



Exploratory Studies on Occurrence and Potential Benefits of High Fruit Yielding Reproductive Phenophasic Variants of *Pithecellobium dulce* in Augmenting Fruit Based Semi-arid Agro Forestry Systems

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background: *Pithecellobium dulce* is a multipurpose legume tree species of high ecological significance. Several reports indicate existence of huge genetic diversity within the natural population of the species. Various morphological parameters have been used to assess intra species genetic diversity, however, there is no report available so far on diversities in reproductive phenology of *P. dulce* of semi-arid or arid climate.

Methods: The present paper is based on assessment of diversity in flowering phenophases through construction of phenological calendar incorporating temporally observable morpho-phenotypic descriptors for reproductive behaviour of individual trees comprising natural population of the species at designated study sites during a period of three years. The study was carried out during September, 2015 and March, 2019 at Ajmer, Rajasthan, India. Data were periodically obtained and statistically analyzed.

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Results: Majority of trees of flowering stage (89.94%) exhibited normal or winter blooms in which flowering was initiated in almost synchronized manner during December; 10.05% of normal blooming trees inconsistently showed additional early or autumn blooms during either September or October or November; 2.58% of trees exhibited early blooms in which flowering was consistently initiated in the month of September in addition to normal blooms. The overall production and availability of fresh fruits in early-cum-normal episodic blooming trees were greater as compared to only normal blooming trees. Though, the numerical fruit productivity in winter blooms was superior to autumn, the pomometric characteristics were inferior in former in early-cum-normal episodic blooming trees.

Conclusion: The methodology is a novel experimental approach to assess diversities in reproductive phenology of seed raised population of high value fruit bearing economically important tree species. The present work explores agronomically valuable variants of *P. dulce* and also reports causes of appearance of persistent fruits and sporadic *in-situ* germination of seeds. Based on above findings, the paper also makes evolutionary predictions that how a shift in flowering phenology of *P. dulce* as exhibited by the elite variants could have long-term implications for reshaping of future landscapes of semi-arid or arid climate. Conclusively, Winter to autumn phenophasic shift in flowering in *P. dulce* is economically productive, evolutionarily progressive, and environmentally well cued partial retrieval under semi-arid conditions. Moreover, the phenophasic variants could serve as candidate plus trees that deserve out of the season floral management and clonal propagation, thereby the species may get a fillip to status up gradation from minor fruits to major fruit category.

Keywords: Phenology; *Pithecellobium dulce*; genetic diversity; fruit persistence; semi-arid.

1. INTRODUCTION

Pithecellobium dulce (Roxb.) Benth. belongs to the sub-family Mimosoideae of family Fabaceae. The species is native to the tropical America (Mexico, USA, Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Surinam, Uruguay and Venezuela), but has now become naturalized in India, China, Cambodia, Malaysia, Myanmar, Philippines, Puerto Rico, Sudan, Tanzania, Thailand, Vietnam and cultivated there as economic and ornamental trees [1,2]. In India, *P. dulce* is present throughout the country except Jammu and Kashmir, Himachal Pradesh, Sikkim, Arunachal Pradesh, Assam, Nagaland, Meghalaya, Manipur, Tripura and Mizoram [3]. The species have wide ecological amplitude with respect to soil types and annual rainfall, in India it grows well in semi-arid regions characterized by mean monthly temperatures ranging from 7 to 8°C in January to 40 to 42°C in May and June. It is generally considered heat and drought resistant tree world over [4]. *P. dulce* is a medium-sized fast growing tree commonly attaining a height up to 10-15 metres. Branching pattern is irregular and leaves are bi-pinnate with thin spines in pairs at the base of leaves that range from 2 to 15 mm in length. The growth of new leaves coincides with the loss of old leaves which give the tree an evergreen appearance. Inflorescences are panicles that differentiate

axillarily or terminally from the leafy branches of the tree. Flowers are borne on short panicles 10 to 20 cm in length, often as terminal compound inflorescences. Each panicle is composed of 20 to 30 floral units potentially capable of fertilization. Individual flowers are greenish-white, sessile present in small stalked heads consisting of 10-15 floral buds; calyx and corolla small, single thread like tubular pistil longer than stamens, pink in colour with capitate stigma; and stamens are fifty in numbers that give hairy appearance. Because of nitrogen fixing ability and high ecological values, *P. dulce* trees are often planted on waste land for reclamation, shelter belt plants and roadside ornamentals [5,6]. They are extensively planted for production of protein rich live stock fodder and fuel wood in Asia, Latin America and Africa [7,8,9]. *P. dulce* has been designated as minor fruit trees and considered one of the most suitable trees for alley cropping. Fruits are plump, constricted between the seeds, spirally coiled and spongy white arils surround 5 to 12 shiny black seeds within the pod. Arils are sweet and acidic in taste so consumed by birds which also disperse seeds in the process of eating [10]. The seed contains 20% oil which is used in soap industries, refined seed oil is edible. Flowers attract honey bees and significantly contribute to honey making. Trees have great potential for rearing lac insects. In addition to ecological significance of trees and economic values of fruits, *P. dulce* has been

reported to possess many pharmacological properties and clinical applications that include *inter alia* Pitheduloside extracted from the plant are anti-inflammatory, anti-microbial, anti-tubercular, spermicidal, protease inhibitor, anti-venom and abortifacient [11,12]. *P. dulce* extracts have been demonstrated to exhibit synergistic actions in combination with conventional allopathic antibiotics in checking bacterial infections thereby ameliorating the side effects of antibiotics [13,14]. In folklore, *P. dulce* is being used to cure leprosy, venereal diseases, peptic ulcers, sores, toothache, convulsions, dyspepsia and dysentery [15]. Leaf extract has been used to relieve pain, convulsions, indigestion and diabetes. Arils are being used in preparation of beverages. Extracts of fruits, seeds, leaves, and bark have been demonstrated to possess antioxidant properties potentially capable of curing ulcers, cancers and gastrointestinal ailments [16,17,18,19,20]. In view of vast utilities of *P. dulce*, it is pertinent to explore, identify, and select the variations with superior agronomic traits from the population. Reports are available on genetic diversity in *Pithecellobium elegans* [21], as well as selections of agronomically desirable genotypic variants of *P. dulce* from natural population with large pods containing small seeds and sweet less astringent arils, and variegated plants with green and white leaves. They are being clonally propagated and grown in gardens in Thailand, Philippines, and Hawaii [22,23]. Quite recently, Goyal et al., 2014 [24], have made assessment of genetic diversity in *P. dulce* germplasm of Rajasthan state using RAPD and ISSR markers. Their findings indicate a high level of morphological and genetic diversity in *P. dulce*. Their selections were based on the phenotypic assessment of various characters viz., branching habit, branching pattern, canopy size, number of primary branches, colour of bark, height of tree, leaf shape, number of flowers per inflorescence, pod size, number of seeds per fruit, leaf size and percent seed germination. There has been no report so far on the diversity in flowering and fruiting phenology of the species. Therefore, the present paper attempts to identify agronomically advantageous variations in flowering-fruiting phenophases of *P. dulce* growing in semi-arid climate of Ajmer (RIE campus, Nag Pahar hillocks and along Ajmer-Pushkar road side) with prime objective to enhance production and prolong the availability of fruits respectively. Ecological phenomena of persistence of fruits throughout the year and sporadic *in situ* germination of seeds under specific

