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SEED WEIGHT VARIATION IN SIX SAFFLOWER CULTIVARS OF PAKISTAN

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ABSTRACT

Variation in single seed weight in six cultivars of safflower (viz. Thori-78, Gilla, S-208, SAF- 65, Pawari-85 and SPS-6912) grown at Regional Agricultural Research Station, Bahawalpur, Pakistan is described. One hundred seeds of each cultivars was weighed individually. The single seed weight (mg) of these cultivars averaged to as follows:

SAF- 65: 49.67± 1.477, CV: 19.75%, SPS-6912 = 45.64 ± 1.173, CV: 25.7%, Gilla = 43.43 ± 1.304, CV: 30.22%, Thori-78 = 43.43 ± 0.995, CV: 23.02%, S-208 = 42.36 ± 1.029, CV: 24.30%, Pawari = 41.20 ± 1.05, CV: 25.49%. Seed weight of pooled sample (N = 600) averaged to 44.32 ± 0.495, CV: 27.34%). Seed weight in each variety distributed asymmetrically. The compositional similarity amongst the germplasms varied substantially (60 to 89%).

There were only two broader groups on the basis of agglomerative cluster grouping extracted at < 5 % of dissimilarity. Cluster A was composed of Varieties. Thori, Pawari, SPS 6912, Gilla and SAF-65. Var. S-208 alone stood as Cluster B.

The mean seed weight in cluster - A was 44.713 ± 0.5556 mg, N = 500 varying from 15 to 107 mg (CV: 24.30%). The mean seed in cluster-B was lower amounting to 42.363 ± 1.0296mg, N = 100 varying from 15 to 107 (CV: 27.78%). The seeds of cluster B (Cultivar S-208) were relatively lighter in weight as compared to the cluster A merely by a magnitude of 2.35 mg. Average seed size data for many safflower cultivars, lines and populations are presented for comparison.

Key Words: *Carthamus tinctorius* L., Safflower cultivars of Pakistan, Seed weight distrubtion, Agglomerative clustering.

INTRODUCTION

Safflower (*Carthamus tinctorius* L., Family Asteraceae, chromosome number = 2N = 24), is an annual oil-seed crop mainly produced for high quality edible oil (non-allergenic), biodiesel and birdseed. It bears several vernacular names – False saffron, saffron bátard, saffloer in Dutch, farbertistel in German, Aspir in Turkish, and Kesumba in Indonesian (Hauze *et al.,* 2015).

It is well-adapted to arid and semiarid regions because of its tolerance to drought, salinity and heat stress (Kaya, 2014). It yields red (carthamin) and yellow (carthamidin) dyes (Zohary and Hopf, 2000) and exhibits antioxidant property (Sung *et al.,* 2018). Its domestication is said to begin c 4000 years ago in Fertile Crescent (Pearl *et al.,* 2014). According to Walsh *et al.* (2008), safflower is cultivated since ancient times in China, Egypt and India. It was grown in Europe, Central America and South America in Middle Ages and its cultivation in USA began in 1925. It is now cultivated in over 60 countries (Omidi *et al.,* 2009). India is the largest producer of safflower – c 206,000 tons of seeds annually (Rajvanshi, 2005). It has highly been advised for the regions suffering from rainfall scarcity where a traditional crop rotation of wheat-fallow is necessarily applied to increase oil production (Singh *et al.,* 2016). Gilla, US-10, S-208, Thori – 78 and Pawari-95 are some of the safflower varieties in Pakistan (Baloch *et al.,* 2015). Fawad *et al.* (2020) reported the best 20 genotypes, out of 94 accessions from 26 countries, and experimented in Pakistan and Turkey. These cultivars were Pakistan -7, Egypt -3, Egypt -5, Iran -1, Jordon -1, Jordon – 2, Portugal -4, China -1, Turkey – 4, Pakistan -8, Pakistan -9, Jordon -3, Jordon -4, Jordon – 5, Israel - 4, Hungary -1, Turkey – 9, China -3, China -4 and China -5. The average 100-seed weight (g) of these genotypes averaged to 3.3287 ± 0.5933 (SD) varying from 2.165 -5.3195g. There are also several competitive superior lines. PI-405995, PI – 253566, PI- 205077, PI – 405-990, and PI – 195895 have been reported to surpass commercial check variety, Thori-78, in some vegetative or reproductive agronomic traits (Baloch *et al.,* 2015).

