, as well as energy and essential fatty acids, which are vital for normal and healthy human development and growth (Xia *et al*., 2021). Edible oil is a rich source of sterols, phospholipids, triglycerides, and fatty acids, which are important for energy provision and regulating essential cellular activities such as gene expression, cell and tissue repair, and signal transduction (Zhang *et al*., 2019). Most edible fats and oils are derived from the fruits and seeds of oleaginous plants. Sunflower (Helianthus annuus L.) is an economically significant oilseed crop cultivated globally, including in Pakistan, due to its versatile applications in the food, feed, and oil industries (Jan *et al*., 2016). In Pakistan, both traditional (rapeseed, mustard, groundnut, sesame, cotton) and non-conventional (sunflower, soybean, safflower) edible oilseed crops are grown. Due to high levels of erucic acid, rapeseed and mustard oils are rarely used as standard cooking oils; only 5% of these oils are blended to make "ghee," or clarified butter. According to Khan *et al*. (2003), sunflower oil can significantly contribute to reducing the gap between Pakistan's production and consumption of edible oils, with sunflower seeds containing 35–45% oil. With the global population increasing, oil demand has risen worldwide. Russia is the largest producer of sunflower oil, showing the highest projected production volume of sunflower globally in the 2022-23 crop year (Statistica, 2023). In Pakistan, only 18-

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

20% of the necessary edible oil is produced locally; the remaining 80-82% is imported, with sunflower contributing about 11% of locally produced oil. As a developing country, Pakistan's economy bears a heavy burden due to the import of tea, pulses, and oil. It is crucial to increase domestic production to meet the growing demand for edible oils. This requires significant changes in traditional cultivation methods or the development of new oilseed crops. Achieving high crop yields and quality is a primary concern for farmers and researchers. Genetic compatibility plays a crucial role in the success of sunflower breeding programs, influencing traits like seed quality, yield, and adaptability to local environmental conditions (Iqbal *et al*., 2013).

Diallel crosses, a robust breeding technique, are widely used to assess genetic compatibility among different sunflower genotypes (Rauf *et al*., 2018). This method involves reciprocal crosses between multiple parental lines to analyze the inheritance pattern of important traits, providing insights into the genetic architecture and compatibility of parental lines (Gupta *et al*., 2019). As a cross-pollinated crop, sunflower is evaluated using combining ability analysis to produce high-quality composites, synthetic varieties, and hybrids. Due to cross-pollination and high heterotic value, hybrids often yield more and perform better than common varieties (Karasu *et al*., 2010). Early maturing hybrids are particularly well-suited to current cropping patterns and local conditions (Habib *et al*., 2006). To complement traditional breeding methods, advanced molecular techniques like Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) profiling have been employed in sunflower research (Nadeem *et al*., 2014). SDS-PAGE allows the separation and visualization of proteins, which provides information about genetic diversity and relatedness among sunflower genotypes (Chen *et al*., 2018). By analyzing protein profiles, researchers can identify specific proteins associated with traits of interest, such as disease resistance or oil content, aiding in the selection of compatible parental lines for breeding programs.

In Pakistan, where sunflower is a vital crop, integrating diallel crosses and SDS-PAGE profiling offers a promising approach to enhance the genetic compatibility of sunflower varieties. The current study aimed to use analytical techniques to compare sunflower genotypes under investigation and to evaluate diversity based on specific proteins. The fundamental principle of sunflower breeding is to improve yield and oil content. For enhanced yield, it is essential to evaluate and improve agronomical traits. For better oil content, genetic potential and diversity are key factors, with genetic diversity being crucial for evaluating germplasm.

Material and Methods

Three trials were conducted for this study: two in the field and one in the laboratory at the University of Agriculture, Faisalabad. The field studies were carried out at Raja Wala during the autumn and spring seasons of 2022–2023, while the laboratory experiments were conducted at the UAF Centre of Biochemistry and Biotechnology.

Field Experiments

The experimental material for this study was provided by the Department of Plant Breeding and Genetics at the University of Agriculture Faisalabad and was imported from the USDA. Four paternal lineages were used in total. Using Griffing's diallel numerical technique, these genotypes were planted in a 4×4 pattern in the autumn of 2022. The plants were sown using a Randomized Complete Block Design (RCBD) with three replications. The distance between rows was 1.5 to 2 ft, and the distance between plants was 6-9 inches. Crosses were made before 9 a.m. following manual emasculation, considering pollen viability and dehiscence. Emasculation and crossing of seed were done manually. The seeds from the parents and their crosses were carefully collected when fully developed and stored for future sowing.

