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VARIATION IN PSEUDOCARP- AND SEED-SIZE AND SEED PACKAGING COST IN *CHIMONANTHUS PRAECOX* (L.) LINK. (FAMILY CALYCANTHACEAE) FROM CHENGDU, SICHUAN PROVINCE, CHINA

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ABSTRACT

Quantitative phenotypic traits of pseudocarps and seeds of *Chimonanthus praecox* (L.) Link. are described from a road-side tree in Chengdu, Sichuan province of China. The parameters included 1. Air dried pseudocarp weight (PW), 2. Seed Yield per pseudocarp (SYP), 3. Number of seeds per pseudocarp (NSP, the brood size), 4. weight of Empty pseudocarp (WEP), 5. Mean single seed weight (MSSW) in each of 131pseudocarps, 6. Seed weight variation and distribution in a composite sample of 468 seeds, 7. Seed packaging cost per seed (SPC_1 = WEP / NSP and seed packaging cost per g seeds (SPC_2 = WEP / SYP). The phenotypic traits of seeds and pseudocarps were rich in variation and exhibited great deal of co-linearity amongst them. The pseudocarp weight (PW) averaged to 1.4726 ± 0.047g and seed wt. averaged to 0.2455 ± 0.002688 g. The weights of pseudocarps and seeds were found to vary by 39.96 and 23.73%, respectively. Around 16% pseudocarps had weight ≤ 1.0g, and those in the size class of 1.01 to 2.0g were 65.7% in number. Seed packaging cost per seed (SPC_1) was 0.1417 ± 0.00566g and on the basis of one g seeds (SPC_2), 0.5933 ± 0.02826g. SPC_1 and SPC_2 varied by 47.75 and 54.52%, respectively. The results are compared and discussed on the basis of available literature.

Key Words: *Chimonanthus praecox* L. (Link.), Pseudocarps and seed phenotypic traits, seed weight variation, Correlation amongst the traits, seed packaging cost

INTRODUCTION

Chimonanthus praecox (L.) Link. (Family Calycanthaceae) is a deciduous shrub native to China and known as ‘làmei’ or ‘La Mei Hua’ in Chinese and ‘röbai’ in Japanese and Korean. It is cultivated in China for more than 1000 years for its medicinal importance and highly fragrant flowers. Flowers have essential oils – elemene, muurolene, caryophyllene, cardinal and spathulenol. They are natural source of antioxides and biocides (Jin-shun *et al.* 2012). Vernacularly, it is known as Winter Sweet (http://en.wikipedia.org; Kew Science: Plants of the World *online*). The genus is derived from Greek Cheimon = winter and anthos = flower. It has USDA hardiness of 7-9. Two well-known varieties are 1. *C. praecox* ‘Grandiflorus’ and 2. *C. praecox* ‘Luteus’. One hundred and fifty six cultivars of *Chimonanthus praecox* of Hannan province have, however, been placed in three groups by Lu *et al.* (2012) – 1. *Ch. praecox* Concolor group, 2. *Ch. praecox* intermedius group and 3. *Ch. Praecox* Patens group. Oil is prepared from extremely scented waxy flowers and used in Chinese traditional medicines.

C. praecox forms many populations in montane forests (500-1100m) and distributed in several provinces of China – Anhui, Fujian, Guizhou, Henan, Jiangsu, Jiangxi, Shanxi, Shandong, Sichuan, Yunan and Zhejiang . Nicely (1965) have published a comprehensive monograph on Calycanthaceae. Zhou *et al.* (2006) have described the pollination biology of this plant. It is an entomo-pollinated plant. Foliar micro-morphology of the plant has been described by Ye and Pingtas (1999) - foliar epidermal layer cells are with sinuate anticlinal walls with paracytic stomata. Four populations of *C. praecox* (Population 1, Longwangkan of Fuyong, Population 2, Bidougshan of Fuyong, Population 3, Wujianshan of Fuyong and Population 4, Fangshan of Linán of Zhejiang province) have been studied for variation in phenotypic traits of fruits and seed by Huicong *et al.* (2018).

The present paper describes the results of studies with pseudocarps and seeds of this species from inland locality of Chengdu of Sichuan province some 1500 km away from southwestern coastal province of Zhejiang, to estimate variation in seed and pseudocarp weights and the seed packaging cost.

MATERIALS AND METHODS

The pseudocarps of *C. praecox* were collected from a road-side cultivated tree in Chengdu (30^o 5’ – 31^o 26’ N latitude and 102.54^o to 104^o 33’ E longitude, Sichuan province (southwestern part of China in June, 2018) (Fig. 1). Chengdu is situated in a big plain and surrounded by mountains all around (www.quora.com/what-is-the-climate-of-chengdu-china). It is an area where temperature may vary from - 4.6 to 33 ^{o}C (http://en.wikipedia.org/wiki/Chengdu;

http://www.china highlights.com/Chengdu/weather.htm). The habit of plant and the forms of the pseudocarps and seeds are presented in Fig. 2 and 3).

Climate

Chengdu climate is classified as warm and temperate, summer precipitating (maximum rainy days in July – c. 17) and least rain in December. It has an altitude of 499 m. January is the coldest month and June, July and August the hottest (c. 25 ^{o}C). Total annual rains amounts to 991mm per year (http://en.climate-data.org/asia/china/ Sichuan /Chengdu-2239/ --). It is 685 miles away from the Sea. Average humidity is high fluctuating around 80% (www.weather-and-climate.com). Winds in Chengdu are from NNE or SSW predominantly. (Sichuan Giant Panda Sancturies, China, http:// sites.google. com. site/geo 121 wikispring 2012/ --). According to Köppen-Geiger system, the area falls in cfa type of climate (Köppen and Geiger, 1954; Peel *et al.,* 2007).

Fig. 1. Location of the site of collection of *C. praecox* pseudocarps -Chengdu, Sichuan province of China.

The pseudocarps were air-dried for around 60 days in laboratory. Pseudocarps and seeds were weighed on electronic weigh meter with a least count of 0.0001g. To determine biomass investment in seeds and seed packaging cost following parameters were determined (Mehlman, 1993; Chen *et al*., 2010; Khan and Zaki, 2012; Khan *et al.*, 2014) - The parameters included 1. Air dried pseudocarp weight (PW), 2. Seed Yield per pseudocarp (SYP), 3. Number of seeds per pseudocarp (NSP, the brood size), 4. Weight of Empty pseudocarp (WEP), 5. Mean single seed weight (MSSW) in each of 131pseudocarps, 6. Seed weight variation and distribution in a composite sample of 468 seeds, 7. Seed packaging cost per seed (SPC_1 = WEP / NSP and seed packaging cost per g seeds (SPC_2 = WEP / SYP). The weight of each seed recovered from the pseudocarps was recorded and seed weight distribution was determined.

The data were analyzed (Zar, 2010) for descriptive high order statistics. The statistical package employed was SPSS ver. 17. Normality of data distribution was tested with, Kolmogorov-Smirnoff test (K-S test*) corrected for Lilliefors significance correction (Dallal and Wilkinson, 1986; Neter *et al.,* 1988) and Shapiro-Wilk test (Shapiro and Wilk, 1965). Thode (2002) has opined that KS-z suffers from its low power to detect normality and should no more seriously be considered for testing normality. It should be used with Lilliefors significance correction.