There is considerable level of variability in genetic material of safflower (Shinwari *et al.,* 2014). N_2 application and irrigation during drought increases grain yield (Santos *et al.,* 2018). Chaudhary (1990) pointed out that safflower agronomic traits like plant height, leaf number, primary branches per plant, seeds (achenes) per capitulum, and 1000-seed weight had positive effects on seed yield. Furthermore, he suggested a selection criteria combining seeds per capitulum, capitula per plant, and 1000-seed weight to be efficiently used in selecting high yielding genotypes

during the selection process. Irrigated safflower yields were 93% higher as compared to dry land yields when three irrigations were applied in addition to the 7.3 mm rainfall during growing season (Armstrong, 1981). The biochemical analysis of safflower is presented by Mailer *et al.* (2008). Velasco *et al.* (2005) reported that seeds are rich in Zn, Cu, Mn and Fe, vitamins (Thiamine and β- carotene) and tocopherols (α, β and γ).

The present paper describes the variation in single seed mass of six common safflower cultivars viz. Thori-78, Gilla, S-208, SAF- 65, Pawari-85 and SPS-6912 grown at Regional Agricultural Research Station, Bahawalpur, Pakistan.

MATERIALS AND METHODS

One hundred seeds, randomly drawn from each of the lots of six safflower cultivars (supplied by Regional Agricultural Research Station, Bahawalpur, Pakistan), were weighed individually (pappus removed) on an electronic balance with an accuracy of 0.1 mg. The location and dispersion parameters for each germplasm were calculated. The symmetry, skewness and kurtosis were calculated (Sokal and Rholf, 1995). Normal distribution of seed mass data was tested by Kolmogorov-Smirnoff test (KS-z test) with Lilliefors significance correction and Shapiro-Wilk test. These tests assess whether the observations could reasonably have come from the population following normal distribution. The compositional similarity amongst the varieties was calculated on the basis of % frequency distribution of seeds of the six varieties following the method of Brock (1977). The germplasms were compared on the basis of seed size and they were linked by hierarchical cluster analysis by Ward's (1963) method using Euclidean distances with respect to the seed masses. The statistical analyses were performed with software viz. 'SPSS version '19'.

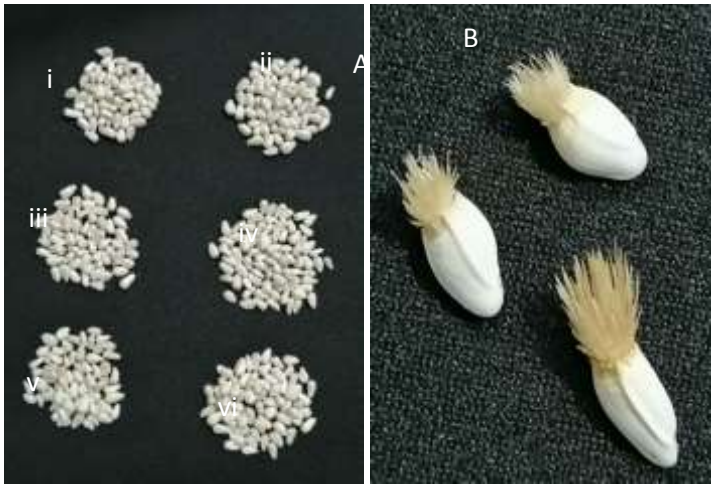

Fig. 1. A) Seeds of Safflower varieties provided by Regional Agricultural Research Station, Bahawalpur, Pakistan. i, Gilla; ii, S-208; iii, SAF-65; iv, Thori-78; v, Pawari -95 and vi, SPS-6912. Achenes of all these cultivar are milky white in colour and each achene is provided with three ridges running longitudinal

B) Achenes with persistent fibrous pappus attached on the broader apical end.