environmental conditions have also been studied. Undoubtedly, such exploration and selections would help plant breeders to design appropriate strategies for multiplication and conservation of the elite variants or germplasm and also pave the way for further genetic improvement of cultivars. Additionally, these phenophasic variants could serve as potential candidate plus trees for out of the season floral management through biotechnical interventions. If successfully employed the species may get a fillip to status up gradation from minor fruits to major fruit category. Nevertheless, the greater understanding of diversity in reproductive strategies of *P. dulce* in conjunction with time and environmental dynamics would provide better insights into predictive evolutionary processes that might impact on future reshaping of semi-arid or arid landscapes.

2. MATERIALS AND METHODS

Three study sites of naturally growing populations of *P. dulce* were identified within 10-15 kilometres range at Ajmer, Rajasthan, India. Study sites: RIE campus, Nag Pahar hillocks and Ajmer-Pushkar road side barren land were marked as site – I, II and III respectively. Phytosociological survey was initially conducted at all study sites to identify and enlist species of tree communities, species of *Pithecellobium* and numbers of individuals of *P. dulce* during August-September, 2015 at all study sites. Periodical observations were made during August 2015 and December 2018 to collect data on phenology of *P. dulce* with particular focus on flowering and fruiting events to explore diversity in phenophases with respect to onset and completion of flower initiation, flowering continuum, fruit initiation, fruit maturation and fruit ripening. Annual records of number of trees with persistent fruits and number of persistent fruits per trees were also maintained for all study sites during the period under study. Phenological observations on flowering frequencies of all tree species constituting the plant community of the habitat under study were also made to classify four levels of species: Continual species, episodic or sub-annual species, annual species, and supra-annual species.

Data pertaining to comparative account of two blooms, viz., early or autumn and normal or winter with respect to number of branches bearing flowers, position of inflorescence on trees, number of inflorescences per branch, number of floral units per inflorescence, number

of fruits per inflorescence, number of seeds per pod, average length and diameter of fruits, and degree of synchrony in flowering of elite variants were separately maintained and statistically analysed and presented in tabular forms. Data recording on flowers necessitates elaboration on structure of inflorescences to conceptualize experimental use of floral units equivalent to flowers potentially capable of fertilization. Inflorescences are panicles that differentiate axillarily or terminally from the leafy branches. Each panicle produces small clusters of 2 to 5 heads all along its length of 10-20 cm, at the distance of 1.0 cm in spiral manner. Clustered heads originate from a single point asynchronously. Each head consists of about 10 to 20 small floral buds connected to the point of origin through a stalk. All buds of a head open synchronously, therefore, anthesis is of long duration when about fifty of hair like stamens in a flower grows further along with long thread like single pink coloured pistil. So, clustered flowers give hairy appearance with fragrance and extra floral nectaries attract honey bees and other insect pollinators. If all floral buds of all clustered heads of each inflorescence are counted before anthesis, about 3000-4000 flowers should have been produced in each inflorescence. However, fruit setting in each inflorescence never exceeded 10-15 in our observations thereby making the fruit to flower ratio as low as 0.003. Hence, the huge numbers of flowers per inflorescence posed a big challenge in experimental data recording for numbers of flowers per inflorescence. To overcome such obstacle, we examined many samples of inflorescences from different blooms as well as trees and found that majority of floral buds constituting an inflorescence are not functional in reproduction because of absence of pistils in flowers, improper orientation of stigmas, partially drying up of long tubular pistils, non-opening and drying up of many floral buds. Data on numbers of functional flowers capable of fertilization was obtained after long observation on numbers of floral heads showing anthesis per day or a week, duration of anthesis and blossoming till fruit setting. Therefore, floral units in observation table correspond to numbers of functional flowers capable of reproduction per inflorescence. Data were also utilised in construction of flowering-fruiting phenophasic calendar of *P. dulce* for specified population during the study period. Vaucher specimens and seeds of agronomically advantageous flowering-fruiting phenophasic variants or genotypes were deposited in herbaria of Botany Department of RIE, Ajmer.

3. RESULTS AND DISCUSSION

3.1 Diversities in Flowering Fruiting Phenophases in Natural Population of *P. dulce*

Species of tropical plant communities vary considerably with respect to flowering onset timings viz., winter, spring, summer or autumn; frequency of flowering, several times in a year, or once in several years; and duration of flowering from a few days to the whole year. Thus the onset timing, duration, and frequency of flowering define reproductive phenological patterns of species which interact with each other in order to display spectacular diversities in flowering patterns what we observe in nature [25,26,27, 28]. Furthermore, phenological patterns in tropical trees particularly flowering and fruiting are determined through interactions between shortterm environmental events and evolutionary forces. Alternatively, flowering phenology is both under genetic control and is plastic to environment, that means changes in climatic conditions may trigger the expression of phenotypic responses currently hidden [29]. Different geographic locations comprise their own edaphic and climatic conditions such as soil nutrients (level of N in soil), variation in rainfall, changes in atmospheric temperature, photoperiod, irradiance and sporadic environmental events that serve to provide environmental cues in triggering floral phenological events in tropical plants [30,31,32,33,34,35,36,37,38,39,40,41,42].

