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INTER - AND INTRABERRY VARIATION OF SEED SIZE IN A SPINELESS PRICKLY PEAR, *OPUNTIA FICUS-INDICA* (L.) MILLER (FAMILY CACTACEAE)

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ABSTRACT

The present paper reports the intra- and interberry variation of seed size in 30 mature dry berries of *O. ficus-indica* (L.) Mill. grown in Karachi as an ornamental plant. The berries of *O. ficus-indica* averaged to 3.84 ± 0.09 cm in length (varying from 2.95 to 5.2 cm; CV: 12.75%) and 2.26 ± 0.063 cm in width (varying from 1.60 to 2.90 cm; CV: 15.11%) between the widest points. A considerable number of abortive ovules (seeds) were present in the berries varying greatly from zero to 153 (mean = 57.7 ± 5.64, CV = 53.39). The number of normal seeds per berry (NS) varied from 8 to 85 (mean: 50.40 ± 3.61). The magnitude of NS in 14 berries was higher than the mean value and in 16 berries was lower than the mean value. The mean single seed weight (MSSW) in berries varied from berry to berry. Seed size ranged from 10.1 to 36.1mg. MSSW, in our studies, was found to be distributed symmetrically. Amongst the thirty berries inspected seed weight distribution was asymmetrical in 10 berries, symmetrical in 17 berries. The coefficient of variability amongst seeds of a berry ranged from as low as 7.9% to as high as 22.8%. The individual seed weight for a sample of 1512 seeds, averaged to 22.78 ± 0.1054 mg varying from 10.7 to 36.1 mg (CV = 17.98%) showing substantial variation in seed size. There was more variation in seed weight among the berries (61.4%) than the within-berry variation (38.6 %). The significant magnitude of Chi square indicated to the asymmetric distribution in the pooled sample. Agglomeration of 30 berries on the basis of seed weight distribution yielded 14 groups which could further be classified into nine categories if characterized on the basis of MSSW and CV.

Most of the berries represented high diversity and low dominance concentration. The seed weight diversity was positively linearly related with evenness (r = 0.772) and richness (r = 0.522). The seed weight diversity and its components of evenness and richness significantly related to the NS in a quadratic manner. Increased NS caused increase in evenness in the seed size. The variability of seed size in berries (CV %) declined with increased NS. The results are discussed in view of available literature.

KEYWORDS: Seed size and abortive ovule variation; *Opuntia ficus-indica*.

INTRODUCTION

Genus *Opuntia* has around 200 species. Few species of *Opuntia* are so far reported from Pakistan growing in wild in arid areas - *Opuntia monocantha* from Kabal valley, Swat (Ilyas *et al.*, 2013 and Chhumbi –Surla wild life sanctuary (Chaudhary *et al.*, 2001), *Opuntia hemifusa* from District Gujrat (Majeed *et al.*, 2011), *Opuntia dillenii* from Allai valley, Battagram (Haq *et al.*, 2012), Senhsa, District Kotli, Azad Jammu & Kashmir (Ahmad *et al.*, 2012), from Chagharzai valley (Sher *et al.*, 2011), from Nandiar valley (Haq *et al.*, 2010), and from Khanpur dam (Qureshi *et al.*, 2014), *O. littoralis* from Tehsil Katlang, District Mardan (Khan *et al.*, 2003), *Opuntia stricta* from near River Ravi, Lahore (Mukhtar *et al.*, 2015) and *Opuntia ficus indica* from Northern areas (Mirza and Bukhari, 1996), from Takht-e-Nasrati District Karak (Khan *et al.*, 2013a) and from Bannu (Khan *et al.*, 2013b). *O. ficus-indica* is a dull blue-green shrub with flattened egg to oblong shaped pads larger c. 40 x 20 cm, dark green, spineless, thick and fleshy. Flowers yellow. Tepals c 18 in number, bristles present on inner as well as outer surface (Sparse), outer green and inner yellow and larger. Flowers c 5 cm across. All tepals provided with small bristles. Anthers free, yellow, filament c 1cm long. Stamens numerous, sensitive to touch. Anthers are small and dithecous. Gynoecium tricarpellary, syncarpous, stigma bifid (six in number). Style coming out of a well-like depression. Style c 1.5 cm long. Ovary inferior. Fruit berry, purplish red in colour. The basic chromosome number of Opuntia is 11. Polyploidy is common up to octaploid (N = 88*) (http://www.opuntiads.com).* There exists much variation in *O. ficus-indica* and in the strictest sense, it is not a natural species (Kiesling, 2013) rather a complex of cultivars and naturalized clones.

O. ficus-indica (Barbary fig, Indian fig, Sweet prickly pear) originated and domesticated in Mexico (Bravo, 1978, USDA, 2017)) where the highest number of cultivars is found. In outside world its name is Indian fig because of its supposed resemblance to Ficus fruit (Barbera, 1995). Its origin is in Mexico (Bravo, 1978). There is still much to know about the Systematics relationship of *Opuntia ficus-indica* (Reyes-Agüero *et al.*, 2005). Opuntias are grown ornamentally around the world and cultivated in several countries. In Karachi, *O. ficus-indica* is seldom grown as ornamental. Some cultivars of *O. ficus-indica* are spineless. Opuntias are very important economically and put to several uses. *O. dillenii* is used in fencing, hedge making and in folklore medicine (Haq *et al.*, 2012). The fruits and seeds of *O. dillenii* are medicinal. Fruit is reported to be antidiabetic (Zhao *et al*., 2011) besides antioxidant and cardio-protector properties (Kavitha *et al.*, 2012). *O. ficus-indica* is also biologically very active species (Chauhan *et al.*, 2010) and a traditional medicine for several ailments (Halmi *et al.*, 2013). *Opuntia ficus-indica* is a multipurpose underexploited arid shrub (Singh *et al.*, 2005). On the basis of proximate analysis of fruit pulp, fruit skin and seeds, Kossori *et al.* (1998) rated is as a neglected nutritional source. El Finti *et al.* (2013) have reported several agro-technological parameters of 13 cultivars of *O. ficus-indica* to vary amongst the cultivars. Larger is the fruit weight, larger is the seed yield. Fruit with larger diameter and length are heavier.

Spineless cactus was introduced at CAZRI Jodhpur in 1970s. The spineless cactus *(Opuntia ficus indica)* has oblong to elliptic joints, yellow flowers, pale, green, red and yellow fruits and is not affected by wild cochineal insect *Dactylopius* spp.) under Indian conditions. It is highly drought-resistant and can grow in areas of 200 mm rainfall on deep sandy soils and also in areas with 120 to 150 mm annual rainfall if supplemental run-off is available. Fronds of the cactus are succulent and palatable (Singh *et al.*, 2005).

Cactaceae of Pakistan has not been so far revised (Pak. Plant Data base: www. tropicos.org). The taxon was, however, identified on the basis of Field identification Guide (Potter and Rutherford (2013); The Flora of China (2007). The present paper reports the intra- and interberry variation of seed size in *O. ficus-indica* (L.) Mill. grown in Karachi as an ornamental plant.

