

## Heritability, Genetic Advance, Correlation Analysis of Fatty Acid Profile and Economically Important Traits of Sunflower Genotypes

Tilahun Mola\*, Dereje Fekadu and Yadesa Abeshu

Department of Holeta Agricultural Research Center, Holeta, Ethiopia

### Abstract

In any plant breeding program, genetic variability and related parameters study in a given population is a pillar to identifying and selecting elite breeding materials to develop ideal varieties. Two hundred twenty sunflower genotypes including checks were evaluated for important agronomic traits and their Fatty acid profile contents using an Alpha Lattice design with two replications. A significant genetic variability and its related parameters were observed for all the traits under study. Descriptive statistics analysis shows a remarkable phenotypic variation among sunflower genotypes. The high genotypic and phenotypic variance was observed for stearic acid content, Oleic acid content, linoleic acid content, days to maturity, days to flowering, and chlorophyll content. A significant phenotypic variance was observed for all studied traits, especially for selected fatty acid Profiles. Fatty acid profile, palmitic acid, stearic acid, and Oleic acid content and 100 seed weight showed high PCV and GCV values. The Highest heritability coupled with high genetic advance as a percent of mean was observed by palmitic acid content, stearic acid content, Oleic acid content, days to flowering, days to maturity, hundred seed weight, and oil content. Highly negatively correlated and positively correlated responses of the traits were also observed. Exhaustive work should be done in Ethiopia supported by molecular data is recommendable for future breeding. The output of this study can be used for information, and reference and will also be helpful in the amplification of future sunflower improvement at the national level.

**Keywords:** Sunflower; Correlation; Fatty acid profile; Genetic advance; Heritability

### Introduction

The cultivated sunflower (*Helianthus annuus L.*) is a member of the family Compositae (Asteraceae) and has originated in the USA. It was introduced to Europe during the 16th century and by the early 1880's it was developed into an important source of oil in Russia. Sunflower ranks 2nd next to soybean among the annual crops grown for edible oil in the world. It is a temperate crop widely cultivated worldwide; in Romania, Turkey, Russia, Spain, Argentina, China, Hungary, Bulgaria, USA, France, India, Australia, South Africa, and Serbia. The success of the Russian breeders in improving the oil content from less than 30% to 50% was a key factor for sunflower to become one of the world's major oilseed crops (Fick & Miller, 1997) [1]. Sunflower performs best when grown in the mid-altitude areas of 1300-2200 m.a.s.l with mean temperatures ranging from 16 0C to 25.0 0C but also due to its elastic behavior it can perform up to 800 m.a.s.l and 2400 m.a.s.l (Hiruy, 1989). Sunflower is adapted to a wide range of soil conditions due mainly to its deep and prolifically branching root system. Deep, fertile, and well-drained soils with near-neutral reactions are preferable [2]. Protein, oil contents, and fatty acid profile composition (Flagella et al., 2002) in sunflower are significantly influenced by season mainly by the temperature, and growth durations which are particular characteristics of seasonal changes (Killi, 2004). The longer maturity period and warm temperature at the time of seed formation and seed filling stage are favorable for high oil contents (Kaleem et al., 2010). According to Bukhsh, (2011), Sunflower varieties exhibited significant genetic variability in the fatty acid profile, protein content, and oil content of the seed as well as the unsaturated to saturated ratio of fatty acids of sunflower varieties (Kaleem et al., 2010; Omid et al., 2010; Flagella et al., 2002; Hassan et al., 2012). The recommended unsaturated fatty acids to saturated fatty acids ratio is 2:1 for best human consumption (Bukhsh et al., 2010). Currently, Sunflower edible oil is highly used by heart patients and also higher-class communities because of its premium; very low cholesterol concentration, and high fatty acid concentration (Putnam et al., 1990; Flagella et al., 2002; Omid et al.,

2010) [3,4].

Sunflower was introduced to Ethiopia nearly 170 years ago, but production was started very recently following introductions of germplasm material in the 1980s, on State farms and also by some farmers in potential areas (Hiruy, 1989).

