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SEED MASS VARIATION IN SEED LOTS OF NINE CULTIVARS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Seed mass variation in seed lots of nine sunflower cultivars viz. S-278, local, Hybrid 1, Hysun 33, Hysun 39, N K Armoni, Aussie gold 04, Aussie gold 61 and Aussie gold 62 is described. Within individual cultivars seed mass varied substantially from 1.91-folds in cultivar S-278 to 3.68-folds in Hybrid 1. The variation in terms of coefficient of variation was also the lowest in cultivar S-278 (13.91 %) followed by N K Armoni (15.99%). It was the highest (24.62 %) in cultivar 'local'. In pooled sample, variation was 26.1% (heaviest / lightest seed ratio 3.96). Cultivars varied in mean seed mass which was 50.52 ± 1.05 mg in Hysun 39 to 81.79 ± 1.14 mg in S-278. Mean seed mass of N K Armoni was 74.82 ± 1.196 mg. In other varieties mean seed weight was between 51 and 58 mg. Mean seed mass of the pooled sample was 60.12 ± 0.523 mg. The distribution of mean seed mass around the grand mean was negatively skewed. The coefficient of variation among means was 17.74% i.e., the variation was 1.62-folds. The seed masses exhibited significantly positive skewness in four cultivars local, Hysun 39, Hybrid 1, and Aussie gold 62 and pooled sample. In cultivar NK Armoni, seed masse distribution was significantly negatively skewed. The distribution was characterized with significantly positive kurtosis (leptokurtosis) in cultivars local and Hybrid 1, insignificant leptokurtosis in Aussie gold 62 and Hysun 33 and insignificantly negative kurtosis (platykurtosis) in S-278, Hysun 39, NK Armoni, Aussie gold 61, Aussie gold 04 and the pooled sample of seeds. The normality of distribution was tested with Shapiro-Wilks test. The distribution of individual seed mass was found to be normal in six cultivars viz. S-278, local, Hysun 39, Hysun 33, Aussie gold 61 and Aussie gold 04 and Non-normal in NK Armoni, Hybrid 1, Aussie gold 61 and the pooled sample of all cultivars. Hierarchical clustering, on the basis of seed mass, discretely classified varieties into two groups. Cultivars S-278 and N K Armoni were heavier seed cultivars and cultivars local, Hybrid 1, Hysun 33, Hysun 39, Aussie gold 04, Aussie gold 61 and Aussie gold 62 were substantially lighter seed varieties.

Key Words: Sunflower (*Helianthus annuus* L.) cultivars, Seed mass variation and distribution.

INTRODUCTION

Literature survey of the subject indicates that there exists considerable variation in seed weight within and among species, cultivars of a species, within individual plant and even within and between the fruits of a plant (Black, 1959; Harper, 1977; Janzen, 1977; Sachaal, 1980; Thompson, 1984; Stanton, 1984; Mazer, 1987; Thomson and Pellmyr, 1989; Hendrix, 1984; Hendrix and Sun, 1989; Zhang and Maun, 1990; Kang *et al.*, 1992; Zhang, 1998; Shaukat *et al.*, 1999; Shaukat and Burhan, 2000; Susko and Lovett-Doust, 2000; Cardazzo, 2002; Halpern, 2005; Busso and Perryman, 2005; Cahill and Ehdaie, (2005); Fasoula and Boerma, 2007; Aziz and Shaukat, 2010; Guo et al., 2010; Típscar Oliver and Borja (2010); Ghosh and Singh, 2011; Anis *et al.*, 2011). The plasticity in seed weight appears to be regulated by the internal and external environments of the mother plants (see Krannitz, 1997) and the genetic reasons (Alonso-Blanco *et al.*, 1999; Doganlar *et al.*, 2000).

Alexander *et al.* (2001) have reported the seed weight of sunflower crop, hybrid F1 and wild genotype. Anis *et al.* (2011) have reported seed weight variation in sunflower cultivar Aussie gold 61. In this paper we record mean seed weight and seed weight variation in nine sunflower cultivars available in Pakistan in view of their importance in agriculture and the oil economy.

MATERIALS AND METHODS

Seed Mass variation: One hundred seeds randomly drawn from each of the lots of nine sunflower cultivars supplied by Federal Seed Certification Department, Malir Halt, Karachi, were weighed individually on an electronic balance with an accuracy of 0.1 mg. The box plot distribution of seed masses was constructed and location and dispersion statistics were calculated. The symmetry, skewness and kurtosis were calculated (Sokal and Rohlf, 1995). Normal distribution of seed mass data was tested by Shapiro-Wilks W test. This test assesses whether the observations could reasonably have come from the normal distribution. The varieties were compared on the basis of seed size and they were linked by hierarchical cluster analysis with respect to their compositional similarity on the basis of seed masses. The statistical analyses were performed with softwares viz. 'SPSS version '10' and 'Statistica' (edition 99).

RESULTS

Seed mass variation in seed lots of nine sunflower cultivars viz. S-278, local, Hybrid 1, Hysun 33, Hysun 39, N K Armoni, Aussie gold 04, Aussie gold 61 and Aussie gold 62 is presented in Fig.1 and Table 1.