Newstrom et al., 1994 [43], and Engel et al., 2005 [44], have described four levels of flowering frequency: continual species that continuously flower throughout the year, episodic species that flower more than once a year, annual species that flower once a year, and supra-annual species that flower less frequently than once a year. Based on above classification, *Cordia crenata*, *Commiphora wightii*, *Manilkara zapota*, and some varieties of *Morus alba* constitute the continual species; about 60% of the population of *Prosopis cineraria*, *Acacia nilotica*, and few individuals of *Pithecellobium dulce*, *Psidium guajava*, *Cordia dichotoma*, *Azadirachta indica*, *Grewia asiatica* constitute sub-annual or episodic species; while some cultivars of *Mangifera indica*, *Bamboos*, and *Agave americana* constitute supra-annual species; whereas remaining majority of plants constitute annual species as observed in the habitat under study. Similar observations on frequency of flowering in above mentioned species growing in different

habitats have also been reported by [45,46, 47,48,49,50]. *P. dulce* generally flowers and fruits between December to May and February to August respectively in its native habitat [2]. In Indian tropical deciduous forests, *P. dulce* flowering occurs in January and February and fruiting in April and June respectively [51]. However, a bit altered flowering and fruiting phenologies have been observed in Philippines and West Java where they flower in October-November and April-June respectively. While fruiting at these two locations occurs in January-February and July to August respectively [1]. In Puerto Rico fruiting has been observed throughout the year [2]. Therefore, *P. dulce* exhibits three levels of reproductive phenological patterns based on annual flowering frequency as annual flowering at most of the geographic locations world over, continual flowering in Puerto Rico and episodic flowering by some individuals at semi-arid habitat under study. In present study at Ajmer, India, intra species flowering-fruiting phenology at three designated study sites – I, II, III have been consistently observed during September, 2015 and December, 2018 for flower initiation, fruit initiation, maturation and ripening of fruits. About 90% of flowering individuals of natural population exhibited almost synchronous behaviour for onset of flowering during winter whereas, approximately 10% of them showed alterations. Out of 10% alterations, 7.47% alterations were not consistent with respect to floral initiation timings, 2.58% alterations were consistently observed throughout the study duration (Tables 1 & 2).

In other words, these individuals exhibited off-season flowering, i.e.; two blooms, viz., autumn and winter occur consecutively in same tree thereby the flowering and fruiting durations are prolonged (Fig. 1- A & B). They belong to the episodic or sub-annual species level based on flowering frequency as described by Newstrom et al. 1994 [43]. Similar phenophasic change in flowering of *Prosopis cineraria* or Khejari has been reported by Krishnan et al., 2015, in Indian Thar desert [49]. On account of prolonged flowering and fruiting in *P. dulce*, the overall quantitative and qualitative productivity and time duration of availability of fruits are greater in early-cum-twice blooming trees as compared to normal and single blooming trees (Fig. 3&4). However, early formed fruits during December and January are highly vulnerable to frugivores since very few other trees with fruits are available in vicinity as observed in the habitat

under study. Table 3 provides comparative account of flowering and fruiting in autumn and winter blooms of early and twice flowering individuals of *P. dulce*. It is evident that winter or normal bloom is superior to autumn or early bloom with respect to numbers of inflorescences, flowers and fruits, also winter bloom is more synchronous as compared to autumn blooms in flower initiation timing, blossoming period and fruit-setting, notwithstanding that fruits produced through autumn blooms are more robust with respect to length, diameter, and number of seeds (Fig. 1-D & Fig. 4). Rodriguez et al. 2016 [52] has reported similar observations that during flowering off-peak, episodic variants flowering for shorter periods with even less synchronicity proportionally set more fruits since they were less affected by fluctuations in biotic and abiotic factors. During the three years study period, about 17% of trees of the population of *P. dulce* remained in non-flowering state. They were not the same trees rather different trees did not flower either first or second or third year. However, about 34% of all non flowering trees showed late flowering with onset timings either in February or March. We consistently observed flowering abnormalities in most of these trees since they produced a very low number and much smaller size of inflorescences. Their flowering durations and fruit setting were also very low probably because of non availability of resources such as water, high level of air pollution or very old age of trees. Data have not been included in this paper since they serve separate topic of research. In view of the natural episodic flowering in *P. dulce*, the species is a potential candidate tree that deserve out-of-season management of flowering to avoid detrimental harsh weather conditions of April, May, and June that hamper fruit development of normal or winter blooms through biotechnical engineering in flowering phenology. Phenological engineering of reproductive behaviour of fruit trees through biotechnological interventions involving exogenous applications of plant growth regulators and adoption of appropriate agronomic practices such as pruning, trimming, and chopping of branches have been successfully employed to induce off-season flowering for enhanced fruit yield in mango [53], lychee [54], longan [55], custard apple [56], apple & sweet cherry [57], and guava [48,58,59]. It would not only increase the fruit yield rather prolong availability of fruits to take advantage of market opportunities. Genetic improvement of tree species through conventional selection methods of plant breeding is highly laborious and

time consuming. As suggested by Narayan, biotechnological *in vitro* cultures and propagation techniques can circumvent the bottlenecks through by passing the unwanted juvenile phase of tree species as performance trouble-shooter. Therefore, phenologically plus trees such as *P. dulce* need to be put under prioritised objectives of *in vitro* propagation technology in addition to endemic, threatened, endangered, and elite germplasm not only to multiply and genetically modify them but also to maintain *in vitro* biodiversity [60,61,62].

3.2 Flowering Phenophasic Shift to Autumn Could be an Evolutionary Event towards Better Establishment of *P. dulce* under Semi Arid or Arid Climate

In spite of quantitative inferiority of autumn blooms of *P. dulce* with respect to morphological descriptors of reproductive behaviour (extent of flowering and fruiting) as compared to winter blooms, pomometric characteristics of autumn bloom fruits were observed to be impressive as depicted through graphical representations in Fig. 4. Robust growth of autumn bloom fruits could be attributed to abiotic and biotic components of the prevailing environmental complex. Favourable abiotic environmental conditions of winter with respect to mild temperature, bright sun light, low or mild wind velocity, adequate moisture level in soil and plant body, and low rate of evapo-transpiration in conjunction with biotic factors such as availability of highly specialised pollinators- bees, sun birds and bulbuls that concentrate on *P. dulce* since very few flowering trees were present in vicinity; provided congenial conditions for fruit setting and development. However, concentration of frugivores was also observed which proved to be highly detrimental to developing fruits that warranted special protection to them. Moreover, brief winter shower coupled with greater monthly mean temperature during winter months were growth promoting for fruits of autumn blooms whereas, same conditions were growth retarding for flowering in winter blooms as noticed during 2016-17. In view of above observations in the light of research findings by [26,63,64] that species that flower several times a year or episodically as exhibited by some variants in present study should be more abundant than either annually or supra-annually flowering species. They postulated that multiple clutches may have been selected to reduce reproductive failure from fluctuating populations of pollinators.