In the second experiment, the seeds obtained from the various crosses were sown again during the spring season. Data on pre- and post-harvest traits were recorded. Data was collected from five random plants from each replication for evaluation, including plant height, number of leaves per plant, leaf area, stem diameter, head diameter, internodal distance, oil content, and protein content. Oil content and fatty acid analysis were performed at the Center of Advanced Studies, UAF (CASS), using a random and cleaned seed sample. The oil content (%) and protein content (%) were estimated using a Nuclear Magnetic Resonance (NMR) Spectrometer (Oxford MQA-7005) and Nuclear Infrared Resonance (NIR). The recorded data of various traits were analyzed using analysis of variance following Steel *et al*. (1997), to determine the variation among different sunflower genotypes. A detailed analysis was conducted to ascertain the general and specific combining abilities of the parents and crosses using Griffing's diallel approach, Method 1, Model 1 (Griffing, 1956).

Lab Experiment

Protein Extraction

Seeds from all four parental lines were used to evaluate seed storage proteins. For protein extraction, the seeds were grounded using a mortar and pestle. Then, 0.5g of seed powder was placed into an Eppendorf tube, and protein extraction buffers (lysis,

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

washing, and solubilizing buffers) were added. The lysis buffer consisted of 20 ml acetone, 2g trichloroacetic acid, and 40 µl 2-mercaptoethanol. The washing buffer was made with 20 ml acetone and 10 µl 2-mercaptoethanol. The solubilizing buffer included 10 µl 2-mercaptoethanol, 200 mg urea in 20 ml water, and 240 ml Dh2O in 20 ml water.

SDS gel electrophoresis

Electrophoresis was performed using resolving and stacking gels. The 15% resolving gel was prepared by mixing 3.3 ml of H2O, 4.0 ml acrylamide, 2.5 ml of 1.5M Tris-HCl (pH 8.8), 0.1 ml of 10% SDS, 0.1 ml of 10% APS, and 8 µl TEMED. The stacking gel was prepared with 3.4 ml H2O, 0.83 ml acrylamide, 0.63 ml of 0.5M Tris-HCl, 10% SDS, 10% APS, and 5 µl TEMED. Both gels were poured into gel-holding plates. The extracted protein samples were then mixed with the loading dye, which was made by dissolving 0.05g of bromophenol blue in 100 ml of H2O. The running buffer was prepared by adding 72g of glycine, 15.1g of Tris base, and 5g of SDS to 400 ml of water. Protein samples of 10 to 12 microliters were loaded into the wells. Electrophoresis was conducted at 90V for approximately 2.5 to 3 hours until the blue marker reached the bottom of the gel. The molecular weights of the distinct protein bands were compared to a standard protein ladder (Invitrogen) ranging from 10 to 220 KDa. The gels were then stained with Coomassie blue solution for 40 to 60 minutes. Subsequently, the gels were destained for over two hours using a destaining solution composed of distilled water, acetic acid, and methanol in a ratio of 5:20:75 (v/v).

Results and Discussion

Field Experiment

The results indicated that different parents contribute variably to various traits in sunflower plants, underscoring the importance of selecting parents with desired General Combining Ability (GCA) effects in breeding programs. Not all characters exhibited significant differences between locations, and no interaction was found between location and hybrid. Table 1 displays the combined abilities for various agronomical and qualitative traits, including plant height, number of leaves per plant, leaf area, head diameter, stem diameter, internodal distance, oil content, and protein contents. Plant height is largely influenced by additive genetic effects, as evidenced by the significant GCA values for some parents. According to Lai *et al*. (1997), plant height has a strong favorable genetic correlation with seed output and a positive direct effect on seed yield. For the number of leaves and leaf area, F-48 shows the highest positive GCA effect (0.9375) for leaf number, indicating its contribution to more leaves per plant, and the highest positive GCA effect (5.8029) for leaf area, suggesting it contributes to larger leaf areas. No parent has a highly significant GCA effect on head diameter, although F-44 and F-54 have positive values, suggesting they may slightly increase head diameters. F-48 has a positive GCA effect (0.2750), indicating it contributes to greater internodal distances. F-44, F-54, and F-58 also have positive GCA effects, suggesting they play a role in increasing internodal distance. Sawargaonkar and Ghodke (2008) reported positive GCA results for yield, head diameter, and oil content in earlier studies.