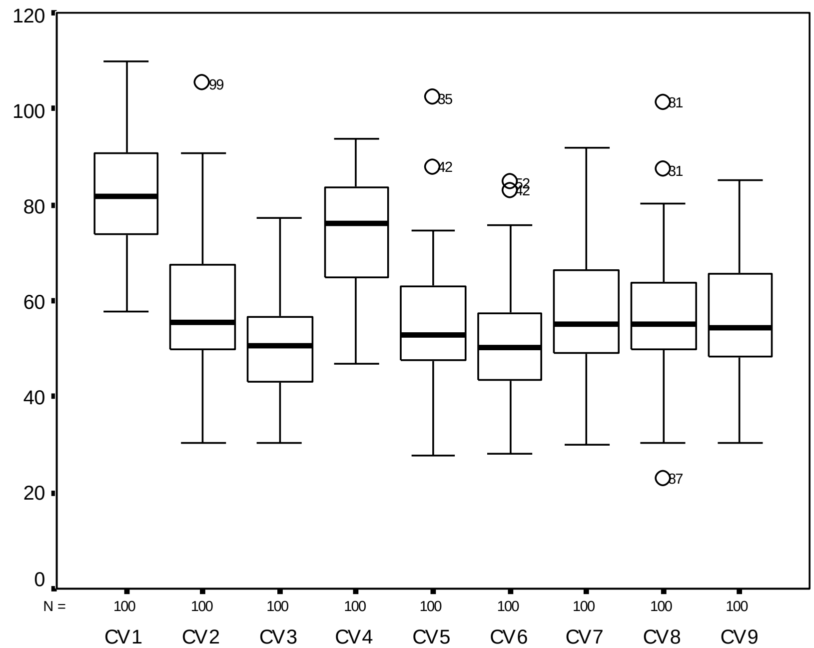

Fig. 1.Box plot distribution of seed weight (mg) of sunflower cultivars. Cv1, S-278; cv2, Local; cv3, Hysun 39; cv4, NK Armoni; cv5, Hybrid 1; cv6, Hysun 33; cv7, Aussie gold 61; cv8, Aussie gold 62; cv9, Aussie gold 4. The box plot shows median and inter-quartile range. The solid line within a box represents Q_2 (median) and box vertical lower and upper limits represent Q_1 and Q_3, respectively. The capping line represents 10 and 90 percentiles. Circles represent the data points outside the 10-90 percentiles.

Table 1. Location and dispersion parameters of seed mass (mg) in nine sunflower cultivars.

Parameters	SUNFLOWER CULTIVARS									
	S-278	Local	Hysun 39	NK Armoni	Hybrid 1	Hysun 33	Aussie gold 61	Aussie gold 62	Aussie gold 04	Cultivars Pooled
N	100	100	100	100	100	100	100	100	100	900
Mean	81.794	57.729	50.519	74.823	54.648	51.402	57.164	56.659	55.95	60.119
SE	1.1374	1.4212	1.0549	1.1963	1.2492	1.145	1.298	1.1480	1.134	0.5233
CV(%)	13.90	24.62	20.75	15.99	22.86	22.27	22.70	21.22	20.27	26.11
Median	81.60	55.50	50.40	76.10	53.00	50.40	54.95	55.20	54.55	57.50
g1	0.157	0.532	0.870	-0.384	0.556	0.462	0.252	0.759	0.023	0.458
g2	-0.390	0.397	-0.252	-0.693	1.267	0.243	-0.490	1.710	-0.685	-0.251
Min.	57.7	30.4	30.2	46.7	27.80	28.0	30.0	30.5	30.40	27.80
Max.	110.0	105.3	72.4	93.9	102.2	84.6	91.7	101.3	85.0	110.0
Max / Min	1.91	3.46	2.40	2.01	3.68	3.02	3.06	3.32	2.80	3.96
Sh-W	0.9802	0.9764	0.9818	0.9608	0.9737	0.9782	0.9790	0.9677	0.9796	0.9793
p <	0.1373	0.0692	0.1826	0.0046	0.0428	0.0964	0.0911	0.0148	0.1244	0.00001

G1, skewness; g2, kurtosis; St. error of skewness (Sg1) = 0.241; St. error of kurtosis (Sg2) = 0.478; Sh-W, Shapiro-Wilks test.

Within individual cultivars seed mass varied substantially from 1.91 fold in cultivar S-278 and 3.68 fold in Hybrid 1. The variation in terms of coefficient of variation was also the lowest in cultivar S-278 (13.90 %) followed by N K Armoni (15.99%). It was the highest (24.62 %) in cultivar ‘local’. In pooled sample seed mass variation was 26.1% and the heaviest / lightest seed mass ratio was 3.96. The seed weight varied among and within cultivars significantly. The seed mass varied among cultivars by 41% and within cultivar by 59% (Table 2).

Table 2. One Way ANOVA for seed masses of nine sunflower cultivars.

Source	SS	df	MS	F	p	% variance accounted for
Between	91108.807	8	11388.601	77.56	< 0.0001	41.05
Within	130831.513	891	146.837			58.95
Total	221940.32	899	-			100

In most of the cultivars mean seed mass was larger than median by only a little fraction of mass ranging from 0.194 to 2.30 mg (mean: 1.29 mg)In cv NK Armoni, however median was larger than the mean by 1.28 mg. There were a few outliers only – one in cv. Local and two outliers each in Hybrid 1 and Hysun 33 and three outliers in Aussie gold 62 (Fig. 1).

