

SEED-SIZE DISTRIBUTION PATTERN WITHIN PODS OF ALBIZIA LEBBECK (L.) BENTH. - POSITIONAL EFFECT

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ABSTRACT

Seed size distribution pattern within pods of *Albizia lebeck* (L.) Benth. is investigated with respect the position of the seeds within pods. The composite healthy pod sample of 73 pods gave brood size of 9.38 ± 0.215 seeds per pod. The average mass of a seed for healthy pods from three mother plants varied significantly around the grand mean of 119.90 ± 0.352 (N=30), 134.52 ± 0.565 (N=30) and 135.57 ± 0.668 mg (N=13), respectively. On an average seed weight in pods of mother plants A, B and C varied by 22.43 ± 2.14 , 26.31 ± 1.75 and 23.19 ± 3.17 %, respectively. The mean individual seed weight in healthy pods (N=73) was 129.898 ± 1.5508 mg. Seed size varied with the position of the seed within the pod. The maximum number of seeds in any healthy pod of *A. lebeck* was 11. The seeds (I to XI) varied in weight with CV = 25.42 to 35.48 %. The pattern of within-pod seed size distribution indicated that the weight of the first two seeds (proximal ones) was quite lesser in magnitude than that of the subsequent seeds. The first two seeds weighed 120.53 ± 0.00422 and 118.48 ± 0.00487 mg, respectively. Most of the subsequent seeds (number III to XI) were generally larger in size by a quantum of c 7 to 19 mg. It was evident by the t-test that seeds # I and II did not vary significantly from each other but were significantly lower ($p < 0.05$) in size than distal seeds (seed # III, VI, VII, VIII and X). The seed size of the first two proximal seeds exhibited significantly lower magnitude in comparison to distal seeds # IV, V and IX at $p < 0.10$. The results are discussed in the light of the eco-physiological literature.

Key Words: *Albizia lebeck* (L.) Benth., Brood size, seed size variation, within pod position of seed and seed size

INTRODUCTION

Seed size variation may result due to several internal and external factors - moisture availability (Martin *et al.*, 1980; Winn, 1991; Busso and Perryman, 2005), age of the walnut plant (Stromberg and Paton, 1990), plant height in *Ranunculus acris* (Totland and Birks, 1996), sites differences and environmental heterogeneity (Krannitz, 1997; Janzen, 1977), among and within individuals of a species (Krannitz, 1997; Howe and Richter, 1982; Sachaal, 1980; Thompson, 1984; Khan and Zaki, 2012; Khan and Sahito, 2013a and b; Afsaruddin and Khan, 2015, 2016; Khan *et al.*, 2016), location of fruits on the plants of *Arum italicum* (Méndez, 1997), position of seeds within a fruit (Lee, 1984) and resource allocation trade off with number of seeds (Venable, 1992). Alonso-Blanco *et al.* (1999) have identified several gene loci responsible for natural genetic variation in seed size in *Arabidopsis thaliana*. Seed size variation has been shown to have several important ecological implications. It is associated with seed germination (Baskin and Baskin, 1998; Navarro and Guitan, 2003), seedling vigour and survival both across species and within species (Manga and Sen, 1996; Shaikat *et al.*, 1999; Walters and Reich, 2000; Vaughan and Ramsey, 2001; Halpern, 2005) presumably reflecting the amount of reserves available for early growth (Castro *et al.*, 2006). Heavier seeds produce healthier seedlings with rapid pre-photosynthetic growth (Unival *et al.*, 2008 Espahbodi *et al.*, 2007; Wulff, 1986). Larger seeds of *Telfaria occidentalis* are reported to be better adapted to cotyledon damage (Iortsuun *et al.*, 2008). In short, seed size variation produces variation in seedling fitness and thus the survival of the species.

Afsar Uddin and Khan (2016) studied pod and seed characteristics of *Albizia lebeck*, a useful tropical tree, with reference to such parameters as pod length, pod width, pod weight, inter-seed distance, brood size, seed yield per pod, pod weight, seed classification, variation of seed weight and seed packaging cost. In the present paper, seed size distribution pattern within pods of this species is investigated with respect the position of the seeds within pods.