Sunflower is an important Oilseed crop having good potential to support the domestic edible oil requirements in Ethiopia. It has the lion's share in vegetable oil consumption of daily oil intake worldwide as well as in our country (Anonymous, 2008-9) [5]. The edible oil scenario in Ethiopia is a major headache and a big issue in our country to allocate millions of dollars for the import of edible oil. The limited number and low Productivity of open-pollinated varieties are also becoming a serious issue for the national oilseeds program. The demand and supply of sunflower raw materials for emerging oil factories in Ethiopia are below 50% needed by the industries. Not only the quantity but also the quality of edible oil is a high concern for health issues in the current scenario. The demand for sunflower seed has been increasing for the last five years and it's mainly due to the above reasons. This all points out and forwards the issue and need for sunflower oil to the national sunflower improvement program at the end. So, breeding for quality traits of sunflower improvement must be a future target and base for the research and development of the national oilseed crop program. So, doing breeding work like screening

\*Corresponding author: Tilahun Mola, Department of Holeta Agricultural Research Center, Holeta, Ethiopia, Email: tilahun235@gmail.com

**Received:** 02-June-2024, Manuscript No: acst-24-140423, **Editor Assigned:** 05-June-2024, pre QC No: acst-24-140423 (PQ), **Reviewed:** 19-June-2024, QC No: acst-24-140423, **Revised:** 23-June-2024, Manuscript No: acst-24-140423 (R), **Published:** 30-June-2024, DOI: 10.4172/2329-8863.1000710

**Citation:** Mola T (2024) Heritability, Genetic Advance, Correlation Analysis of Fatty Acid Profile and Economically Important Traits of Sunflower Genotypes. Adv Crop Sci Tech 12: 710.

**Copyright:** © 2024 Mola T. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

sunflower genotypes for traits of interest like oil content and fatty acids profile is an important task for future breeding programs. This study was initiated to generate information on variance components and correlation analysis for important agronomic traits and selected fatty acid profiles of promising sunflower genotypes. This is a pillar and starting point for identifying and selecting genotypes of high-yielding and recommendable Fatty acids for edible oil from promising genotypes to develop the best sunflower varieties in general [6,7].

## Materials and Method

An experiment was conducted at Holeta (HARC), using 220 sunflower genotypes during 2021 the rainy season. The trial site is located at 9° 00' N latitude and 38°30' E longitude with an altitude of 2400 m.a.s.l and with a mean annual rainfall of 1144 mm and temperature ranges from 6°C-22°C with a rainy season from June to September. The dominant soil type is well-drained Red Nitosols characterized by soil pH 5.2-6.0 and 0.16% Nitrogen content with low organic carbon content of 1.18% (Mekonen and Tilahun, 2019) (Figure 1) [8].

### Experimental design and field management

The experiment was done using an 11x20 Alpha lattice design with two replications. The genotype was planted 25x75 cm<sup>2</sup> spacing between plants and rows respectively. Fertilizer and other agronomic and management practices were applied as per the recommendation for trial. For Fatty Acid Profile Analysis 30gm of seed sample from 220 genotypes were prepared and reading was taken using Near-infrared spectroscopy (NIRS) machines [9]. The near-infrared spectra were collected with a monochromator (FOSS NIR System 5000), by scanning at 1108 – 2492nm spectral range with an 8nm step. All spectra and reference data were recorded and managed with the WinISI version II software (Infrasoft International, Port Matilda, PA, USA). Oil content analysis of the same amount of seed was used to read the oil content using Nuclear magnetic resonance (NMR) machines (Oxford Analytical instrument, Newport analyzer). Chlorophyll content was measured using a SPAD meter from ten leaves of tagged experimental plants [10].

### Data collection and analysis

**The following data were collected for quantitative traits:** Days to flowering DF (days), Days to maturity DM (Days), Petiole Length PL (cm), Head Diameter HD (cm), 100 seed weight HSWT (g), Oil content OC (%), Chlorophyll Content Ch. C (%), Fatty Acid profile in percentage (Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1), Linolenic acid (C18:3) and Linoleic acid (C18:2) (IBPGR, 1985).

