



Evaluation of confectionary sunflower germplasm accessions and their derived hybrids

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Abstract Confectionary sunflower (*Helianthus annuus* L.) has gained popularity due to its utilization in roasting as snacks, poultry, bird food, and bakery products. However, seed quality of confectionary sunflower differs from non-oil seed types. In the present study, 71 sunflower accessions were selected for the evaluation of seed quality traits. Significant genetic variability was observed for traits such as 100-seed mass and seed length, as indicated by high genotypic coefficients of variability. Genotypic correlation analysis revealed a positive association between 100-seed mass and seed size, protein content, and oleic acid content. Path coefficient analysis based on genotypic and phenotypic correlations suggested that accessions with high sugar content may be selected to develop superior germplasm for confectionary products. Biplot analysis was conducted to identify suitable accessions with favorable confectionary traits. Accessions ‘Hybrid 100’, ‘GOR101’, and ‘Odesskij112’ exhibited high sugar content, while

‘Yawn’ demonstrated comparatively higher oleic acid content. Accessions ‘Vinimik 6931’ and ‘HA 305’ displayed high 100-seed mass, while seed length was greater in accessions ‘Comet’ and *H. × multiflorus*. Combining ability analysis were performed to assess the breeding value of accessions. ‘Comet’ exhibited the highest general combining ability (GCA) effects for seed yield per plant, head diameter and 100-achene mass; while ‘G.OR.104’ had positive GCA for achene yield per plant but negative combining ability for other traits. ‘Tenissiei’ displayed positive GCA for all traits. Among the testers, accession ‘Universal’ showed positive GCA for seed yield per plant, while ‘HA-292’ exhibited positive GCA for head diameter. The accessions with higher values for confectionary traits and positive general combining ability effects for yield related traits may be further exploited in confectionary sunflower breeding programs as parents.

Keywords Achene · Correlation · Combining ability · Oleic acid · Sugar contents

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Introduction

Sunflower (*Helianthus annuus* L.) is a versatile crop cultivated for its oilseed, confectionary products, snacks, and as food for birds and small animals. It ranks as the fourth largest oilseed crop globally, with cultivation spanning over 70 countries (Rauf 2019).

According to FAO data from 2022, the total harvestable area dedicated to sunflower cultivation reached 29.25 million hectares, yielding a total production of 54.3 million metric tons. Notably, countries with the largest cultivated areas of sunflower include the Russian Federation, Ukraine, Argentina, China, India, and the USA (FAO 2022).

Sunflower cultivars are categorized into distinct types, broadly classified into two groups: oilseed types and non-oilseed types. Non-oilseed types encompass confectionary cultivars characterized by large seed and low oil content. Confectionary sunflower is prominently cultivated in Eastern European countries such as Turkey, Russia, Romania, Bulgaria, and Ukraine (Aldemir et al. 2016).

Sunflower seeds are commonly crushed to extract oil, leaving behind seed meal that serves as a byproduct. This meal can be repurposed for various applications such as animal feed or incorporated directly into human consumption in confectionary and baking products. However, sunflower meal is frequently considered of low value due to its relatively low energy and protein content, alongside the presence of anti-nutritional components (González-Pérez 2015).

Comparative analysis across various species indicates that sunflower meal typically contains a lower percentage of protein (approximately 30% crude protein) compared to cotton (approximately 42%) and soybean (approximately 50%). Consequently, there arises a necessity to enhance the protein content in sunflower seeds, possibly at the expense of polysaccharides, in order to increase the value of the hull contents. Improving hulling efficiency by reducing fiber content not only augments protein levels but also positively impacts oil extraction.

Research indicates significant variability in protein and oil contents among different sunflower elite germplasm, with maximum protein levels ranging from 35 to 50% (Warburton et al. 2017). This variability is primarily attributed to differences in hull contents. Thus, enhancing protein content entails reducing fiber content and improving hull composition. Additionally, within sunflower germplasm, variation is observed concerning anti-nutritional components such as chlorogenic acid. High protein content is correlated with a higher kernel-to-hull ratio and reduced fiber content, which in turn enhances hull digestibility

(Demir 2021). Improving the nutritional value of sunflower meal by increasing its protein content is a pivotal breeding objective. While sunflower meal provides all essential amino acids except lysine, efforts can be directed towards enhancing lysine content by leveraging initial variations within elite or breeding lines (Nenova & Drumeva 2012).

Confectionary sunflower, characterized by large seed size, low oil, and high polysaccharide and protein contents, serves various purposes. Its seeds are suitable for roasting or snacks when inshelled, and for baking, bird feed, and flour used in various bakery products when shelled. Roasted sunflower seeds offer a cost-effective alternative to nuts and are often served during social gatherings. Confectionary sunflower seeds can be identified based on their seed coat color, which may be albino, striped, or colored, and they are typically larger in size. Confectionary sunflower seeds exhibit a higher 100-seed mass, approximately 8–13 g per 100 seeds, compared to oilseed types, which typically contain only 4–6 g per 100 seeds. Dehulling of confectionary sunflower seeds is easier compared to oilseed types, and the kernels are loosely packed. Sunflower breeders generally select confectionary lines based on criteria such as seed yield potential, protein content, ease of dehulling, hull/kernel ratio, among others. Key characteristics of confectionary sunflower cultivars are seed yield potential (approximately 6 metric tons per hectare), plant height (approximately 175 cm), protein content (>25%), hull ratio (<25%), kernel ratio (>60%), oil content (30–35%), ease of dehulling, and sweet and nutty taste (Rauf et al. 2019). Diversity among the confectionary sunflower accessions based on morphological traits was 0.71, thus showing high morphological variation (Hladni et al. 2017). High diversity was estimated for floral characteristics, medium to high for seed related traits, while the lowest for the leaf descriptors (Hladni et al. 2017). Correlation and path analyses of 8 confectionary accession showed a positive relationship between yield, morphological traits and protein contents (Sincik & Gokosoy 2014). Sunflower oil quality is majorly dictated by the fatty acid, and conventional sunflower cultivars had high linoleic acid, which is a polyunsaturated fatty acid and may decrease the shelf life of seed and oil. Contrary, mono-unsaturated fatty acid oleic acid has