Since the distribution of almost all traits was non-normal, the relationships amongst the phenotypic traits of pseudocarp and seeds were calculated on the basis of Spearman Rank correlation (‘rho’). The predictive regression equations were, however, determined on the basis of usual simple linear or power, or curvilinear models and multiple linear regressions.

RESULTS AND DISCUSSION

The pseudocarps and seeds of C. *praecox* were studied for their quantitative phenotypic traits which are described as follows.

The pseudocarps

The pseudocarps and seeds of *C. praecox* are depicted in Fig. 2 and 3. The pseudocarps of *C. praecox* are urceolate (like an urn), ovoid-ellipsoidal or obovoid-ellipsoid in shape, mustard-brown in colour and dry subwoody. They have swollen body like pitcher but contracted at the orifice with persistent appendages. Apex appendages are varying in number. Inside early pseudocarp, there is apocarpous ovary with 5-15 individual carpels.

In a sample of 10 pseudocarps, the pseudocarp averaged to 2.93 ± 0.2634 cm (1.5-3.8 cm) in length and 1.79 ± 0.4005 cm (0.9 – 2.3 cm) in width at the widest part.

The weight of mature pseudocarps (inclusive seeds inside) averaged to 1.4726 ± 0.04709g varying from 0.4156 to 3.1109g (N = 131; CV = 39.96%). It significantly tended to deviate from normality as suggested by Shapiro-Wilk test (0.977, $p < 0.026$) (Fig. 4). Pseudocarps of ≤ 1.0 g in weight were 16.1% and those in the size class of 1.01 to 2.0g were 65.7% in number. Pseudocarps in larger weight classes were substantially lesser in frequency.

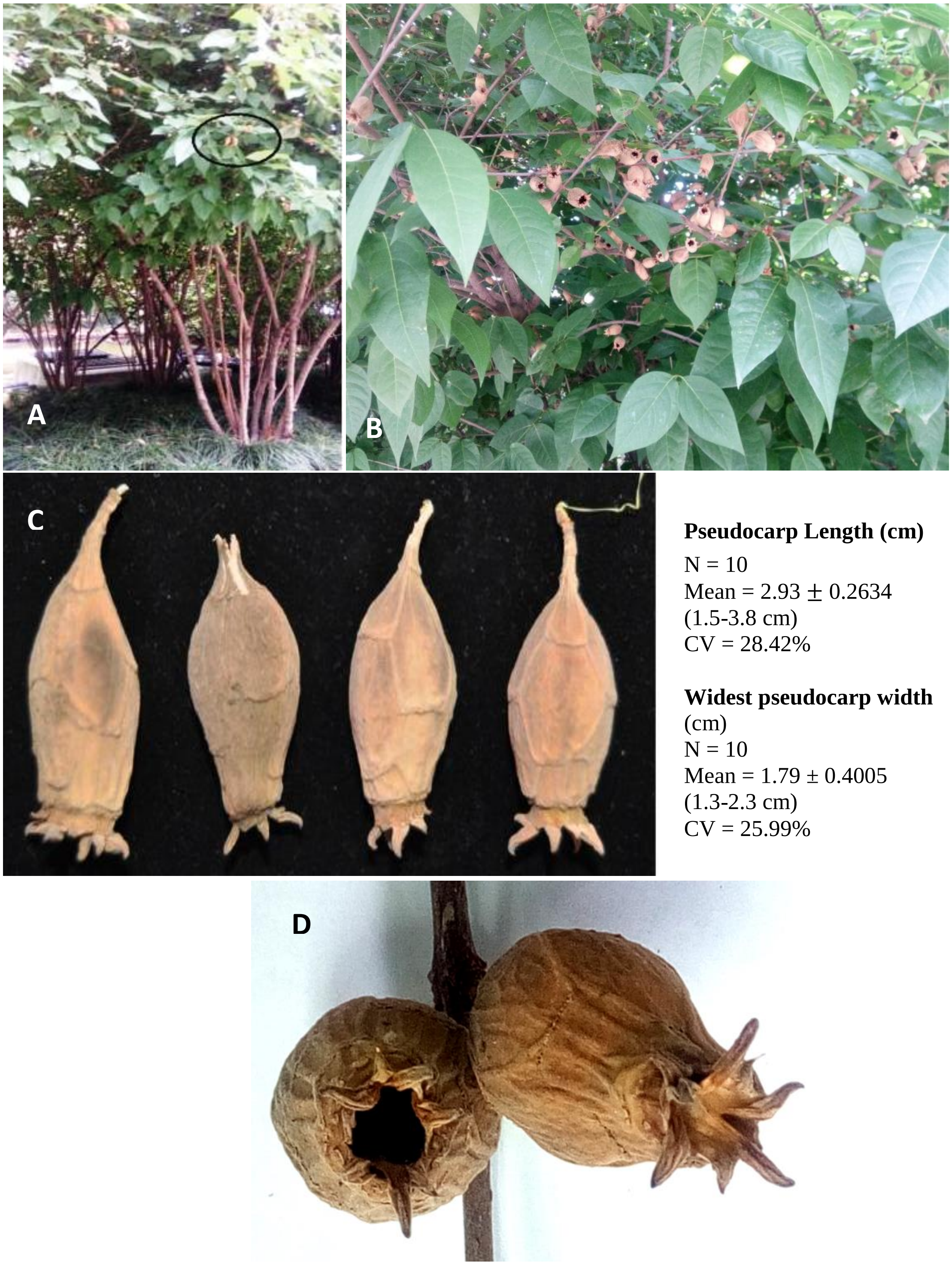


Fig.2. *Chimonanthus praecox.* A, Habit; B, Canopy showing foliage and pseudocarps; C and D, Pseudocarps and their close up view – persistent appendages near orifice ; Pseudocarp dimension (cm) – length excluding pedicel and width measured between the widest points.

The Seeds

The seeds are chocolate brown in colour, more or less elliptical but laterally little compressed to form depression. They are formed out of apocarpous carpels. The seed coat is bitegmic – testa (one layer of exotesta, several layers of mesotesta and one layer of endotesta) and tagmen (two-layered) Paudel and Heo, 2018a). Seeds were 12.90 ± 0.2464 mm in length (CV = 14.17%; 8.5 – 16.0mm) and 5.19 ± 0.1127 mm in width (CV = 16.10%; 2.5 – 6.5 mm) in width (Table 1). Seeds are at times curved. Seed surface is hairy and hairs are unicellular, bending, whitish, and easily removable (Fig. 3). Seed surface cells are pentagonal. The seed hairiness has also been previously reported by Pandel and Heo (2018b).

The viability of freshly collected seeds is reported to be high (97%) but they are dormant. Scarification accelerates water uptake. Germination temperature optimum is 23^{o}C. Tang and Tian (2010) have discussed the dormancy breaking procedure in detail. The alkaloids from seeds of *C. praecox* (D-calaycanthine and L. folicanthine) were significantly inhibitory to fungi such as *Exserothium turcicum, Bipolaris waydis, Alternaria solani, Sclerotinia sderotiorum* and *Fusarium oxysportium* (Zhang *et al*., 2009). Alkaloid calaycanthine is isolated from the seeds of *C. praecox*. They are not edible (Schumacher (2019).

