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Breeding strategies for improvement of ber (*Ziziphus sp.*)

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Abstract

A significant diversity is available in ber germplasm in India as well in other countries which can be exploited through apposite breeding approaches. There is urgent need to develop varieties which are tolerant to fruit fly and powdery mildew. Earliness is desirable particularly in dry regions for growing ber under rainfed or limited irrigation conditions. There is a need to develop dwarfing, high yielding varieties/rootstocks, varieties suitable for high density planting and early bearing varieties having a better quality of fruits with more storage life. The variety should yield consistently with the local requirements of maturity time, tolerant to biotic and abiotic stresses- resistance to frost, insect pests, diseases, and suitable for post-harvest uses (processing, dehydration, candying). The germplasm suitable for canopy management methods and cultural practices is to be introduced and developed. There are two main bottlenecks in ber breeding programme; polyploidy and incompatibility in its cultivars. Besides these, other constraints in improvement are typical floral morphology, short period of anthesis and stigma receptivity and limited period of fruits availability, owing to these, fruit setting is very less (<10%). The hybridization/crossing protocol need to be standardized, which is also a challenge in ber breeding.

Key words: Ber, germplasm diversity, flower biology, selection, hybridization

Introduction

Ber (*Ziziphus mauritiana* Lamk) is grown in the varying agro-climatic conditions in central Asia, which is the centre of origin of ber (De Candolle, 1886). In India, it has been grown from 4000 years, traditionally from ancient times (Prakash, 1961). However, according to archaeological studies, it is found more than 7000 years ago in China (Qu *et al.*, 1989). It is distributed worldwide in tropical and subtropical regions of south-east Asia, China, Africa, Australia, America and Mediterranean region. Few species are also found in temperate regions. However, it is cultivated in the dried/arid region of the globe. The maximum number of species occurs in Asia, very rare in Oceania and Europe *i.e.* 5.3 and 2.9 per cent, respectively (Liu, 2006). In India, arid part of Rajasthan, Haryana, UP, MP, Gujarat, Maharashtra, Bihar, Andhra Pradesh and Tamil Nadu are major ber growing states (Kumari *et al.*, 2015). It is generally grown in the unproductive, underutilized, inferior soil having pH up to 9.0 in arid and semi-arid regions. The ber plants can be grown in the area where other fruit crops cannot be grown easily. Ber fruits are rich in carbohydrates, minerals, proteins, vitamins and amino acids, aspartic acid, glutamic acid, aserine, asparagine, glycine, and threonine (Bal, 1981). It is richer to apple in nutrients (protein, calcium, phosphorus, vitamin C and carotene) (Bakhshi and Singh, 1974) and oranges in carbohydrates, vitamin C, phosphorus, iron and calorific value. It is a common notion among the people that the ber is a poor man's fruit, but now a days the quality fruits of improved varieties are not in reach of poor people due to its high market rate. The diploid genotypes were observed resistant to the powdery mildew and octaploid were seedless (Azam-Ali *et al.*, 2006). However, some diploid species were found susceptible to powdery mildew. Diversity in chromosome karyotypes was exhibited higher in Chinese jujube.

Germplasm conservation

The long term conservation of jujube seeds has not given much attention and cross-pollination in this species also reflects higher heterozygosity in seeds. Storage of the *Ziziphus* seeds is not easy to handle and regeneration of seedlings require more time to fruiting. Seeds of *Ziziphus* had orthodox behavior after drying and storage at low temperature. The gene bank of International Center for Underutilized Crops (ICUC) has considered jujube seeds (cultivars, selection and mutants) for collection and maintenance of germplasm to support improvement efforts. These germplasm may be tested at different locations for introduction at new places away from their origin. International Plant Genetic Resource Institute also conserves the germplasm of different species.

In-situ conservation

In *in-situ* conservation, the germplasm is conserved in their natural habitat. The habitats protected from human interference are natural parks, gene sanctuaries, wildlife sanctuaries or biosphere reserve. The areas of the centre of origin or micro centre within the centre of origin preferably the best option for the gene sanctuaries (Azam-Ali *et al.*, 2006). The sanctuary conservation of existing germplasm allows the new germplasm, which appears with the passes of time. But it is very

difficult to maintain and establish new germplasm in the areas of infrastructure development and population pressure areas. It is required to develop the gene sanctuaries of *Z. mauritiana*, *Z. nummularia* and *Z. rotundifolia* in India and *Z. jujuba* in China as a natural habitat or biosphere reserve. In habitat, the targeted species are maintained through natural reserves, wildlife sanctuaries, natural parks, managed forest, protected area, preservation plot and farm conservation through agro-ecosystem.