high thermos-oxidative stability and may replace the linoleic acid (Shah et al. 2023). Moreover, over sunflower stearins based on fractionation of high oleic and high stearic acid can be substitute for cacao butter equivalent (Bootello et al. 2018).

The primary objectives of confectionary sunflower breeding align with those of oilseed types; however, there are distinct differences in seed morphology and biochemical traits. Confectionary types exhibit characteristics such as larger seed size, higher protein contents, ease of dehulling, and colorful testa (Feng et al. 2022). The testa of confectionary sunflower is easily dehulled, and the kernel is removed intact during shelling (Feng et al. 2022). Experimental confectionary hybrids have demonstrated significant commercial heterosis, particularly for traits like seed yield, head diameter, and seed weight (Pekcan et al. 2015). Notably, two confectionary hybrids (09 TRÇ 003 and 09 TRÇ 004) were submitted for registration based on their superior performance in terms of seed yield and uniformity (Pekcan et al. 2015). Considering this understanding, a research study was initiated with the objectives, to assess genetic variation in sunflower accessions concerning traits relevant to confectionary sunflower, and selection of suitable accessions and their potential utilization based on their general combining ability in a breeding program. This initiative aims to capitalize on the distinct characteristics of confectionary sunflower to develop improved hybrids with desirable traits.

Materials and methods

In 2023, experiments were conducted at the Department of Plant Breeding & Genetics, College of Agriculture, University of Sargodha (Pakistan). The germplasm used in the study was obtained by ordering through the online order on Germplasm Resources Information Network (GRIN) operated by the USDA-NPGS of the United States in 2020. The germplasm was multiplied and maintained for two years before being subjected to evaluation trials. The list of germplasm utilized in the study is provided in Table 1.

Plant material and sowing of experiments, crop husbandry and maintenance of germplasm

Experimental trials were conducted by initiating a field trial on 20th February 2022 at the College of Agriculture, University of Sargodha. On 20th February 2022, a total of 71 germplasm accessions from various countries were sown to screen potential breeding lines with useful genetic variations related to confectionary sunflower at the College of Agriculture, University of Sargodha. These selected plants may be further utilized in the development of inbred lines and hybrid breeding. Additionally, they may be exploited in the breeding program to estimate the general combining ability (GCA) in sunflower.

The experiments were sown on loam soil (pH 7.2 ± 0.2 ; EC 1.17 ± 0.12 dSM⁻¹; organic matter $0.74\% \pm 0.23$; phosphorus 6.1 ± 0.17 mg Kg⁻¹) raised beds with a plant-to-plant distance of 24 cm and a row distance of 75 cm. Weed control was implemented using pre-emergence pesticide (dual gold®). Each breeding line was sown in a single row spanning approximately 6 m. Initially, three seeds were manually dibbled into each hole, with subsequent thinning to a single plant per hole upon germination (approximately 14 days after sowing). The breeding material received fertilization with 50 kg acre⁻¹ of diammonium phosphate and 25 kg acre⁻¹ of sulfate of potash. To prevent insect damage, a recommended pesticide, lufenuron with dichlorobenzene at a rate of 30 ml per 20 L, was sprayed after anthesis (70 days after emergence). The experiments were irrigated with canal water to prevent water stress, and insect control was implemented with the recommended insecticide following pest scouting conducted by an entomologist. Experimental trial received a rainfall of 93.5 mm during entire growth cycle. Each plant was individually tagged and covered with a net bag to prevent pollen contamination from pollinators. To maintain a breeding line, pollen from two selected neighboring plants was collected and used for pollination, through a process known as sib mating. Selected plants were pollinated until all the anthers were withered, ensuring