Table 1. Single-seed weight (mg) distribution in six varieties of safflower.

Parameter	Gilla	Thori-78	Pawari	SAF-65	S-208	SPS -6912	Pooled
N	100	100	100	100	100	100	600
Mean	43.43	43.43	41.20	49.67	42.363	45.64	**44.32**
SE	1.3040	0.9996	1.0500	1.4774	1.0296	1.1734	0.4947
CV(%)	30.22	23.02	25.49	19.75	24.30	25.70	27.34
Median	41.50	44.00	40.00	45.00	42.0	44.00	43.00
Skewness	0.637	-0.012	0.843	0.958	0.731	1.003	0.898
SE of skewness	0.241	0.241	0.241	0.241	0.241	0.241	0.100
Kurtosis	0.256	0.366	1.215	1.613	2.177	1.795	1.3843
SE of Kurtsosis	0.478	0.478	0.478	0.478	0.478	0.478	0.199
Minimum	18	18	17	24	18	15	15
Maximum	78	71	77	107	79	86	107
KST*	0.093	0.063	0.099	0.144	0.092	0.102	0.088
P	0.032	0.200	0.016	0.0001	0.037	0.013	0.0001
Shapiro-Wilk	0.965	0.993	0.958	0.947	0.952	0.933	0.957
p	0.010	0.876	0.003	0.001	0.001	0.0001	0.0001
Distrbution	AS	S	AS	AS	AS	AS	AS

*, Kolmogorov-Smirnoff test of normalcy with Lillefors correction for significance; Shapiro-Wilk, Shapiro0-Wilk test of normalcy. S, Symmetrical; AS, Asymmetrical.

RESULTS AND DISCUSSION

The seeds of inhand six safflower varieties were oval in shape and milky white in colour (Fig. 1). The fibrous pappus is attached on the broader end which was removed while weghing. The location and dispersion parameters and seed weight of six varieties along with the pooled sample is presented in Table 1 on the basis of weight of single seed.

Table 2 . % Frequency distribution of single-seed weight (mg) for uniform seed weight class interval.

Class size mg	Gilla	Thori	Pawari	SAF-65	S-208	SPS-6912	Pooled
A (≤ 20)	2	1	1	zero	1	1	1.0
B (21-30)	15	8	10	6	7	2	7.8
C (31-40)	27	26	40	22	35	37	31.7
D (41-50)	29	40	32	28	43	32	34.0
E (51-60)	16	17	12	23	9	18	15.7
F (61-70)	6	5	3	12	3	5	5.8
G (71-80)	5	1	2	7	2	3	3.3
H (81-90)	-	-		Zero	-	2	0.4
I (91-100)	-	-	-	1	-	-	0.1
J (> 100)	-	-	-	1	-	-	0.1