Brood size

The brood size was defined here following Uma Shaanker *et al.* (1988) - as the number of seeds per pseudocarp. It averaged to 3.94 ± 0.139 varying from 1 to 10 per pseudocarp (N = 131; CV = 40.28%). Wiart (2012) has also reported 3 to 10 seeds per pseudocarp. The brood size distribution tended to be asymmetrical as suggested by significant values of K-S test and Shapiro-Wilk test (Fig. 5). The distribution was positively skewed (g1 = 0.894, Sg1: 0.212) and leptokurtic (g2 = 1.171; Sg2: 0.420). The brood size in size class 2 – 6 seeds per pseudocarp was predominantly high – 93.89% of the total pseudocarps. The modal class was, however, 3-4 seeds per pseudocarp occupying a proportion of 49.6% of the total pseudocarps. There were only two pseudocarps which had one seed each.

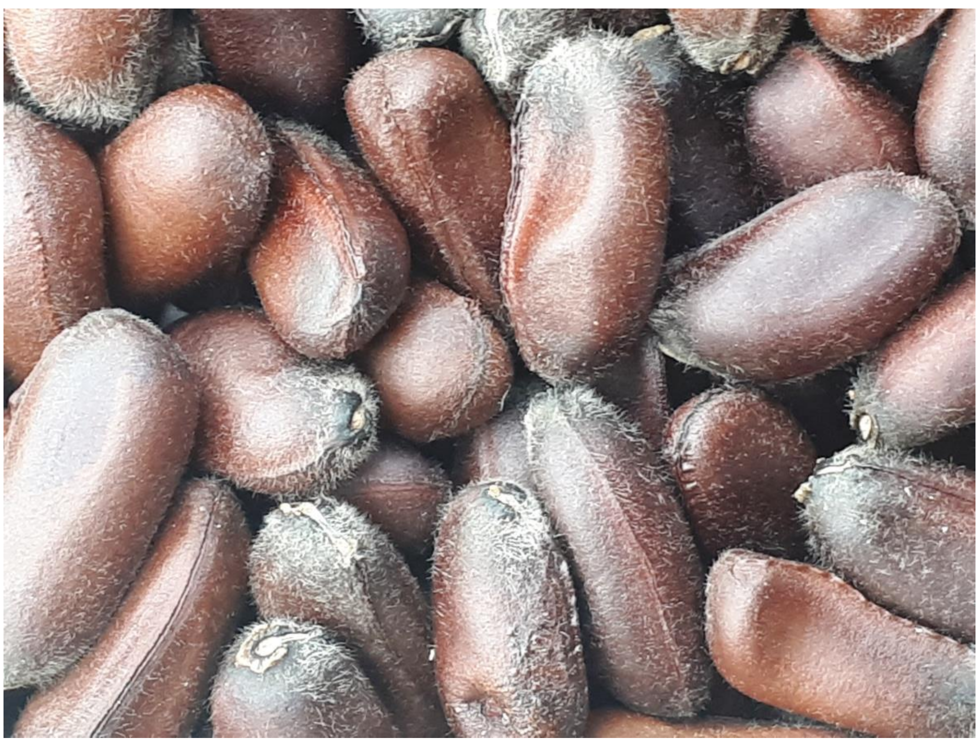


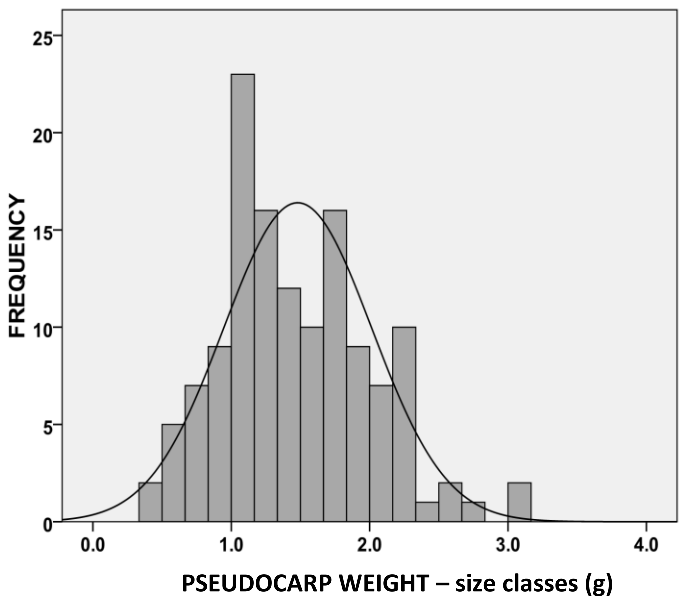
Fig. 3. Seeds of *C. praecox* – shining, chocolate brown in colour and elliptical in shape and bearing hairs on the surface. The hairs may easily be removed during handling.

Distribution of brood size has been described variously in different species. The brood size was normally distributed in *Bauhinia racemosa and B. ungulata (*Uma Shaanker *et al.,* 1988*)* and *in Cassia fistula* (Khan and Zaki (2012). The distribution of brood size has, however, been reported to also significantly vary from normal in several plants. This trait may be positively skewed (PSD) or negatively skewed (NSD). The pattern of brood may probably come up due to differences in the developmental history specific to the individual fruits in the environmental context. PSD in brood size is induced when a minority of ovules develops into mature seeds in most fruits and seed to ovule ratio is low i.e. < 50 % and as a result fruits are one to few-seeded (Uma Shaanker *et al.,* 1988). Some species accomplish brood size NSD through a maternally regulated pre-fertilization inhibition of pollen grains germination by the stigma. (Ganeshaiah *et al.,* 1986, 1988. This leads to NSD of fertilized ovules (Ganeshaiah *et al.*, 1986). NSD of seeds in pseudocarps is said to be a common feature of majority of multi-ovulate species (Lee and Bazzaz, 1982). There is a need to investigate reasons for NSD in brood size in *C. praecox.*

Weight of Empty a pseudocarp (WEP)

Pseudocarps of *C. praecox* are mustard- brown in colour. It is chemically enriched with a number of essential oils (Schumacher, 2019). WEP averaged to 0.5032 ± 0.0178g per pseudocarp. It varied from 0.1568 to 1.2351g (CV = 40.5%). This parameter was positively-skewed and leptokurtic (Table 1). WEP increased with the pseudocarp size (PW) ($r = 0.714$, $F = 134.18$, $p < 0.001$) and related through the following equation.