Therefore, the following evolutionary predictions of future reproductive success of episodically flowering variants of *P. dulce* may prove true in course of time for the habitat of semi-arid climate currently dominated by Acacia species. A shift in flowering phenophase from winter to autumn may perhaps be the first evolutionary step of the species towards better perpetuation and establishment to the semi arid or arid climate characterized by low rainfall, high temperature, dryness of air, intense sun light and high velocity wind for most of the period in a year. According to Elberling et al., 2001 [65], and Anderson and Hill, 2002 [66] findings that in out crossing insect pollinated plants, flowering during harsh seasons (winter or early spring-December, January and February) may decrease reproduction compared with flowering during favourable periods. In consonance with their findings we noticed a long lag phase in reproductive processes after induction of flowering during December in winter blooms till mid February when pollination and fruit setting started because of increase in number of pollinators, whereas, pollination and fruit setting immediately begins with floral induction in case of autumn blooms of *P. dulce* (Table 2). Currently, fruit maturation and ripening period of winter or normal bloom, viz., April-June period is extremely harsh, hence detrimental to aril and fruit development. Despite good tastes, nutritious and high medicinal values of fruits, *P. dulce* has not gone up beyond the level of minor or underutilized fruits in many countries including India, however, the price of its fruits in Indian market is costlier than imported American apples, and cherries which range between Rs. 300 to 400 per Kg. They are also in high demand, however, the supply is abruptly constrained due to low production. Harsh climatic conditions of summer in semi-arid or arid regions adversely affect fruit development in wild habitats. Many fruits because of poor development of aril in the absence of adequate moisture level in plant body and high atmospheric temperature get dry up and become hard, in some cases that persist throughout the year attached with the parent trees (Fig. 1- E, F). Similar findings have been reported by Mohandass et al., 2018, for woody tree species of tropical seasonal forest in China [67]. Low atmospheric humidity triggers splitting of pods and subsequent shrinkage (Fig. 1-C). Dryness induced leaf fall in May and June render top fruiting branches leafless thereby exposing the fruits to frugivores that indiscriminately chop off developing or immature fruits. It would also aid to spirally coiled hanging fruits pose resistance to

the high velocity wind during extreme summer and get easily detached from the trees (Fig. 2-B). Consequently, overall fruit yield is drastically reduced and fruit to flower ratio is very poor. Gradual shift of reproductive events of winter bloom towards autumn bloom through internal genetic mechanisms in response to evolutionary forces to be perceptive to new environmental cues of adequate moisture level in plant body, decreasing day length, and mild temperature regime as happens during autumn (September-October) to trigger flowering phenophase. In course of time, if natural population of *P. dulce* acquire such characteristics and flowering and fruiting phenophases completely shift from winter blooms to autumn blooms, all reproductive events of the species including fruit dehiscence and seed dispersal ought to be completed by the end of March, thereby a complete avoidance of harsh weather conditions of April, May and June months of semi-arid and arid climate may occur. Under new phenophase, January, February and March months would be a period of fruit development, maturation and ripening. Congenial climatic conditions of spring would provide favourable conditions for proper fruit development in *P. dulce*. Undoubtedly, the overall productivity in terms of quantity and quality of fruits would be enhanced. *P. dulce* would be able to compete with other species in attracting varieties of frugivores thereby wide seed dispersal would ensure enhanced multiplication and better establishment of the species in semi-arid or arid climate. Encinas-Viso, 2014 [68]; Lavabre et al. 2016 [69], and Garcia, 2018 [70] have also emphasised on importance of frugivore diversity in reproductive success of tree species. The phenological shift in flowering is outcomes of interactions between pollinators and other biotic and abiotic factors that led to evolution of long duration flowering in *P. dulce*, which is exclusively pollinated by insects, therefore, in agreement with Elzinga, 2007 [71], and Munguia-Rosas, 2011 [72], it would be appropriate to state that insect pollinated plants flower for longer durations as compared to abiotically pollinated plants (Fig. 2-A).

3.3 Persistence of Fruits and Sporadic *In situ* Germination of Seeds under Specific Environmental Conditions

Flowering trees naturally shed their fruits when they are fully developed and ripened. Fruit ripening in many tropical trees is completed

within few months, however, some trees such as *Cassia fistula*, *Thespecia populnea* shed their fruits after one or two years of fruit inception since their fruit development and ripening processes are highly prolonged. Fruits remain attached with the parent plant for more than one year and two generation fruits with distinct developmental stages could be viewed at any point of time unless older generation fruits are mechanically detached from the trees. Fruit persistence in *P. dulce* primarily seems sheer mechanical and environmental phenomena, however, the influence of biotic factors cannot be ruled out. Least number of trees about 16.20% of site-I (Man managed ecosystem) showed persistent fruits during the period of study, whereas, a greater percentage of trees (51.32 and 58.82) of sites-II and III exhibited persistent fruits. These two sites were apparently deficient in soil moisture content during the period of fruit development, however, number of persistent fruits per tree were maximum at site- I (RIE campus), and site- II (Nag Pahar hillocks) on account of dense tree top canopies and structural barriers to high velocity wind through buildings and hill stones at site-I and II respectively. Sites-I&II showed the maximum number of persistent fruits per tree since trees were sparsely scattered and directly subjected to intense sun light and many of them were heavily infested with horned tree hoppers (Table-1). Studies on mode of persistence of fruits in *P. dulce* reveal that those fruits whose developmental pace is slow due to low level of moisture content and spirally coiled fruits get entangled with the spines or small branches that remain under the cover of dense leaves (Fig. 1-E&F). Drying up of such fruits is slow and fruit stalk is protected from frugivores while some parts of pods are eaten away, such fruits remain attached with the parent trees for one or two years (Fig. 1-F&G). These persistent fruits slowly split into two halves exposing their seeds which in most of the cases serve as microhabitat for breeding of insects such as horned plant hoppers, butter flies and plant spiders which serve as pollinators for *P. dulce* as well as neighbouring plants. Some of the persistent fruits sporadically showed *in situ* germination of seeds under high humid conditions during rainy season, July and August (Fig. 2-C). About 7-10% of persistent fruits were observed to exhibit *in-situ* germination of seeds in trees at sites-I and II probably due to high humidity and shade conditions maintained through underneath decaying thick leaf debris and stagnant wind around persistent fruits on account of structural

Table 1. Distribution, number of trees with persistent fruits and average number of *in-situ* germination in persistent fruits of *P. dulce* at three study sites during September 2015 – December 2018