Table 1 List of the germplasm accessions used in the study

#	PI #	Accession	Origin	#	PI#	Accession	Origin
1	PI 650422	Comet	Transvaal, South Africa	37	PI 505836	HA 288	North Dakota, United States
2	PI 296288	USSR Franslever	Former, Soviet Union	38	PI 505836	HA 292	North Dakota, United States
3	PI 296290	Laan	Transvaal, South Africa	39	PI 505836	HA 305	North Dakota, United States
4	PI 296292	Short Russian MN 34% oil	Transvaal, South Africa	40	PI 505836	RHA 273	United States
5	PI 296293	Universal	Transvaal, South Africa	41	PI 505836	RHA 294	North Dakota, United States
6		A 9345	France	42	PI 505836	RHA 298	North Dakota, United States
7	PI 297476	C 1957	France	43	PI 505836	RHA 299	North Dakota, United States
8	PI 297438	Tennissei	France	44	PI 505836	RHA 801	North Dakota, United States
9	PI 297480	V 8883	France	45	PI 505836	HA 61	Texas, United States
10	PI 298632	Saratovski MN 49% oil	Former, Soviet Union	46	PI 505836	HA 89	Texas, United States
11	PI 299992	No. 513	South Africa	47	PI 505836	HA 304	North Dakota, United States
12		No. 524	Turkey	48	PI 505836	RHA 271	United States
13	PI 307832	I-7999-V. 56	Uruguay	49	PI 505836	RHA 293	North Dakota, United States
14	PI 650430	Beacon	South Africa	50	PI 505836	‘Peredovik’	Bulgaria
15	PI 307835	I-29444	Uruguay	51	PI 505836	‘Novi Sad 61’	Former S&M
16	PI 307936	Yugovostok	Former Soviet Union	52	PI 505836	Vniimk 6540	Former, Soviet Union
17	PI 307937	Peredovick	Former Soviet Union	53	PI 505836	‘Vniimk 8931’	Former, Soviet Union
18	PI 650784	Vniimk 6540	Former Soviet Union	54	PI 505836	‘Zelenk 61’	Uncertain
19	PI 345613	Vniimk 8883	Former Soviet Union	55	PI 505836	‘VR Bulgarian’	Bulgaria
20	PI 307945	Chernianka 35	Former Soviet Union	56	PI 505836	‘DDR 1’	Uncertain
21	PI 318468	Girassol	São Paulo, Brazil	57	PI 505836	‘MN17’	North Dakota, United States
22	PI 318600	Peredovic	Former Soviet Union	58	PI 505836	Jupiter	Zimbabwe
23	PI 323281	Black Sayar	Pakistan	59	PI 505836	‘Yawne	Zimbabwe
24	PI 323588	<i>H. × multiflorus</i> 33	Portugal	60	PI 505836	Russian Giant	Zimbabwe
25	PI 331176	Manfredi INTA (3-WAY X)11	Argentina	61	PI 505836	Comet	Zimbabwe
26	PI 650386	USSR Vniimk 6540/66	Former Soviet Union	62	PI 505836	G.O.R. 104	Zimbabwe
27	PI 340781	USSR Armavirskij3497/66	Former Soviet Union	63	PI 505836	G.O.R. 101	Zimbabwe
28	PI 650783	USSR Vniimk 8931/66	Former Soviet Union	64	PI 650426	803,495	Zimbabwe
29	PI 505836	B-12	Former Serbia and Montenegro (S&M)	65	PI 650427	803,496	Zimbabwe
30	PI 505836	D-75–10	Former S&M	66	PI 650428	Hybrid 100	Zimbabwe
31	PI 505836	N 3/2–1	Former S&M	67	PI 650429	803,504	Zimbabwe
32	PI 505836	PO 6/4–2	Former S&M	68	PI 650432	‘Sunrise’	Zimbabwe
33	PI 505836	R-201/4	Former S&M	69	PI650433	‘Dukn’	Zimbabwe
34	PI 505836	V 8931 2/2–1	Former S&M	70	PI 505836	Peredovik ul	Former Soviet Union
35	PI 505836	Odesskij 19	Former Soviet Union	71	PI 505838	Voronezskij 151	Former Soviet Union
36	PI 505839	Odesskij 113	Former Soviet Union				

controlled pollination and genetic purity within the breeding lines.

Crossing for combining ability test and development and evaluation of half sib progenies

Parental lines were carefully selected based on all traits related to 100-seed mass, sugar contents, protein contents, and seed length which were evaluated in spring season (February, 2022) (Table 2). Data of all 71 accessions in the study has been provided in the supplementary file (S1–S7).

Six breeding lines ('HA-305', 'VNIIMIK 8931', 'TENISSIE', 'Odeskij 113', and 'Vniimk89') were mated with three male lines ('HA-292', 'I-29444', and 'Universal') during the autumn season (12th August, 2022). This mating strategy aimed to combine desirable traits from the female and male parental lines, thereby creating potential hybrids with improved characteristics for further evaluation and selection in the breeding program.

They were developed through the process of crossing the female lines with the male lines. Prior to sunrise, the female lines were manually emasculated to remove their anthers, ensuring that only the desired pollen from the male lines would be used for pollination. Both the female and male lines' capitula were covered with net bags to prevent pollen contamination by insect pollinators. The female lines were pollinated until all the stigmas were withered. After pollination, seeds from each crossed head were manually

threshed once the plants reached physiological maturity. This was indicated by the heads turning brown. The seeds were then dried and cleaned to remove all chaff and stored at room temperature until reaching physiological maturity. In total, there were 18 crosses made, resulting from combinations of six female lines and three male lines. These crosses aimed to create a diverse set of hybrids for further evaluation and selection in the breeding program.

The offspring were sown during the autumn season on 18th August 2023. The trial was conducted in loam soil (pH 7.3 ± 0.18 ; EC 1.35 ± 0.17 dSM⁻¹; organic matter $0.84\% \pm 0.19$; phosphorus 7.3 ± 0.23 mg Kg⁻¹), which was well-prepared beforehand. Raised beds were utilized for sowing the half-sib offspring. The soil was fertilized with 12 kg of diammonium phosphate and 5 kg of sulfate of potash. All materials were sown in single rows spanning 6 m, with a plant-to-plant distance of 22 cm and a row-to-row distance of 60 cm. Each seed was manually dibbled into the soil. To control weed growth, the field was sprayed with the pre-emergence herbicide "Dual Gold" (metolachlor). Irrigation was administered when the soil moisture content fell below the field capacity, which was approximately 18% on a weight basis. Additionally, the crop was sprayed with chlorpyrifos at a rate of 250 g acre⁻¹ (R1 stage about 54 days after emergence) to prevent insect infestation in the field.