F-48 exhibits the highest positive GCA effect (1.09 and 0.80) for oil and protein contents in sunflower seeds. The GCA values of parents F-48 and F-58 were highly significant for qualitative traits, showing diverse results for both oil and protein contents. F-48 appears to be a promising parent for improving plant height, leaf number, leaf area, oil content, and protein content. F-58, while contributing to shorter plants, may be valuable for increasing protein content and reducing stem diameter. Among all four parents, F-48 would be the most suitable for improving both yield and oil-related parameters, as it showed highly significant results for plant height, leaf area, internodal distance, oil content, and protein content. The outcome suggests that additive gene activity is significant for yield, head diameter, and oil content in these lines, similar to findings by Kaya and Atakisi (2004) and Mijic *et al*. (2008), who also discovered that GCA variance for these qualities was greater than SCA variance. Parents F-48 and F-58 showed highly significant results for GCA of multiple characters, indicating their suitability for variety development. GCA effects can help breeders make informed decisions about which parents to use in sunflower breeding programs to enhance specific traits of interest. These findings should be considered along with other factors, such as Specific Combining Ability (SCA) effects, heritability, and environmental factors, to make comprehensive breeding decisions.

Table 1 Estimates of general combining ability effects of parents for different characters in sunflower

Parents	Plant Height	Number of leaves per plant	Leaf area	Head diameter	Stem diameter	Intermodal distance	Oil contents	Protein contents
F-44	-00625	-0.3125	4.6508	0.2208	0.1042*	-0.1458	0.56	-0.36*
F-48	3.5208**	0.9375	5.8029*	-0.0250	-0.0833*	0.2750*	1.09**	-0.37*
F-54	-0.5625	-0.2708	-0.5771	0.1917	0.0542	0.3250*	-0.75*	-0.06
F-58	-2.895**	-0.34542	-9.87**	-0.3875*	-0.0750	-0.4542**	-0.90**	0.80**

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

The SCA effect of component lines is utilized to evaluate a cross's potential for harnessing heterosis. According to Sprague and Tatum (1942), non-additive gene activity regulates the SCA. The SCA effect is a critical factor in assessing hybrids. Table 2 provides estimates of specific combining ability (SCA) effects for various traits in sunflowers resulting from crosses between different parent combinations. SCA measures the non-additive genetic effects that arise from the interaction between specific parent pairs. Several notable patterns emerge from the data: Crosses involving F-48 consistently exhibit positive SCA effects for plant height, leaf number, leaf area, and oil content, indicating that F-48 has strong complementarity with other parents in enhancing these traits. Conversely, crosses with F-58 often lead to negative SCA effects for stem diameter, suggesting that combining F-58 with other parents tends to reduce stem thickness. Additionally, some crosses, such as F-44×F-58 and F-54×F-44, show substantial positive SCA effects for oil content and protein content, implying that these specific parent combinations hold the potential for improving sunflower nutritional characteristics. Overall, the SCA effects underscore the importance of considering specific parent combinations in sunflower breeding programs to achieve desired trait enhancements through non-additive genetic interactions. Among the crosses, F-44×F-58, F-48×F-58, F-44×F-58, and F-48×F-54 were identified as the best general combiners due to their high SCA effects. In contrast, F-44×F-48, F-48×F-58, F-54×F-58, and F-44×F-54 showed high RCA effects. Additionally, F-44×F-48, F-44×F-54, F-44×F-58, and F-58×F-44 demonstrated significantly better or mid-parent heterosis. Gene action analysis revealed that plant height and internodal length exhibited additive gene action, while the remaining traits displayed dominant gene action. The best-performing parental accessions and crosses can be utilized for various breeding programs. Non-additive gene actions are generally less predictable and stable, whereas additive gene actions or complementary epistatic gene interactions are more reliably transferable (Iqbal *et al*., 2007).