Climatic Peculiarities of the area

The climate of Karachi is of BWh type and bio-climate type as determined by Holdridge's system falls in the category of Tropical Bush Formation. The rainfall is irregular and averages below 200mm, mostly received in summer (Khan *et al.*, 2006). Annual evapo-transpiration is 1750 mm (Zubenok, 1977). Summer (May-October) and winter (November-April), are two climatic extremes. Summer is hot and winter is warm. Solar radiation is maximum in summer months of May and June and substantially lower in winter months (Ahmad *et al.*, 1991).

MATERIALS AND METHODS

Three plants of *A. lebeck* were selected in Karachi University Campus for the collection of pods and designated as mother plant A, B and C. The morphometric parameters such as height stem diameter, canopy height,

canopy diameter, etc. and characteristics of soil from underneath of each tree have been reported in Afsaruddin and Khan (2016).

One hundred pods of current year growth were collected from each individual plant in February 2012. The sampling of pods was random – selecting them from all four sides of the canopy. The pods were air dried in the laboratory for around sixty days. The healthy pods (Fig. 1), in terms of seeds normalcy (no insect infestation, no fungal infection and no occurrence of shriveled seeds in the pods), from each mother plant were employed to study effect of position of seed on the seed size. In three *Albizia* mother plants, 61, 45 and 13 % of the pods, respectively, were normal in the above sense. In all, 73 pods of *Albizia* were studied for such parameters as distribution of seed-size in pods, seed weight variation in normal pods and the brood size. The shape of each pod was drawn on the graph paper and seeds positions were located for each pod on graphs. The seeds were weighed individually according to their position from proximal to distal point of pod and noted on the graph. An electrical balance with an accuracy of 0.1mg was used to weigh the seeds.



Fig.1.The images of the healthy mature pods of *A. lebbek*.

To investigate the seed size relations with the position of the seed within normal pods, four approaches were employed.

1. In the first approach, a pod was divided into two parts- above and below the middle seed (s) and the middle seed weight was compared with the mean weight of the seeds falling above the middle seed (s) or below the middle one.

2. In the second approach, the weight of the first proximal seed was compared with the mean of the rest seeds falling below the proximal seed until the last distal seed.

3. The third approach was converse to the second one. The weight of the most distal seed was compared with the mean of the seeds present above the distal seeds until the most proximal seed.

4. In the fourth approach, average weight of i^{th} seed starting from the first (proximal) seed to the ultimate (distal most) seed was determined in healthy pods (30 pods each in case of mother plants A and B and 13 in case of mother plant C). To detect a pattern of the seed size distribution within pods, average weight of seeds numbered sequentially from proximal to distal end were plotted against the seed number. The t-test for various pairs of seeds was performed to test significant difference among the seeds.

The location and dispersion parameters of data were calculated and the frequency distributions were characterized with skewness (g1) and kurtosis (g2). Kolmogorov-Smirnov z test (KS-z test) was performed to detect normal distribution, if necessary (Sokal and Rohlf, 1995). KS-z test assesses whether the observations could reasonably have come from the normal distribution. t-test comparisons were employed to determine significant difference between means. The data was analyzed on canned statistical packages such as costat, mstat and SPSS version 12.

RESULTS AND DISCUSSION

A substantial variation in seed weight was recorded among and between the healthy pods collected from three mother plants of *A. lebbek*. A discrete pattern of variation was also recorded in weight of seeds located at various

positions in the pods. There was significant variation in brood size in the pods studied. This may be described as follows:

Table 1. Average single seed weight (mg) for the seeds recovered from normal pods of three *Albizia* mother trees.