Data were subjected to statistical analysis according to Gomez and Gomez (1984), using SAS version 9.3 (SAS Institute, 2014) computer software. Considering the block term as nested in the replication. Best linear unbiased predictor (BLUP) means were estimated using multivariate mixed model (REML) spatial analysis considering the Block/Rep + treatment as a random effect for special correction of the nearest block errors to avoid the biased estimate of variance components at 5% level of significance (Panse and Sukhatme, 1985 and Fisher, 1992) [11].

**Heritability and genetic advance:** Broad sense heritability (H<sup>2</sup>) and genetic advance as a percent of the mean (GAM) were also estimated according to the formula (Allard, 1960). The heritability percentage was categorized as low, moderate, and high as suggested by Johnson et al. (1955). Genetic advance in an absolute unit (GA) and as a percent of the mean (GAM), assuming the selection of a superior 5% of the genotypes was estimated in accordance with the methods illustrated by Johnson et al. (1955). The GA as a percent of the mean was categorized as low, moderate, and high as suggested by Johnson et al. (1955). Phenotypic and genotypic variance and coefficient of variation, heritability, and genetic advance were computed using the Microsoft Excel program [12].

**Genotypic and phenotypic variance:** Phenotypic, genotypic, and environmental variance components and their coefficients of variation were estimated based on the methods detailed in (Burton & Devane, 1953). According to Siva Subramanian and Menon (1973), genotypic coefficients of variance (GCV) and phenotypic coefficients of variance (PCV) Values greater than 20% are high, less than 10% are low and between 10% and 20% are medium.

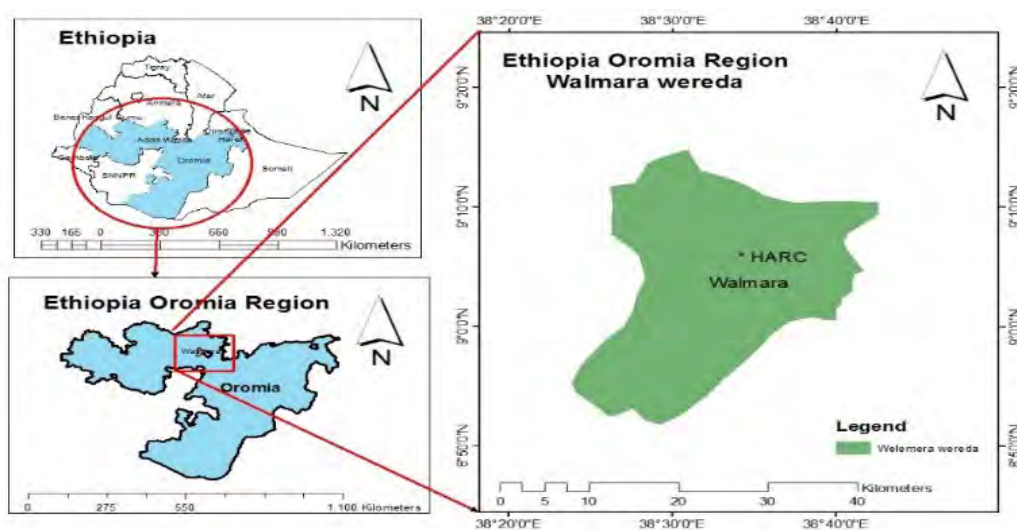


Figure 1: Map of the study area.

## Results and Discussion

### Range and mean performance

Descriptive statistical analysis was done for traits of interest and presented in Figure 2. The sunflower genotypes depicted that palmitic acid (C16:0) content ranges between 3.68% to 13.68% with a mean value of 7.15% for the sunflower population. The maximum Oleic acid (C18:1) content was 33.08% and the minimum was 3.05%. maximum Linolenic acid (C18:3), Linoleic acid (C18:2), and stearic acid (C18:0) were 21.60%, 66.61%, and 30.19% respectively. Generally, sunflower genotypes having high unsaturated fatty acids like Oleic acid, Linolenic acid, and Linoleic acid content are promising breeding elite materials for future breeding for quality traits. Head diameter, oil yield and content, early flowering and maturing, and short and medium sunflower genotypes with above indicated fatty acid content are the future targets of sunflower breeding in Ethiopia. This result concedes with the results of Kefale & Sisay (2017) (Figure 2) [13].