The skewness appearing from unequal whiskers in box plot representation was ascertained by calculating g1 and standard error of g1 (Sg1). Kurtosis was ascertained by calculating g2 and standard error of kurtosis (Sg2). The seed masses exhibited significantly positive skewness in four cultivars local, Hysun 39, Hybrid 1, and Aussie gold 62 and pooled sample. In cultivar NK Armoni, seed masse distribution was significantly negatively skewed. The distribution was characterized with significantly positive kurtosis (leptokurtosis) in cultivars local and Hybrid 1, insignificant leptokurtosis in Aussie gold 62 and Hysun 33 and insignificantly negative kurtosis (platykurtosis) in S-278, Hysun 39, NK Armoni, Aussie gold 61, Aussie gold 04 and the pooled sample of seeds. The normality of distribution was tested with Shapiro-Wilks test. The distribution of individual seed mass was found to be normal in six cultivars viz. S-278, local, Hysun 39, Hysun 33, Aussie gold 61 and Aussie gold 04 and Non-normal in NK Armoni, Hybrid 1, Aussie gold 61 and the pooled sample of all cultivars (Table 1 and Fig.2).

Mean seed mass was the lowest (50.52 ± 1.05 mg) in cultivar Hysun 39 and the highest (81.79 ± 1.14 mg) in cultivar S-278. Mean seed mass of N K Armoni was 74.82 ± 1.196 mg. In other varieties mean seed mass was between 51 and 58 mg. The average seed mass in the pooled sample for all cultivars (N = 900) was 60.12 ± 0.523 mg (Table 1). Thus, S-278 appeared to be the heaviest seeded cultivar and Hysun-39 the lightest seeded cultivar – difference in mean seed mass being 31.27 mg.

Figure 3 portrays the distribution of seed masses of the cultivars amongst the nine size classes as standard (ranging from 20 to 110 mg with an interlude of 10 mg) so that the cultivars may be compared with respect to their seed size spectra. The seed weight distribution was substantially among the cultivars. Following text compares the cultivars.

1. cv. S-278 – The modal class extended from 81-90 mg occupying 35% of the seeds. Some 89% of the seeds fall in size of 61-70 mg. Some 17% of the seeds were in the size category of 71-80 mg and 10% of the seeds had mass > 100mg. There was no seed below 50 mg of mass.
2. cv. NK Armoni – Modal class extending from 71-80 mg occupying c 25% of the seeds. Some 89% 0f the seeds fall in the category of seed mass between 71 to 100 mg. Some 10% of the seeds were in 91-100 mg category. There was no seed below 40 mg of mass.
3. cv. Local – Modal class extended from 51-60 mg with 26% of the seeds. Around 46% of the seeds fall under the category of 41 to 60 mg. Some 10% of the seeds occupied the lower category of 31-40 mg and only 1% of the seeds had mass greater than 110 mg.
4. cv. Hysun – 39 - Some 34% of the seeds fall in the Modal class, 41-50 mg. There was no seed weighing above 80 mg. Ninety per cent of the seeds had weight between 41 to 70 mg. Some 14% of the seeds weighed below 40 mg.
5. cv. Hysun - 33 – Modal class was the same as in Hysun – 39 occupying 34% of the seeds. No seed weighed above 90 mg and 12% of the seeds had weight below 40 mg.

6. cv. Hybrid-1 – Modal class was 51-60 mg with c 30% of the seeds. One per cent of the seeds weighed below 30 mg and 1% above 100 mg. Some 74% of the seeds belonged to the category of 41-70 mg categories.