***In-vitro* conservation**

In this technique, more number of planting material can be preserved in a limited place under controlled conditions using biotechnological tools. Tissue culture methods are suitable for cryopreservation of tissue and/or germplasm. *In-vitro* storage technique for *Z. jujuba* was developed in the Nikitsky Botanical Gardens, Ukraine (Mitrofanova *et al.,* 2002). The explants growth can be retarded by physical (culture condition, light and temperature), chemical (cultural medium) and physiological (crop growth stage, plant age and dormancy) factors.

Germplasm centres

Several ber germplasm including species, cultivars and other types have also been collected at different research stations in the country and are being maintained in the field gene bank centres namely CIAH, Bikaner, NBPGR, Jodhpur, MPKV, Rahuri, CCS HAU, Hisar, CCS HAU, RRS, Bawal, CAZRI, Jodhpur, GAU, SK Nagar. At CIAH, Bikaner highest collections (338) have been made in the National Field Repository (Vashistha, 2006). Most of the leading germplasm in India and China were selected from the heterogeneous population or wild forest. The superior types of germplasm/cultivars were multiplied vegetatively and cultivated on a widespread area e.g. Umran and Gola (Indian jujube) and Sui Men (Chinese jujube). In India, about 300 varieties of ber are being cultivated and few of them become commercially important (Pareek and Nath, 1996), but 180 varieties are mentioned in the literature (Pareek, 2001). In China, about 700 cultivars of jujube are reported by Qu and Wang, 1993. These are divided into two groups- sour and sweet type. Sour type is mostly used as rootstock, animal fodder and medicinal purpose, while sweet type is used as a scion for fruit consumption (Ciminata, 1996).

Resistant germplasm

Powdery mildew: Jamadar *et al*. (2009) studied different germplasm of ber for powdery mildew and reported Mundia and Jogia resistant to powdery mildew, Banarsi Kadaka was moderately resistant, while Umran was reported as susceptible. Godhara *et al*. (2002) studied different germplasm, among them Darakhi-1, Darakhi-2, Safeda Rohtak, Villaiti, Guli and Seedless were reported free from disease. However, Kaithali, Umran, Sandhura Narnaul and Illaichi were susceptible to powdery mildew.

Fruit fly: Thirty five germplasm of ber were screened against fruit fly. On the basis of per cent infestation these were categorized into four categories, i.e. resistant (infestation less than 1.0 %), less susceptible (infestation from 1 to 10%), moderate susceptible (infestation from 10 to 20 %) and highly susceptible (infestation more than 20%). Among these germplasm, BS 1 was found free from fruit fly infestation whereas Gola, Umran, Mudia Murhara, Laddu and Kaithali were found highly susceptible to fruit fly. Mann and Bindra (1976) reported the damage of fruit fly in cultivars Sanaur-1, Safeda Selected, Illaichi, Mirchia, ZG-3 and Umran. However, Singh (1984) observed that infestation was varied from cultivars to cultivars and reported 6.7% in Tikadi to 73% in Gola.

Genetic erosion

The growing of clonally or vegetatively propagated plants are the main sources of genetic erosion in addition to this the rejuvenation and top working also converted the local germplasm into the improved cultivars (Yadav, 1991). The primary centre of origin becomes the secondary centres (Tropical Africa for *Z. mauritiana,* South-west Asia, Central Asia and parts of Africa for *Z. jujuba*), whilst the population pressure and developmental activities caused the conversion of wildlife sanctuary into the residential area and manufacture unit. Rural community is using the *Ziziphus* for many purposes (windbreak, shelterbelt, fencing, firewood and timber) which caused genetic erosion.

Variability

The physical and morphological variability in germplasm can be studied as recommended in the descriptor of NBPGR (Mahajan *et al.*, 2002) and guidelines for DUS testing of PPV&FRA (Anonymous, 2016). However, the biotechnological tools are the best to find genetic variability.