Pods	Mother Plant A		Mother Plant B		Mother Plant C	
	Mean weight	CV (%)	Mean weight	CV (%)	Mean weight	CV (%)
p1	126.22(11)*	30.17	135.12(10)	31.51	165.86(11)	9.88
p2	115.57(10)	41.57	167.39(10)	23.86	147.99(7)	17.47
p3	107.72(9)	25.63	157.23(9)	37.13	121.76(11)	40.25
p4	107.32(10)	42.95	157.68(10)	22.86	123.47(6)	33.77
p5	113.81(7)	36.55	170.64(10)	20.83	126.02(9)	7.14
p6	145.49(11)	5.66	198.55(10)	14.92	90.3(11)	42.83
p7	125.05(10)	9.62	150.81(8)	29.90	151.98(11)	16.09
p8	120.88(9)	38.35	148.16(11)	32.05	131.24(11)	24.98
p9	114.75(8)	32.20	157.97(10)	19.30	124.29(11)	20.94
p10	124.8(11)	19.65	128.46(5)	5.50	130.15(11)	16.41
p11	85.04(8)	28.47	159.66(11)	28.87	110.83(11)	18.08
p12	110.77(9)	17.73	109.32(10)	37.57	175.59(10)	18.12
p13	101.77(7)	24.04	96.13(11)	40.95	162.93 (9)	35.57
p14	155.21(9)	14.04	155.78(10)	33.25	<div> Brood size per healthy pod N (pods) = 73 Mean = 9.38 SE = 0.2146 Q2 = 10.0 CV = 19.8% g1 = -1.008 Sg1 = 0.281 g2 = 0.014 Sg2 = 0.555 Min = 5.0 Max = 11.0 KS-z = 2.100 P < 0.0001 </div>	
p15	111.63(9)	18.35	111.55(6)	26.49		
p16	137.62(11)	8.37	151.36(11)	15.38		
p17	151.15(10)	14.97	162.55(6)	21.91		
p18	90.34(5)	53.02	123.22(5)	13.84		
p19	127.63(11)	11.42	119.18(5)	34.90		
p20	137.63(11)	17.16	145.12(10)	24.47		
p21	142.94(8)	9.29	171.64(8)	34.01		
p22	136.96(11)	17.06	71.86(10)	39.91		
p23	84.48(10)	17.40	109.84(10)	45.66		
p24	77.84(8)	34.52	117.94(8)	23.33		
p25	115.70(8)	11.07	96.08(5)	22.03		
p26	124.83 (7)	14.05	75.31(9)	24.87		
p27	131.31(10)	18.49	84.41(7)	23.73		
p28	126.72(11)	20.79	146.36(11)	10.54		
p29	118.94(11)	25.15	131.75(10)	17.74		
p30	126.94(11)	15.30	124.46(10)	30.09		
Grand Mean ± SE	119.90 ±0.352	22.43 ±2.14	134.52 ±0.565	26.31 ±1.75	135.57 ±0.668	23.19 ±3.17
Av. CV (%) ± SE						
CV (%) Range	5.56-53.02		5.50-45.66		7.14-42.83	

p1 to p30, the pod number; *, SN i.e. number of seeds per pod (Brood size).

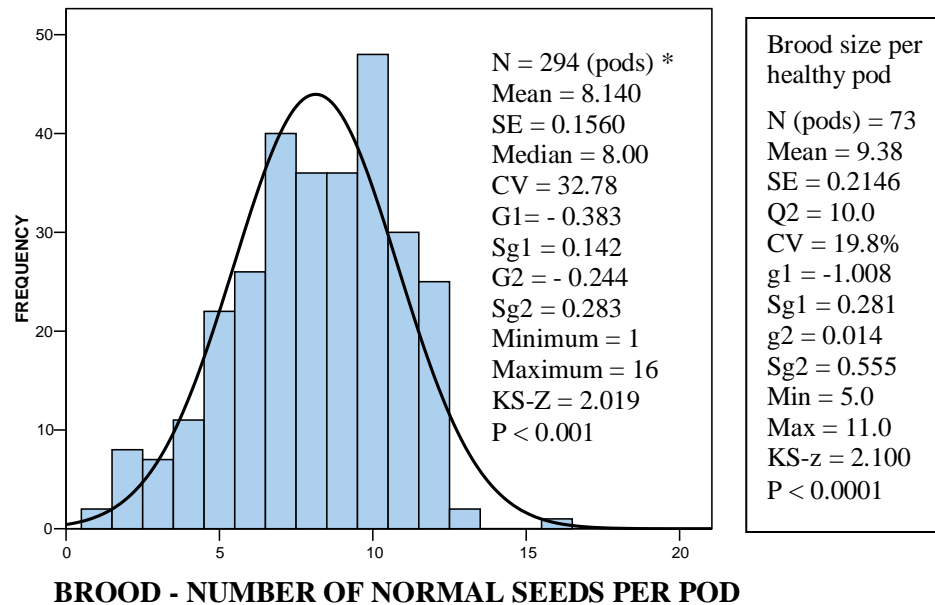


Fig 2. Distribution of number of seeds per pod (Brood size) in pods (N = 294) of *A. lebbek*. *, pods yielding no normal seed were excluded.