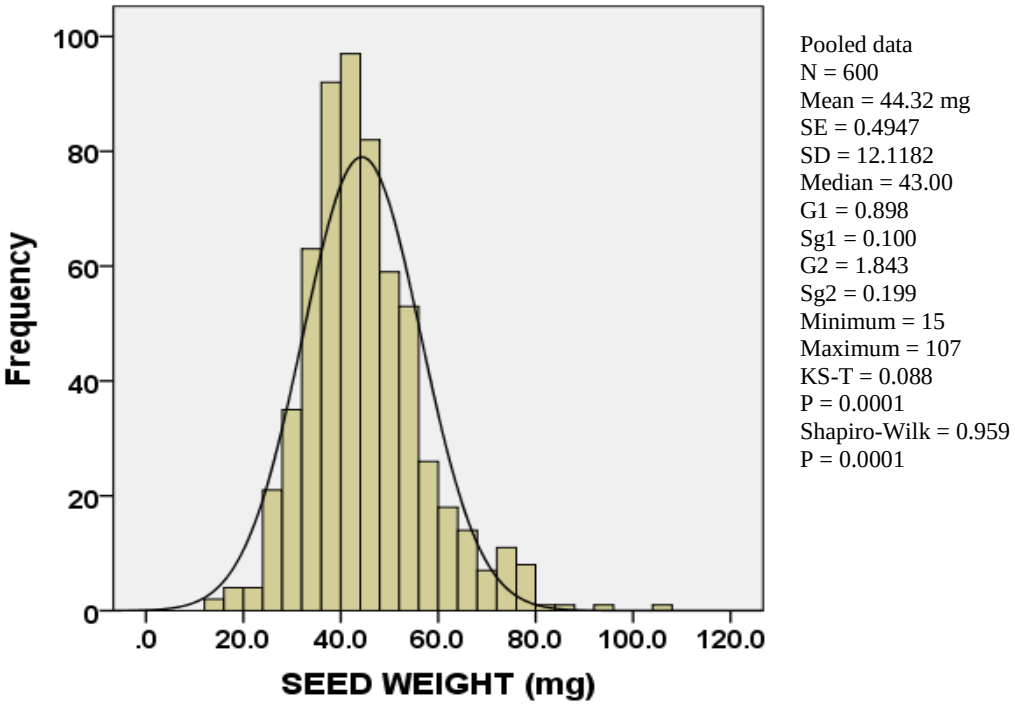

Fig .2. Single-seed weight distribution of pooled seeds of six varieties of safflower.

The single seed weight (mg) of the cultivars averaged to as follows:

SAF- 65: 49.67 ± 1.477, CV: 19.75% > **SPS-6912** = 45.64 ± 1.173, CV: 25.7% > **Gilla** = 43.43 ± 1.304, CV: 30.22% > **Thori-78** = 43.43 ± 0.995, CV: 23.02% > **S-208** = 42.36 ± 1.029, CV: 24.30% > **Pawari** = 41.20 ± 1.05, CV: 25.49%. Seed weight of pooled sample (N = 600) averaged to 44.32 ± 0.495, CV: 27.34%). Seed weight in each variety as well as pooled sample distributed asymmetrically (Table 1 and Fig. 2).

The frequency distribution of single see weight data amongst among various seed classes (Table 2) indicated that three seed size classes were important i.e. classes, C: 31-40mg, D: 41-50mg and E: 51-60mg, respectively, amongst which class D was more prominent. Collectively these lasses occupied a proportion of 72% in Gilla, 83%

in Thori, 84% in Pawari, 73% in SAF-65 and 87% in S-208 and SPS-6912 each. In pooled sample these three classes occupied a proportion of 81.4%. Other classes were relatively much lower in proportion except CV. Gilla.

Table 3. Percent compositionsl similarity of seed weights (mg) amongst the safflower varieties on the basis of single seed weight frequency distribution as calculated following Brock (1977).

SIMILARITY MATRIX

A	A						
B	76	B					
C	77	77	C				
D	73	83	63	D			
E	67	87	70	89	E		
F	72	84	60	89	84	F	
G	72.8	90.5	81	89.5	87.7	90.8	G

Acronyms:
Varieties: A, Gilla; B, Thori - 78; C, SAF-65; D, Pawari; E, S-208; F, SPS 6912, and G, Pooled sample for six varieties.

Similarity amongst germplasms on the basis of single seed weight composition

The similarity matrix amongst the six germplasms was calculated on the basis of seed weight % frequency distribution following Brock (1977) is outlined in Table 3. The compositional similarity amongst the germplasms varied substantially (60 to 89%). There was no case of similarity amongst the germplasms lesser than 63 %. Variety S-208 and Pawari were highly similar in compositional similarity, 89%.