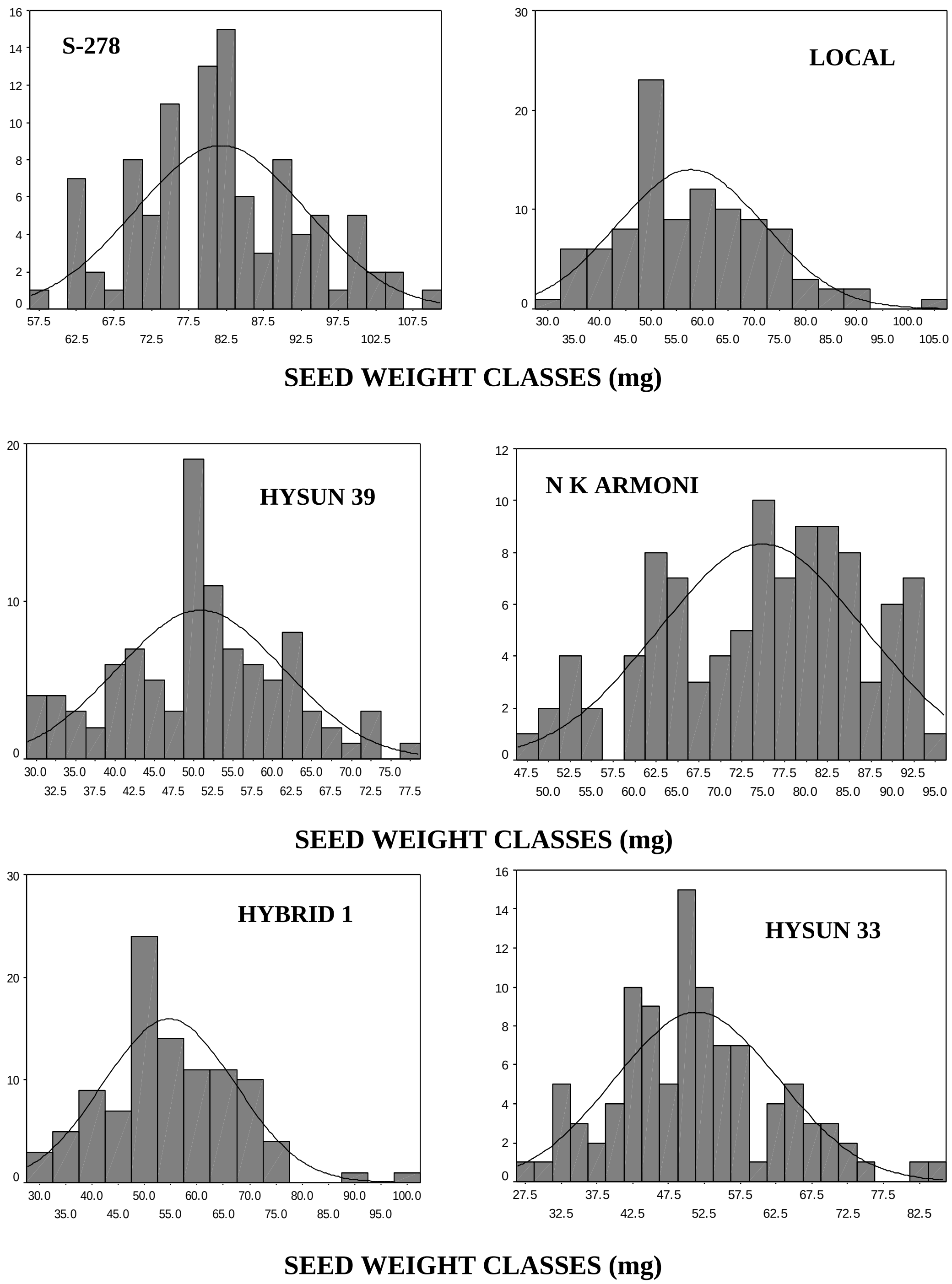

Fig. 2. Seed weight distribution in 9 sunflower varieties and their pooled data. (Continues on the next page)

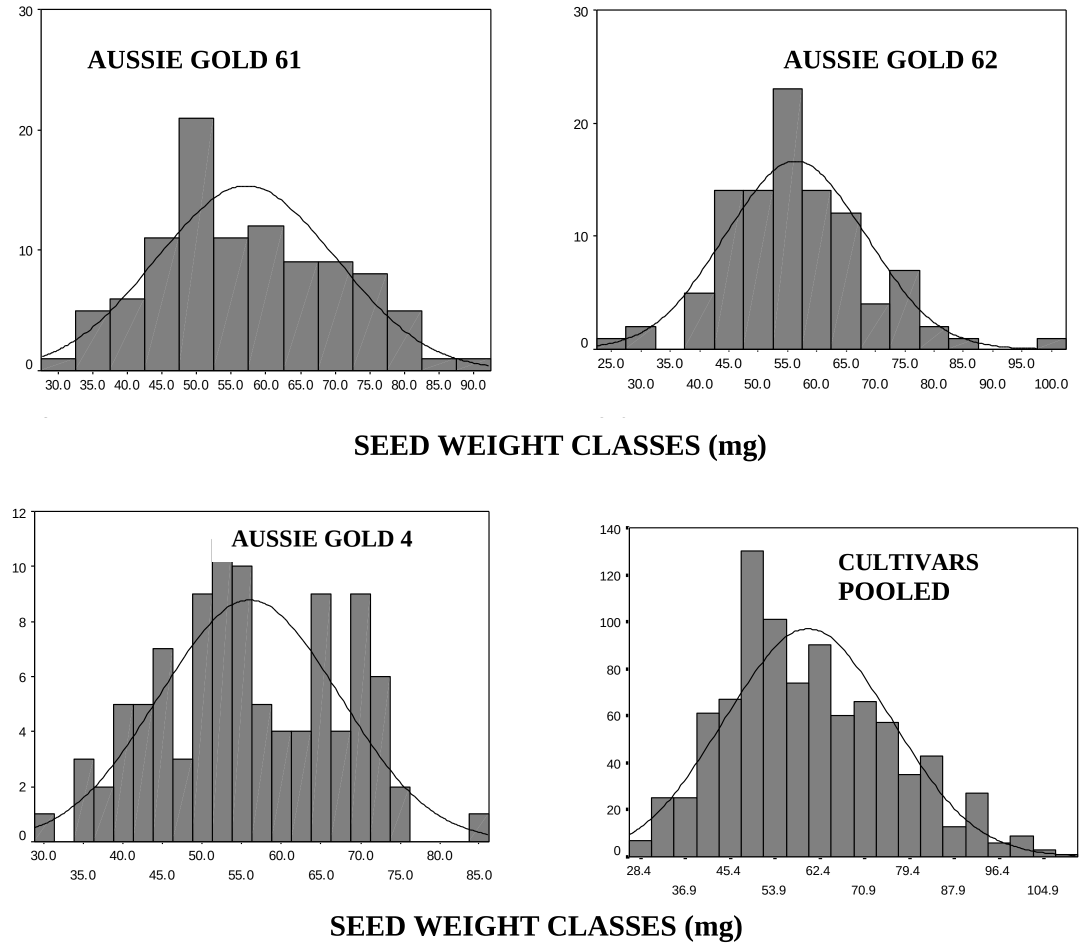

Fig. 2 (continued). Seed weight distribution in nine sunflower varieties and their pooled data.