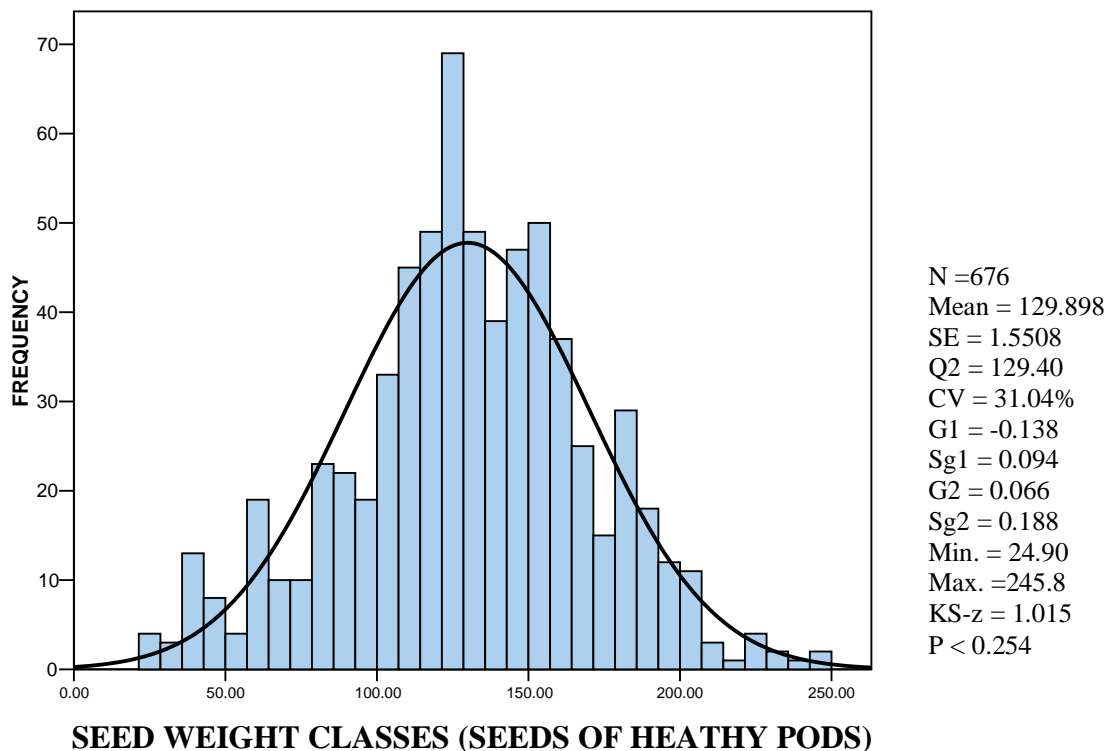


Fig. 3. Seed weight (mg) distribution in 73 healthy pods collected from sample mother plants A and B and C of *Albizia lebbek*.

Brood Size

The brood size in healthy pods of three mother plants A, B and C averaged to 9.6 ± 0.294 (N = 30; CV = 17.2%), 8.87 ± 0.374 (N = 30; CV = 23.1%) and 9.92 ± 0.473 (N = 13; CV = 17.2%) seeds per pod, respectively (Table 1). The composite healthy pod sample of 73 pods gave brood size of 9.38 ± 0.215 seeds per pod which was significantly larger than the mean brood size from the pod crop of 294 seed-yielding pods collected from the three

mother plants (8.14 ± 0.156 per pod) (Fig. 2). The brood size in healthy pods varied from 5-11. It was found to be negatively skewed in healthy pods of all the three plants (Fig. 2). Uma Shaanker *et al.* (1988) have reported brood size in *A. lebbeck* to be 6.88 ± 2.73 seeds per pod from India (site and environment not mentioned) which is not significantly different from our statistics ($t = 0.913$, NS). The negatively-skewed brood size in *Albizia* as observed here is a common feature in many species with multi-ovulated fruits (Lee and Bazzaz, 1982).

Variation of mean single seed weight (MSSW) in pods around the grand mean value of seed weight

The weight of individual seeds varied within and among the pods substantially. The average mass of a seed for healthy pods of the mother plants varied significantly in mother plant A, B and C around the grand mean of 119.90 ± 0.352 , 134.52 ± 0.565 and 135.57 ± 0.668 mg, respectively (Table 1). On an average seed weight in pods of mother plants A, B and C varied by 22.43 ± 2.14 , 26.31 ± 1.75 and 23.19 ± 3.17 %, respectively (Table 1). The mean seed weight in a pod (129.9 mg) was larger than the grand mean seed weight for all the healthy pods in case of the pods of plant A and B. In rest of the pods of these plants, the major proportion of 43.3 to 46.6% was occupied by seeds of weight lesser than the respective grand means and in 3.3 to 6.6% of the pods the mean weight was nearly equal to the grand means. In plant C the seeds were of weight lesser than the grand mean in 61.54% of the cases.