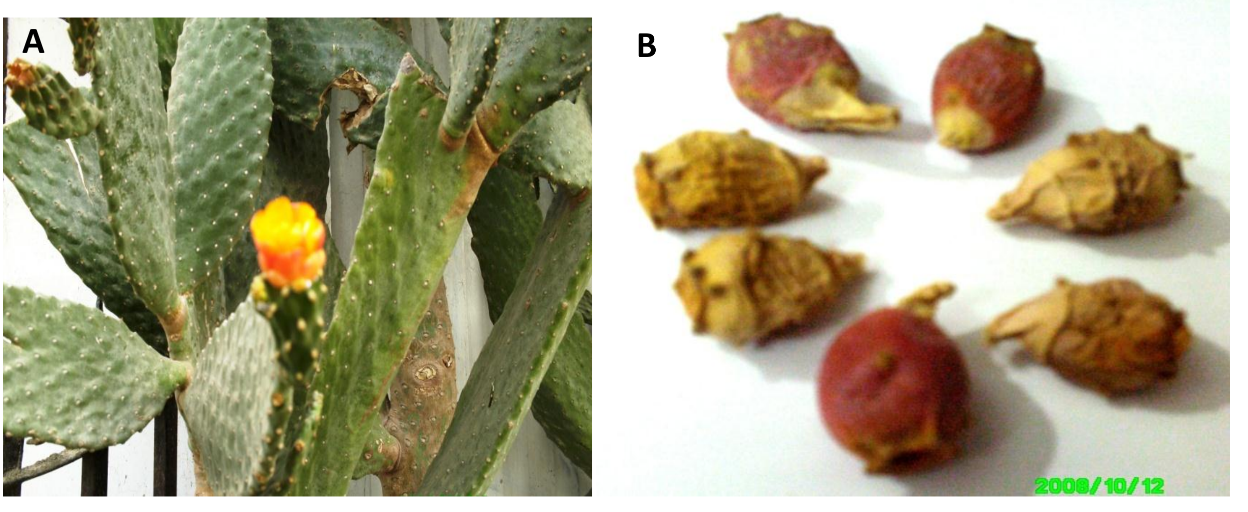

Fig. 1. A) Cultivated plant of *Opuntia ficus-indica* (Karachi-2010). B) Fresh (scarlet) and dried (brown) berries.

MATERIALS AND METHODS

Thirty mature berries were collected from an individual of *Opuntia ficus indica* (Fig. 1) in Karachi. The dry berries were opened and the contents recovered from inside were kept in a brown glass bottles for some time for further studies. The number of seeds and abortive ovules from each berry were counted.

Seed weight: The seeds from each berry were weighed individually on an electrical balance with an accuracy of 0.1mg. Seeds below 10 mg in weight were considered smaller.

The seeds were classified into various sizes as per criterion given below:

Deformed, ill-filled or very small seeds in size: ≤ 10mg
Small seeds: 11-20 mg
Medium seeds: 20.1-30mg
Large seeds: > 30mg

Statistical analysis: The location and dispersion parameters of data were calculated and the frequency distributions were characterized with skewness (G1) and kurtosis (G2) and their errors (SG1 and SG2, respectively) following Shaukat and Khan (1979). Kolmogorov-Smirnov z test (KS-z test) and X^2 test were performed to detect normal distribution (Zar, 2010). The ANOVA analysis was performed to distinguish main and interactive effects within and between the factors and compare the means. The data was analyzed on canned statistical packages such as costat and SPSS version 12. Agglomeration of berries was performed on the basis of seed weight distribution using Ward (1963) clustering method of Euclidean distances between berries.

Seed weight diversity: A number of diversity measures have been proposed by several workers (Magurran, 2004) and there has been some discussion on the superiority of diversity indices (Shaukat and Khan, 1979; Shaukat *et al.*, 1981; Dhanmoanonda and Sahunalu, 1988; Magurran, 2004). The dominance and diversity and its components (species richness and evenness) were calculated using the weights of the seeds in a berry by following formulae as given in

Ludwig and Reynolds (1988). Diversity was measured by the information theory function H' (Shannon and Weaver (1963) and also by McIntosh diversity measure Mc (McIntosh, 1967) as these indices have been reported to be suitable for desert vegetation (Shaukat and Khan, 1979; Shaukat *et al.*, 1981). The measure of species richness (d) was calculated by Menhinick (1964) index and equitability was measured by e (Pielou, 1975). These measures of diversity have been employed by many ecologists to ascertain diversity under various conditions (Shaukat *et al.*, 1981; Niazi *et al.*, 2007; Nazim *et al.*, 2010). The dominance concentration within berries was ascertained by Simpson's (1949) index (C). The formulae employed for diversity and dominance estimation were as follows:

Species Richness, $d = NS / \sqrt{WN}$ (Menhinick, 1964)

Diversity = $H' = -\sum_{i=1}^{NS} pi. \ln pi$ (Shannon-Wiener Index)

McIntosh diversity measure = $Mc = 1 - \frac{\sqrt{\Sigma wni^2}}{WN}$ (McIntosh, 1967),

Dominance = $C = -\sum_{i=1}^{NS} pi.^2$ (Simpson, 1949) and

Equitability = $e = H' / H'_{max} = H' / \log NS$ (Pielou, 1975).

Where NS is the number of seeds in a sample berry and WN is the total weight of seeds in a berry, pi is the proportion of weight of i^{th} seed (wni) in a berry to the total seed weight (WN) in the sample berry. While applying above formulae the berries were considered analogous to assemblages (communities) and seeds analogous to species of an assemblage and seed weight as Biostatistical attribute to the seeds (species).

RESULTS AND DISCUSSION

Studies conducted with respect to interaberry and interberry variation in seed size of *O. ficus-indica* are described as follows:

Table 1. Location and dispersion parameters of number of seeds per berry (brood size) and abortive ovules or seeds in *O. ficus-indica*.

Parameters	Seeds			Abortive ovules
	Large + medium	Smaller	Total seeds	
N (berries)	30	30	30	30
Mean	50.40	6.333	56.733	57.7
SE	3.6105	1.3107	3.9395	5.64
Median	49.50	3500	57.50	49.50
CV (%)	39.24	113.36	38.03	53.39
G1	-0.330	1.386	-0.497	1.290
Sg1	0.427	0.427	0.427	0.427
G2	0.241	0.734	-0.189	2.408
Sg2	0.833	0.833	0.833	0.833
Minimum	8.0	Zero	8.00	Zero
Maximum	85.0	23.0	89.0	153
KS-z	0.742	1.499	0.522	0.892
p	0.640	0.022	0.948	0.404

Berries: Prickly pear is said to be prolific seed producer. A pad can produce one to several fruits. Each fruit contains seeds. Lindheimer prickly pear fruit collected from Crockett County, Texas in 1988 contained an average of 288 seeds per fruit. (Pers. Comm. of Steve Whisenant, Texas A & M University to S.W. Ueckert). The seeds of prickly pear are rich in polyphenols (*pollinator.org./assetsFile s/Opuntia.draft.pdf*). Prickly pear is considered to be a fat burning slimming-fruit. To reach full maturity, berries take 98-112 days (Duru and Turker, 2005).

In our studies, the berries averaged to 3.84 ± 0.09 cm in length (varying from 2.95 to 5.2 cm; CV: 12.75%) and 2.26 ± 0.063 cm in width (varying from 1.60 to 2.90 cm; CV: 15.11%) between the widest points. The berries averaged to 15.838 ± 0.9854 cm^3 in volume ranging from 7.49 to 27.59 cm^3 (CV= 34.08%). Younger berries were green which turned to scarlet on maturity and became brown when dry (Fig. 1). The berries were spheroid in shape and provided with a well-like depression at the top formed due to abscission of floral parts (calyx, corolla and styles. Berries had small bristles on the surface. The berries of *Opuntia* spp. are reported to be biologically active and they are used in indigenous system of medicine for the treatment of Asthma, inflammatory diseases, ulcer and diabetes (Chauhan *et al.*, 2010). Fruits of *Opuntia* are reported to be 6.1 cm in length and 3.1cm in width and seed content about 125 per fruit. Seeds are 4-5 mm wide. (*http://florafaunaweb.nparks.gov.sg/special-pages/plant-detail.apx?id=2282*). The fruits *of Opuntia ficus-indica* are antidiabetic (Zhao *et al.*, 2011).

Reis *et al.* (2017) have presented data on morphology and bioactive compounds in fruits of *Opuntia* spp. The fruits is reported to weigh 50.1g fresh. The fruits contain large amount of total phenol (2579.3 – 3790.3 mg per kg, ascorbic acid to be as high as 896.9 mg per kg. Gallic acid equivalent, DPPH 0.06-0.47g/L, Betaxanthins 35.51- 778.5 and Betacyanins 117.9-1675.36 mg / L. Nadia *et al.* (2013) reported that in Algeria the pH of the fruit pulp of *O. ficus-indica* (green, yellow, orange and red) varied from 6.2 to 6,6. And orange variety was richer in phenolic compounds. Fruit pulp has glucose 35%, fructose 29%, calcium 2009% and K 3.4% (Kossori *et al.*, 1998).