Table 2 Estimates of specific combining ability effects of parents for different characters in sunflower

Crosses	Plant Height	Number of leaves per plant	Leaf area	Head diameter	Stem diameter	Intermodal distance	Oil contents	Protein contents
F-44×F-48	2.4792	1.9792	11.40**	0.7500*	0.0208	0.3458	2.61**	0.57
F-44×F-54	2.8958	0.6875	0.3583	-0.4667	-0.2333**	-0.1208	-2.23**	-0.34
F-44×F-58	5.7292**	2.6042*	0.7579	-0.2542	-0.0208	0.1250	1.81**	1.88**
F-48×F-54	1.6458	-0.2292	-2.6304	-0.0375	0.1208	0.6750**	1.61**	0.76*
F-48×F-58	-4.854**	2.3125*	1.3708	0.6583**	0.0500	-0.1292	-2.28**	0.19
F-54×F-48	-2.7708	1.8958	1.0858	0.0750	0.2125**	-0.4292	-0.73	2.35**
F-48×F-44	5.8333**	0.8333	24.67**	0.8000	-0.1000	-0.2167	-0.70	-0.55
F-54×F-44	2.8333	0.6667	19.76**	1.3667**	0.3167**	0.6333*	2.60**	0.17
F-54×F-48	4.0000	0.8333	16.833**	0.8333*	0.2333*	0.1667	1.93*	-0.60
F-58×F-44	0.8333	1.000	-3.1667	0.4500	0.167	-0.4167	3.48**	-0.73
F-58×F-48	4.333**	2.5000	6.9017	-0.0667	0.1167	0.1667	2.06**	-1.80*
F-48×F-54	4.667**	2.5000	1,9000	0.1333	-0.1833	-0.0833	2.76**	0.50

Table 3 provides insight into the genetic diversity for heterobeltiosis in various sunflower crosses across different traits. Heterobeltiosis measures the superiority of hybrids over their parents and is crucial in plant breeding for selecting high-performing hybrids. In this context, "ns" denotes non-significant differences, while asterisks indicate the significance levels of the heterobeltiosis values. When considering crosses involving the same parent (F-44×F-44 or F-48×F-48), the heterobeltiosis values for most traits are not statistically significant (ns), suggesting that using the same parent in the cross does not lead to substantial improvement in these traits. However, there are exceptions; for instance, in the F-44×F-44 cross, there is a significant improvement in oil and protein contents, indicating heterosis in these traits. Notably, some crosses show significant positive heterobeltiosis values, indicating that the hybrid offspring outperform their parents in certain traits. For example, the F-44×F-54 cross exhibits significant positive heterobeltiosis for leaf area, stem diameter, oil content, and protein content, indicating the potential for improvement in these traits through hybridization. Conversely, some crosses demonstrate negative heterobeltiosis values, suggesting that hybrid offspring may perform worse than their parents in certain traits. For instance, the F-48×F-58 cross shows negative heterobeltiosis for plant height, stem diameter, and oil content, indicating potential challenges in improving these traits through hybridization with these specific parent combinations. Overall, the heterobeltiosis values provide valuable

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

information for sunflower breeders, guiding them in selecting the most promising crosses for enhancing specific traits of interest while considering the genetic diversity inherent in different parent combinations. These results are supported by the earlier findings of Sugoor *et al*. (1996), Gangappa *et al*. (1997), Sessiikumar and Gopalan (1999), and Jayalakshmi *et al*. (2000), who also reported positive mid-parent heterosis for plant height in sunflower.