Seed weight Variation in healthy pods

Afsar Uddin and Khan (2016) have reported seed weight variation in three mother plants of *A. lebbeck*. The weight of individual seed in three mother plants of *Albizia* (A, B and C) was reported to average to 120.0 ± 1.11 (N = 822 seeds), 129.31 ± 0.17 (N = 726 seeds) and 120.42 ± 1.38 mg (N = 845 seeds), respectively with a variation of 26.55, 35.67 and 33.38%, respectively. Distribution was skewed in plant A and C and platykurtic in case of mother plant B. The maximum / minimum seed mass variation in three plants was between 8.44-fold to 17.5-fold. The seed weight distribution from composite sample of 2383 seeds was reported by them to be somewhat negatively skewed with mean seed weight 123.026 ± 0.1811 mg. The seed weight varied by 32.2% (ranging from 14 to 245.0 mg (maximum / minimum variation: around 17.5-fold).

The mean seed weight (for seeds recovered from 73 healthy pods (N = 676 seeds) was found to be 129.898 ± 1.5508 mg (Fig. 3). Seed weight varied from 24.9 to 245.8mg (CV = 31.04%) and distributed normally as suggested by insignificant value Kolmogorov-Smirnoff statistics (KS-z = 1.015, $p < 0.254$, NS).

Positional effect on the seed size

To investigate within-pod positional effect on the seed size, four approaches were employed. 1) comparison of weight of the first proximal seed with the mean weight of the rest of the seeds in the pod, 2) comparison of the weight of the last (distal seed) with the mean weight of the seeds lying above the distal seeds, 3) comparison of the weight of middle seed (s) of the pod with the mean weights of seeds lying proximally or distally and 4) the mean seed weight of each seed in healthy pods was determined sequentially from proximal to distal seed and compared with each other through t-test.

Table 2. Mean weight of middle seed (s), mean weight of seeds above (proximal) the middle seed (s) and mean weight of seeds lying below the middle seed (s) in 13 normal healthy pods of mother plant C of *A. lebbeck*.

Seed Location	Mean weight (g)	SE
Seeds above (proximal) the middle seed (s)	0.135692	0.0072702
Middle seed (s)	0.139815	0.0083772
Seeds below (distal) the middle seed (s)	0.136345	0.0075835

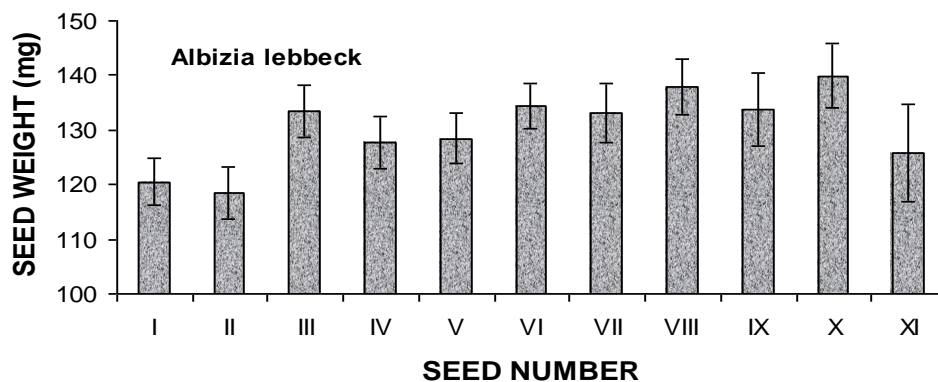
Table 3. Comparison of mean weight of middle seed (s), seeds proximally above the middle seeds (s) and weight of seeds distally below the middle seed (s).