7. cv. Aussie gold 61 – The modal class (41-50 mg) contained c 295 of the seeds. A great proportion of seeds (72%) had weight between 51-70 mg. One seed had mass below 30 mg and one above 90 mg.
8. cv. Aussie gold 62 – Thirty eight per cent of the seeds belonged to the modal class (51-60 mg). A sizeable proportion of seeds (62%) weighed between 41 and 60mg and 31% between 61-80 mg. Eighty three per cent of seeds had mass between 41 and 70 mg.
9. cv. Aussie gold 04 - Thirty eight per cent of the seeds belonged to the modal class (51-60 mg) and 33% of seeds weighed between 61-80 mg. There was no seed above 90 mg of weight and there were no seeds below 40 mg.
10. Pooled Sample – Around 25 % of the seeds fall within the modal class of 51-60 mg. There were 1.44 % seeds above 100 mg in mass and around 0.33% of the seeds had masses below 30 mg and 7.56% between 312 and 40 mg. Some 32.7% of the seeds weighed between 61-80 mg (Fig. 4).

The distribution of mean seed mass around the grand mean was negatively skewed. The coefficient of variation among means was 17.74% i.e., the variation was 1.62-folds (Fig. 5).

Hierarchical clustering, on the basis of seed mass, discretely classified varieties into two groups. Cultivars S-278 and N K Arconi were heavier seed cultivars and cultivars local, Hybrid 1, Hysun 33, Hysun 39, Aussie gold 04, Aussie gold 61 and Aussie gold 62 were substantially lighter seed varieties (Fig.6).

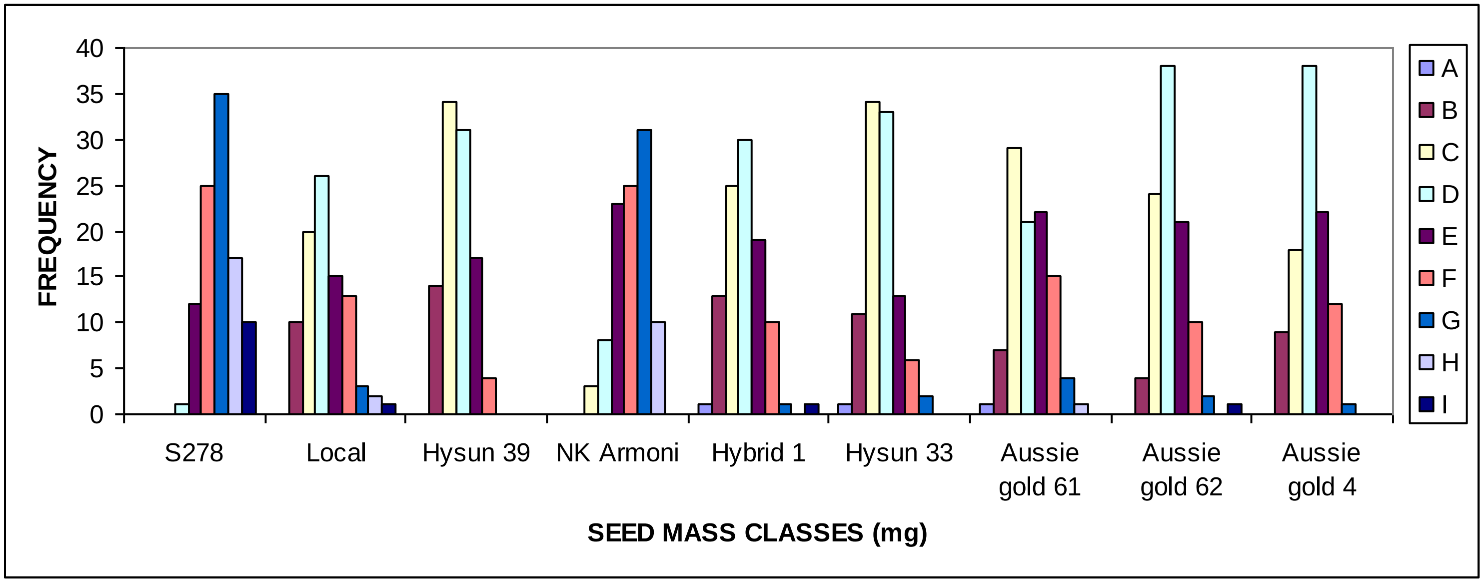

Fig.3. The frequency distributions of seed masses of nine sunflower cultivars prepared with a constant class interval magnitude of 10 mg. Key to the classes. A, 20 < X ≤ 30mg; B, 30 < X ≤ 40mg; C, 40 < X ≤ 50 mg; D, 50 < X ≤ 60 mg; E, 60 < X ≤ 70 mg; F, 70 < X ≤ 80; G, 80 < X ≤ 90; H, 90 < X ≤ 100 and I, 100 < X ≤ 110 mg.