Study sites	Duration	No. of tree species	No. of species of <i>Pithecellobium</i>	No. of individuals of <i>P. dulce</i>	No. of trees with persistent fruits	No. of persistent fruits/tree Mean ± SE	% of persistent fruits showing <i>in-situ</i> germination
Site-I	2015-16	76	1	105	18	3.33 ± 0.442	8.34
	2016-17	75	1	95	14	4.5 ± 0.626	9.52
	2017-18	75	1	90	15	6.26 ± 0.407	9.58
Site-II	2015-16	26	1	31	15	4.26 ± 0.589	9.38
	2016-17	24	1	25	12	4.16 ± 0.548	10.01
	2017-18	21	1	20	12	4.5 ± 0.657	7.4
Site-III	2015-16	21	1	19	11	1.63 ± 0.278	-
	2016-17	20	1	17	10	1.6 ± 0.266	-
	2017-18	17	1	15	9	1.66 ± 0.235	-

Table 2. Phenological calendar depicting month-wise flowering-fruiting phenophases of individual trees of *P. dulce* natural population during September 2015 – December 2018 at three study sites

Study sites	Duration	Flowering-fruiting phenophasic individuals	J	F	M	A	M	J	J	A	S	O	N	D	
Site-I	2015-16	EFIs	Fr.m-3+2, Fc-1, Fc-6	Fr.m-3+2, Fr.i-1, Fc-6	Fr.r-1+2, Fr.m-1, Fr.i-6	Fr.r-1, Fr.m-6	Fr.r-6	Fr.r-6				Fi-3	Fc-3, Fi-2	Fr.i-3, Fc-2, Fi-1	Fr.m-3, Fr.i-2, Fc-1, Fi-6
		NFIs	Fc-86	Fc-85	Fr.i-82	Fr.m-78	Fr.r-75	Fr.r-70	Fr.r-10	Fr.r-5					Fi-90
	2016-17	EFIs	Fr.m-3, Fc-3+1+4	Fr.m-3, Fr.i-3+1, Fc-4	Fr.r-3, Fr.m-3+1, Fr.i-4	Fr.r-3, Fr.m-3+1+4	Fr.r-3+1+4	Fr.r-3+4	Fr.r-1+2			Fi-3	Fc-3	Fr.i-3, Fi-1	Fr.m-3, Fc-3+1, Fi-4
Site-II	2015-16	NFIs	Fc-80	Fc-80	Fr.i-70	Fr.m-70	Fr.r-50	Fr.r-40	Fr.r-12	Fr.r-7				Fi-84	
		EFIs	Fr.m-3, Fr.i-2, Fc-5	Fr.r-3, Fr.m-2, Fc-5	Fr.r-3+2, Fr.i-5	Fr.r-2, Fr.m-5	Fr.m-5	Fr.r-5	Fr.r-2			Fi-3	Fc-3, Fi-2	Fr.i-3, Fc-2	Fr.m-3, Fc-2, Fi-5
	2016-17	NFIs	Fc-73	Fc-70	Fr.i-65	Fr.m-62	Fr.m-62	Fr.r-60	Fr.r-9	Fr.r-6				Fi-75	
Site-III	2015-16	EFIs	Fr.m-4, Fc-2+6	Fr.m-4, Fr.i-2, Fc-6	Fr.r-4, Fr.m-2, Fr.i-6	Fr.r-4, Fr.m-2+6	Fr.r-3, Fr.m-6	Fr.r-6	Fr.r-2			Fi-4	Fc-4, Fi-2	Fr.i-4, Fc-2, Fi-6	
		NFIs	Fc-12	Fc-12	Fr.i-10	Fr.m-10	Fr.r-10	Fr.r-8	Fr.r-4	Fr.r-2				Fi-16	
	2016-17	EFIs	Fc-5, Fr.i-5	Fr.m-5, Fc-5	Fr.m-5, Fr.i-5	Fr.r-5, Fr.m-5	Fr.r-1, Fr.m-5	Fr.r-4					Fi-5	Fc-5, Fi-5	
Site-I	2017-18	NFIs	Fc-8	Fc-8	Fr.i-7	Fr.m-7	Fr.m-5	Fr.r-3						Fi-9	
		EFIs	Fr.m-3, Fc-3	Fr.m-3, Fc-3	Fr.r-3, Fr.i-3	Fr.r-3, Fr.m-3	Fr.m-3	Fr.r-3	Fr.r-1			Fi-3	Fc-3	Fr.i-3, Fi-3	
	2015-16	NFIs	Fc-7	Fc-7	Fr.i-7	Fr.m-6	Fr.m-6	Fr.r-5	Fr.r-2					Fi-7	
Site-II	2015-16	EFIs	Fr.m-2, Fc-2	Fr.m-2, Fc-2	Fr.r-0, Fr.i-2	Fr.m-2	Fr.m-2	Fr.r-2			Fi-2	Fc-2	Fr.i-2	Fr.m-2, Fi-2	
		NFIs	Fc-12	Fc-10	Fr.i-10	Fr.m-7	Fr.m-6	Fr.r-3	Fr.r-1					Fi-13	
	2016-17	EFIs	Fr.m-2, Fc-2	Fr.m-2, Fr.i-2	Fr.r-2, Fr.m-2	Fr.r-2, Fr.m-2	Fr.r-1+2	Fr.r-1				Fi-2	Fc-2	Fr.i-2, Fi-2	
Site-III	2016-17	NFIs	Fc-11	Fc-10	Fr.i-10	Fr.m-10	Fr.r-9	Fr.r-9	Fr.r-3					Fi-11	
		EFIs	Fr.m-1, Fc-1+2	Fr.m-1, Fr.i-1, Fc-2	Fr.r-1, Fr.m-1, Fr.i-2	Fr.r-1, Fr.m-1+2	Fr.r-1, Fr.m-2	Fr.r-2			Fi-1	Fc-1, Fi-1	Fr.i-1, Fc-1, Fi-2		
	2017-18	NFIs	Fc-8	Fc-8	Fr.i-7	Fr.m-6	Fr.m-5	Fr.r-4						Fi-8	

Abbreviations: EFI – Early Flowering Individuals; NFI – Normal Flowering Individuals; Fi – Flower Initiation; Fc – Flowering Continuum; Fr.i – Fruit Initiation; Fr.m – Fruit Maturation; Fr.r – Fruit Ripening. Inside grids, digits denote number of individual trees passing through a particular phenophase. + sign is used to denote cumulative addition of trees undergoing the same phenophase. Bold letters under EFIs denote exclusively winter or normal blooming individuals

Table 3. Comparative account of morphological descriptors of flowering-fruiting in autumn and winter blooms of early-cum-normal episodic flowering individuals of *P. dulce* during three years period (September, 2015- December, 2018)