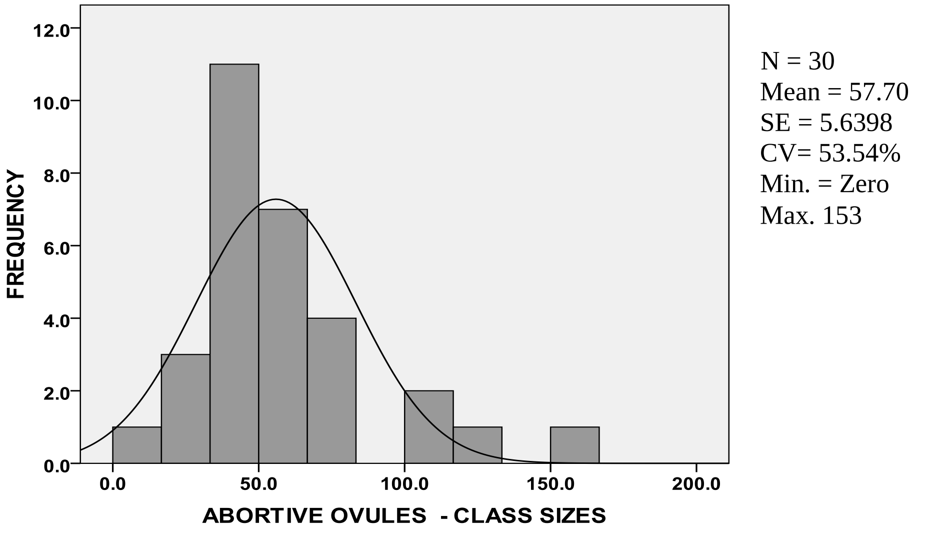

Fig. 2. Distribution of abortive ovules = seeds in 30 berries of *O. ficus-indica.*

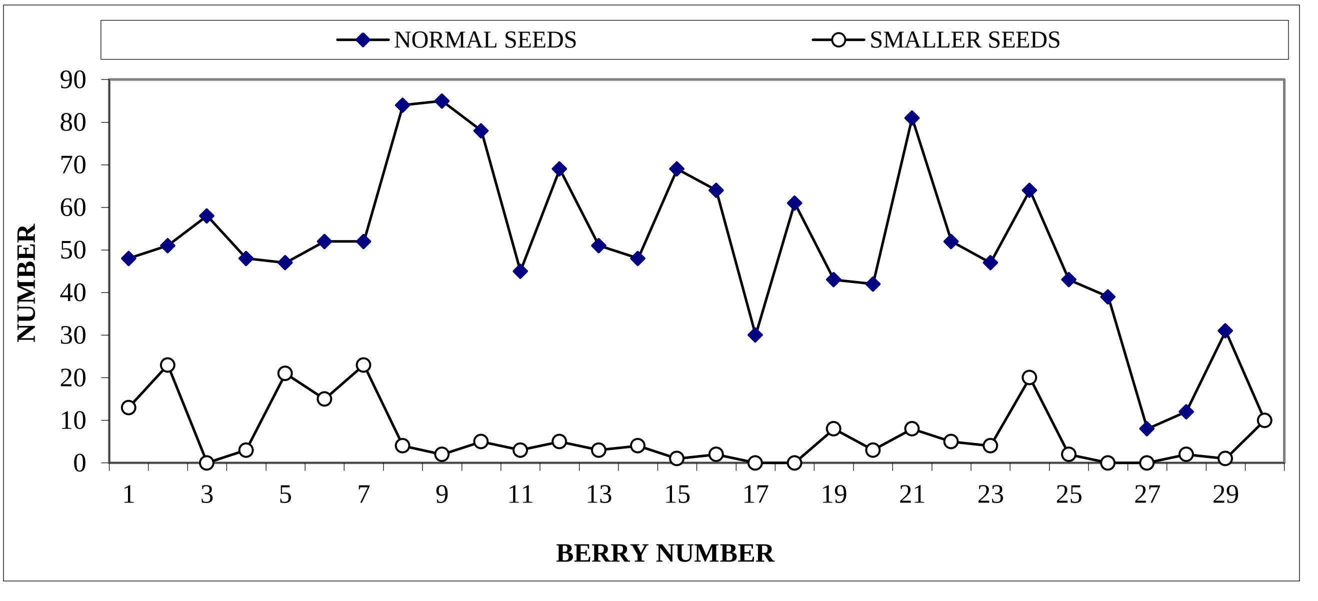

Fig. 3. Number of normal seeds and smaller seeds (< 10mg) recovered from the berries.

Total abortive ovules or seeds: Table 1 indicated that a considerable number of abortive ovules (seeds) were present in the berries varying greatly from zero to 153 (mean = 57.7 ± 5.64, CV = 53.39%). Some 50% of the berries had 26-50 abortive ovules, some 30% of the berries had 51-75 abortive ovules and only 6.7% of the berries had 76-100 abortive ovules. There were only 3.3% of the berries without any abortive ovules. The distribution was asymmetrical (positively skewed) with only few berries with larger number of abortive ovules (Fig. 2). Variation in abortive and viable seeds in a berry has been reported in *Opuntia* spp. with respect to the localities in the Eastern Mediterranean region of Turkey from 96 to172 and 78 to 131, respectively (Karababa *et al.*, 2004).

Table 2. Per cent frequency distribution of weights (mg) of healthy seeds in the berries of *O. ficus-indica.*

Seed Wt. (CMP)	**SEED SIZE RANGE: 10 – 36.1 mg**														
	BERRY NUMBER														
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**	**B13**	**B14**	**B15**
10.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.5	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	1.5
12.5	-	-	-	-	-	-	1.9	0	-	-	-	-	-	-	0
13.5	-	-	-	-	-	-	0	0	2.4	-	-	-	-	-	0
14.5	-	-	-	-	-	-	0	0	0	-	-	1.5	-	-	0
15.5	-	-	-	2.1	-	-	0	7.1	1.2	-	-	13.0	-	-	1.5
16.5	-	-	-	2.1	-	1.9	0	15.5	1.2	-	2.2	15.9	-	2.1	1.5
17.5	2.08	-	-	0	-	0	1.9	**21.4**	2.4	-	13.3	**18.8**	2.0	2.1	0
18.5	0	-	-	0	2.1	0	0	16.7	5.9	1.3	20.0	17.4	0	2.1	0
19.5	0	-	-	0	0	0	0	13.1	5.9	3.9	22.2	14.5	0	10.4	0
20.5	2.1	-	-	12.5	0	5.8	0	14.3	**27.1**	10.3	**24.4**	13.0	0	18.8	0
21-5	10.4	-	-	16.7	0	1.9	7.7	7.1	17.7	7.7	2.2	1.5	7.8	**22.9**	7.3
22.5	12.5	-	-	**27.1**	4.3	11.5	3.9	1.2	22.4	19.2	8.9	4.4	7.8	**22.9**	17.4
23.5	10.4	1.9	-	14.6	12.8	**23.1**	5.8	1.2	10.6	15.4	6.7	-	5.9	10.4	14.5
24.5	12.5	3.9	3.5	10.4	14.9	11.5	**13.5**	1.2	1.2	**19.2**	-	-	5.9	6.3	**21.7**
25.5	**16.7**	1.9	6.9	8.3	**21.3**	11.5	7.7	-	2.4	10.3	-	-	**25.5**	-	17.4
26.5	8.3	5.9	3.5	6.3	12.8	7.7	9.6	-	-	6.4	-	-	17.7	-	5.8
27.5	10.4	17.7	5.2	-	4.3	7.6	9.6	-	-	3.9	-	-	13.7	-	8.7
28.5	8.3	17.7	1.7	-	10.6	5.8	11.5	-	-	0	-	-	5.9	-	1.5
29.5	2.1	5.9	5.2	-	4.3	3.9	9.6	-	-	1.3	-	-	5.9	-	0
30.5	4.2	**23.5**	17.2	-	6.9	7.8	5.8	-	-	1.3	-	-	3.9	-	1.5
31.5	-	7.8	17.2	-	4.3	-	7.7	-	-	-	-	-	0	-	-
32.5	-	5.9	**25.9**	-	2.1	-	0	-	-	-	-	-	2.0	-	-
33.5	-	5.9	3.5	-	-	-	3.9	-	-	-	-	-	-	-	-
34.5	-	2.0	8.6	-	-	-	-	-	-	-	-	-	-	-	-
35.5	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-
36.5	-	-	1.7	-	-	-	-		-	-	-	-	-	-	-
	LOCATION & DISPERSION														
Mean	24.9	29.2	30.7	22.6	26.1	24.9	26.4	18.4	21.0	23.5	19.6	18.0	25.7	21.2	24.0
SE	0.39	0.34	0.36	0.32	0.41	0.40	0.55	0.22	0.23	0.26	0.26	0.22	0.38	0.33	0.33
CV%	10.9	8.2	8.8	9.7	10.8	11.6	15.1	11.1	10.0	9.6	8.6	10.0	10.6	10.5	11.4
g1	-0.2	0.003	-0.7	-0.6	0.14	0.01	-0.9	-1.9	-1.2	0.28	0.6	0.38	-0.2	-2.0	-1.6
g2	2.97	3.04	2.93	4.3	3.2	3.3	4.8	4.2	5.6	3.1	2.7	2.5	3.8	10.0	8.4
Min	17.2	23.1	24.3	15.8	18.2	16.2	12.2	11.0	13.1	18.5	16.6	14.8	17.4	11.0	11.8
Max	30.5	34.9	36.0	26.9	32.1	30.5	33.5	24.2	25.2	30.0	23.3	22.4	32.9	24.8	30.0
X^2	2.67	11.6	11.2	4.3	6.6	8.6	1.2	2.48	17.3	5.1	15.6	1.61	7.8	7.3	10.4
p	0.75	0.04	0.04	0.5	0.25	0.13	0.94	0.78	0.003	0.4	0.008	0.90	0.06	0.19	0.06
NS	48	51	58	48	47	52	52	84	85	78	45	69	51	48	69
Dist.	**S**	AS	AS	**S**	**S**	**S**	**S**	**S**	AS	**S**	AS	**S**	**S**	**S**	**S**