### Variance component

According to Variance analysis, useful for the characterization and classification of genotypes evaluated for traits of interest, all the genotypes showed a significant genetic variability based on twelve target characters of the Fatty Acids profile and important agronomic traits. In this study, the Variance component and correlation analysis were computed.

### Phenotypic and genotypic variance

The highest  $\sigma^2g$  and  $\sigma^2p$  were recorded for days to maturity, days to flowering, oleic acid (C18:1) content, and stearic acid (C18:0) content. In contrast, Palmitic acid (C16:0), Linolenic acid (C18:3), Petiole Length, Head Diameter, and hundred Seed Weight showed a low record. Days to maturity, days to flowering, oleic acid (C18:1), and Stearic acid (C18:0) showed the highest  $\sigma^2p$  and  $\sigma^2g$ . Generally, a remarkable difference of phenotypic ( $\sigma^2p$ ) variance was recorded for traits of interest both on fatty acid profile and important agronomic characters [14].

The assessed genotypic variance was higher than the corresponding environmental variance for most of the traits of interest except for petiole length and chlorophyll content. This indicates the presence of genetic variability among genotypes for traits of interest. This result concedes with the results of Bukhsh et al. (2011) and Lira et al. (2017), indicating that the estimates of phenotypic variance were higher than genotypic variance for elite sunflower genotypes in Pakistan and Brazil [15].

### Genotypic and phenotypic coefficients of variance (GCV & PCV)

Both, variation indicates the relative variability calculated as percentage, which measures how much variability exists for future selection. The phenotypic coefficients of variance (PCV) showed between 9.24% to 68.63% for Linolenic acid (C18:3) and Stearic acid (C18:0) respectively although genotypic coefficients of variance (GCV) ranged from 5.31% to 67.99% for Linolenic acid (C18:3) and Stearic acid (C18:0) respectively. PCV was higher than the GCV value for the twelve traits of interest. This shows that the environmental impact on sunflower genotypes was highly expression. More or less similar results were also reported by scholars (Sivasubramanian and Menon, 1973; Bukhsh, et al. 2011, Baraiya et al., 2018 and Lagiso et al., 2021). The highest PCV was recorded for Stearic acid (C18:0) and the least PCV was recorded for Linolenic acid (C18:3). The remaining traits showed moderate PCV values. The highest GCV was recorded for Stearic acid (C18:0), the lowest GCV was recorded for Linolenic acid (C18:3), and the moderate PCV was recorded for the remaining traits of interest. For low GCV and PCV values, selection based on those traits will not be successful. However, GCV value only provides how much genetic variability is present in a trait of interest and it can't predict heritable portion of the traits simply by using GCV. So, GCV with heritability value could predict the amount of advance to be expected from selection (Burton and Devane, 1953) [16].

### Heritability, genetic advance, and genetic advance of mean

Heritability ( $H^2$ ) and genetic advance as the percentage of the mean (GAM) at 5% selection intensity and variance component are presented in Table 1. The highest and lowest broad sense heritability was 3.11% to 99.48% for Chlorophyll content and Days to Maturity respectively. High heritability was recorded for the majority of traits and the medium was recorded for petiole length. Therefore, Selection based on the phenotypic performance of highly heritable traits should enhance and fasten the pre-breeding program. Traits having low and medium heritability are highly influenced by the environment. So, selection based on the phenotypic performance of those traits may lead to a wrong direction, unsuccessful (Table 1).