Trees	No. of flowering branches/tree MM ± SE	Position of inflorescence on trees	No. of nflorescences /branch M ± SE	No. of floral units/ inflorescence M ± SE	No. of fruits/inflorescence M ± SE	No. of seeds/pod M ± SE	Average length of fruit M ± SE (cm)	Average diameter of fruit M ± SE (cm)	Degree of synchrony in flower initiation
AUTUMN BLOOM OR EARLY FLOWERING									
1 (Site-I)	6.66±1.45	Top	7.07 ± 1.09	12.92 ± 1.17	2.16 ± 0.19	8.37 ± 0.40	11.14 ± 0.64	1.28 ± 0.06	+
2 (Site-I)	7.33±1.85	Top	6.23 ± 0.66	11.87 ± 1.87	1.94 ± 0.39	8.25 ± 0.49	11.19 ± 0.28	1.36 ± 0.12	+
3 (Site-I)	7.0±1.52	Top	7.12 ± 1.22	11.15 ± 1.83	1.98 ± 0.36	8.15 ± 0.50	10.89 ± 0.28	1.22 ± 0.05	+
WINTER BLOOM OR NORMAL FLOWERING									
1 (Site-I)	28.33±4.40	All over	9.18 ± 0.54	17.42 ± 0.93	3.04 ± 0.17	6.48 ± 0.96	7.43 ± 0.42	0.97 ± 0.06	+++
2 (Site-I)	35.0±2.88	All over	8.61 ± 0.83	15.64 ± 0.49	2.70 ± 0.16	6.98 ± 0.99	7.66 ± 0.48	0.93 ± 0.03	+++
3 (Site-I)	37.33±7.21	All over	8.18 ± 0.54	15.32 ± 0.62	2.89 ± 0.17	7.55 ± 0.97	8.50 ± 0.29	0.96 ± 0.02	+++

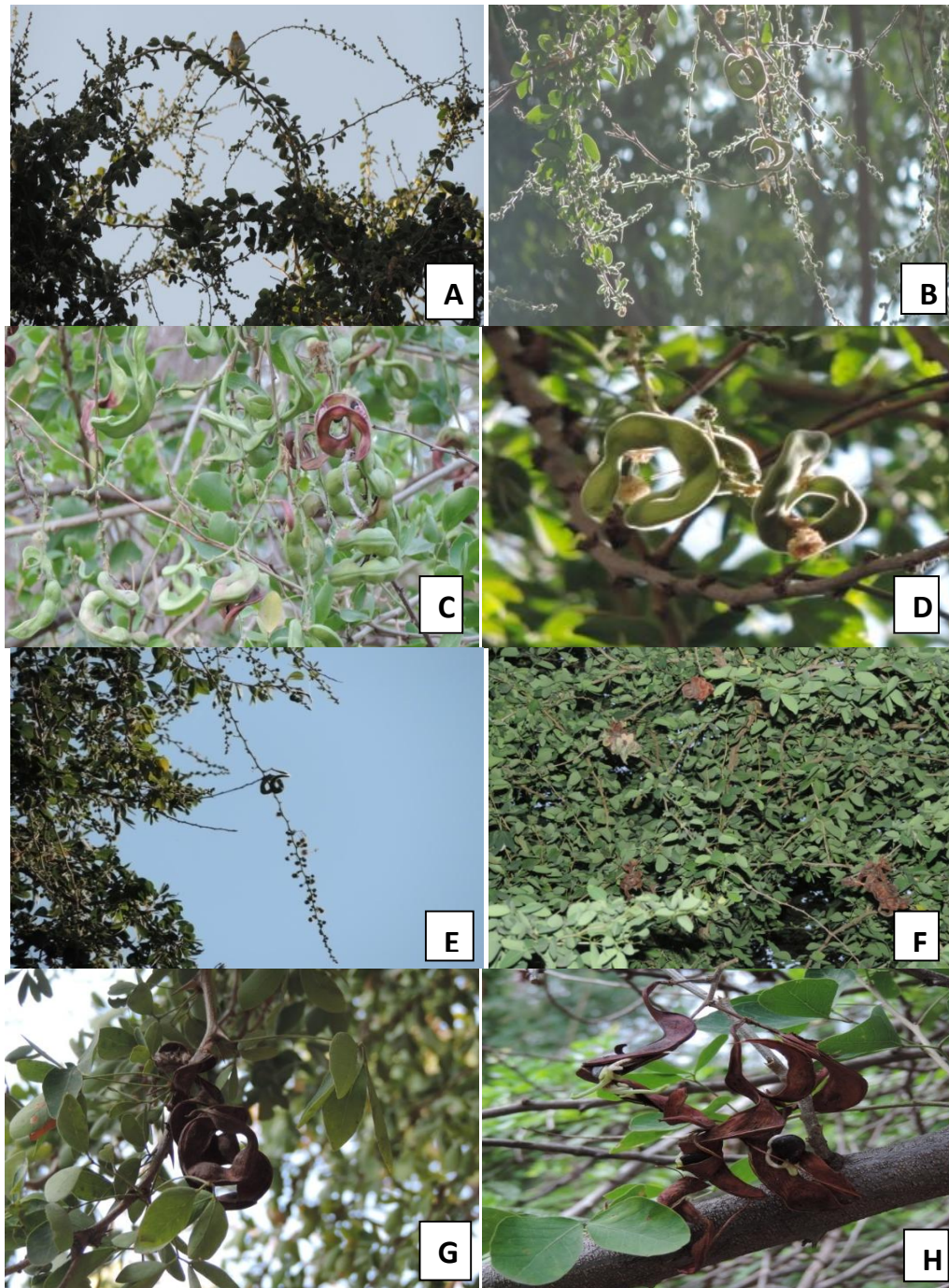


Fig. 1. Morphological descriptors of reproductive behaviour of *Pithecellobium dulce* of semi-arid climate

A. Single winter blooming tree showing synchronized floral initiation during December B. Early-cum-twice blooming tree showing fruits of autumn blooms along with synchronized floral initiation of winter blooms during December C. Shrinkage and splitting up of fruits of winter blooms during harsh summer D. Well developing plump fruits of autumn blooms E. Autumn bloom fruits entangled with winter bloom inflorescence F. Persistent fruits that remain attached with the parent tree throughout the year G. Dried persistent fruit with seeds and thin substratum within the cavity of the fruit H. In situ germination of seeds within the persistent fruit during high humid rainy season