Table 2. (Cont'd).

	BERRY NUMBER														
Seed Wt. CMP	**B16**	**B17**	**B18**	**B19**	**B20**	**B21**	**B22**	**B23**	**B24**	**B25**	**B26**	**B27**	**B28**	**B29**	**B30**
10.5	-	-	-	-	-	-	-	-	-	-	-	12.5	-	3.2	-
11.5	1.6	-	-	-	-	-	-	-	-	-	2.6	0	-	3.2	10.0
12.5	0	-	-	-	-	-	-	-	-	-	0	12.5	-	3.2	0
13.5	0	3.3	-	-	-	-	-	2.1	-	2.3	0	0	-	0	0
14.5	1.6	0	-	-	-	-	-	0	-	0	0	0	-	3.2	0
15.5	1.6	6.7	-	-	-	1.2	1.9	0	-	4.6	0	12.5	-	**22.6**	0
16.5	4.7	0	-	2.3	-	0	1.9	0	-	0	10.3	0	-	**22.6**	0
17.5	4.7	0	-	9.3	-	0	1.9	0	10.9	7.0	5.1	12.5	-	6.5	0
18.5	10.9	3.3	-	**18.6**	2.4	0	5.8	0	20.3	**18.6**	18.0	12.5	-	12.9	0
19.5	14.1	3.3	-	4.7	11.9	1.2	9.6	0	9.4	16.3	10.3	0	-	16.1	10.0
20.5	**17.2**	26.7	0	11.6	14.3	2.5	17.3	0	**23.4**	**18.6**	**20.5**	**25.0**	8.3	0	0
21-5	10.9	**30.0**	14.8	2.3	11.9	0	**25.0**	10.6	17.2	14.0	12.8	0	16.7	3.2	10.0
22.5	10.9	16.7	**26.2**	4.7	**23.8**	11.1	21.2	10.6	6.3	9.3	10.3	0	8.3	3.2	20.0
23.5	12.5	6.7	23.0	0	14.3	8.6	13.5	25.5	7.8	7.0	5.1	12.5	0	-	**30.0**
24.5	4.7	3.3	11.5	0	16.7	**22.2**	1.9	10.6	4.6	2.3	2.6	-	**25.0**	-	10.0
25.5	3.1	-	13.1	2.3	4.8	13.6	-	**27.7**	-	-	0	-	16.7	-	0
26.5	1.6	-	3.3	2.3	-	18.5	-	12.8	-	-	2.6	-	8.3	-	0
27.5	-	-	4.9	11.6	-	9.9	-	-	-	-	-	-	8.3	-	0
28.5	-	-	3.3	7.0	-	9.9	-	-	-	-	-	-	0	-	0
29.5	-	-	-	14.0	-	1.2	-	-	-	-	-	-	0	-	0
30.5	-	-	-	2.3	-	-	-	-	-	-	-	-	8.3	-	10.0
31.5	-	-	-	7.0	-	-	-	-	-	-	-	-	-	-	-
32.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LOCATION& DISPERSION														
Mean	20.6	20.9	23.7	23.6	22.2	25.3	21.2	21.1	20.3	20.0	20.0	17.5	24.5	16.8	23.2
SE	0.35	0.44	0.24	0.77	0.218	0.26	0.25	0.31	0.25	0.35	0.44	1.5	0.77	0.46	1.6
CV%	13.6	11.4	7.9	21.2	8.1	9.2	8.5	8.8	9.6	11.4	13.5	22.8	10.3	15.0	21.7
g1	-0.6	-1.6	0.8	0.14	-0.06	-0.9	-0.9	-2.3	0.28	-0.5	-0.4	-0.3	0.4	-0.3	-0.9
g2	3.6	5.5	2.99	1.36	1.9	5.11	3.8	12.1	2.34	3.51	4.1	1.9	2.6	3.5	4.6
Min	11.5	13.5	21.0	16.8	18.7	15.9	15.7	13.8	17.1	13.3	11.7	10.9	20.8	10.7	1.5
Max	26.1	24.7	28.3	31.7	25.1	29.6	24.3	26.9	24.9	24.1	26.5	23.4	30.0	22.4	31.8
X^2	5.0	15.3	11.8	31.2	5.6	12.3	4.9	12.8	10.8	3.3	2.2	-	-	6.4	-
p	0.41	0.01	0.04	0.001	0.35	0.03	0.42	0.03	0.06	0.65	0.82	-	-	0.27	-
NS	64	30	61	43	42	81	52	47	64	43	39	8	12	31	10
Dist.	**S**	AS	AS	AS	**S**	AS	**S**	AS	AS	**S**	**S**	-	-	**S**	-

S, Symmetrical; AS, Asymmetrical distribution; g1, Skewness; g2, Kurtosis

On little calculation, it was found that on an average 50.98 ± 3.38% of the ovules in a berry underwent successful maturation into normal healthy seeds. The maturation percentage, however, varied greatly with the berries from as low as 7.21 to 100%. This parameter followed a normal distribution pattern (KS-z: 0.678, p< 0.748, NS). In 56.6% of the berries, 41 to 60 % of the ovules underwent development to apparently normal healthy seeds and in 24.4% of the berries; 61-80% of the ovules underwent successful maturation. Barbera *et al.* (1994) have reported that number of aborted seeds was not related to fruit and core weight in two cultivars – Gialla and Ross.