### Correlation

A Pearson correlation number is found between -1 and +1 which shows how much the two traits are directly related. It is usually used for quantitative traits of relationships between traits of interest. It predicts the amount and degree of the relationship between two traits of interest. So, understanding the correlation between two traits of

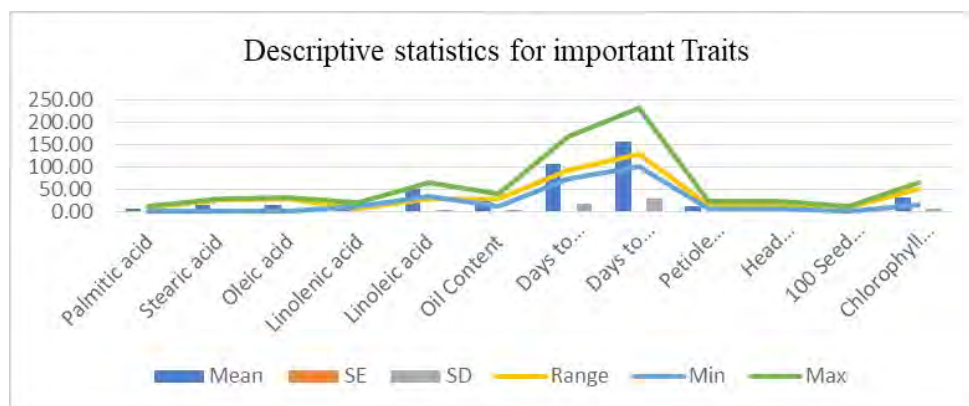


Figure 2: Distractive statistics of 12 important traits of sunflower.

**Table 1:** Heritability, genetic advance, and coefficients of variations sunflower genotypes.

Traits	Mean±SD	$\sigma_2g$	$\sigma_2e$	$\sigma_2p$	GCV	PCV	H <sup>2</sup>	GA	GAM
C16:0	8.03 ± 0.27	5.63	0.04	5.67	29.54	29.74	98.7	4.86	60.47
C18:0	12.11 ± 1.13	67.76	0.64	68.4	67.99	68.63	98.14	16.8	138.7
C18:1	21.14 ± 0.82	72.8	0.34	73.14	40.36	40.55	99.08	17.5	82.76
C18:3	18.46 ± 1.40	0.96	0.98	1.94	5.31	9.24	33	1.16	6.28
C18:2	50.77 ± 1.26	28.26	0.79	29.05	10.47	10.76	94.68	10.66	20.99
DF	106.61 ± 1.60	350	1.29	351.28	17.55	17.61	99.27	38.4	36.02
DM	158.4 ± 2.12	864.08	2.25	866.33	18.56	18.61	99.48	60.4	38.13
PL	13.15 ± 3.70	3.07	6.83	9.9	13.32	31.09	18.35	1.55	11.75
HD	14.98 ± 3.03	6.89	4.58	11.47	17.52	26.73	42.94	3.54	23.65
HSWT	6.03 ± 0.49	3.15	0.12	3.27	29.44	30.54	92.9	3.52	58.45
Ch. C	33.98 ± 10.13	3.29	51.27	54.56	5.34	30.27	3.11	0.66	1.94
OC	27.19 ± 0.47	28.8	0.11	28.91	19.73	19.81	99.25	11.01	40.5

NB. C16:0-Palmitic Acid, C18:0-Stearic Acid, C18:1-Oleic acid, C18:3-Linolenic acid, C18:2-Linoleic acid, DF-Days to Flowering, DM- Days to Maturity, PL- Petiole Length, HD-Head Diameter, HSWT-100 Seed Weight, Ch. C- Chlorophyll Content, OC- Oil Content

**Table 2:** Person correlation coefficients for important traits of sunflower.

Traits	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linolenic acid (C18:3)	Linoleic acid (C18:2)	DF (days)	DM (days)	PL (cm)	HD (cm)	HSWT (g)	Ch. C (%)	OC (%)
C16:0												
C18:0	0.44											
C18:1	-0.38	-0.24										
C18:3	-0.7	-0.46	0.47									
C18:2	0.03	-0.12	-0.66	-0.11								
DF	-0.44	-0.39	0.13	0.34	0.13							
DM	-0.48	-0.41	0.15	0.37	0.17	0.88						
PL	-0.1	-0.25	0.02	0.17	0.08	0.19	0.26					
HD	-0.04	-0.09	-0.05	0.02	0.05	-0.01	0.04	0.42				
HSWT	0.03	-0.02	-0.04	0.01	0.06	0.01	0.02	0.19	0.15			
Ch. C	-0.14	-0.12	0.02	0.19	0.06	0.16	0.17	0.28	0.18	0.09		
OC	0.31	0.12	-0.11	-0.26	0.03	-0.26	-0.2	0	-0.01	-0.07	-0.04	