Genetic variability

Ploidy level: Molecular marker-based study is used to find genetic diversity as compared to conventional/ morphological method. AFLP has been used in limited accessions to study the genetic diversity of different germplasm of *Ziziphus sp.* (Singh *et al*., 2006). The variability in ploidy level varied from tetraploid to octaploid (x=12) and natural alloploides also created a large variability (Khoshoo and Singh, 1963). However, Indian jujube usually appears polyploidy, n = 12, 20, 24, 30, 36, or 48. Khoshoo and Singh (1963) also observed variation in cultivars as n = 24 (most of the cultivars), n = 48 (two cultivars) and n = 30 (one cultivar), however, Nehra *et al.* (1983) observed n = 4 (wild material). Only limited wild species have been counted particularly *Z. lotus (n=10), Z. nummularia (n=12)* and *Z. oenoplia (n=36)* and *Z. lotus, Z. oenoplia* and *Z. nummularia* were observed as diploid, tetraploid and polyploidy, respectively. The possibility of genus exists as tribasic with x = 10, 12, or 13 (Darlington and Wylie, 1955). Diploid, triploid, tetraploid, pentaploid and octoploid are the range of polyploids observed in Indian jujube, while Chinese represented polyploid series 2n = 45, 60, 90 (Mehetre and Dahat, 2000).

Morphological variability

A wide variation exhibited in growth, flowering, yield and quality parameters of *Ziziphus* is due to cross-pollination. The variability in morphological and physicochemical traits has also been reported by Shobha *et al*. (2001). As per DUS study conducted by Krishna *et al*. (2006), the maturity of fruits varied as early (Gola), mid season (Banarasi Karaka, Banarasi Pewandi, Chhuhara, Chhuhara Bawal, Illaichi, Kaithali, Mundia, Narma, Reshmi, Safeda Selection, Seb), while (Dharki No.1, Gularivasi, Jogia, Kala Gola, Katha Phal, Lakhan, Mehrun, Safeda Rohtak, Sanaur-5, Tikadi, Umran, ZG-3) were late-maturing varieties. Out of 24 cultivars have anthocyanin blush on immature fruits *i.e.* Katha Phal and Sanaur-5, while no blush on development was observed on other 22 varieties. Vegetative characters such as leaf area and branching habit are the most appropriate characters of classification, while fruit apex, stalk, shape and stylar flesh cavities are dependable characters (Bal, 1992). Some cultivated and wild species have serrated leaf margin *Z. mauritiana*, *Z. nummularia and Z. rotundifolia,* except *Jhar ber* and *Desi ber (*Gupta *et al.,* 2003). Higher coefficient of variation was observed in leaf (39.4%) than edible to the inedible portion of fruit (2.7%); variation was also observed in fruit shape, seed and flower bud opening time.

Reproductive variability

Flowering and fruit set: In North Indian conditions the tree shed leaves during March to April and starts vegetative growth from June onwards and flowering on the current season growth during August-September. Flowers are borne on axillary cluster/ cymes. The ber flowers are greenish-yellow, faintly fragrant, pentamerous, hermaphrodite, calyx with deltoid lobed, hairy outside, glabrous within petals 5, sub spathulate, concave, reflexed stamens 5; ovary 2-celled, styles bifid, disc 10-lobed or grooved (Azam-Ali *et al*., 2006). Flower sepals are dorsally tomentose, disc diameter (3 mm), ovary is 2-celled, disc is immersed, styles are two having 1 mm length. Nehra *et al.* (1984) recorded the initiation of flowering in ber cultivars from September to November under Hisar condition. Kundi *et al.* (1989) observed the time of flowering from 1st week of October to 1st week of November in various cultivars of ber in Pakistan. Flowers emerge from mid-May to late June in northern China (Zeng *et al*., 1959), however, flowering from June to July in Korea (Cheong and Kim, 1984), May to August in California, USA (Ackerman, 1961). Flowers bear in the cluster and ranged from 10-14 and 16 to 28, varied with agro-climatic conditions (Josan *et al*., 1980). Umran had recorded the highest hermaphrodite flower (22.2%), followed by Gurgaon Gola (20.1 %) (Singh and Jindal, 1980).

Flowering duration: The variation in the duration of flowering varied with the cultivars, it ranged from 68 to 94 days (Babu and Kumar, 1998) and 57 to 75 days (Dhaliwal and Bal, 1998). The duration of flowering was observed as 71 ± 5.62 days in Gola, 68 ± 4.49 days in Seb, while shortest flowering duration (47 days) was observed in Tikadi and longest (71 days) in Umran.

Flower order: Flowers on the bearing shoots emerge simultaneously. Flowers emergence on the middle part of the bearing shoot than the upper and middle part of the bearing shoot, fruits also mature early in the middle of the shoot as compared to apex and base (Qu *et al*., 1989a).