Table 2 Mean values of the selected accessions used in the line \times tester analysis

Accessions	100-seed weight	Days to flowering	Oleic acid (%)	Oil percentage	Protein content (%)	Seed length (cm)	Sugar content (mg g ⁻¹)
'Comet'	9.37	65.33	41.67	27.00	15.33	2.27	114.99
'Universal'	8.73	59.33	40.33	25.33	18.00	2.40	102.23
'Tennissei'	9.37	54.67	46.33	27.00	18.33	1.40	113.00
'Odeskij 113'	6.17	59.00	52.33	42.67	15.33	0.97	135.00
'HA 292'	14.50	80.67	34.33	26.00	18.67	1.40	124.00
'HA 305'	12.87	61.00	43.33	25.67	18.33	1.03	116.00
'Vniimk 8931'	11.23	61.33	52.67	20.00	19.33	1.30	106.00
'Comet'	7.30	62.33	41.33	30.33	15.33	1.23	123.67
'I-29444'	8.57	74.67	47.67	25.33	18.67	1.63	109.67

Trait evaluation

The following traits were evaluated in all accessions according to the details provided:

Each of the above traits was evaluated meticulously to gather comprehensive data for further analysis and selection of superior accessions in the breeding program.

Days to 50% flowering

Each row was tagged, and the number of days to anthesis for 50% of the plants in each row was observed and recorded.

Head diameter

The diameter of the capitula was measured using a measuring tape from the center of the capitula to the front side, and all data were recorded in centimeters. This measurement provides valuable information about the size and morphology of the capitula, which can influence seed production and overall plant performance.

At physiological maturity, the following traits were evaluated in the half-sib offspring for further combing ability analysis:

Achene yield head⁻¹

The heads were manually harvested and dried under shade to ensure proper drying without exposure to direct sunlight. After drying, the heads were manually threshed to separate the seeds from the heads. The threshed seeds were then cleaned to remove any debris or chaff. Subsequently, the cleaned seeds were placed in kraft paper bags and dried to achieve a moisture content of 12%. This drying process helps preserve the quality of the seeds during storage. To calculate the seed yield per head, all the harvested seeds from a single head were measured using a digital balance. This measurement provided accurate data on the quantity of seeds obtained from each individual head, allowing for the assessment of seed yield potential.

100-achene mass

A total of 100 seeds were counted using a digital counter to ensure consistency in the sample size. Subsequently, the mass of these 100 seeds was measured using a digital balance to obtain accurate data on seed mass. This process allows for precise assessment of seed size and weight, which are important factors in determining seed quality and yield potential.

Achene oil content

Achenes weighing 10 g were utilized to determine the oil contents using a Soxhlet apparatus. Kernels were crushed gently and placed in a thimble for oil extraction through hexane until all oil was extracted from the seed lot. The oil contents of the kernels were calculated using the formula:

$$\text{Achene oil contents} = \frac{[(\text{Achene mass before extraction}) - (\text{Achene mass after oil extraction})]}{[\text{Achene mass}]}$$

Fatty acid profile

Oil was extracted manually using a small expeller from a 40 g seed sample, and the expelled oil was cleaned to remove any seed material. Oil samples were thereafter refrigerated at 4 °C for further analysis. A 50 µL sample of this oil was methylated using 4 ml KOH for one hour at room temperature. Methylated fatty acids were extracted with hexane. Oleic acid contents were determined through gas chromatography (M-3900, Varian, USA). Oil samples were methylated using KOH, and methylated fatty acids were extracted with hexane. Analysis was performed on a fused capillary column with a flame ionizing detector. Analysis was done by using the fused capillary column, flame ionizing detector and nitrogen gas carrier @ 3.5 ml min⁻¹. Injector and detector temperature were set at 260 °C, while column oven temperature was set at 222 °C.

Sugar content

It was determined using the phenol–sulfuric acid method. Samples were treated with phenol–sulfuric acid solution and incubated for 30 min before being cooled to room temperature. Standard curves of glucose and sucrose were prepared, and absorbance was measured using a spectrophotometer (Shimadzu, UV-1900i) at 490 nm.

Data and biometrical analysis

All data was analyzed on “R” computer based software. Analysis of variance was carried out in randomized complete block design (RCBD) with 3 replications. RCBD analysis was done in library “doebioresearch” and function “rcbd”, while correlation and path analysis was carried out in library “variability” and function “ancova”, “geno.corr” and “geno.path” were used to estimate the correlations and path analysis value. Principle component analysis and multi-trait biplot analysis were done using “ggplot2”, “gridExtra”, “ggbiplot” and “factoextra”, while line×tester analysis was done in library “gpbStat” and function “lrc” (RStudio Team 2020). Genotypic and phenotypic coefficient of variation was calculated (Burton and Vane 1953) on the excel sheets using following formula:

$$\text{Genotypic coefficient variation} = \frac{\sqrt{\sigma^2 \text{Genotypic}} \times 100}{\text{Grand mean}}$$

$$\text{Phenotypic coefficient variation} = \frac{\sqrt{\sigma^2 \text{phenotypic}} \times 100}{\text{Grand mean}}$$

$$\sigma^2 \text{Genotypic} = \frac{\text{MSG} - \text{MSE}_r}{r}$$

$$\sigma^2 \text{Phenotypic} = \sigma^2 \text{Genotypic} + \sigma^2 \text{Environment}$$