Table 3 Genetic diversity for Heterobeltiosis in different Sunflower crosses

Crosses	Plant Height	Number of leaves per plant	Leaf area	Head diameter	Stem diameter	Intermodal distance	Oil contents	Protein contents
F-44×F-44	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	6.32*	6.49**
F-44×F-48	6.03 ns	3.45 ns	82.55 ns	17.78 ns	-15.79 *	10.65 ns	0.64	9.48*
F-44×F-54	14.37 ns	15.49 ns	45.45 ns	6.33 ns	-6.32 **	2.56 ns	7.71	19.39**
F-44×F-58	19.51 ns	23.94 ns	31.22 ns	0.00 ns	-6.32*	3.03 ns	8.98*	11.05**
F-48×F-44	-11.56 ns	-2.30 ns	-7.84 ns	-3.56 ns	-9.47 *	18.34 ns	9.86	7.73**
F-48×F-48	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	-0.51	4.56**
F-48×F-54	-3.52 ns	-3.45 ns	-3.72 ns	-2.95ns	3.66 ns	5.13 ns	-9.30	8.06*
F-48×F-58	-11.56 **	-5.75 ns	5.01 ns	24.57 ns	15.49 ns	3.55 ns	-3.35	13.81**
F-54×F-44	4.19 ns	9.86 ns	-28.90 ns	-28.27 ns	-26.32 **	-16.92 ns	1.02	27.30**
F-54×F-48	-6.03 ns	-10.34 ns	7.88 ns	-14.35ns	2.44 ns	17.95 ns	0.26	24.47**
F-54×F-54	0.00 ns	0.00 ns	0.00 ns	0.00ns	0.00 ns	0.00 ns	6.32*	19.48**
F-54×F-58	0.00 ns	28.17 ns	-14.11 ns	-13.50 ns	-0.00 ns	-3.03 ns	-9.55	23.06**
F-58×F-44	2.40 ns	16.90 ns	-36.23 ns	-22.22 ns	-21.05 *	-2.37 ns	1.44	8.06*
F-58×F-48	4.88 ns	-22.99 ns	-20.27 ns	26.86 ns	5.63 ns	-15.38 ns	-8.51	9.17**
F-58×F-54	-24.62 **	7.04 ns	-21.25 ns	-10.13 ns	13.41 ns	0.00 ns	10.28	4.56**
F-58×F-58	-14.37 ns	0.00 ns	-0.00 ns	0.00 ns	0.00 ns	-3.03 ns	0.71	9.48*

Lab Experiment; SDS-PAGE Analysis

To stabilize production and increase yields amidst disease epidemics and environmental changes, as well as to harness genetic resources for enhancing germplasm performance, genetic diversity is imperative. In assessing the diversity of seed proteins in crop germplasm, SDS-PAGE electrophoresis is commonly employed. The Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method is frequently utilized for the separation of seed storage proteins. The standard SDS-PAGE protocol was adhered to, utilizing a 15% resolving gel and a 10% stacking gel. Protein analysis via PCR was conducted at a constant voltage of 80 volts for two hours. Four of the best-performing crosses from the breeding material were selected for protein analysis.