Pairs for comparison	Pair difference		t	df	p
	Mean difference	SE mean difference (pair)			
Upper seeds – Middle seed (s)	0.00412310	0.0096679	0.426	12	0.677 (NS)
Upper seeds – Lower seeds	0.00065380	0.0067469	0.097	12	0.924 (NS)
Middle seed (s) – Lower seeds	0.00034692	0.010956	0.340	12	0.740 (NS)

Table 4. The comparison of first (proximal) and distal seed mass with the average mass of the rest (lower or Upper) seeds in the pods in terms of per cent frequency of occurrence

Parameter	COMPARISON BY SEED WEIGHT					
	Proximal to lower seeds			Distal to upper seeds		
	Plant A N =30	Plant B N =30	Plant C N =13	Plant A N =30	Plant B N=30	Plant C N =13
Percent Occurrence						
NE (%)	0	3.33	0	6.66	6.66	0
H (%)	36.67	30.37	30.77	33.33	43.33	61.53
L (%)	63.3	66.7	69.2	60.00	50.00	38.46
Composite Sample (N =73)						
Percent Occurrence						
NE (%)	1.37 ± 1.37 a			4.44 ± 2.22 a		
H (%)	32.43 ± 2.75 b			46.06 ± 8.25 b		
L (%)	66.41 ± 1.72 c			49.48 ± 6.22 b		

Key to the acronyms: NE, the first proximal or distal most seed is near equal in mass to the mean mass of the rest seeds in the pod. H, the first proximal or distal most seed's mass is higher in magnitude than the mean mass of the rest seeds. L, The first proximal or distal most seed mass is lesser in magnitude than the mean mass of the rest of the seeds. The dissimilar alphabets indicate significant difference at least at $p < 0.05$.



Seed #	N	Min	Max	Mean (g)	SE	CV (%)
I	73	0.0401	0.2023	0.120526	0.004218	29.95
II	73	0.0249	0.1999	0.118479	0.004867	35.10
III	73	0.0402	0.2458	0.133285	0.004771	30.50
IV	73	0.0250	0.2231	0.127739	0.004705	31.47
V	73	0.0413	0.2280	0.128473	0.004670	31.06
VI	68	0.0428	0.2272	0.134262	0.004131	25.42
VII	65	0.0408	0.2130	0.133249	0.005388	32.60
VIII	60	0.0332	0.2028	0.137892	0.004979	27.97
IX	52	0.0266	0.2426	0.133779	0.006582	35.48
X	43	0.0597	0.2240	0.139898	0.005670	26.57
XI	23	0.0362	0.1886	0.125830	0.008955	34.13

Figure 4. Mean weight (g) for individual seeds for specific position in pod sequentially from proximal to distal end in healthy pods (N = 73) of *A. lebbbeck*. The Table below the graph describes the weight statistics of seeds occupying various positions sequentially from proximal to distal positions in 73 healthy pods.

Table 5. t-test for significance of seed weight of seeds located at different positions sequentially from proximal to distal position in healthy pods (N = 73) of *A. lebbeck*. *, values of 't' in bold are significant at least at $p < 0.05$.

S I	S I											
S II	0.4	S II										
S III	-2.6	-2.72	S III									
S IV	-1.53	-1.71	0.99	S IV								
S V	-1.62	-1.88	0.88	0.14	S V							
S VI	-2.67	-2.52	0.001	-1.09	-0.85	S VI						
S VII	-2.36	-2.73	0.39	-0.96	-1.35	0.3	S VII					
S VIII	-3.02	-3.34	-1.16	-1.56	-1.45	-0.32	-0.68	S VIII				
S IX	-1.67	-1.66	1.01	0.75	-0.21	0.49	0.3	0.43	S IX			
S X	-2.77	-2.29	0.36	-1.6	-0.42	-0.01	0.12	-0.04	-1.34	S X		
S XI	0.21	0.005	1.19	0.35	0.73	0.9	1.36	0.92	0.15	1.9	S XI	

The seed size of middle seed (or the mean seed size of two middle seeds in case of pods with even number of seeds) and mean seed size of seeds lying proximally or distally to the middle seed are given in Table 2 and compared with each other in Table 3 through t-test. As regard to this approach no clear cut pattern of seed weight distribution emerged as the all the comparisons (Table 3) were found to be insignificant. The comparison of proximal seed size with mean seed size of lower seeds or distal seed size with mean seed size of upper seeds provided some interesting results in terms of per cent frequency of occurrence of seed sizes in three classes – nearly equal in size (NE), higher in size (H) or lesser in size (L) (Table 4). The frequency of proximal seed to be nearly equal to mean size of distal or lower seeds was quite low (3.33%). Distal seed size was nearly equal to mean size of upper seeds in 6.66 per cent of cases. In majority of cases of composite sample (N = 73), proximal seeds belonged to the lesser class (66.41%) when compared with the mean seed size of lower seeds. In a similar comparison, distal seed size was more or less equally divided in H ($46.06 \pm 8.25\%$) and L ($49.48 \pm 6.22\%$) classes.