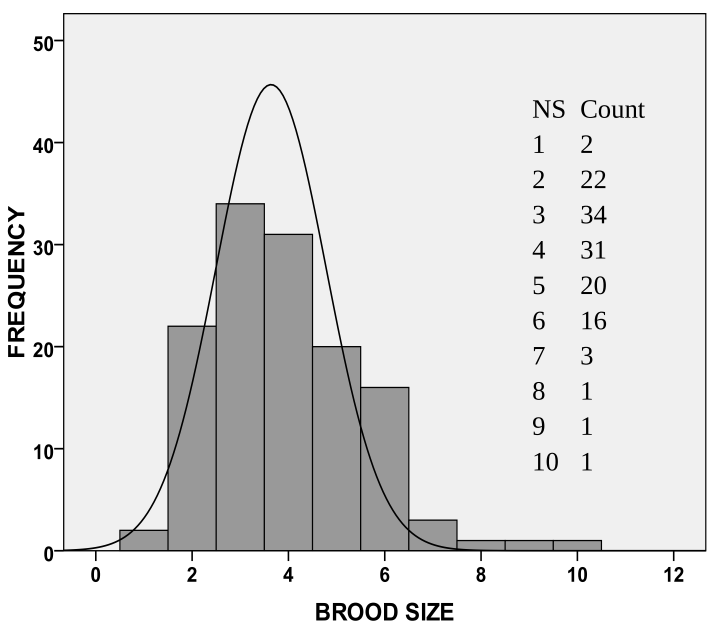
$$WEP\ (g) = 0.105 + 0.270\ PW\ (g) \pm 0.143$$



PSEUDOCARP WEIGHT (g)

N = 131
Mean = 1.4726 g
SE = 0.047096
Median = 1.4204
CV = 39.96%
Skewness = 0.504
SE skewness (Sg1) = 0.211
Kurtosis (Sg2) = 0.082
Sg2 = 0.420
Minimum = 0.4156
Maximum = 3.1109
K-S-test * = 0.088
P < 0.0.015
Shapiro-Wilk = 0.977
P < 0.026

Fig. 4. Frequency distribution of individual pseudocarp weight (g) of *C. praecox.* *, Kolmogorov-Smirnoff test with Lilliefors correction.



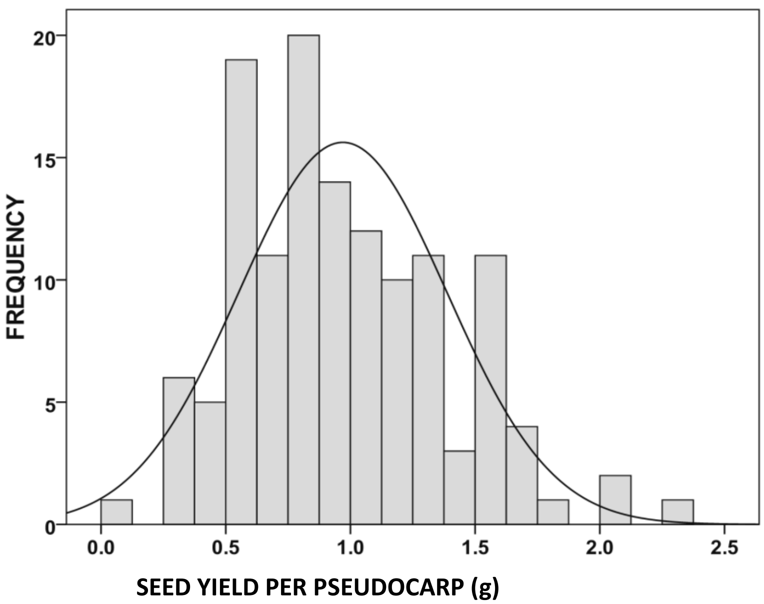
BROOD SIZE

N = 131
Mean =3.94
SE = 0.139
Median = 4.00
CV = 40.28%
Skewness= 0.8940
SE skewness (Sg1) = 0.212
Kurtosis = 1.171
SE kurtosis (Sg2) = 0.420
Minimum = 1
Maximum = 10
K-S test * = 0.166
P < 0.0001)
Shapiro-Wilk = 0.923
P < 0.0001)

Fig. 5. Frequency distribution of brood size (NAP). *, Kolmogorov-Smirnoff test with Lilliefors correction.

Seed yield per pseudocarp

Seed yield varied considerably from as low as 0.1142 per pseudocarp to 2.3201g per pseudocarp averaging 0.9695 ± 0.0365g (CV = 43.12%). The parameter deviated significantly from normal distribution (Fig. 6). Seed yield from 76.3% of the pseudocarps varied between 0.5 to 1.5g per pseudocarp.



SEED YIELD (g)
per pseudocarp

N = 131
Mean = 0.96951
SE = 0.03653
Median = 0.8871
CV = 43.12%
Skewness = 0.604
SE skewness (Sg1) = 0.212
Kurtosis = 0.189
SE kurtosis (Sg2) = 0.420
Minimum = 0.1142
Maximum = 2.3201
K-S test * = 0.082
P < 0.031)
Shapiro-Wilk = 0.972
P < 0.0019)

Fig. 6. Frequency distribution of seed yield (g) per pseudocarp. *, Kolmogorov-Smirnoff test with Lilliefors correction.

Individual Seed weight (pooled sample)

The weight of individual seed in the pooled sample of 468 seeds was negatively skewed (g1 = - 0.859, Sg1 = 0.113) and leptokurtic (g2 = 1.9701, Sg2 = 0.225) – deviating from the normality significantly (Fig. 7). *C. praecox* produced smaller seeds than expected from normal distribution of seed weight as also reported in *Purshia tridentata* (Krannitz, 1997).

Seed weight in *C. praecox* averaged to 245.52 ± 2.688mg varying around 23.73%. Around 82.9% of the seeds belonged to the seed weight size class of 201 – 300mg. The average seed weight of 0.2455 ± 0.002688g was found not to be significantly different from the grand mean value of MSSW (Table 2) of 0.2463 ± 0.00415g (t = 0.1627, NS).

Intraspecific variation in seed mass is common in tropical species (Janzen, 1977; Foster and Janson, 1985; Khan *et al.*, 1984; Murali, 1997; Marshall, 1986; Upadhaya *et al.*, 2007) and even cultivars of a species (Khan *et al.*, 2018). The variation may be many-fold in magnitude (Zhang and Maun, 1990). 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.

Similar to *C. praecox*, the distribution of seed weight was also found to be negatively skewed and leptokurtic in *Delonix regia* (Khan and Sahito, 2013) and *C. fistula* (Khan and Zaki, 2012). In another publication, seed weight distribution was reported to be normal in six cultivars of sunflower and skewed in three cultivars (Khan *et al.*, 2011). Zhang (1998) has reported seed mass variation in *Aeschynomene americana* from its 72 populations to be normally distributed in 9, positively skewed significantly (p < 0.05) in 14 and negatively skewed in 49 populations. Seed weight is known to vary within a species with site quality (Busso and Perryman, 2005).

The variation in seed size may be the result of many factors (Fenner and Thompson, 2005; Wulff, 1986). 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). 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). 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. It has been suggested that producing seeds of different sizes can be an evolutionary stable strategy in spatially or temporally heterogeneous habitats (Geritz, 1995). Alonso-Blanco *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.

The variation in weight of seed of *C. praecox* was observed to be lesser (CV: 23.09%) than that of the brood size (CV: 40.28%). It is in agreement with Harper's (1961) contention that there is lesser variation in seed size than the seed number. It has strongly been supported by Smith and Fretwell's (1974) model of resource optimization, according to which parents maximize their fitness producing seed with a homogenous optimal size. Variation around the optimal size within an individual or a population may, however, be related to variation in parental size or quality of resources (McGinley, 1988), physiological, developmental or morphological constraints (McGinley *et. al*., 1987), parent offspring conflict and sibling rivalry (Uma Shankar *et al*., 1988; Ganeshaiah and Uma Shankar, 1988; Ganeshaih and Uma Shaanker, 2003). Since Smith-Fretwell model 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 as well extended to fruits of an individual tree where different optima may occur for different fruits produced on a tree as may be postulated here from the high degree of variation of mean single seed weight (MSSW) amongst the pseudocarps of an individual tree of *C. praecox*. A reproductive potential of a pseudocarp obviously should be a function of its developmental history based on both its external and internal environments (Khan and Sahito, 2013).