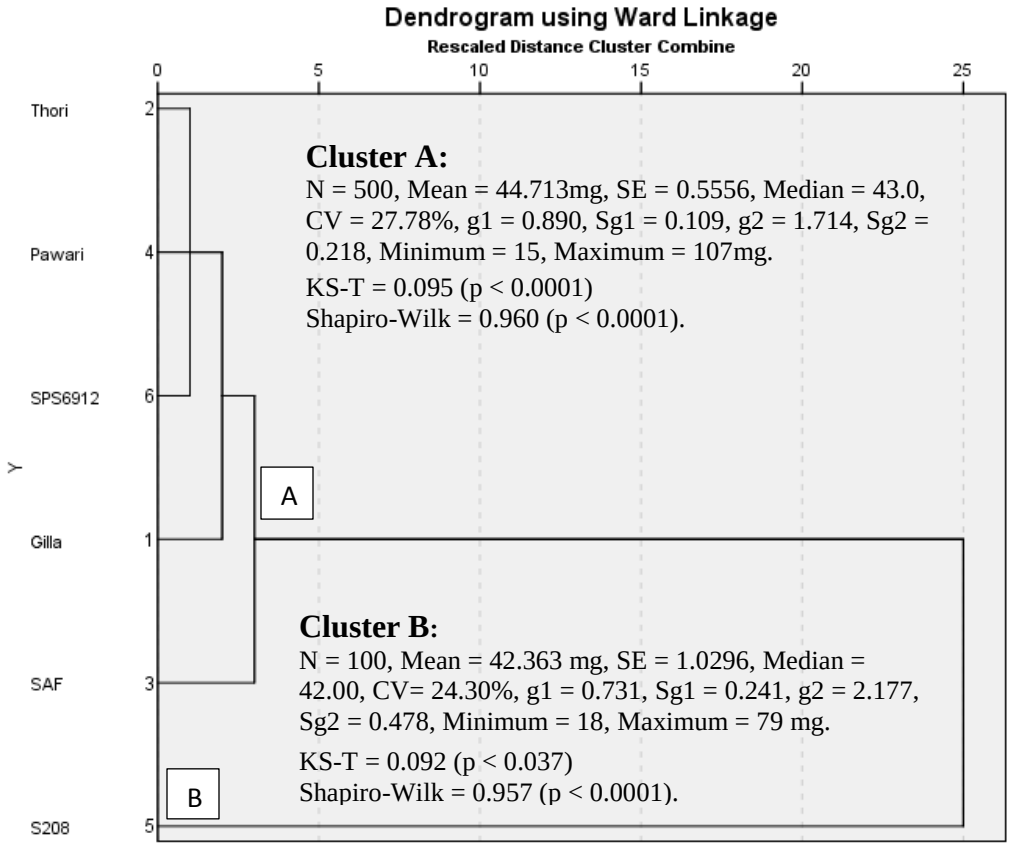

Fig. 3. Agglomerative dendrogram of seed weight data of safflower varieties. Cluster A was constituted by cultivars Thori, Pawari, SPS-6912, Gilla and SAF-65. The cluster B was represented by cultivar S208. The mean single seed weight of cluster A was higher significantly than the mean single seed weight of cluster B at $p < 0.05$ ($t = 2.009$) by an average magnitude of 2.35mg.

Agglomerative clustering of germplasms on the basis seed weight distribution of the varieties

There were only two broader groups on the basis of group extraction at < 5 % of dissimilarity (Fig. 3).

CLUSTER A: Var. Thori, Var. Pawari, Var. SPS 6912, Var. Gilla and Var. SAF-65.

CLUSTER B: Var. S-208, alone)

The mean seed weight in cluster - A was 44.713 ± 0.5556 mg, N = 500 varying from 15 to 107 mg (CV: 24.30%). The mean seed in cluster-B was significantly lower amounting to 42.363 ± 1.0296mg, N = 100 varying from 15 to 107 (CV: 27.78%) Table 4.

Table 4. Seed weight characteristics of the two clusters separated at ≤ 5% distance.