NB. C16:0-Palmitic Acid, C18:0-Stearic Acid, C18:1-Oleic acid, C18:3-Linolenic acid, C18:2-Linoleic acid, DF-Days to Flowering, DM- Days to Maturity, PL- Petiole Length, HD-Head Diameter, HSWT-100 Seed Weight, Ch. C- Chlorophyll Content, OC- Oil Content

interest is essential to speculating selection criteria (Table 2) [17].

Anticipated correlations were observed between fatty acids and important agronomic and between fatty acid traits of sunflower. Unfortunately, most of the traits show negative and minimal correlation with palmitic acid content, only stearic acid, linolenic acid, hundred seed weight, and oil content showed positive correlation [18]. Palmitic acid content and linolenic acid content showed highly negatively correlated responses to each other and oleic acid content intermediately negatively correlated to palmitic acid content. Stearic acid content and palmitic acid content have positive and intermediate correlations, its nearly directly related traits. And that of oil content and palmitic acid content showed a positive intermediate correlated response. Surprisingly, late flowering and maturing genotypes showed a negative intermediate correlation with both palmitic and stearic acid content. Oleic acid content and linoleic acid content showed a positive low level of correlation with both days to flowering and maturity. Both days to flowering and maturity showed a positive intermediate correlation with linolenic acid content. Nearly the Stearic acid content showed a negative low level of correlation among the whole traits of interest for sunflower genotypes. A negative and nearly high correlation was observed between Oleic acid content and linoleic acid content; increasing one of the traits may decrease the other one. This result is more or less similar to Bukhsh, et al. (2011) who reported that increasing oil contents, palmitic acid concentration, and reducing

protein contents in achenes without affecting stearic, oleic, linoleic, and linolenic acid concentration in achenes [19].

## Conclusion and Recommendation

Considering genetic variability and related parameters among sunflower genotypes may be a pillar and beginning point to distinguish and select high-yielding, tolerant to biotic and abiotic factors genotypes in addition to quality traits to develop ideal and resilient sunflower varieties. Determining genetic variability, heritability, genetic advance and correlation of traits among sunflower genotypes were the intentions of the study. Descriptive statistics analysis shows the existence of a remarkable phenotypic variation among sunflower genotypes. A high  $\sigma_2g$  and  $\sigma_2p$  were recorded for stearic acid content, Oleic acid content, linoleic acid content, days to maturity, days to flowering, and chlorophyll content although palmitic acid content, linolenic acid content, petiole length, head diameter, and hundred seed weight showed the low  $\sigma_2g$  and  $\sigma_2p$ . A significant amount of phenotypic ( $\sigma_2p$ ) variance or variability was observed for all studied traits of interest especially for the fatty acid content of sunflower seed. Regarding fatty acid contents, palmitic acid, stearic acid, and Oleic acid content and hundred seed weight showed high PCV and GCV values. The Highest heritability coupled with high genetic advance as a percent of mean was observed by palmitic acid content, stearic acid content, Oleic acid content, days to flowering, days to maturity, hundred seed weight, and oil content. Low heritability and genetic advance as a percent of mean was also recorded for chlorophyll



content showing that the trait is highly affected by the environment and the trait is highly governed by non-additive gene action. The study also showed highly negatively correlated responses of the traits; that increasing the contents of one may decrease the other traits and vice versa. Positive correlated responses were also observed from the study between palmitic and stearic acid content and also between days to flowering and maturity. By adjusting the maturity and flowering days we can increase the contents of linolenic acid content at the intermediate level and also at the low level for linoleic acid and Oleic acid contents of the sunflower seed. By developing late-maturing sunflower varieties we can decrease the palmitic and stearic acid contents of seed. Generally, intensive work on all available sunflower genotypes in Ethiopia supported by molecular data is recommendable to identify novel genes responsible for economically important traits like maturity, oil content, plant height, head diameter, and fatty acid profile analysis.