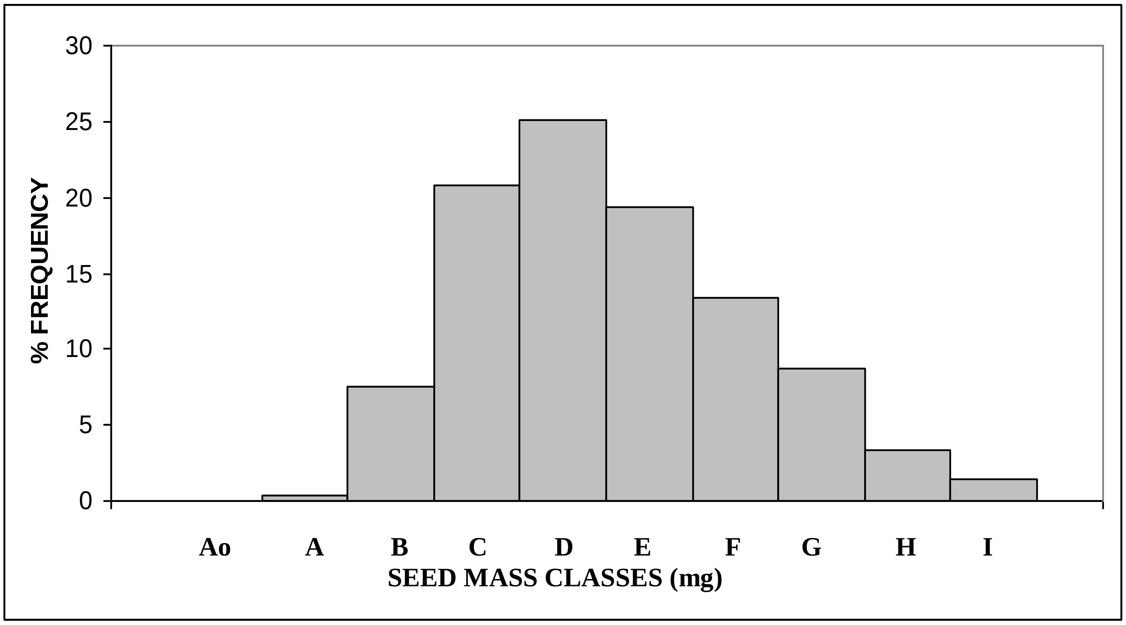

Fig. 4. The frequency distribution pooled seed mass data of all cultivars studied. Key to the classes: A0, $10 < X \leq 20$ mg, A, $20 < X \leq 30$mg; B, $30 < X \leq 40$mg; C, $40 < X \leq 50$ mg; D, $50 < X \leq 60$ mg; E, $60 < X \leq 70$ mg; F, $70 < X \leq 80$; G, $80 < X \leq 90$; H, $90 < X \leq 100$ and I, $100 < X \leq 110$ mg.

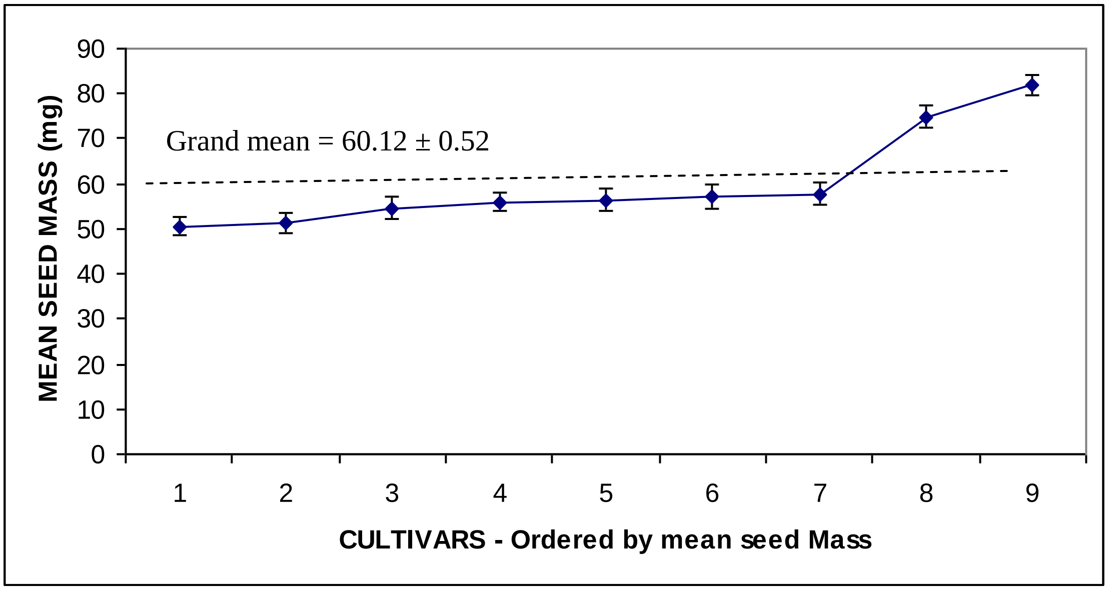

Fig.5. Variation of mean seed masses of nine sunflower cultivars ordered in ascending mean seed mass. The SEs shown are two-times magnified. Key to the cultivars: 1, Hysun 39; 2, Hysun 33; 3, Hybrid 1; 4, Aussie gold 4; 5, Aussie gold 62; 6, Aussie gold 61; 7, Local; 8, NK Armoni; 9, S-278. Grand mean mass is drawn as broken line.