Seed Weight Parameters	Cluster A	Cluster B
N	500	100
Single seed Wt. (mg)	44.713	42.363
SE of mean	0.5556	1.0296
Min-Max Range (mg)	15-107	18-79
CV (%)	27.78	24.30

Obviously, the seeds of cluster B (Cultivar S-208) were relatively lighter in weight as compared to the cluster A.

Table 5. Single-achene mass (mg) data adapted and calculated for some safflower cultivars from various sources.

S. No.	Germplasm Cultivars / Lines / Populations	Locality of origin or collection	Achene mass (mg)	Reference
CULTIVARS				
	A C Sterling	USA CV, cult. In Turkey	42.57	Sirel and Aytac (2016)
	Balci	Turkey	40.91	Arslan and Culpan (2018)
	Benno	Albania (cultivated)	44.79	Vorpsi *et al.* (2010)
	Black Sun	Turkey	34.2	ADA Rahim (2014)
	CB - 32	Pakistan	**53.0**	Akmal *et al.* (2002)
	CB 1221	Romania	42.8	Dobrin and Marin (2015)
	CW 74	Collection: ICARDA, etc.*	36.1	Beyyavas *et al.* (2011)
	CW 99 OL	Argentina	35.91 (2008) 41.87 (2009).	Franchini *et al.* 2012).
	CW 4440	Collection from ICARDA, etc. *	33.7	Beyyavas *et al.* (2011)
	Cyprobren	Collection from ICARDA, etc. *	37.4	Beyyavas *et al.* (2011)
	Dholka Sindh	Pakistan	54.0	Akmal *et al.* (2002)
	Dinçer	Collection from ICARDA, etc. *	34.7	Beyyavas *et al.* (2011)
	Dinçer	Turkey	36.3	ADA Rahim (2014)
	Dinçer	Turkey	47.08	Arslan and Culpan (2018)
	Dinçer **	Turkey	36.2	Kizil *et al. (*2008)
	Gilla	Australia (Irrigated)	34.0 -39.2	Armstrong (1981)
	Gila	Collection from ICARDA, etc. *	36.9	Beyyavas *et al.* (2011)
	Girard	Pakistan	**53.0**	Akmal *et al.* (2002)
	Goldasht	Iran	31.1	Omidi *et al.* (2012).
	Finch	USA CV, cult. In Turkey	36.68	Sirel and Aytac (2016)
	I.L III	Iran	41.7	Nikabadi *et al.* (2008)
	Hartinan	Collection from ICARDA, etc. *	36.4	Beyyavas *et al.* (2011)
	Isfahan	Iran	30.1	Nikabadi *et al.* (2008)
	K.W2	Iran	25.7	Omidi *et al.* (2012)
	KS-06	Turkey	40.2	ADA Rahim (2014)
	L-221	Pakistan	47.0	Akmal *et al.* (2002)
	Linas	Turkey	44.11	Arslan and Culpan (2018)
	MKH -8	Collection from ICARDA, etc. *	40.0	Beyyavas *et al.* (2011)
	MKH - 9	Collection from ICARDA, etc. *	33.5	Beyyavas *et al.* (2011)
	Montola 2000	Albania (cultivated)	43.6	Vorpsi *et al.* (2010)
	N5	USA CV. Cult. In Turkey	43.87	Sirel and Aytac (2016)
	Olas	Turkey	46.36	Arslan and Culpan (2018)
	Ole	USA CV, cult. In Turkey	40.23	Sirel and Aytac (2016)
	Oleic Leed	USA CV, cult. In Turkey	42.58	Sirel and Aytac (2016)