Results

Analysis of variance showed significant variation due to accessions for all traits under study. Table 3 presents mean values of various traits, ranges, genotypic and phenotypic coefficient of variation (GCV and PCV) showing variability among the accessions for all traits. The highest variation was observed in seed length, with GCV and PCV estimates of 29% and 31% respectively. The range for seed length was 1.39–2.40 cm among the accessions. Considerable variation was also observed for 100-seed mass, with a range of 3.4–14.5 g and GCV and PCV estimates of 27%. Sugar content exhibited the lowest variation, with GCV and PCV estimates of 9%. Seed sugar contents ranged between 120 and 139 mg g⁻¹. Seed oil contents range was 34–49%, with GCV and PCV estimates at 19%. Protein contents range was 16–19%, while oleic acid percentage range was 39–53%, thus indicating that the accessions were generally suitable for confectionary purposes. Moreover, the range of oleic acid was low to medium, and no high oleic acid accessions were present within the exotic germplasm.

Trait associations

Table 4 presents the estimated genotypic correlations among various traits. The 100-seed mass exhibited a positive relationship with protein contents (0.40) and seed length (0.28), indicating that higher seed mass tend to have higher protein contents and longer lengths. Conversely, it showed a negative relationship with oil percentage (−0.58), suggesting that accessions with higher seed masses tend to have lower oil percentages. Days to flowering displayed highly significant ($P \leq 0.01$) negative correlations with oil percentage (−0.23) and positive correlations with

Table 3 Germplasm statistics of seed quality traits based on 71 accessions

Statistics	100-Seed mass (g)	Days to flowering	Oleic acid (%)	Oil content (%)	Protein content (%)	Seed length (cm)	Sugar contents (mg g ⁻¹)
Mean	7.78	62.95	39.30	33.85	16.10	1.39	120.37
Maximum	14.50	98.00	52.67	49.00	19.33	2.40	138.67
Min	3.40	46.33	23.33	20.00	11.33	0.67	102.00
GCV	26.73	12.20	18.47	19.20	13.61	28.84	8.88
PCV	27.40	12.20	20.63	19.61	14.72	31.42	9.30

GCV genotypic coefficient variation, PCV phenotypic coefficient of variation

Table 4 Genotypic correlation coefficient among traits related seed quality

Traits	100-SM	DTF	OA	Oil%	PC%	SL
100-SM	1.00	0.06 ^{ns}	0.16*	−0.57**	0.40**	0.28**
DTF	0.06 ^{ns}	1.00	−0.18 ^{ns}	−0.23**	0.31**	0.16**
OA	0.16*	−0.18 ^{ns}	1.00	−0.−0.13 ^{ns}	0.09 ^{ns}	−0.07 ^{ns}
Oil%	−0.57**	−0.23**	−0.13 ^{ns}	1.00	−0.59**	−0.28**
PC	0.40**	0.31**	0.09 ^{ns}	−0.59**	1.00	0.17*
SL	0.28**	0.16*	−0.07 ^{ns}	−0.28**	0.17*	1.00
SC	0.01 ^{ns}	−0.07 ^{ns}	0.06 ^{ns}	0.34**	−0.48**	−0.05 ^{ns}

^{ns}, * and ** indicate non-significant ($P \geq 0.05$), *significant ($P \leq 0.05$) and highly significant ($P \leq 0.01$) genotypic correlations, respectively. They were tested using a two-tail t test with degree of freedom as number of genotypes−2. 100-SM: seed mass; *DTF* days to flowering; *OA* oleic acid percentage; *Oil%* oil percentage; *PC* protein content percentage; *SL* seed length

protein contents (0.310 and seed length (0.16). These correlations suggest that accessions suitable for confectionary purposes tend to mature later.

Phenotypic correlations showed that seed length showed highly significant ($P \leq 0.01$) positive relationships with 100-seed mass (0.25) and protein contents (0.37), indicating that longer seeds tend to have higher masses and protein contents. The 100-seed mass exhibited significant ($P \leq 0.05$) positive relationship with oleic acid (0.14) and protein contents (Table 5), thereby suggesting that larger seeds tend to have higher oleic acid and protein contents. It also displayed a negative relationship with oil percentage (−0.55), reinforcing the earlier observation that accessions with higher seed masses tend to have lower oil percentages. Days to flowering had a positive relationship with both protein contents and seed length, while displaying negative relationships with oil percentage. This indicates that accessions with later flowering times tend to have higher protein contents and seed lengths, but lower oil percentages.

Path analysis

In the partitioning of genotypic correlations, it was found that sucrose contents, followed by protein contents, had the strongest direct effect on 100-seed mass. However, oil percentage exerted the highest negative direct effect on 100-seed mass. Additionally, protein contents exhibited positive indirect effects on seed mass via oil percentage, indicating that accessions with higher protein contents may indirectly be selected through lower oil percentages. Similarly, path analysis based on phenotypic correlations revealed that sucrose contents, followed by protein contents, had positive direct effects on 100-seed mass (Supplementary S8). Conversely, oil percentage exerted a direct negative effect on 100-seed mass, suggesting that breeding lines with high oil content may be selected through genotypes with lower 100-seed mass (Supplementary S9).