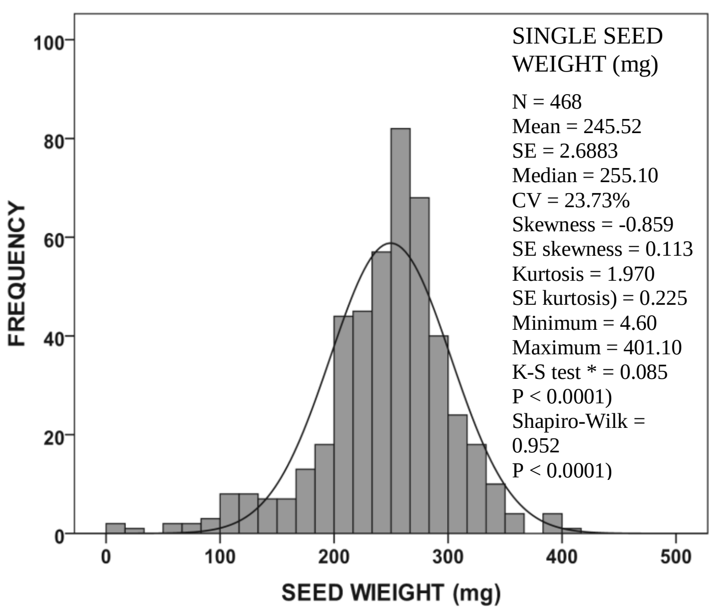


Table 1. Seed length and width (mm).

Statistics	Length	Width
N	55	55
Mean	12.91	5.191
SE	0.2464	0.1127
g1	-0.730	-0.736
g2	-0.126	0.413
Minimum	8.50	2.5
Maximum	1.60	6.5
K-S test *	0.164	0.930
p	0.0001	0.003
Shapiro-Wilk	0.179	0.898
p	0.0001	0.0001

*, Kolmogorov-Smirnoff test with Lilliefors correction.

Fig. 7. Frequency distribution of single seed weight (mg). *, Kolmogorov-Smirnoff test with Lilliefors correction.

Mean Single seed weight (MSSW) for pseudocarps

The parameter of MSSW for 131 pseudocarps is presented in Table 2 and Fig. 8. It varied around 19.31% (from 0.089 to 0.390g). This parameter scattered around the grand mean value (0.2463 ± 0.00415g) in an interesting manner. MSSW was lower than the grand mean in 48.85% pseudocarps and higher than the grand mean in 48.09% pseudocarps. In 3.05% of the pseudocarps, MSSW was equal to the grand mean value. Khan *et al.* (2108) have also reported considerable variation in mean single seed weight in pods of *Vachellia nilotica* ssp. *indica.*

The parameter exhibited slightly negatively-skewed distribution with mesokurtosis (Table 2). Kolmogorov-Smirnoff test with Lilliefors correction indicated that the variable significantly deviated from normal distribution where as Shapiro-Wilk test ranked the parameter to be normally distributed (Table 1).

The seed size variation amongst the seeds of a pseudocarp, as determined by coefficient of variability amongst the seed weights (CV, %) for 129 pseudocarps containing not less than two seeds (excluding single-seeded pseudocarps) was variable from pseudocarp to pseudocarp substantially (Fig. 9). The distribution of such variation in 129 pseudocarps exhibited positive skewness and high leptokurtosis with an average CV value of 16.0 ± 1.385%. A size class of 5-10% was, however, the modal class. The mean magnitudes of CV for pseudocarps classified according to brood size (1-10) presented no clear-cut pattern except that the magnitude of CV for pseudocarps with highest number of seeds (10 seeds per pseudocarp) was significantly higher (Fig. 10). It appears that packaging of more than 9 seeds per pseudocarp causes more variation amongst the seed sizes presumably due to the increased competitive intensity among the developing seeds and differential availability of nutrients to the seeds. Within-fruit seed weight variation is reported in several species (Stanton, 1984; Mendez, 1997).

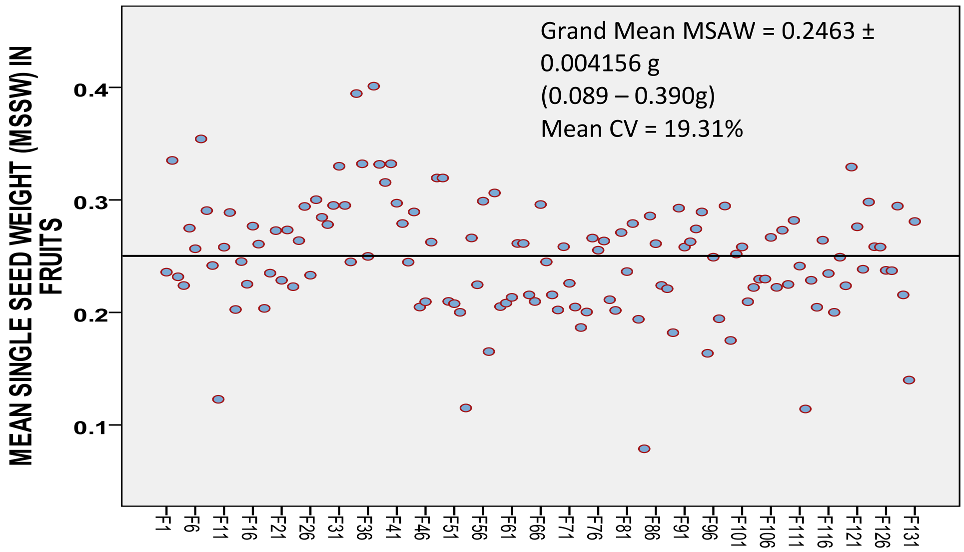


Fig. 8. Mean single seed weight (MSSW) for 131 pseudocarps of *C. praecox.* The horizontal line represents the grand mean value. The brood size varied from 1 to 10.

In our studies the MSSW in a pseudocarp correlated significantly positively with pseudocarp size in a power law model (Fig. 11) in form of best fit equation, MSSW = 0.222.pseudocarp weight $^{0.263}$ ± 0.188. That is to say that larger the pseudocarp weight, larger is the Mean single seed weight in a *C. praecox* pseudocarp.

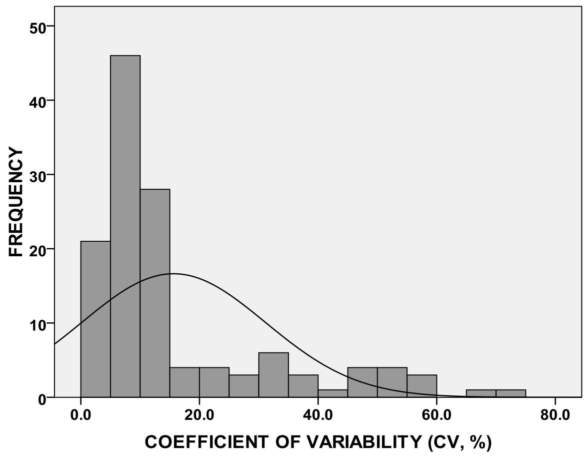
Table 2 Location and dispersion parameters of pericarp mass (g), Mean single seed weight in a pseudocarp (MSSW), and seed packaging costs (SPC1 and SPC2) in *C. praecox*.