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                 H I E R A R C H I C A L C L U S T E R   A N A L Y S I S
                           Dendrogram using Ward Method
                          Rescaled Distance Cluster Combine

Berry          0         5         10        15        20        25
Number*        +---------+---------+---------+---------+---------+
B14           -+
B22           -+-+
B9            -+ +-----------+
B17           ---+           |
B10           -+-+           +---------------------+
B20           -+ |           |                     |
B4            ---+---+       |                     |
B18           ---+   +-------+                     |
B30           -------+                             +-----------+
B24           -+-+                                 |           |
B25           -+ |                                 |           |
B16           -+-+---------+                       |           |
B26           -+ |         |                       |           |
B8            ---+         +-----------------------+           |
B11           ---+         |                                   |
B12           ---+-----+   |                                   |
B29           ---+     +---+                                   |
B19           -------+-+                                       |
B27           -------+                                         |
B2            -------+-----------+                             |
B3            -------+           |                             |
B1            -+-+               |                             |
B15           -+ +-+             +-----------------------------+
B6            ---+ +-+           |
B23           -----+ |           |
B5            ---+   +-----------+
B21           ---+-+ |
B7            ---+ +-+
B13           -----+ |
B28           -------+
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*, see Table 2 for enumeration of berries.

Fig. 4. Dendrogram constructed by clustering 30 berries of *Opuntia* on the basis of *per cent* frequency distribution of weights of normal seeds recovered from each of the berries (Table 2).

Hierarchical clustering of berries in relation to their seed weight distribution: An agglomerative clustering of the berries based on the frequency distribution of the seed weights of normal seeds recovered from each of the 30 berries was employed using Euclidean distances between the berries and grouping them following Ward (1963) method. Some 14 groups of berries were extracted in the dendrogram (Fig. 4) when groups were extracted on the basis of separation of groups at a distance of <10% dissimilarity as given below. The fourteen groups were, however, found to be discretely classifiable into nine categories on the basis MSSW and CV (%) characterizing the groups as outline in Table 3. The results imply that each berry has its own ecological history of development as they are regulated with a large number of intrinsic and extrinsic factors.

Table 3. Groups extracted from the dendrogram constructed by clustering of berries using Euclidean distances calculated on the basis of seed weight frequency distribution (See Figure 4). Groups extracted at distance scale at 10.

Groups	Berry #	CV (%)*	MSSW (mg)	Remarks
Group I	14	10.5	21.2	
Group II	22	8.5	21.2	Low CV & Moderate MSSW
Group III	9,17,10,20, 4 &18	9.45	21.7	
Group IV	30	21.7	23.2	High CV & moderate MSSW
Group V	25,16,26,24,8,11,12,&29	11.5	24.5	Moderate CV & Moderate MSSW
Group VI	27	22.8	24.5	High CV & moderate MSSW
Group VII	2,3	8.5	30.0	Low CV & high MSSW
Group VIII	1	10.9	24.0	
Group IX	15	11.5	24.03	Low CV & moderate MSSW
Group X	6,23 & 5	10.4	25.3	
Group XI	21	9.7	26.4	Low CV & moderately High MSSW
Group XII	7	15.1	25.7	Moderate CV & high MSSW
Group XIII	13	10.6	25.7	Low CV & moderate MSSW
Group XIV	28	10.3	24.7	

*, Within group variation in seed weights. MSSW, Mean single seed weight in the group (mg)

Seeds and seed weight distribution: Seeds of *Opuntia ficus-indica* are pale brown in colour and somewhat rounded (i.e. subglobose) in shape. Seed oil of *Opuntia ficus-indica* is useful and contains linoleic acid 58.79%, palmitic acid 11.18% and stearic acid 1.50%. Vit. E. content is 1.23% of the total lipids (Ghazi *et al.,* 2013). Linoleic acid was also the dominant fatty acid in pulp (Ramadani and Mörsal (2013).

The number of seeds recovered from the berries varied considerably (Fig. 3). The number of normal seeds per berry (NS) varied from 8 to 85 (mean: 50.40 ± 3.61). The magnitude of NS in 14 berries was higher than the mean value and in 16 berries was lower than the mean value (Tables 1 and 2). Prickly pear fruit in 9 cultivars of *O. ficus-indica* in Morocco is reported to contain 100-300 seeds (Dehbi *et al.,* 2008). The amount of ripe seeds per fruit is reported to vary with the three cultivars of yellow prickly pear (4.98 to 6.32g per pear; CV: 11.89%) (El-Ghares *et al.,* 2006). Seed content of about 125 per fruit of *Opuntia* were reported by Chauhan *et al.* (2010).

Of the total recovered seeds from 30 berries (abortive seeds excluded) there were 190 very small seeds (≤ 10mg), 350 small seeds (11-20mg in size), 1063 seeds in medium size class of 20-30 mg and 99 seeds in large size class i.e. > 30 mg. Thus, excluding the very small seeds, 1512 seeds were recovered of which 88.84 % fall in the medium size class.
Per cent frequency distribution of seed size (weight) in 30 berries is presented in Table 2 with location and dispersion parameters of seed weight for each berry. Amongst the thirty berries inspected seed weight distribution was asymmetrical in 10 berries, symmetrical in 17 berries and it was inconclusive due to lesser number of seeds in these berries (8, 10 and 12 seeds in berry # 27, 28 and 30, respectively), being insufficient sample size to determine distribution pattern. The mean single seed weight varied from berry to berry.

The modal class of seed weights for each berry varied substantially. The lowest modal class had the class midpoint (CMP) of 15.5mg in berry # 29 and the highest modal class was observed with CMP of 32.5mg observed in berry # 3. In three berries (# 14, 25 and 29) a phenomenon of more than one modal class was apparent. The most prevalent modal classes of seed weight distribution were with CMP 20.5mg observed in seven berries i.e., 23.3 % of the berries studied. This was followed by the modal classes of CMP 24.5, 22.25 and 25.5g in five, four and four berries (16.6, 13.3 1nd 13.3 % of the berries respectively. That is, the classes with CMP 20.5, 22.5, 24.5 and 25.5mg collectively occupied or represented 66.6% of the berries. In short, the berries varied significantly in seed size composition and associated with differentially varying seed size (Mean single seed weight in a berry, MSSW) which ranged from 17.5 ± 1.5mg to 30.7 ± 0.36mg. Similarly the coefficient of variability amongst seeds of a berry ranged from as low as 7.9% (berry # 20) and as high as 22.8% (berry # 27). Such a variation was below 10% in 13 berries and ranged from 10.1-15.1 % in 14 berries. Three berries showed high variability of seed weight (21.2 to 22.8%). Andres-Augustin *et al.* (2006) have reported high range of variation in seed size of *O. ficus-indica* in terms of major and minor length. According to them coefficients of variation of 12% or less are acceptable in characterizing plant organ (s) in horticultural species and it would be desirable to increase the sample size if this ratio is higher. In our studies most of berries had variability below or near equal to 15%. Only three of berries showed higher variability. This may be attributed to the differential developmental histories of the berries and some random events specific to them.

Mean single seed weight per berry: The mean single seed weight (MSSW) in a berry varied from berry to berry (Table 2; Fig. 5). Seed size ranged from 10.1 to 36.1mg. MSSW, in our studies, was found to be distributed symmetrically being higher than the grand mean weight in 14 berries and lower than the grand mean weight in 14 berries. Under controlled environmental conditions, Thompson (1984) has reported the distribution of mean seed weight in *Lamatium grayi* (Umbelliferae) around the grand mean of seed to be non-skewed and significantly leptokurtic. However, differences in mean seed weight in different fruits has been suggested due to differences in environmental conditions e.g., nutrients, light, water or salinity level to which individual mother plants could have been subject during period of floral development and growth and seed development and maturation (Gutterman, 1992). Drought during pod filling significantly affected seed weight in *Acacia* species (Gaol and Fox, 2002). In brief, resource availability commonly limits fecundity (Fenner and Thompson, 2005). Since Smith and Fretwell (1974) model in relation of seed size to seed number predicts optimum seed size expected in a particular ecological context, different optima for different individuals of a species may be expected. This concept may probably be extended to fruits of an individual tree where different optima may occur for different fruits produced on a tree over a period of time and internal and external environmental forces may differentially interact with different fruits developing over time as suggested by Khan and Sahito (2013a, b).