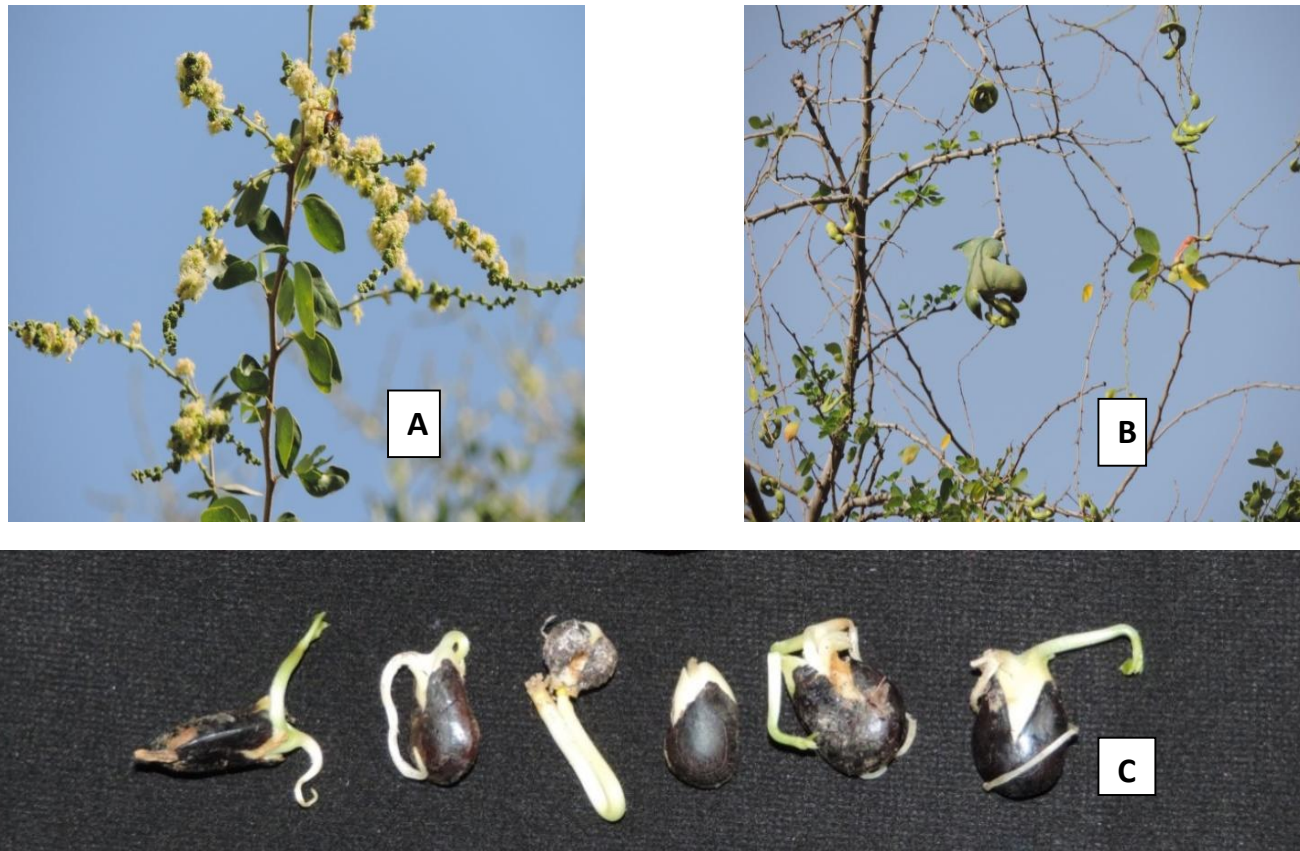


Fig. 2. Morphological descriptors of reproductive behaviour of *Pithecellobium dulce* of semi-arid climate
A. Winter bloom flowers with pollinators B. Dryness induced defoliation of branches with exposed fruits along with frugivores during summer months C. Seedling abnormalities showing constrained growth of plumules and radicles that coil around seeds during in situ germination within the walls of dried persistent fruits during high humid rainy days

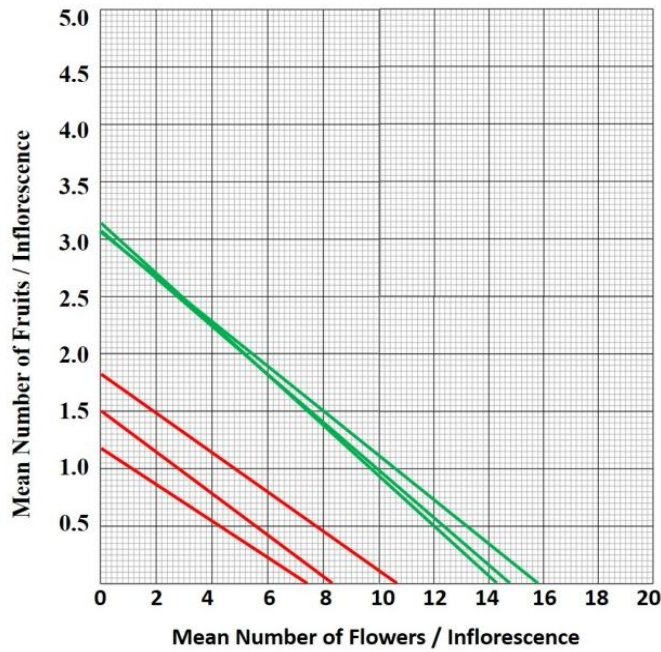


Fig. 3. Graphical representation of fruit to flower ratio of winter and autumn blooms exhibited by three individual twice-flowering trees. Autumn blooms and winter blooms have been represented through red and green lines respectively