Table 5 Phenotypic correlation coefficient among traits related seed quality

Traits	100-SM	DTF	OA	Oil%	PC%	SL
100-SM	1.00	0.04 ^{ns}	0.14*	−0.55**	0.37**	0.23**
DTF	0.04 ^{ns}	1.00	−0.13 ^{ns}	−0.22**	0.27**	0.14**
OA	0.14*	−0.13 ^{ns}	1.00	−0.13 ^{ns}	0.06 ^{ns}	−0.06 ^{ns}
Oil%	−0.55**	−0.22**	−0.13 ^{ns}	1.00	−0.54**	−0.25**
PC%	0.37**	0.27**	0.06 ^{ns}	−0.54**	1.00	0.14*
SL	0.25**	0.14*	−0.06 ^{ns}	−0.24**	0.14*	1.00
SC	0.01 ^{ns}	−0.07 ^{ns}	0.05 ^{ns}	0.31**	−0.41**	−0.03

^{ns}, * and ** indicate non-significant ($P \geq 0.05$), *significant ($P \leq 0.05$) and highly significant ($P \leq 0.01$) genotypic correlations, respectively. They were tested using a two-tail t test with degree of freedom as number of genotypes−2. 100-SM: seed mass; *DTF* days to flowering; *OA* oleic acid percentage; *Oil%* oil percentage; *PC* protein content percentage; *SL* seed length

Biplot analysis

The genetic variation within the 71 germplasm accessions was characterized into several components based on the traits under study. The first component accounted for 34.5% of the total genetic variation, followed by the second component carrying about 17.8% of the variation (Supplementary Fig. 1). Hence, it was concluded that the first two factors cumulatively carried 52% of the total genetic variation, thus highlighting the importance of selecting key traits within each component to capture genetic variability within the germplasm. Supplementary Fig. 2 illustrates the relative contribution of various traits within each component. Percent oil and protein contents were relatively important traits in the first component, followed by 100-seed weight. In contrast, oleic acid, days to flowering, and 100-seed mass were traits with

high relative contribution in the second component. Furthermore, traits were partitioned into two major groups (Fig. 1). The first group included traits such as 100-seed mass, seed length, percent oil, and days to flowering, which may have positive relationships. On the other hand, the second group included sugar contents and percent oil, suggesting that accessions with higher sugar content may also contain accessions with higher oil percentage. Biplot analysis conducted using R software further characterized the accessions based on their traits (Fig. 1). For instance, accessions such as RHA-298 (49%), ‘HA-271’ (46.60%), ‘Peredovik’ (45.33%), and ‘HA-89’ (42.50%) exhibited high oil contents, while accessions like ‘Hybrid 100’ (128.67 mg g⁻¹), ‘GOR101’ (135.33 mg g⁻¹), and ‘Odesskij 112’ (135 mg g⁻¹) had high sugar contents. Accession ‘Yawn’ (48.87%) showed comparatively higher oleic acid contents than others. Additionally,

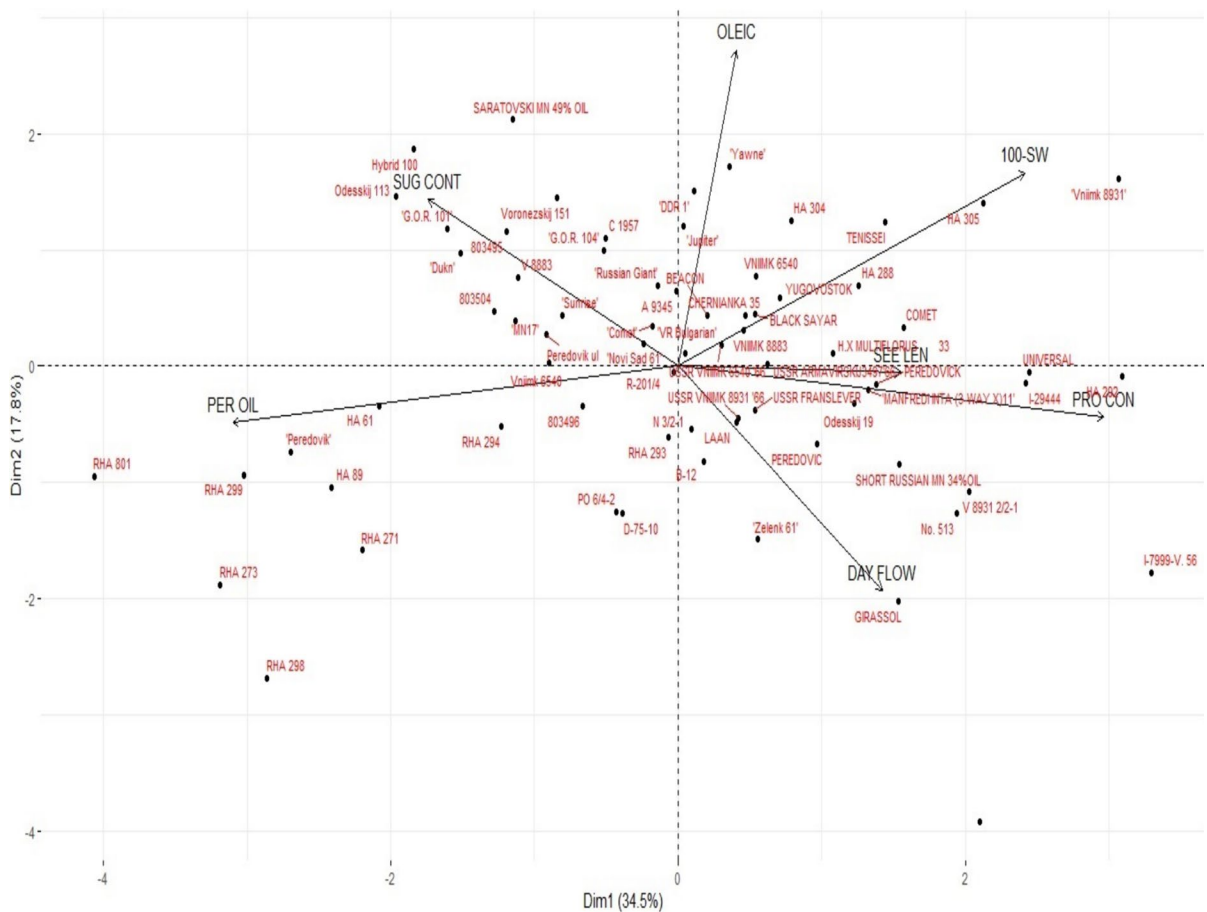


Fig. 1 Biplot analysis partitioned accessions on the basis of their relative importance with respect to the traits