### Acknowledgments

The author would like to express his thanks to Holeta Agriculture Research Centre (HARC) for Providing all research facilities and laboratory services to carry out this research work.

### Conflict of Interest

The authors declare no conflict of interest.

### Data availability statement

All the data are available.

### References

1. Anonymous (2008-09) Economic Survey, Govt. of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad.
2. Baraiya VK, Patel PJHR (2018) Genetic variability, heritability, and genetic advance for seed yield in sunflower (*Helianthus annuus* L.). *Indian J Crop Sci* 6: 2141-2143.
3. Bukhsh MAAHA, Iqbal J, Kaleem S, Wasaya A, Ishaque M, et al. (2011) Qualitative analysis of spring-planted sunflower hybrids as influenced by varying nutritional areas. *Pakistan Journal of Nutrition* 10: 291-295.
4. Burton GW, Devane DE (1953) Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material 1. *Agronomy Journal* 45: 478-481.
5. Fick GN, Miller JF (1997) Sunflower breeding. *Sunflower technology and production*. 35: 395-439.
6. Fisher RA (1992) Statistical methods for research workers. In *Breakthroughs in Statistics* (pp. 66-70).
7. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. John Wiley & Sons.
8. Hassan SM, Iqbal MS, Rabbani G, Shabbir G (2012) Genetic variability, heritability, and genetic advance for yield and yield components in sunflower (*Helianthus annuus* L.). *Electronic Journal of Plant Breeding*, 3: 707-710.
9. Johnson HW, Robinson HF, Comstock RE (1955) Estimates of genetic and environmental variability in soybean. *Agron J* 47: 314-18.
10. Kaleem S, Hassan FU, Farooq M, Rasheed M, Munir A, et al. (2010) Physio-morphic traits as influenced by seasonal variation in sunflower: A review. *International Journal of Agriculture and Biology* 12: 468-473.
11. Kefale B, Sisay A (2017) Evaluation of basic quality parameters within sunflower and safflower varieties from Holeta, Ethiopia. *MOJ Biorg Org Chem* 1: 50-52.
12. Killi FA (2004) Influence of different nitrogen levels on productivity of oilseed and confection sunflowers (*Helianthus annuus* L.) under varying plant populations. *International Journal of Agriculture and Biology*, 4: 594-598.
13. Lagiso TM, Singh BCS, Weyessa B (2021) Evaluation of sunflower (*Helianthus annuus* L.) genotypes for quantitative traits and character association of seed yield and yield components at Oromia region, Ethiopia. *Euphytica* 217: 1-18.
14. Lira EG, Amabile RF, Fagioli M, Montalvão APL (2017) Genetic parameters, phenotypic, genotypic and environmental correlations and genetic variability on sunflower in the Brazilian Savannah. *Ciência Rural*, 47(8).
15. Mekonen H, Tilahun M (2019) Seed coating; Genius Coat Cereal Disco AG L-439; effect on seed yield and yield related traits of malting barley (*Hordeum vulgare* L.) in central highlands of Ethiopia. *International Journal of Agriculture and Biosciences* 8: 141-147.
16. Omidi H, Tahmasebi Z, Badi HAN, Torabi H, Miransari M, et al. (2010) Fatty acid composition of canola (*Brassica napus* L.), as affected by agronomical, genotypic, and environmental parameters. *Comptes rendus biologiques* 333: 248-254.
17. Panse VG, Sukhatme PV (1985) Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi 359.
18. Putman JD, David C, Molinillo JMG, David M, Varela RM, et al. (1990) Synthesis of heliannane skeletons. Facile preparation of heliannol D. *Tetrahedron* 59: 1679-1683.
19. Sivasubramanian S, Menon N (1973) Heterosis and inbreeding depression *Genet.* 17: 381-387.