The maximum number of seeds in a healthy pod of *A. lebbeck* was 11. The seeds (I to XI) varied in weight with magnitude of 25.42 to 35.48 %. The fourth approach to investigate the pattern of within-pod seed size distribution indicated that the weight of the first two seeds (proximal ones) was quite lesser in magnitude than that of the subsequent seeds. In *A. lebbeck*, the first two proximal seeds weighed 120.53 ± 0.00422 and 118.48 ± 0.00487 mg, respectively. The most of the subsequent seeds (number III to XI) were generally larger in size by a quantum of c 7 to 19 mg. It was evident by the t-test that seeds # I and II did not vary significantly from each other but were significantly lower ($p < 0.05$) in size than distal seeds (seed # III, VI, VII, VIII and X) (Fig. 4, Table 5). The seed size of first two proximal seeds exhibited significant lower magnitude in comparison to distal seeds # IV, V and IX at $p < 0.10$ (t_s values varying from 1.5 to 1.88). Comparatively lesser seed size of proximal seed (s) than other seeds of the pod, as observed here, is also reported in other mimosacean species (*Vachellia nitotica* and *Leucaena leucocephala*) by Afsar Uddin and Khan (2015) and Khan *et al.* (2016), respectively. In the composite samples of healthy pods of these species, first proximal seed was also found to be lesser in size than distally located seeds.

The pattern of seed size variation in pods as seen here may be thought to be related with the disparity of nutrition availability to the seeds in multi-seeded fruits (Mendez, 1988). Within fruit seed mass variation i.e. unequal seed mass partitioning among seeds in a fruit is considered to result from differential parental supply related to the genetic quality of seeds (Timme, 1986), the position effect within a fruit or in inflorescence (Lee, 1988; Waller, 1982; Méndez, 1997), parent-offspring conflict (Lloyd, 1992) or sibling rivalry (Uma Shaanker *et al.*, 1988).

The distal ovules in the ovary of *A. lebbeck* are nearer to the stigma and by virtue of their position these ovules are relatively more prone to little earlier fertilization and initiation of their development into seeds. Ovules located in ovary proximally should require relatively longer pollen tubes for effective fertilization and likely fertilize with some delay. As ovary develops into pod, it attains an average length of 21.96 ± 0.253 cm stays in hanging position on the tree. In longer pods, as in *A. lebbeck*, the role of gravity in enhancing availability of photosynthates / nutrients to relatively larger proportion to lower seeds (distal seeds) may not totally be ignored. Obviously, it should be more important in elongated, multi-seeded and hanging pods provided with single vascular supply along the ventral suture. Considering a pipe-line analogy, the photosynthates moving through phloem under gravity should tend

presumably be lesser available to the proximally developing seeds and more available to the subsequent seeds. Phloem unloading and transport, however, from source (leaves) to sink (developing seeds) may change with assimilate production at the source which may directly affect assimilate availability to the developing seeds by changing the pressure differences that governs photo-assimilate movement (Patrick, 1997). Ganashaiah and Uma Shaanker (1994), using the concept of fluids flow, assumed that probability of any given sink getting the resource molecule should be the function of 1) sink drawing ability and 2) amount of resource molecule already moved to that sink. That is to say that any molecules moving to a sink auto-catalytically increases the probability of the later molecule to move to the sink. In an elongated pod, thus differentially favourable sinks may develop. Ganashaiah and Uma Shaanker (1994) have demonstrated that in such scenarios seed abortion may also take place even under the conditions of resource abundance which may often be seen in several legumes with elongated multi-seeded pods (Khan and Zaki, 2012; Afsar Uddin and Khan, 2015; Khan *et al.*, 2016).

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PS: Srimathi *et al.* (1992) have reported that seeds recovered from middle part of the pods of *Albizia saman*, *Cassia fistula* and *C. hybrida* showed higher seed weight in comparison to the proximal or distal seeds when pods were equally divided into three segments – proximal, middle and distal. In *Delonix regia*, seeds farther from the stalk were, however, found to be heavier. They suggested that such a pattern may be due to time lag differences of fertilization leading to the differences in developmental stages and the nutrient disparity. Larger seeds produced better vigour and seedling establishment.

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