Parameter	WEP (g)	MSSW (g)	SPC_1(g)*	SPC_2 (g) **
N	131	131	131	131
Mean	0.5032	0.2463	0.1417 67	0.59326
SE of Mean	0.017804	0.0041553	0.005663	0.028262
Median	0.4764	0.253020	0.12167	0.49127
CV (%)	40.50	19.31	45.75	54.52
Skewness	1.277	-0.392	1.034	2.683
SE of skewness	0.212	0.212	0.212	0.212
Kurtosis	2.303	0.414	0.297	0.11996
SE of Kurtosis	0.420	0.420	0.420	0.420
Minimum	0.1568	0.1019	0.0305	0.1849
Maximum	1.2351	0.3769	0.3045	2.6392
K-S test*** (p)	0.120(0.0001)	0.087(0.017)	0.148 0.0001)	0.182(0.0001)
Shapiro-Wilk test (p)	0.914(0.0001)	0.984(0.126)	0.896(0.0001)	0.769(0.0001)

*, SPC1 (g pericarp per seed); **, SPC2, g pericarp per g seeds; ***, Kolmogorov-Smirnoff test with Lilliefors correction.

Comparison of phenotypic traits of *C. praecox* from Zhejiang and Sichuan provinces

Comparing the phenotypic traits' quantitative data from four *C. praecox* populations from Zhejiang province (Huicong *et al.*, 2018) with that of the Chengdu sample from Sichuan province (the present study) (Table 3), it was apparent that pseudocarps were larger in length but lesser in width in Zhejiang as compared to the Chengdu sample. This was probably the reason that pseudocarp weight was not significantly different at two places. The seed length and width were shorter in Sichuan sample than Zhejiang. However, the brood size was significantly larger in Sichuan sample and probably it was the reason for lower mean single seed weight in Sichuan.



N = 129
Mean = 16.008
SE = 1.38495
Median = 9.86
SD = 15.73
Skewness (g1) = 1.744
SE Skewness (Sg1) = 0.213
Kurtosis = 2.283
SE Kurtosis (Sg2) = 0.423
Minimum = 0.20
Maximum = 73.15
K-S t* = 0.274
$p < 0.0001$
Shapiro-Wilk = 0.751
$p < 0.0001$

Fig. 9. Distribution of magnitude of CV (%) among weights of seeds produced in the pseudocarps (N = 129) of *C. praecox* excluding the pseudocarps containing one seeds only.

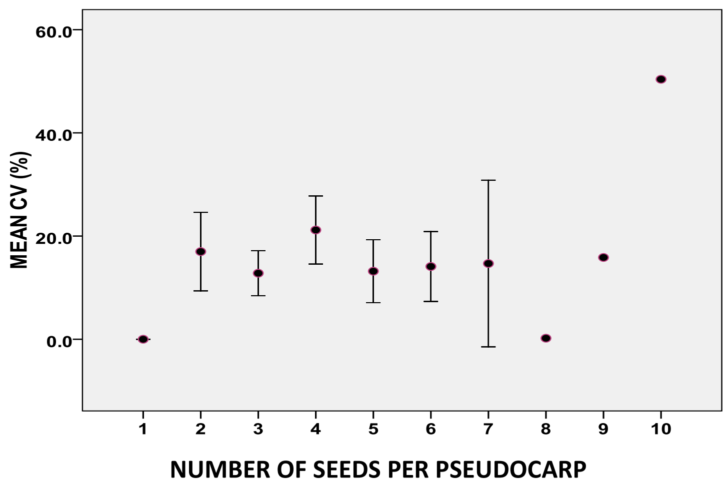


Fig. 10. Variability of weights of seeds produced in a pseudocarp (N=131pseudocarps) of *C. praecox* in terms of coefficient of variability (CV %) as the function of the brood size.

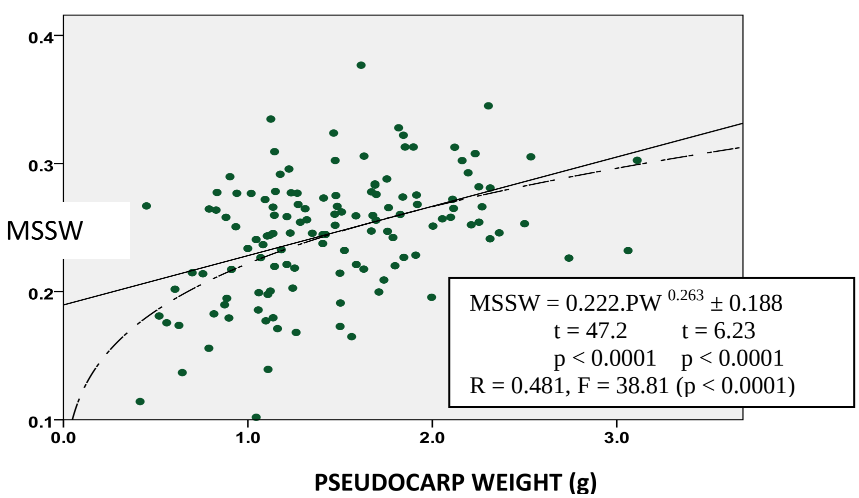


Fig. 11. Relationship of MSSW in pseudocarp with pseudocarp size. The above-given power model was the best fit model than the linear model:

MSSW (g) = 0.190 + 0.038 Pseudocarp weight ± 0.43

(R = 0.436, F = 30.23($p < 0.0001$), N =131.

Table 3. Comparative account of phenotypic traits of pseudocarps and seeds of *C. praecox* from the Province of Zhejiang* (mean of four populations) and Chengdu sample from Sichuan province of China.

Data of Huicong *et al.* (2018)		Data of the present study	
Phenotypic trait	Zhejiang province	Traits	Chengdu sample (Sichuan province)
Fruit length (mm)	49.82 ± 3.30 (CV= 18.92%)	P Length, mm	29. 3 ± 0.02634 (28.42%) (t = 6.22) *
Fruit width (mm)	15.76 ± 0.70 (CV = 18.72%)	P width, mm	17.9 ± 0.0400 (CV = 25.99%) (t = 3.01)*
Fruit weight (g)	1.48 ± 0.23 (CV = 41.05%)	PW (g)	1.4726 ± 0.04704 (CV = 39.96%) (t = 0.030) NS
Pericarp weight (g) per Fruit **	0.58 ± 0.05 (CV = 36.37%)	WEP (g)	0.5032 ± 0.01780 (CV = 40.50%) (t = 1.45) NS
Seed length (mm)	15.48 ± 0.42 (CV = 7.25 %)	Seed length, mm	12.90 ± 0.2464 (CV: 14.32%) (t =5.62) *
Seed width (mm)	6.94 ± 0.16 (CV = 7.72%)	Seed width, mm	5.191 ± 0.1127 (CV: 16.24%) (t = 10.46) *
1000-grain wt. (g)	325.24 ± 11.43 (CV = 6.67%)	-	Not determined
Number of seeds per fruit	2.70 ± 0.50 (CV = 45.72%)	NSP	3.94 ± 0.139 (CV = 40.28%) (t = 2.39) *
Single seed wt. (g)	0.325 ±0.0114	Weight of a seed (g)	0.2455 ± 0.02688 (CV = 23.69%) (t = 2.52) *
Seed yield per fruit (g)	-	SYP (g)	0.9695 ± 0.03653 (CV = 43.12%)
-	-	SPC_1 (g)	0.1417 ± 0.00566 (CV = 54.52%)
-	-	SPC_2 (g)	0.5933 ± 0.02826 (CV = 54.52%)
SPC (g) calculated***	0.2148	-	-

*, Significant at least at $p < 0.05$). **, We saw Chinese version of Huicong *et al.* (2018) publication; we do not know how they have defined pericarp? In our studies we have determined the weights of empty pseudocarps (WEP) to calculate SPC_1 and SPC_2. Acronyms: P length = Pseudocarp length; P width = Pseudocarp width; PW = Pseudocarp weight (inclusive seeds); WEP = Weight of empty pseudocarp; NSP = number of seeds per pseudocarp; SYP = Seed yield per pseudocarp and seed packaging costs, SPC1 = WEP / NSP and SPC_2 = WEP / SYP. ***, Seed packaging cost calculated from Huicong *et al*. data as pericarp weight per fruit (g) / Number of seeds per fruit.