	Oleic Leed	Turkey	32.60	ADA Rahim (2014)
	Padideh	Iran	30.0	Omidi *et al.* (2012)
	Remzibey	Turkey	33.6	ADA Rahim (2014)
	Remzibey	Turkey	37.71	Arslan and Culpan (2018)
	S-541	Collection from ICARDA, etc. *	37.1	Beyyavas *et al.* (2011)
	S-541-2	Collection from ICARDA, etc. *	36.9	Beyyavas *et al.* (2011)
	Saff (2002)	Albania (cultivated)	43.26	Vorpsi *et al.* (2010)
	Sahuaripa - 88	Mexican CV, cult. In Turkey	45.05	Sirel and Aytac (2016)
	Sironaria	Pakistan	49.0	Akmal *et al.* (2002)
	Syrian	Collection from ICARDA, etc. *	36.5	Beyyavas *et al.* (2011)
	Syrian -1	Collection from ICARDA, etc. *	34.1	Beyyavas *et al.* (2011)
	Syria Hama	Collection from ICARDA, etc. *	41.2	Beyyavas *et al.* (2011)
	Thori -78	Pakistan	**52.0**	Akmal *et al.* (2002)
	UC - 1	USA CV. Cult. In Turkey	42.53	Sirel and Aytac (2016)
	US - 10	USA CV. Cult. In Turkey	42.40	Sirel and Aytac (2016)
	Yenice	Collection from ICARDA, etc. *	33.1	Beyyavas *et al.* (2011)
	Yenice **	Turkey	33.9	Kizil *et al.* (2008)
	Yenice	Turkey	36.63	Arslan and Culpan (2018)
	Zangibar	Romania	44.49	Dobrin and Marin (2015)
	88 OL	Romania	42.75	Dobrin and Marin (2015)
	5-154 **	Turkey	36.6	Kizil et al. (2008).
LINES				
	198290	Collection from ICARDA, etc. *	35.7	Beyyavas *et al.* (2011)
	200536	Collection from ICARDA, etc. *	36.1	Beyyavas *et al.* (2011)
	250537	Collection from ICARDA, etc. *	33.7	Beyyavas *et al.* (2011)
	250540	Collection from ICARDA, etc. *	34.9	Beyyavas *et al.* (2011)
	251982	Collection from ICARDA, etc. *	36.9	Beyyavas *et al.* (2011)
	251984	Collection from ICARDA, etc. *	34.5	Beyyavas *et al.* (2011)
	258417	Collection from ICARDA, etc. *	37.3	Beyyavas *et al.* (2011)
	301055	Collection from ICARDA, etc. *	39.5	Beyyavas *et al.* (2011)
	537636	Collection from ICARDA, etc. *	35.1	Beyyavas *et al.* (2011)
	Line -159	India	31.93	Abd El-Lattief (2012).
	Line- 1697	Cyperus	48.75	Abd El-Lattief (2012).
	Line -1687	Ethiopia	49.13	Abd El-Lattief (2012).
	Accession China (N = 240)	Trial in china	**53.7 ± 8.0 (30.7-83.2)**	Sung *et al.* (2018)
	Accessions, Japan	Trial in china	**49.9 ± 3.90 (47.2-55.8)**	Sung *et al.* (2018)
	Accessions, S. Korea	Trial in china	40.5 ± 7.5 (27.0 – 56.1)	Sung *et al.* (2018)
	Accessions, N. Korea	Trial in china	44.9 ± 4.9 (37.8 -50.8)	Sung *et al.* (2018)
POPULATIONS				
	Afyon	Collection from ICARDA, etc. *	30.4	Beyyavas *et al.* (2011)
	Cyperus	Collection from ICARDA, etc. *	33.1	Beyyavas *et al.* (2011)
	Sivas	Collection from ICARDA, etc. *	35.9	Beyyavas *et al.* (2011)

*, Genotypes collected from Int. Centre for Agriculture in Dry areas (ICARDA), Anatolian Agricultural Res. Institute (Eskisehir) and Dept. Of Field crops of faculty of Agric., Univ. Cukurova. **, At 100 % flowering in 2005-06.