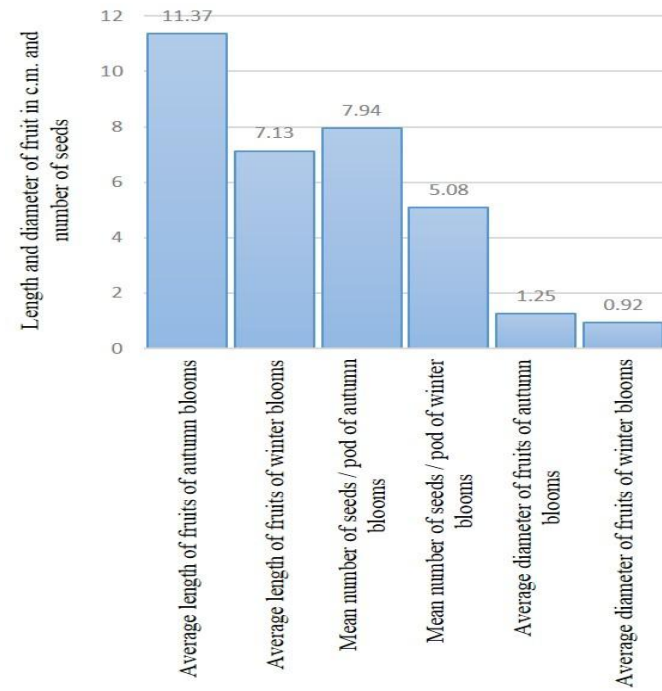


Fig. 4. Histogram showing pomometric characteristics

barriers posed by buildings and hillocks respectively; no *in situ* germination of seeds was observed at site-III where trees are exposed in open space along the road side (Table 1). *In situ* germination of seeds in *P. dulce* is unique in the sense they do not involve hormonal elicitation secreted through maternal fruit tissues since extremely low numbers of persistent fruits under sporadic conditions exhibit such germination behaviour. Therefore, it cannot be equated with either vivipary or crypto-vivipary. *In situ* germination behaviour has been seen in those persistent fruits of *P. dulce* that get entangled with small branches and possess some viable seeds. During hot and windy summer small cracks develop on the walls of the pods which help in deposition of fine dust particles over the dried arils as well as empty spaces within the cavities of fruits. Rain water easily percolates into the fruit cavities through the cracks during July and August. Hygroscopic arils along with fine dust particles form thin substrata within the cavities of pods which retain water for a week thereby helping seeds to absorb water and quickly germinate within the pods since *P. dulce* shows orthodox seed storage behaviour and have wide range of tolerance in germination with respect to temperature and light conditions, even very thin substratum can support germination when seeds are oriented with sideward facing hilum as reported by Vargas-Figueroa et al. 2015 [73]; and Guimaraes et al. 2016 [74]; on the basis of their seed germination studies. However, some of the germinating seeds have been observed to be dropped on to the soil and further grow. Moreover, many seedlings derived from those seeds are abnormally coiled being confined within the walls of fruits (Fig. 2-C). Persistence of fruits is wide spread phenomena in *P. dulce* particularly in semi-arid or arid climate, which commence in response to interactions between abiotic and biotic factors. When drastic changes in atmospheric temperature and humidity are perceived, horned tree hoppers, *Leptocentrus taurus* migrate from exposed branches of deciduous trees to the evergreen branches of *P. dulce* to get reprieve and shelter. They have been frequently observed sucking fruit sap, thereby rendering them highly vulnerable which gradually dry up under high temperature of summer. Being dehydrated, fruits no longer attract frugivores, thus they remain attached with the parent trees for several months in the bunch of dried partially eaten fruits by frugivores particularly parrots and bulbuls. Similar observations have also been reported by Tang et al. 2005 [75]. Hence, it cannot be

regarded as a phenological event of reproductive process, however, it could serve as bio-indicator of high temperature and low humidity. Conversely, low temperature of winter is an essential prerequisite for floral induction in *P. dulce*, thus most of the trees of population respond to the low temperature (5-8°C) during December and exhibit floral induction in a synchronised manner. Therefore, data records on flowering phenology and persistence of fruits for a long period could accurately portray plant responses to climate change. Similar views have been expressed by Tooke and Battey 2010 [76], Siegmund et al. 2016 [77], and Wadgyamar et al. 2018 [78] in their research findings. It would not be surprising to know that how some Japanese climatologists have reconstructed climatic calendar of changes in spring time temperature in Kyoto, Japan for a period of 732 years since 9th Century on the basis of historical flowering phenological data series of Cherry tree, *Prunus jamasakura* [79].

4. CONCLUSION

There are three categories of individual trees of *P. dulce* comprise its natural population of habitat under study of semi-arid climate with respect to their reproductive behaviour. Of all blooming trees, majority of them (89.94%) constitute category-I which exhibits normal or winter bloom in which flowering is initiated in almost synchronised manner during December and all reproductive activities including dehiscence of fruits are completed by June or July months every year. Category-II trees (7.47%) exhibit two blooms consecutively in a year, viz., early or autumn bloom and normal or winter bloom. However, their flowering-fruiting phenological onset timings of early or autumn blooms were inconsistent during the period of study. Category-III individuals (2.58%) of *P. dulce* exhibit agronomically advantageous flowering-fruiting phenophase, early or autumn blooms in these individuals were observed to be conspicuously consistent throughout the study duration. They exhibited very conspicuous early or autumn blooms in which flowering was initiated in the month of September in addition to normal or winter blooms. Overall productivity in terms of number of fruits in this category of trees is greater as compared to other two categories of trees. Though, the numerical value of fruit productivity of autumn blooms was inferior to winter blooms within the same tree, pomometric characteristics of former were superior. Moreover, the availability of fresh fruits in early-

cum- twice blooming tree is also prolonged (February to July). Undoubtedly, Category-III variants or germplasm are horticulturally superior and more desirable that warrant their selection and clonal multiplication. It is evident through consistency in data in phenological calendar that early or autumn bloom in this category of individuals is not influenced by environmental variables alone, i.e., edaphic or climatic since they grow in a similar habitat, however, some age specific flowering was observed. They arose probably by expression of heterogeneity in seeds which differentiated through natural genetic recombination in response to evolutionary forces being operative under prevailing environmental conditions of semi-arid climate. The present paper also makes evolutionary predictions that how a shift in flowering phenology of *P. dulce* as exhibited by the elite variants or germplasm could have long-term implications for reshaping of future landscapes of semi-arid or arid climate through reproductive success, wide dissemination of seeds, consequently better establishment of the species. The concept of reproductive success as postulated in this paper is also expressed by Kudo, 2006 [80], and Elzinga et al., 2007 [71], that individual plants flowering for longer durations have several advantages over other members of the population with respect to higher out crossing rates owing to availability of diversities in pollinators, and more time for fruit development and seed maturation during favourable season as observed in case of autumn blooms of *P. dulce*. The present work identifies and selects agronomically valuable variants or germplasm of *P. dulce* through simple method of construction of phenological calendar. It also reports associated ecological phenomena of persistence of fruits serving as micro-habitat for breeding of insect pollinators and sporadic *in-situ* germination of seeds. Nevertheless, a great effort would be required in resolution of some inherent research possibilities arising with discovery of a new phenophasic variants such as innovation in designing agronomic practices or methods to enhance overall productivity of the cultivars through optimising the yield from early or autumn blooms; development of macro and micro propagation protocols for enhanced multiplication of the elite variant or genotype; genetic modification of the cultivars through biotechnological interventions involving elite germplasm; and deciphering the molecular cascades for reception of altered environmental cues for phenological shift in flowering from normal or winter blooms to early or autumn

blooms are relevant topics of scientific investigations which could further pave the way for molecular engineering of phytophenology as future area of promising research of far reaching consequences.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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