Table 4. Values of Spearman Rank correlation (rho) amongst the phenotypic traits of pseudocarps and seeds of *C. praecox*.

	Pseudocarp weight.						**Spearman 'rho'**
WEP	**0.746**	WEP					
SPC_1	-0.131	**0.440**	SPC_1				
SPC_2	**-0.385**	**0.271**	**0.880**	SPC_2			
NSP	**0.806**	**0.380**	**-0.606**	**- 0.667**	NSP		
MSSW	**0.428**	**0.389**	**0.357**	-0.057	- 0.023	MSSW	
SYP	**0.934**	**0.495**	**-0.413**	**-0.665**	**0.889**	**0.384**	(SYP)

Rho values in bold are significant at least at $p < 0.05$.

The calculated seed packaging cost of 0.2148 g per fruit in Zhejiang from Huicong *et al.* (2018) data was significantly higher than that in Chengdu sample – probably due to the fact that brood size tended to associate with seed packaging cost significantly negatively in this species. The provinces of Sichuan and Zhejiang both have more or less similar climate of cfa type according to Koppen-Geiger's (1954) system of world climate. Zhejiang is a coastal mostly hilly (700-1929m altitude) southeastern province (facing East China Sea) of monsoon season, temperate climate, abundant sunshine and rainfall (1000-1043 mm), humidity and typhoons. Annual temperature is 18^{o}C. The average temperature is 27-30^{o}C in July and 2-8^{o}C in January. Temperature in the month of November is 9-16 ^{o}C in Chengdu and 9-17^{o}C in near Hangzhou (Zhejiang) (www.chinahighlights.com). Sichuan is, however, inland province quite far from Zhejiang (c 1500 km). Chengdu is around 685 miles from the Sea receiving c 991 mm rains annually. The differences in phenotypic traits quantitatively may presumably be more due to environmentally- (edaphically-) or genetically-induced variations in reproductive phenotype than climatic and geographic reasons.

Correlations amongst the phenotypic traits of pseudocarp and seeds

Like Huicong *et al.* (2018), a substantial degree of multi-colinearity was observed amongst the phenotypic traits of *C. praecox* from Chengdu on the basis of Spearman rank correlation (rho) given in Table 4. In our data, maximum degree of positive correlation existed between the brood size and seed yield per pseudocarp (SYP) (rho = 0.889). Similar order of positive correlation was explicit between the two parameters of seed packaging cost, SPC_1 and SPC_2 (rho = 0.880) and between brood size and pseudocarp size (rho = 0.800). WEP exhibited significant positive association with PW, the pseudocarp weight (rho = 0.740). WEP showed no correlation with SPC_1 but substantial correlation with SPC_2 (rho = 0.385) and almost similar order of correlation with MSSW (rho = 0.389).

MSSW is known to vary with brood size in soapnut wherein although seed yield per fruit increased with brood size, the MSSW declined with brood size (Khan, 2018). No such correlation between mean single seed weight with brood size was, however, found in *Erythrina suberosa* (Khan *et al.*, 2014). Insignificant correlation of MSSW with NSP (brood size) in *C. praecox* suggests that variation in seed weights of seeds in a pseudocarp is not as a result of seed number- seed size trade-off in this species. It may be due to some other factor (s) owing to internal or external environment of the plant. MSSW, however, related with pseudocarp size positively.

Seed packaging cost

The seed packaging cost determined on the basis of per seed (SPC_1) and per g seeds (SPC_2) are presented in Table 1. SPC_1 averaged to 0.1417 ± 0.00566g per seed (or 141.7 mg per seed) and SPC_2 averaged to 0.5933 ± 0.02826g per g seed or 593 mg per g seeds) with well-marked variation in magnitude, 45.75 and 54.52 %, respectively. These parameters related highly significantly with each other through following equation with an explanatory power of 73.96%.

$SPC_1 = 0.024 + 0.202\ SPC_2 \pm 0.033$
$t = 3.46 \quad t = 18.67$
$p < 0.001 \quad p < 0.001$; N = 131; r = 0.860, F =359.80 ($p < 0.001$)

Seed packaging cost has been studied by several workers (Willson *et al.*, 1990; Khan and Zaki, 2012; Khan and Sahito, 2013a & b; Khan *et al*., 2013, 2016; 2018). Variation in seed packaging cost in *C. praecox* is in agreement with the fact that seed packaging coast is found to vary not only from species to species but also from fruit to fruit. Willson *et al.* (1990) had recorded a marked variation in average seed packaging investment amongst 28 species surveyed. *Cassia fasciculata* included in their study showed SPC per seed to be 76.47 ± 1.89 mg per seed. Mehlman (1993) also reported SPC to vary significantly in pods of *Baptisia lanceolata.* Seed packaging investment across 62 species of 35 families from China is also shown to vary among species (Chen *et al.*, 2010). The lowest cost was 0.065 mg per seed in *Dicroa febrifuga* (Family Saxifragaceae) and highest 1124.897 mg / seed for *Vernicia fordi* (Family Euphorbiaceae). Highest packaging investment is, however, presented by Willson *et al.* (1990) in case of *Asimina triloba* to be 13,101 mg per seed. Afsar uddin (2012) has reported the packaging investments in dehiscent type of pods of *A. lebbeck* (2327.0 mg per g seed and 281 mg per seed) and *L. leucocephala* (826.0 mg per g seed and 32 mg per seed) and in schizocarpic pods of *A. nilotica* (1725 mg per g seed and 205 mg per seed). SPC is not only species specific but also varies from fruit to fruit even in case of a single individual of a species. It signifies the importance of the environmental history of the fruits at individual level. Khan *et al.* (2016) found much more variation in SPC_1 and SPC_2 within the mother plants as compared to that amongst the mother plants of *Leucaena leucocephala* in Karachi.

The three pseudocarp-related parameters (WEP, SYP and NSP) related significantly with SPC_1 and SPC_2 - WEP related positively and brood size (NSP) and Seed yield per pseudocarp (SYP) related negatively (Eq. 1-6). Equation 7 and 8 represent the multiple linear regressions of seed packaging cost with the three parameters. Clearly, variation in SPC_1 was best accounted for by the linear combination of three independent variables more closely (R = 0.931, Eq. 7).