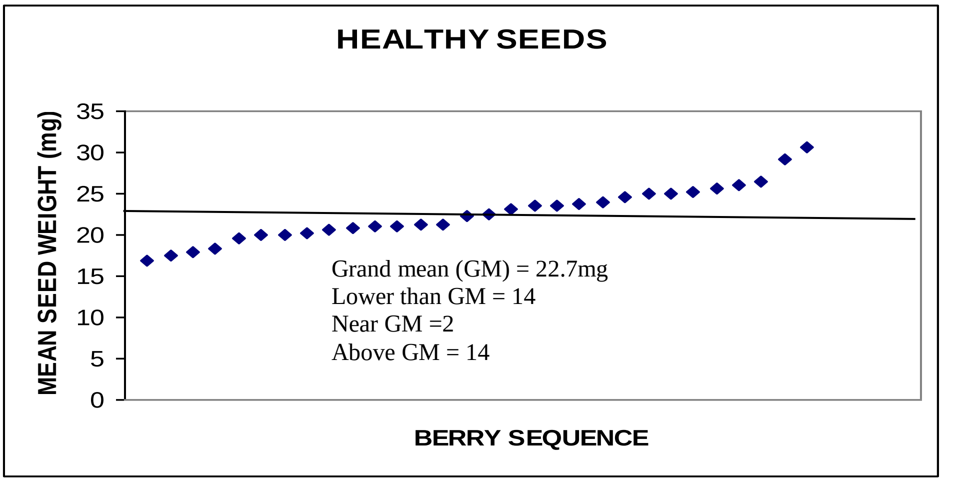

Fig. 5. Mean single seed weight (MSSW) values of healthy (A) recovered from the 30 berries of *O. ficus-indica* arranged in ascending order indicating symmetrical distribution of seeds in berries.

NS related reduction of CV: In the present studies, the coefficient of variability (CV %) of seed size in berry was found to be significantly related linearly- negatively with number of normal seeds per berry (NS) as follows:

CV (%) = 17.068 – 0.107NS ± 3.3816
t = 9.65 t = -3.31
$p < 0.0001$ $p < 0.002$
r = -0.538, $r^2 = 0.289$, F = 11.41 ($p < 0.002$)

This relationship was, however, slightly better given by a quadratic equation (Fig. 6). It was clear that the magnitude of variation in seed weights in a berry declined significantly with the increase of the number of seeds per berry (NS) in other words the uniformity among the seed size increased with NS i.e. within a given range of NS, lesser the number of seeds developing in a berry, larger is the variation in seed size.

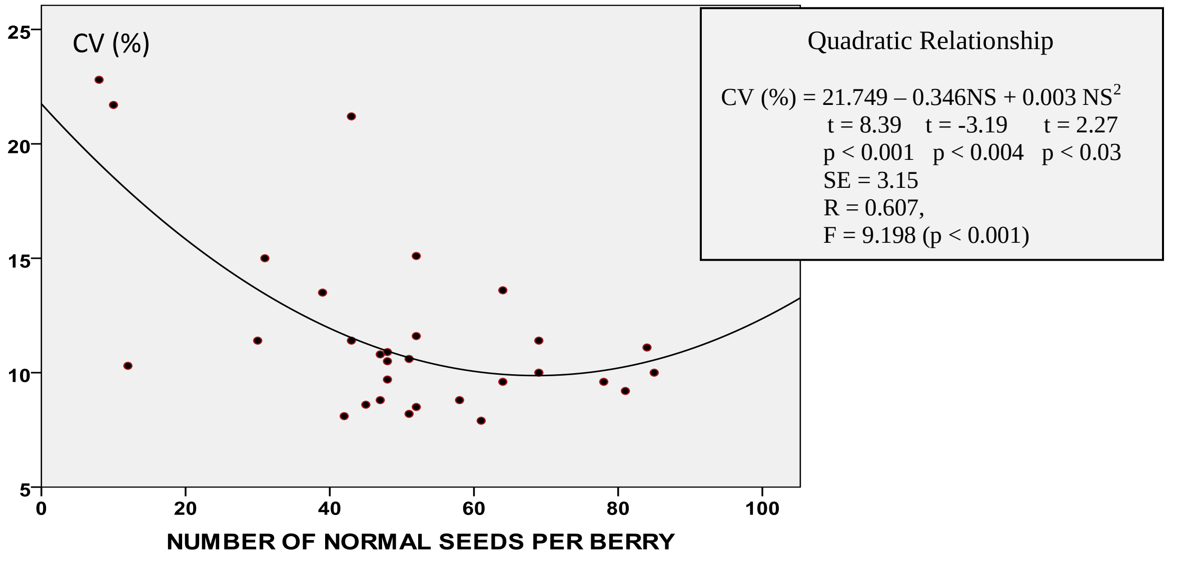

Fig. 6. Relationship of coefficient of variation in seed weight with the number of seeds in a berry.

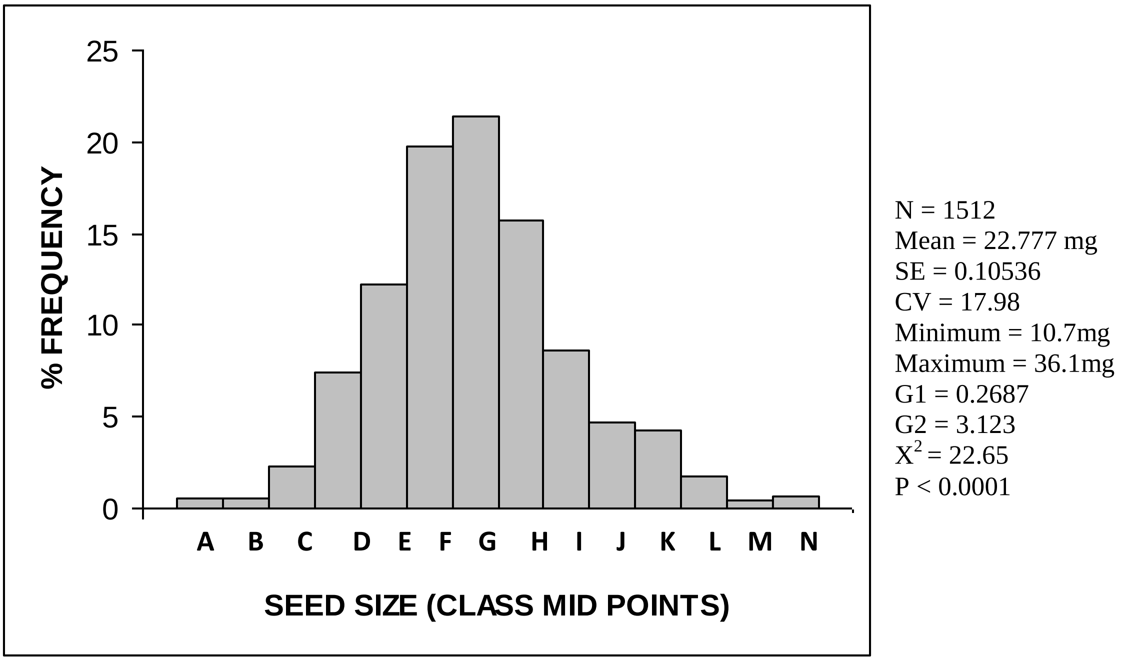

Fig. 7. Frequency distribution of pooled healthy seeds recovered from 30 berries of *O. ficus-indica*. Class mid points: A, 11.5 (11-12 mg); B, 13.5 (13-14mg); C, 15.5; ……..G, 23.5; H, 25.5 (25-26 mg); … M, 35.5 (35-36 mg) and N, 37.5 (37-38 mg). This parameter tended to be somewhat positively skewed and platykurtic. Significant value of Chi square indicated to the asymmetrical distribution. Modal class appeared to be a size class: 22-24 mg. Around 69 % of the total seeds (1045) occupied the central classes: E, F, G and H; CV (%) = *per cent* coefficient of variability; g1, skewness ; g2, kurtosis.