accessions HA-292 (14 g), HA 305' (13 g) and 'Vinimik 8931' (11.23 g) displayed high 100-seed mass, while 'Comet' (1.23 cm) and *H. × multiflorous* (1.16 cm) exhibited higher seed length. Accession 'Garissol' (84 days) showed higher days to flowering, indicating a later maturity. Accessions with better mean values and may be further analyzed for their breeding value in breeding programs. Data of all 71 accessions in the study has been provided in the supplementary file (S1–S7).

Combining ability analysis

This analysis aimed to determine the type of gene action involved in the studied traits and formulate breeding strategies for enhancing target traits. Moreover, GCA effects were estimated to assess the breeding value of the selected plant material. A good general combiner carries lesser genetic load and may produce more uniform offspring when mated with a

tester population. Combining ability analysis of the selected accessions revealed significant variation for all traits due to crosses, lines, and line × tester interactions (Table 6). Significant values indicated that crosses and selected lines significantly differed for the traits under study (Table 6). Analyses of variance showed high magnitude of variance due to lines (Female parents) in all three traits, while mean sum of square variance for line × tester was lower than lines (Table 6). Mean values of progenies obtained after crossing female lines with male testers for achene yield head⁻¹ and head diameter are presented in Table 7. Progenies obtained from 'Comet' lines exhibited the highest achene yield (135 g head⁻¹) and head diameter (27 cm) across all crosses. Comet progenies showed an increase of 17% for achene yield and 25% for head diameter over grand mean of all progenies. Progenies obtained from 'Vniimk 8931' showed the lowest trait values in all crosses (Table 7). 'Odeskij 113' and 'Tenisseiä displayed higher values

Table 6 Analysis of variance for the yield related traits following line × tester mating design for the accessions selected on the basis of traits related to the confectionary sunflower

Source of variation	Degrees of freedom	Mean sum of square		
		100-seed mass	Head diameter	Seed yield per plant
Replications	2	2.07*	2.57 ^{ns}	12.07 ^{ns}
Crosses	17	5.64*	35.05**	780.71**
Lines	5	12.37*	103.46**	2454.63**
Testers	2	0.57 ^{ns}	2.46 ^{ns}	132.91*
Lines × Testers	10	3.28*	7.37*	71.82*
Error	34	1.47	1.86	34.58
Total	53			
Genotypic variance		1.19	10.83	256.21
Phenotypic variance		2.65	12.69	290.80
Environment variance		1.47	1.87	34.58
Heritability (Broad sense)		0.48	0.85	0.88

^{ns}insignificant ($P \geq 0.05$),
*significant when probability ($P \leq 0.05$) and
**highly significant when probability ($P \leq 0.01$)

Table 7 Mean values for the seed yield head⁻¹ and head diameter following the line × tester mating design for the accessions selected on the basis of traits related to the confectionary sunflower

Lines	Seed yield head ⁻¹			Head diameter (cm)		
	Testers			Testers		
	'HA-292'	'I-29444'	'Universal'	'HA-292'	'I-29444'	'Universal'
'Comet'	138.67ab	135.33a-c	143.00a	26.33a-c	27.67a	27.33ab
'G.O.R. 104'	116.37fg	126.67c-e	122.67ef	18.67h-j	20.67e-h	18.33h-j
'HA-305'	115.00fg	111.00g	121.33ef	24.33cd	20.67e-h	22.67de
'Odeskij 113'	110.67g	116.00fg	132.67ef	21.33e-g	19.00f-i	20.33e-h
'Tenissei'	124.00d-f	119.00e-g	132.67b-d	21.67ef	22.33de	25.33bc
'Vniimk 8931'	93.67h	88.33hi	84.67ij	19.33f-i	17.00i-l	16.33j-m

of seed yield when crossed with the tester ‘Universal’ (Table 7). For 100-seed mass, ‘Comet’ displayed the highest mean value across all crosses, while ‘HA-305’ exhibited the highest 100-seed mass following the ‘HA-292’ tester (Table 8). ‘Tenissei’ also demonstrated high 100-seed mass following its cross with tester ‘I-29,444’. The cross of ‘Comet’ with ‘Universal’ also resulted in high seed mass (Table 8).

GCA effects were estimated following a line \times tester design (Table 9). ‘Comet’ showed the highest GCA effects for achene yield per plant, head diameter, and 100-seed mass (Table 9). ‘G.OR.104’ was a positive general combiner for achene yield per plant but a negative combiner for other traits. ‘Tenissei’ exhibited positive GCA for all traits under study. Among the testers, ‘Universal’ displayed positive GCA for seed yield per plant, while ‘HA-292’ exhibited positive GCA for head diameter (Table 9).