Shinwari *et al.* (2014) have investigated 122 safflower accessions. The accessions 16235, 16320, 26733, 26735, 26738, 26741, 26752 and 26769 appeared to be promising with single seed mass more than or equal to 45 mg. An average weight of seed, c 40 mg (0.030 – 0.045g), is reported for safflower germplasms by Australian Government (2019) - some 25, 000 seeds / kg. Abd El-Lattief (2012) reported that of the 25 lines of safflower investigated from Egypt, India, Cyperus and Ethiopia, had 1000-seed weight ranging from 31.93g to 49.13g under arid environment.

The dependence of seed yield with 1000-seed wt. was low (R^2 = 3.0%) but substantially more depended on plant height (R^2 = 30.3% and branches per plant (R^2 = 33.8%) (Saisanthosh *et al.* (2018). The 100-seed weight of 61 genotypes including elite germplasm lines varied significantly among germplasms averaging to 4.18 g (2.17-5.77g). It was, however, quite high for test check (A1) to be 6.68g followed by cultivar Bhima (5.77g). Muhammad *et al.* (2020) investigated different genetic parameters in 200 genotypes, from India, Afghanistan, Iran, Turkey, Pakistan., Egypt, Ethiopia, Australia, Portugal, Sudan, Russia, Iraq, Spain, Germany, China and USA. Their agglomerative clustering on the basis of morphogenetic parameters indicated significant genetic variability and resulted in 8 groups. The 1000-seed weight of these germplasms averaged to 37.78g (CV= 5.367%) i.e., 37.78 mg per seed. Thirty nine genotypes from various countries and six local Turkish cultivars were studied by Arslan and Culpan (2018) for their potential use in germplasm development of safflower. The 1000-seed weight in these germplasms varied from 27.00 to 55.7g. The seed weight for some safflower cultivars from various countries is presented in Table 5. The perusal of this data on average single seed weight indicated that safflower cultivars, lines, or accessions appears to be the trait influenced genetically as well as environmentally and may be classified into four categories – A) ≤ 30.0 mg (such as cultivars K.W2 and Pedideh of Iran), B) 30.1- 40 mg, C) 40.1 – 50.0 mg and D) > 50 mg. Seeds of the most of the cultivars belonged to category B and C. In hand local cultivars with average seed size ranging from 42.73 to 47.86 mg belonged to category C (see Table 1 and 5). Some cultivars such as Dilçer from Turkey or from ICARDA collection and Oleic Leed from Turkey and that from USA cultivated in Turkey, sometimes belonged to category B and sometimes to category C possibly due to environmental reasons (Table 5). There was temporal (seasonal) fluctuation in seed size in CW99 with year of cultivation in Argentina – 35.91mg in 2008 and 41.87 mg in 2009. The germplasms investigated by Akmal *et al.* (2002) such as CB-32, Dholka Sindh, L-221, PI-262424, PI- 279874, PI- 3070600, Sironaria, Thori – 78 varied around 6.8% in 100-seed weight under rain fed conditions. Nutrification with NPK promoted the 1000-grain weight in cultivar 88 OL, CW 1221 and Zanzibar cultivars by 3.32, 3.86 and 4.54%, respectively, over non-nutrified conditions in Southeastern Romania (Dobrin and Marin, 2015).

There were some cultivars with single seed weight more than 50 mg (Table 5). The data of 240 accessions from China (Sung *et al.,* 2018) exhibited heavier seed size averaging to 53.7 ± 8.0 (varying from 30.7 – 83.2mg). Some cultivars from Pakistan such as Thori – 78 (52mg), Girard (53 mg), Dholka Sindh (54 mg) and CW 32 (53mg) also exhibited heavier seed size (Akmal *et al.,* 2002). In present studies, CV. Thori-78 exhibited average seed weight of 43.43 ± 0.995 mg, quite lower than that reported by Akmal *et al.* (2002). Such a disparity may probably be attributed to cultural practices and field environmental conditions of Bahawalpur, Pakistan where in hand cultivars were grown. The germplasms of heavier seed weight may obviously be useful resource in safflower development.

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