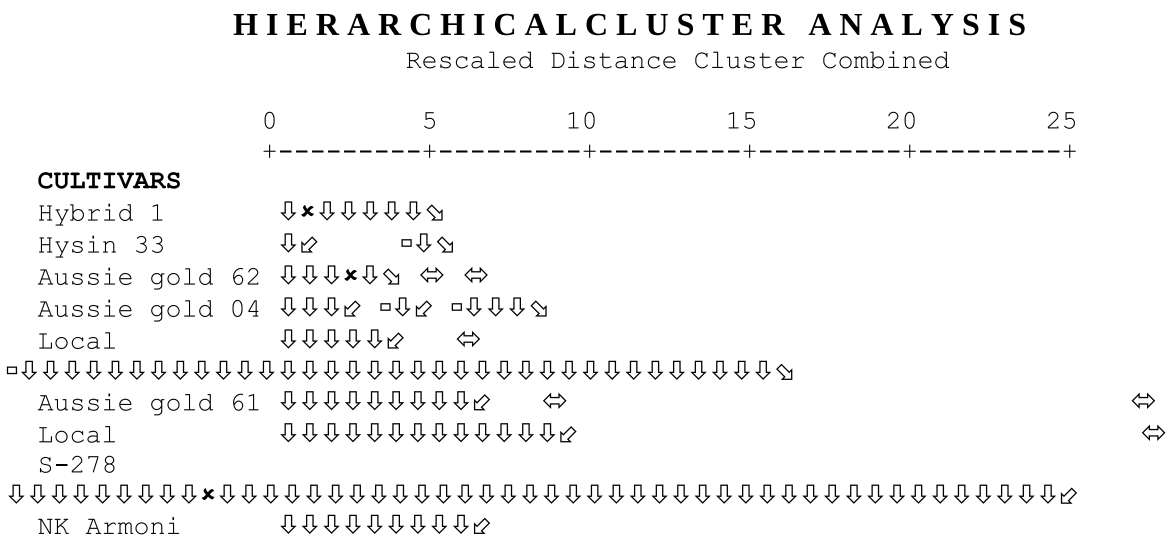

Fig. 6. Hierarchical Cluster Analysis. Dendrogram constructed on the basis of average linkage (Between varieties).

DISCUSSION

Wide intraspecific variations in seed mass have been reported in several tropical species (Janzen, 1977; Foster and Janson, 1985; Khan *et al.*, 1984; Khan *et al.* 1999, 2002; Khan and Umashanjkar, 2001; Murali, 1997; Marshall, 1986; Upadhaya *et al.*, 2007). The mean seed weight of all cultivars in hand was, however, larger than the mean weight reported for sunflower crop by Alexander *et al.*, 2001). The weight of a seed of sunflower cultivar Aussie gold 61 has been reported to average to 56.66 ± 0.8607 mg varying around 21.52% and distributing symmetrically (Anis *et al*., 2011). Mean seed weight of sunflower crop, hybrid (F1) and wild genotype is reported to be 47.92 ± 0.62, 8.85 ± 0.28 and 7.09 ± 0.10 mg. (Alexander *et al.* (2001). The cultivated sunflower seed being larger by 6.76 folds. Mean seed weight of cultivar Hysun 39 and Hysun 33 was somewhat comparable to seed weight of sunflower crop as reported by Alexander *et al.* (2001) and all other cultivars had comparatively heavier mean seed.). S-278 appeared to be the heaviest seeded cultivar and Hysun-39 the lightest seeded cultivar – difference in mean seed mass being 31.28 mg. All cultivars of sunflower are strictly of determinate growth - one plant with one apical capitulum. It is in contrast to the wild forms of sunflower which retain variation in the number of capitula per plant (Harper *et al.*, 1970). Harper *et al*. (1970) suggested that species which vary widely in seed size are generally determinate in flowering while those showing little variation are indeterminate in flowering. Within wild sunflower plants which have retained the variation in number of capitula seed weight may vary 1.25 folds over a 156-fold range of plant densities (Khan, 1967). There are, however, exceptions to it also (Sachaal, 1980).

Seed weight variation in plants may be many-fold in magnitude (Zhang and Maun, 1990). Sachaal (1980) found 5.6 fold variation among 659 seeds collected from a population of *Lupinus texensis*. Khan *et al.* (1984) have reported seed weight variation in desert herbs to be around 6.82 % in *Achyranthes aspera*, 12.91% in *Peristrophe bicalyculata*, 14 % in *Cassia holosericea* and 16.83% in *Prosopis juliflora*, a tree legume. *Opuntia ficus-indica* exhibited seed weight variation c. 18.2% (Khan, 2006). Michaels *et al.* (1988) have examined 39 species (46 populations) of plants in eastern-central Illinois and reported variability (in terms of coefficient of variation) of seed mass commonly exceeding 20% - significant variation being among the conspecific plants in most species sampled. Seed weight variation in sage brush is reported to lie between 26.31 and 31.75% amongst the sites and years of study, respectively (Busso and Perryman (2005). Seed weight is highly variable in *Alliaria petiolata* (8-fold among populations, 2.5 – 7.5-folds within population, two-three folds within individuals and 1.4 – 1.8 folds within fruits Susko and Lovett-Doust, 2000). Halpern (2005) reported seed mass in 5839 seeds of 59 maternal plants of *Lupinus perennis* to highly variable (5-fold variation). Aziz and Shaukat (2010) have reported seed weight variation to be 19.47% in *Ipomoea sindica*, 23.3% in *Cleome viscosa*, and 19.13% in *Digera muricata*. Seed weight variation in *Senna occidentalis* was 18.35% (Saeed and Shaukat, 2000). Seed weight variation in *Thespesia populnea* is around 27% (Zahida N. Gohar, Personal Communication). Sixteen-fold variation in seed mass is reported in *Lamatium salmoniflorum* (Thompson and Pellmyr, 1989). According to Tíscar Oliver and Borja (2010) most variation occurred in seed mass within trees of *Pinus nigra* subsp. *Salzamannii* (c 61%) rather than between them (c 39%). Four-fold variation in seed mass was found ranging from 8 to 32 (-36) mg. Significant variation in seed size exists in *Jatropha* *curcas* in various agro-ecological zones of India (Ghosh and Singh, 2011). Variation among sunflower cultivars in mean seed mass indicates that both environmental and genetic components are involved.