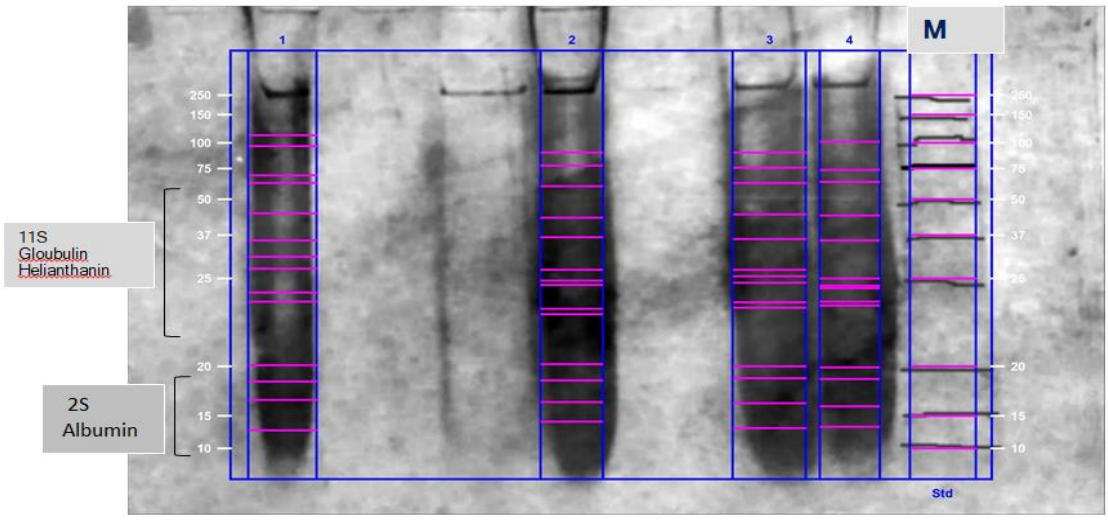

Figure 1; SDS-PAGE patterns of proteins from sunflower genotypes

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

Water-soluble albumins (2S) and salt-soluble globulins (helianthinin 11S) represent the predominant protein subtypes in sunflower seeds. Sunflower albumins (SFAs) are characterized as alkaline proteins with molecular weights ranging from 10 to 18 KDa. Among the various forms of SFAs, methionine-rich 2S proteins (12 KDa) and 16 KDa 2S albumin storage proteins are notable examples. Helianthinin, on the other hand, consists of six spherical subunits arranged in a trigonal antiprism at neutral pH. Each subunit comprises six monomers, consisting of basic and acidic polypeptide chains linked by disulfide bridges, with molecular weights ranging from 32 to 44 KDa. SDS-PAGE analysis revealed significant protein subunits and polypeptides, ranging from 46.2 to 96.4 kDa, indicating possible partial dissociation of helianthinin. The sunflower hybrid P-58 exhibited notably higher levels of 2S albumin compared to other varieties, with kernels from P-58 containing the highest proportion of 2S protein (27.3% of total extracted protein). This abundance of sulfur-rich proteins suggests their potential for enhancing the nutritional value of seeds and vegetative tissues, particularly for ruminant feeding, as sunflower 2S proteins are known to resist degradation by rumen bacteria (Kortt *et al*., 1991).

Despite the significance of these findings, comparisons with existing literature were challenging due to limited published data on the protein composition of sunflower fractions. Difficulties in isolating and purifying the primary protein component of sunflower seeds were noted, with factors such as the presence of phenolic compounds, notably chlorogenic acid, posing obstacles. Phenolic compounds are known to interact with proteins, reducing their digestibility and functionality (Sastry and Rao, 1990). Consequently, the formation of globulins (helianthinin) during seed processing was observed as a secondary by-product. These challenges underscore the complexity involved in characterizing sunflower seed proteins and highlight the need for further research to elucidate their composition and potential applications in diverse agricultural contexts (Durante *et al*., 1989).