Individual seed weight distribution in composite sample: The individual seed weight for a sample of 1512 seeds, averaged to 22.78 ± 0.1054 mg varying from 10.7 to 36.1 mg (CV = 17.98%) showing substantial variation in seed size. The significant magnitude of Chi square indicated to the asymmetric distribution in the pooled sample (Fig. 7). Weight of 100-seeds of *Opuntia* has been reported to vary between 1.03 to 2.61g (mean: 1.66g) (Samah and Valdez-Moetazuma, 2014) corresponding to 10.3 to 26.1 mg per seed (mean: 16.6mg), almost comparable to our seed weight statistics. According to Samah and Valdez-Moetazunma (2014) seed area of *Opuntia* was directly proportional to seed weight. The weight of 100 seeds in *Opuntia* spp. are reported to weigh 1.7 to 2.1g by Reis *et al. (*2017) which correspond to 17 to 21mg per seed.

ANOVA of the weight individual normal seeds recovered from each berry indicated that there was more variation in seed weight among the berries (61.4%). Only around 38.6 % of the variance of seed weight was accounted for within berries (Table 4).

In view of large number of seeds in a pear Caplan (1995) suggested that research should be directed to develop cactus variety with lower number of seeds, softer seeds, and smaller seeds. Seedless variety is, however, impossible to develop because pulp develops from seeds (Pimienta and Engleman, 1985).

Table 4. One-way ANOVA for weights of normal seeds recovered from the berries of *O. ficus-indica*.

Source (Berries)	SS	df	MS	F	p	Variance accounted for
Between	15571.441	29	536.946			61.40
Within	9788.145	1482	6.605	81.30	0.001	
Total	25359.585	1511	-			38.60

Grand mean = 22.77 ± 0.10536 mg

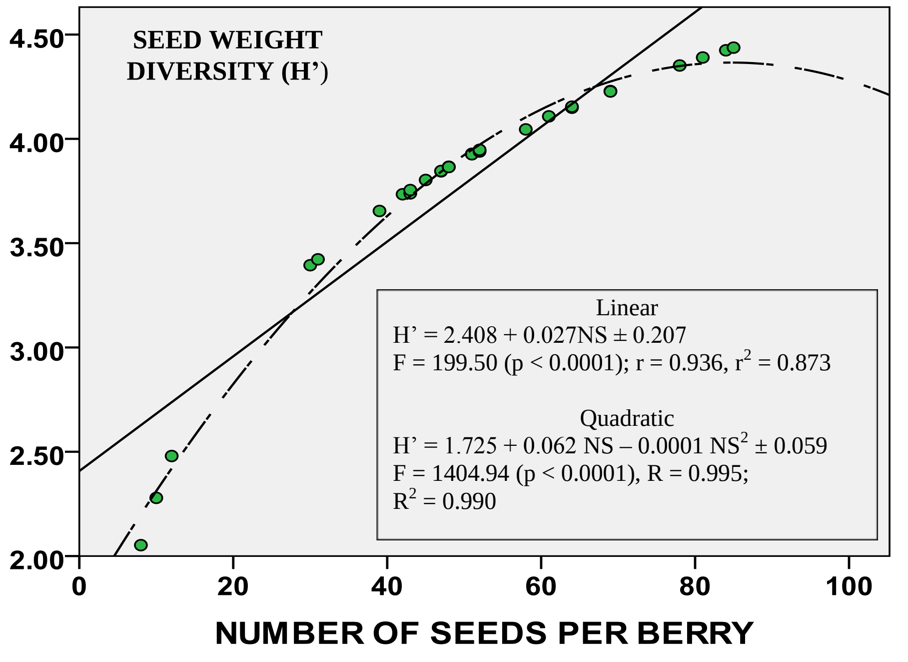

Fig. 8. Relationship between seed weight diversity (H') with number of seeds per berry (NS). As compared to the linear equation, the predictive power in quadratic equation was improved by 11.7%.

Diversity, dominance, equitability and Species richness: Diversity, an important attribute of structure, function and organization of in an assemblage, was calculated on the basis of weight of the individual seeds in a berry. The magnitude of various diversity indices is given in Table 5 along with their mean values for the berries studied. The in-hand fruits had high seed weight diversity and low dominance concentration (0.02775 ± 0.0048). The seed weight diversity (H') for *O. ficus-indica* seeds averaged to 3.7934 ± 0.1060. In 21 berries, it was higher than the mean value and in 9 cases lower than the mean value. Menhinick richness was, however, equally poised. In 15 berries, its magnitude was higher than its mean value and l in 15 berries its magnitude was lower than the mean value of 1.4629 ± 0.0639. The evenness was the least varying parameter (CV= 0.28%) and it was higher than the mean value in 96.6% of the berries. The dominance concentration in seed size recovered from berries varied substantially from 0.01196 to 0.12527, CV: 95.02%).

A great deal of multi-colinearity was apparent from the correlation analysis amongst various diversity indices. H' and MC were generally positively related with equitability and species richness. Contrary, dominance was inversely related with the diversity measures (Table 6). As regard to the dependence among the diversity measures and diversity-dominance relations, our results are in agreement with the results of earlier workers (Shaukat *et al.*, 1978; Saifullah *et al.*, 1984). In our studies, seed weight diversity was found to be positively linearly related with the evenness component, e (r = 0.772) and richness, d (r = 0.522).

Also, there was significant positive relationship between diversity (H') and number of normal seeds in a berry (NS, often used richness parameter). This was best given by a quadratic model (Fig. 8). The evenness salso best related with number of normal seeds in quadratic fashion (Fig. 9) than in linear fashion. The richness component was equally-significantly related with number of seeds (NS) per berry whether expressed linearly or in quadratic fashion (Fig.10). That is to say that larger were the number of seeds in a berry larger was the measure of seed weight diversity and its components. Larger was the number of seeds developing in a berry, there was more evenness in the weights of the seeds in the berry. These results are in agreement also to our above-given finding that larger number of seeds developing in a berry caused decline in the variation of seed size (CV %) in the berry (Fig. 6).

Our results indicated that two components of diversity were not equally important in determining the diversity of these assemblages. Here evenness appeared to control diversity slightly more than the species richness. Tramer (1969) suggested that biological assemblages from rigorous environments (adverse environmental conditions) very in diversity according to their relative abundance component whereas assemblages in non-rigorous environment (biologically controlled environment) is a function of species richness. Our results agree with Smith (1980) that there is no such assemblage as wholly physically controlled or wholly biologically controlled. It is rather influenced by the interaction of the two. Larger number of seeds developing in a berry should experience more intense competition for availability of nutrients besides several other factors the fruits may be subject to during their development.

Table 5. Seed weight diversity and richness and equitability components in 30 berries *O. ficus-indica.*