Table 8 Mean values for 100-seed mass following the line \times tester mating design for the accessions selected on the basis of traits related to the confectionary sunflower

Testers			
Lines	‘HA-292’	‘I-29444’	‘Universal’
‘Comet’	12.33a-d	12.67a-c	13.00ab
‘G.O.R. 104’	8.33g	11.33b-f	9.67e-g
‘HA-305’	13.00ab	11.00b-f	12.00a-e
‘Odeskij 113’	10.33c-g	10.00d-g	10.33c-g
‘Tenissei’	11.67a-e	13.67a	11.00b-f
‘Vniimk 8931’	11.00b-f	10.00d-g	11.00b-f

Table 9 General combining ability effects for yield related traits estimated following the line \times tester mating design for the accessions selected on the basis of traits related to the confectionary sunflower

Lines	Seed yield per plant	Head diameter	100-seed mass
‘Comet’	21.19	5.48	1.43
‘G.O.R. 104’	4.185	−2.41	−1.46
‘HA-305’	−2.04	0.93	0.76
‘Odeskij 113’	−1.82	−1.41	−1.01
‘Tenissei’	7.41	1.48	0.87
‘Vniimk 8931’	−28.93	−4.07	−0.57
<i>Testers</i>			
‘HA-292’	−1.37	0.32	−0.13
‘I-29444’	−1.76	−0.41	0.20
‘Universal’	3.13	0.09	−0.07
LSD value	1.03	0.12	0.14

Discussion

The screening of sunflower germplasm for confectionary traits revealed significant variability among the traits, with GCV ranging between 9 and 29%. Previous research also reported broad genetic variability among confectionary sunflower cultivars (Velasco et al. 2014; Aldemir et al. 2016; Chen et al. 2020), thereby highlighting the potential for further improvement in this crop. A study on the landraces sampled in Spain for confectionary sunflower revealed wide variability within functional molecules within sunflower germplasm (Velasco et al. 2014). The seven accessions that were obtained from Cordoba had high phytosterol contents and obtained accessions were valuable source for improvement of this crop. Another study on newly developed confectionary sunflower types indicated an achene length of 15.2 mm and width of 8.50 mm, with dehulled seed oil contents of 49% and protein contents of 28% (Sandrinelli et al. 2022). The fatty acid profile showed high levels of linoleic acid (56%) followed by oleic acid (34%). Diversity among the confectionary sunflower accessions based on morphological traits was 0.71 which show higher morphological diversity (Hladni et al. 2017). High diversity was estimated for floral characteristics, medium to high for seed related traits, while the lowest for the leaf descriptors (Hladni et al. 2017).

Sunflower oil quality is majorly dictated by the fatty acid, and conventional sunflower cultivars had high linoleic acid, which is a polyunsaturated fatty acid and may decrease the shelf life of seed and oil (Mourad et al. 2016). Sunflower oil has been characterized into high (> 80%), medium (50–60%) and low

oleic acid (<50%). Mono-unsaturated fatty acid oleic acid has high thermos-oxidative stability and may replace the linoleic acid (Shah et al. 2023). Moreover, over sunflower stearins based on fractionation of high oleic and high stearic acid can be substitute for coca butter equivalent (Bootello et al. 2018). It has also been observed that presence of medium or high linoleic acid also improve the taste and impart health benefits on lipid serum quality.

Correlation and path coefficient analyses revealed associations between various confectionary traits. For instance, 100-seed mass was positively correlated with protein contents, seed length, and oleic acid, suggesting that accessions with high 100-seed mass tend to have higher protein contents, seed length, and oleic acid contents. Additionally, genotypes with high sugar contents may be selected to develop confectionary sunflower cultivars with high seed mass.

Previous research emphasized protein contents as a crucial criterion for the selection of confectionary sunflower (Hladni et al. 2015, 2016; Vedmedeva et al. 2023). Path coefficient analysis has been used to determine the direct and indirect effects of seed-related traits on protein yield (Hladni et al. 2015). Improvement in the plant based protein could enhance the meal values for the confectionary purpose. Notably, oil contents showed a significant negative direct effect on protein yield (Hladni et al. 2015). Reinert et al. (2020) showed a negative association between seed length and floret traits such as pollen tube length. Similar, correlation and path analyses of eight confectionary accessions showed a positive relationship between yield, morphological traits and protein contents (Sincik and Gokosoy 2014).

Combining ability analysis of selected confectionary sunflower types revealed high heritability estimates and a preponderance of dominance variance for traits such as 100-seed mass and head diameter, suggesting the potential for recurrent selection to develop breeding lines with high seed mass. Accessions showing positive GCA effects may be preferred for breeding high-yielding confectionary sunflower hybrids (Li et al. 1995; Ćiric et al. 2013; Rauf 2019). For example, ‘Comet’ exhibited the highest GCA effects for seed yield per plant, head diameter, and 100-seed mass. ‘Tenissiei’ also showed positive GCA for all traits under study. Accessions like ‘Universal’ and ‘HA-292’ displayed positive combining ability for specific traits, thus offering valuable insights for

breeding programs aimed at enhancing confectionary sunflower cultivars.

In summary of the above research, it is concluded that accessions with better confectionary traits along with high GCA for yield and components such as ‘Comet’ and ‘HA-305’ may be used as parents in breeding for the confectionary sunflower.

Author contribution SR did the planning and mobilised resources for this research. AL, SR and MN did the field research and data analysis. Results interpretation was done together by AL, SR, MN and RO. SR provided the first draft, whose main editing was done by RO. All authors contributing to this manuscript accept this last version.

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Data availability The data that support the findings of this study are available from the corresponding author SR upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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