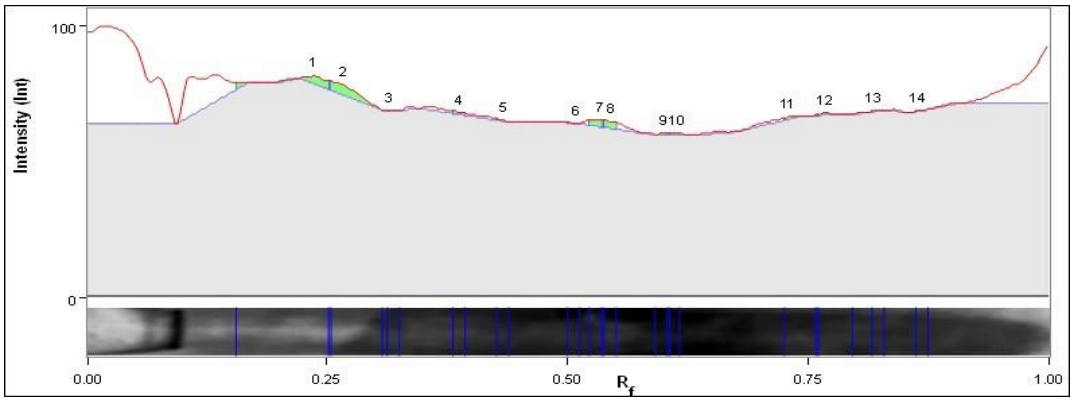

Figure 2 Band Analysis of Parent F-48

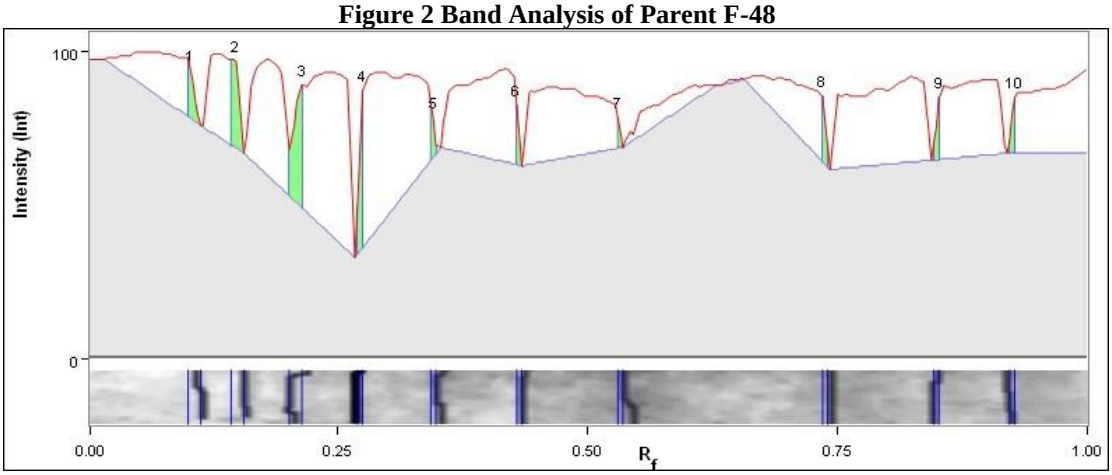

Figure 3 Band Analysis of Parent F-58

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

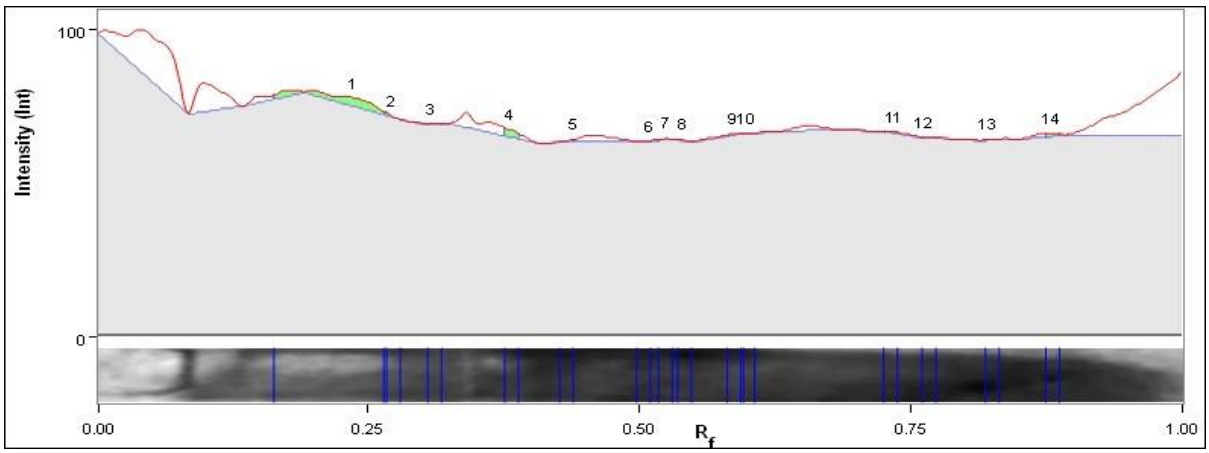

Figure 4 Bio Rad Precision plus Marker

Conclusion

From the previously mentioned experiment, it can be inferred that parental diversity based on morphological and seed storage protein polymorphism resonates with the heterotic manifestations of various crossings' characteristics.

References

Chen, Y., Zhang, Z., Li, J., Zhao, T., & Zhao, J. (2018). SDS-PAGE analysis of storage proteins in cultivated and wild sunflower. American Journal of Plant Sciences, **9**(05): 864-878.

Durante, M., Bernardi, R., Lupi, M.C. and Sabelli, P.A., (1989). Characterization of Helianthus annuus L. storage proteins. J. Agric. Food Chem. 37: 852-855.

Gangappa, E.K.M. Channakrishnaiah, S. Ramesh and M.S. Hariri, (1997). Exploitation of heterosis in sunflower (Helianthus annuus L.). Crop Res., **13**: 339–48.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. biol. sci. 9(4):463-493.

Gupta, V. K., Balyan, H. S., & Gahlaut, V. (2019). Diallel analysis for gene action study in sunflower (Helianthus annuus L.). Current Journal of Applied Science and Technology, **35**(2), 1-10.

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. Pak, J. Agri. Sci. **43**(3-4):131-135.

Iqbal, A., Khan, S., Basra, S. M. A., & Afzal, I. (2013). Genetic variability and path analysis for yield and its components in sunflower (Helianthus annuus L.). Journal of Agricultural Science and Technology, **15**, 1113-1122.

Iqbal, M. J., & Ashiq, M. N. (2007). Adsorption of dyes from aqueous solutions on activated charcoal. *Journal of hazardous materials*, **139**(1), 57-66.

Jan, S. A., Shinwari, Z. K., & Khan, I. A. (2016). Genetic diversity among sunflower (Helianthus annuus L.) genotypes based on molecular and morpho-agronomical traits. Genetics and Molecular Research, **15**(3).