Berry #	NS	CV (%)	d	c	H'	e	Mc
1	48	10.89	1.388711	0.020261	**3.865196**	**0.99845**	**0.854809**
2	**51**	8.24	1.321714	0.019824	**3.928413**	**0.99913**	**0.859497**
3	**58**	8.81	1.38445	0.017051	**4.045305**	**0.99627**	**0.876919**
4	48	9.68	1.45887	0.020123	**3.866389**	**0.99874**	**0.854991**
5	47	10.76	1.34127	0.020726	**3.844348**	**0.99894**	**0.853293**
6	52	**11.59**	1.44567	0.018731	**3.944474**	**0.99828**	**0.85708**
7	52	**14.97**	1.40356	0.018947	**3.939241**	0.99696	**0.85978**
8	**84**	11.12	**2.13533**	0.011413	**4.424574**	**0.99859**	**0.89022**
9	**85**	10.02	**2.01131**	0.021133	**4.437677**	**0.99881**	**0.89096**
10	**78**	9.59	**1.82815**	0.012406	**4.35169**	**0.99885**	**0.88625**
11	45	8.64	**1.51497**	0.021279	**3.80298**	**0.99903**	**0.85037**
12	**69**	10.00	**1.95498**	0.013843	**4.22927**	**0.99886**	**0.87901**
13	**51**	10.60	1.40978	0.019078	**3.92613**	**0.99855**	**0.85919**
14	48	10.54	**1.50486**	0.021020	**3.86511**	**0.99843**	**0.85486**
15	**69**	11.44	**1.69609**	0.014087	**4.22698**	**0.99832**	**0.87883**
16	**64**	**13.63**	**1.76428**	0.015167	**4.14923**	**0.99768**	**0.87384**
17	30	11.42	1.19952	**0.032221**	3.39419	**0.99794**	0.81624
18	**61**	7.88	**1.60382**	0.015815	**4.10783**	**0.99926**	**0.87157**
19	43	**21.25**	1.34922	0.023344	**3.75863**	**0.99399**	0.84409
20	42	8.05	1.37568	0.022916	**3.73442**	**0.99913**	0.84519
21	**81**	9.16	**1.79526**	0.011964	**4.39010**	**0.99901**	**0.88842**
22	**52**	8.45	**1.56743**	0.018477	**3.94756**	**0.99907**	**0.86083**
23	47	8.77	1.40164	0.020570	**3.84595**	**0.99891**	**0.85357**
24	**64**	9.63	**1.77347**	0.015034	**4.15428**	**0.99889**	**0.87442**
25	43	11.36	**1.46654**	0.024188	**3.75457**	**0.99824**	**0.84652**
26	39	13.46	1.39768	0.024853	**3.65423**	0.99745	0.83843
27	8	**22.83**	**0.67588**	0.125273	2.05229	0.98694	0.63735
28	12	10.35	0.69938	0.081105	2.47960	**0.99787**	0.70978
29	31	**14.97**	1.36023	**0.031116**	3.42244	0.99664	0.81839
30	10	**20.67**	0.65597	0.100402	2.27897	0.98974	0.677087
Mean ± SE	50.40± 3.61	11.68± 0.7199	1.46286± 0.063705	0.027753± 0.004815	3.79339± 0.10602	0.99755± 0.00050	0.84177± 0.01100

Table 6. Matrix of Pearson correlation coefficients amongst diversity measures calculated on the basis of weights of the healthy seeds recovered from 30 berries of *O. ficus-indica.*

NS	NS						
d	0.491	d					
c	-0.781	-0.491	c				
H'	0.936	0.522	-0.947	H'			
e	0.621	0.441	-0.848	0.772	e		
Mc	0.859	0.511	-0.989	0.983	0.823	Mc	
CV	-0.538	-0.525	0.673	-0.641	-0.899	-0.668	CV

df = 28; **Acronyms:** NS, Number of normal seeds recovered from a berry; Richness d (Menhinick, 1964); c, Simpson's index of dominance; H', Species diversity (Shannon index); e, equitability (Pielou, 1975); Mc, McIntosh measure of diversity (McIntosh, 1969); CV, Coefficient of variability (%) = (Standard deviation/mean) * 100.

All values of correlation coefficients were significant at least at $p<0.01$.

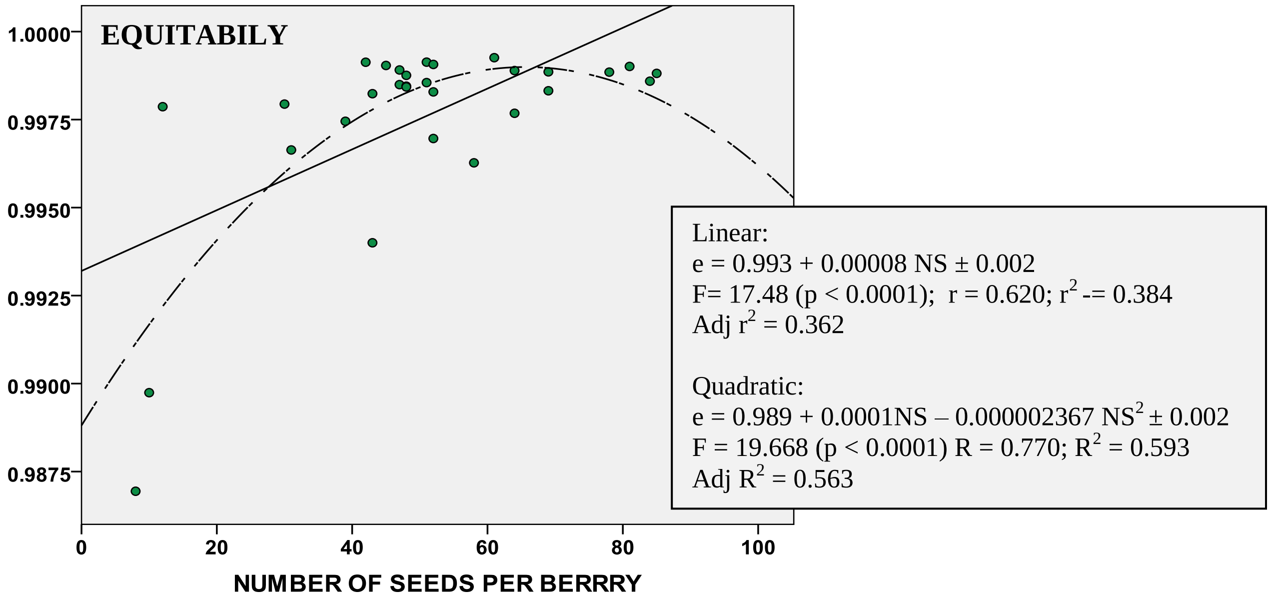

Fig. 9. Relationship between seed weight equitability (e) and the number of seeds (NS) in berries.

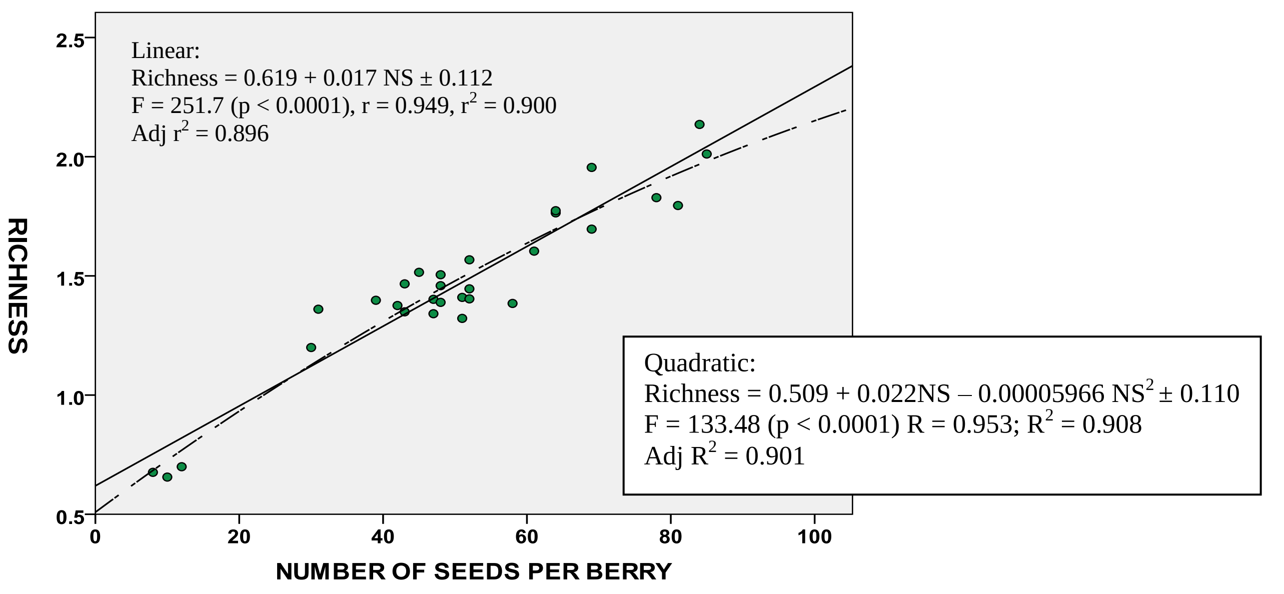

Fig. 10. Relationship between seed weight richness and number of seeds (NS) in berries.

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