Seed weight distribution was found to be normal in six sunflower cultivars in hand viz. S-278, local, Hysun 39, Hysun 33, Aussie gold 61 and Aussie gold 04 and Non-normal in NK Armoni, Hybrid 1, Aussie gold 61 and the pooled sample of all cultivars. Seed mass in a seed lot of sunflower cultivar Aussie gold 61 is reported to normal distribution by Anis et al. (2011). Seed mass was found to be normally distributed in *Blutapason portulacoides* and *Panicum recemosum* but not in case of *Spartina ciliata* (Cardazzo, 2002). Halpern (2005) reported normal distribution of seed mass in *Lupinus perennis*. Zhang (1998) has reported seed mass variation in *Aeschynomene americana* by weighing 150 seeds from each of its 72 populations to be normally distributed in 9, positively skewed significantly ($p < 0.05$) in 14 and negatively skewed in 49 populations. The mass of mature seeds had a normal distribution in two natural populations of *Arum italicum* (Mendez (1997). Seed weight is reported to vary within a species with site quality and year of study – varying from symmetry to skewness, from leptokurtic to platykurtic (Busso and Perryman, 2005). Seed weight distribution was reported to be skewed in *Phlox drummondii* (Leverich and Levin, 1979). Such a high degree of variation in seed mass may be thought to have important ecological implications forming basis of qualitative and quantitative female reproductive fitness so crucial in life history diversification (Braza *et al.* (2010).

The variation in seed size may be the result of myriad of factors (Fenner, 1985; Wulff, 1986). Earlier impression of seed weight constancy in earlier ecological literature seems to be arising primarily from observations of the relative constancy of mean seed mass in some plant species rather than an analysis of the variability among individual seed masses which have demonstrated considerable variability (Obeid *et al*., 1967). The analysis of means alone may, therefore, not realistically uncover the variability of seed masses in natural plant populations (Obeid *et al.,* 1967; Thompson, 1984). Winn (1991) has suggested that plants may not have the capability of producing a completely uniform seed weight simply as a result of variations in resource availability (e. g., soil moisture during seed development). Seed size is significantly reduced under moisture stress in mature trees of walnut (Martin *et al.,* 1980). Seed weight is said to be the direct function of precipitation (moisture availability) and monthly precipitation is reported to explain around 85% of the total variation in seed weight in Wyoming sage brush, *Artemisia tridentata* (Busso and Perryman, 2005). Seed weight is also reported to decline with age in walnut (*Juglans major*) in terrace habitat of central Arizona (Stromberg and Patten (1990). Seed weight has also been reported to be the function of plant height in a population of *Ranunculus acris* (Totland and Birks, 1996). The large variation of seed mass among plants suggests a potential for but not necessarily the presence of genetic control of seed size. This is because maternal parents may influence seed size via both maternal genetics and the maternal environment effect (Roach and Wulff, 1987; Busso and Perryman, 2005). Obviously the seeds collected from the plants might be a mixture of half sibs and full sibs instead of strict half sibs. Seed weight variation in plants thus appears universal which may be due to trade-off of resource allocation between seed size and number (Venable, 1992) or environmental heterogeneity (Janzen, 1977) or the genetic reasons. Alonso-Balnco et al., (1999) have indeed identified several gene loci responsible for natural genetic variation in seed size in *Arabidopsis thaliana*. Doganlar *et al.*, (2000) have presented seed weight variation model in tomato. It may be asserted that within a species, seed mass variation should have both genetic and environmental components. Contrary to it the variation within a plant can only reflect environmental variance due to either development stability or genetically based adaptive variability –very difficult to distinguish (Hickman, 1979).

Seed weight in elite cultivars is generally considered to be highly homogenous as the other traits. Sunflower cultivars, however, exhibited considerable variation in seed weight among and within cultivars. In all cases, the seed mass variation was substantially high as compared to those for a variety of biological traits which Simpson *et al.* (1960) have suggested to usually have a value ≈ 5%. Intra-cultivar variation in seed mass has also been reported by Fasoula and Boerma (2007) in three elite soybean cultivars. They found the magnitude of intra-cultivar variation in seed weight across years between the largest- and the smallest-seeded lines averaged to 36 mg / seed for cultivar Benning, 22mg / seed for cultivar Cook and 45 mg / seed for cultivar Haskell in Soybean. One should agree with their contention that cultivars may not be permanent records with non-existent variation but genetic material that can be upgraded to maintain uniformity in the long-term and further improve desirable agronomic or seed trait characteristics.

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