Jayalakshmi, V., B. Narendra,V. Sridhar and K.R. Devi, (2000). Heterosis in sunflower (Helianthus annuus L.). Agric. Sci. Digest, 20: 114–5

Karasu. A.M. Oz, M. Sincik, A.T. Goksoy and Z.M. Turan, (2010). Combining Ability and Heterosis for Yield and Yield Contents in Sunflower. Not. Bot. Hort. Agrobot. Cluj **38**(3):259-264.

Kaya, Y. & Atakisi, I.K. (2004). Combining ability analysis of some yield characters of sunflower (Helianthus annuus L.). Helia, 27, 75-84.

Khan, S.A., H. Ahmad, A. Khan, M. Saced, S. M. Khan and B. Ahmad. (2009). Using line x tester analysis for earliness and plant height traits in sunflower (*Helianthus annus* L.).Rec. Res. Sci. Tech. **1** (5):202-206.

Kortt, A.A. and Caldwell, J.B., (1990). Low molecular weight albumins from sunflower seed: identification of a methionine-rich albumin. *Phytochemistry* **29**: 2805-2818.

Lal, G.S., V.S. Bhadoriya and A.K. Singh, (1997). Genetic association and path analysis in elite lines of sunflower. Crop Res. Hisar, **13**: 631–4

Mijic, A., Kozumplik, V., Kovacevic, J., Liovic, I., Krizmanic, M., Duvnjak, T., Maric, S., Horvat, D., Simic, G. & Gunjaca, J. (2008). Combining abilities and gene effects on sunflower grain yield, oil content and oil yield. Period biol., **110**: 277-284.

Nadeem, M. A., Shahid, M. Q., & Dasti, A. A. (2014). Protein profiling and genetic diversity among sunflower genotypes using SDS-PAGE markers. Pakistan Journal of Botany, **46**:953-957.

Rauf, S., Khan, A. A., Khan, M. A., Ullah, I., & Ali, A. (2018). Estimation of gene action involved in yield and its attributing traits in sunflower

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]

through diallel analysis. Pakistan Journal of Agricultural Sciences, 55:575-580.

Sastry, M.C.S. and Rao, M.S.N., (1090). Binding of chlorogenic acid by the isolated polyphenol free 11S protein of sunflower (Helianthus annuus L.) seed. J. Agric. Food Chem. **38**: 2103-2110.

Sawargaonkar, S.L. & Ghodke, M.K. (2008). Heterosis in relation to combining ability in restorer lines of sunflower. Helia, **31**, 95-100.

Sessikumar, D. and A. Gopalan, (1999). Heterosis for seed yield and its components in sunflower (Helianthus annuus L.) Madras Agric. J., **86**: 565–7

Sprague, G. F., & Tatum, L. A. (1942). General vs. specific combining ability in single crosses of corn 1. Agronomy journal, **34**: 923-932.

Steel, R.G.D., J.H. Torrie, and D.A. Dickey, (1997). Principles and Procedures of Statistics;Biometrical Approach (2nd Ed). McGraw Hill Book Co. Inc. Singapore.

Sugoor, R.K., K. Giriraj and P.M. Salimath, (1996). Heterosis for yield and earliness in crosses involving induced mutant restorer lines of sunflower. J. Maharashtra Agric. University, **21**: 467–8

Xia, Q., Z. Du, D. Lin, L. Huo, L. Qin, W. Wang, L. Qiang, Y. Yao, and Y. An, (2021). Review on contaminants in edible oil and analytical technologies. Oil Crop Sci. **6**:23-27.

Zhang, L., S. Wang, R. Yang, J. Mao, J. Jiang, X. Wang, W. Zhang, Q. Zhang, and P. Li, (2019). Simultaneous determination of tocopherols, carotenoids and phytosterols in edible vegetable oil by ultrasound-assisted saponification. Food chem. **289**:313-319.

Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

Funding Statement

Not applicable

Conflict of Interest

There is no conflict of interest among the authors regarding this case study.

Authors Contribution

All authors contributed equally.

[Citation: Laique, A., Mustafa, H.S.B., Tanveer, M., Athar, M.H., Hassan, M., Sarwar, S., Shahzad, S., Asghar, S., Qamar, R., Khan, Q.A.T., Altaf, M., Iqbal, M.A. (2024). Diallel Crosses and SDS-PAGE Profiling for Understanding Genetic Compatibility in Sunflower (*Helianthus Annuus* L.). *Biol. Clin. Sci. Res. J.*, **2024***:* 912. doi: https://doi.org/10.54112/bcsrj.v2024i1.912]