$SPC_1 = 0.228 – 0.022$ Brood size ± 0.0548, r = - 0.537, F = 52.39 ($p < 0.0001$)……… ……. Eq.1
$SPC_2 = 0.997 – 0.103$ Brood size ± 0.2805, r = - 0.504, F = 43.82 ($p < 0.0001$) ……… …….Eq.2

$SPC_1 = 0.195 – 0.056$ Seed yield ± 0.0600, r = - 0.359, F = 18.83 ($p < 0.0001$) ……. …….. Eq.3
$SPC_2 = 0.924 - 0.353$ Seed yield ± 0.230, r = - 0.535, F = 50.92 ($p < 0.0001$)….……………. Eq.4

$SPC_1 = 0.045 + 0.188$ WEP ± 0.051, r = 0. 600, F = 71.42 ($p < 0.0001$)…………… ………. Eq.5
$SPC_2 = 0.263 + 0.615$ WEP ± 0.241, r = 0.460, F = 34.77 ($p < 0.0001$) …………... …… ….Eq.6

$SPC_1 = 0.139 – 0.026$ Brood size – 0.026 Seed yield + 0.263 WEP ± 0.2394 ………….. ……........Eq.7
t = 20.41 t = - 8.887 t = - 2.235 t = 22.648
$p < 0.001$ $p < 0.0001$ $p < 0.027$ $p < 0.0001$,
N = 131, R = 0.931, F = 275.25 ($p < 0.0001$)

$SPC_2 = 0.651 + 0.024$ Brood size – 0.725 Seed yield + 1.102 WEP ± 0.1877 ………………… Eq.8
t = 12.20 t = 1.02 t = -7.84 t = 12.11
$P < 0.0001$ $p < 0.308$ (NS) $p < 0.0001$ $p < 0.0001$
N = 131, R = 0.819, F = 86.35 ($p < 0.0001$)
(Note the insignificance of brood size in this equation)

In case of SPC_2, brood size appeared to be an insignificant parameter in defining variation in SPC_2 when viewed through linear combination of three independent variables ($t = 1.02$, $p < 0.308$, NS) (Eq. 8). It appears that in spite of significant correlative association of SPC_1 with SPC_2, the packaging parameter SPC_1 appears to be somewhat better parameter than SPC_2. The differential behaviour of SPC_1 and SPC_2 may probably be attributed to the fact that these parameters were related to the brood size better through power law models (Fig. 11 and 12) than in linear fashion.

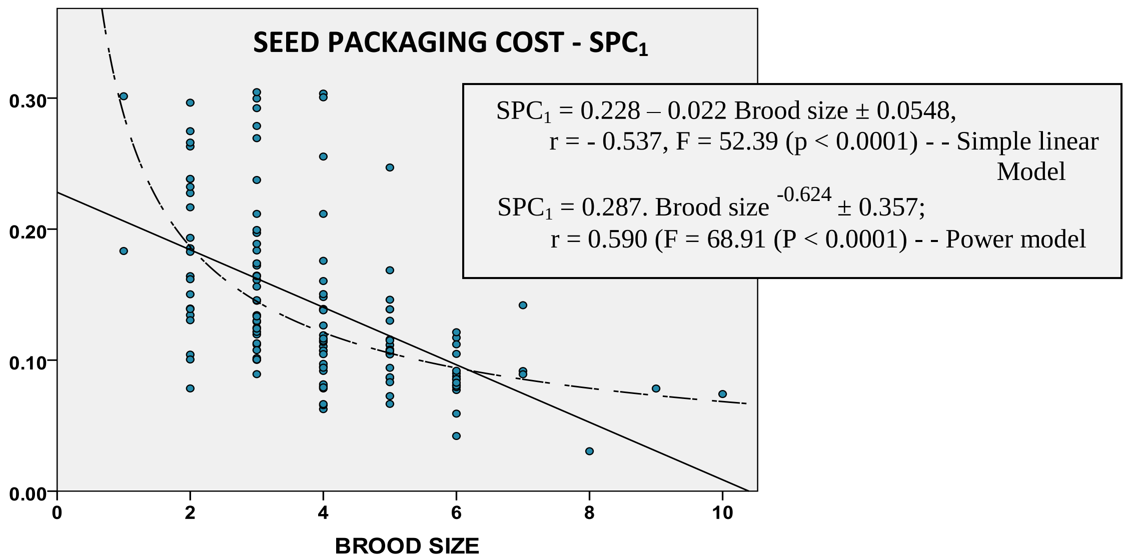


Fig. 11. Relationship of SPC_1 with brood size employing simple linear and power model of curve fitting.

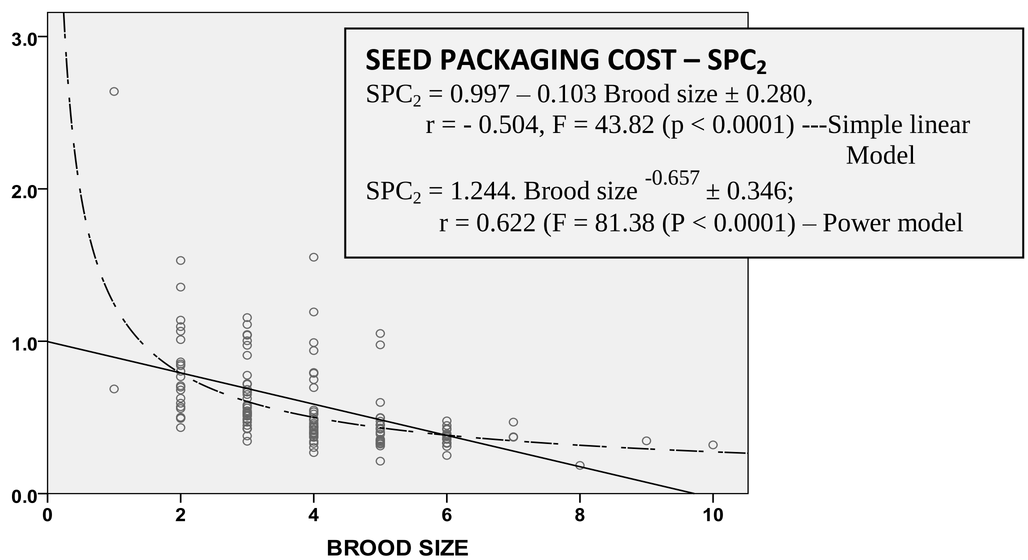


Fig.12. Relationship of SPC_2 with brood size employing simple linear and power model of curve fitting.

In short, on the basis of the results obtained here, there appears rich variation in phenotypic traits of pseudocarps and seeds of *C. praecox* from the Chengdu (Sichuan) sample which is in agreement with Huicong *et al.* (2018) who suggested that there is rich phenotypic variation in fruits (pseudocarps) and seeds traits in wild populations of *C. praecox* in Zhejiang Province. Huicong *et al.* (2018) reported that in Zhejiang the studied four populations of *C. praecox* formed two clusters when extracted on the basis of Euclidean distances calculated with fruits and seeds phenotypic traits. The populations of Fuyong (Longwangkan, Bidongshan and Wujian) agglomerated together showing below 10% dissimilarity whereas the population of Fangshan of Lin'an stood separate at much higher Euclidean distance. Within population variation was found by them to be more predominant variation and hereditary character appeared to the main factor of phenotypic trait variation. The phenotypic traits variation was found to associate with the synthesis action of multiple soil factors also. More intensive studies are, however, needed to further elucidate the reproductive ecology of this species.

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