Selection

Most of the planting materials selected and identified during the survey having better yield and quality traits with good economic returns. The identified germplasm is maintained and multiplied vegetatively. Most of the cultivars are being developed by selection. These cultivars have been developed as a result of cross-pollination (entomophilous) or natural hybrids in the different germplasm of wide genetic base available in the locality (Khoshoo and Singh, 1963). The best performing genotypes are propagated vegetatively for multiplication of true to type planting material. Tikadi has been developed as a result of selection form the *Z. rotundifolia* having ovate fruit shape. This selection has a good pulp stone ratio, TSS, shelf life and resistant to fruit fly (*Carpomyia vesuviana*). Artificial cross-breeding is very difficult in the ber therefore most of the cultivars are developed by selection (Zhu *et al.,* 1998). The great variability exists within the leading cultivars (Luo *et al*., 1997) so the chances to develop new cultivar within the cultivars are more. Gola cultivar has got many variants such as Kala Gola, Kakrola Gola, Gurgaon Gola, Gurgaon Gola-I, II, *etc* (Pareek, 2001). The variety of ber Goma Kirti selected from the population of Umran at CHES, Godhara.

Table 1. Screening of ber germplasm against fruit fly at RRS Bawal (Anonymous, 2020)

S/No.	Varieties/ germplasm	Categories
1	BS-1 (1)	Less than 1.0 % (Resistant)
2	Illaichi, Sanaur No.3, Nauki, Katha Gurgoan, Khathaphal, Banarasi, BS-2, Jhajjar, Tasbataso, Reshmi, Narkali, Khatti, Desi Alwar (13)	1-10% (Less susceptible)
3	Sua, Seo, Jhajjar Special, Sandhura Narnaul, Akhrota, Safeda Rohtak , Seo Bhadurgarh, Popular Gola, Nazuk, Kakrula Gola, Katha Rajasthani, Illaichi Jhajjar, Govidgarh Selection, Gola Gurgoan no.2, Bhadurgarhia (15)	10 to 20 % (Moderate susceptible)
4	Gola, Umran, Thornless, Mundia Murhara, Laddu and Kaithali (6)	More than 20% (Highly susceptible)

Pollen viability: Wodehouse (1960) studied pollen of *Ziziphus*. Later on, Rouhakhsh *et al*. (2014) studies morphology of different species of *Ziziphus*. The pollen of flowering branches was collected between 6.00-7.00 AM during cool hours and reported the 93±2.93% at anthesis. 1.0% of 2, 3, 5- triphenyl tetrazolium chloride (TTC) is used to study the viability of pollen by staining and recorded viable pollen after 2 hours (Shivanna and Rangaswamy, 1992). Pollen viability of most of the Chinese jujube was reported 50% to less than 10% (Wang, 2004). The percentage of pollen viability was recorded in five replications and calculated using the following formula:

Pollen germination (%) = (Total number of germinated pollen/ total number of pollens) x 100

Pollen germination: Pollens are yellowish, sticky with fine mass. Sucrose solution was observed the best medium for pollen germination. Pollen grain germination was ranged from 36.47 to 47.66 per cent in different cultivars of ber. The pollen germination of *Z. sativa* cv. Moodeung and Bokjo increased with increase in concentration of sucrose from 1.0 to 10.0 % and thereafter it decreased with increase in the concentration of sucrose (Park and Yu, 1989) and it further increased with the addition of 35 ppm boric acid (Yun *et al*., 1989). Pollen germination under *in-vitro* conditions was observed optimal in 30% sucrose solution solidified with 1.0% agar (Mchedlidze and Shekiladze, 1986). However, Yun *et al*. (1989) observed 37-39% germination of fresh pollen at 35-30 ºC in 15% sucrose solution solidified with 1.0% agar medium by 24 hours, thereafter it decreased and no germination after 24 hours. Pollen germination was observed maximum (91±2.65%) in 20% sucrose and miimum (35±1.61 %) in 5% sucrose, but observed maximum 48.35±1.75 µm pollen tube length (Dinesh, 2018). The pollen germination (%) was observed using the following formula:

Pollen viability (%) = (No. of stained pollen/ total no. of pollen) x 100

Pollination: Pollination was observed by the honeybee, housefly and ladybird (*Coccinella novemnotata*), however, wind pollination was observed nil in Chinese jujube in California (Ackerman, 1961). The insects remain more active between 11.00 h to 15.00 h. The house fly was observed more efficient and active for a longer period in pollination as compared to the honeybee; both are most active at noon. It is also reported the insect's *viz*., housefly (*Musca domestica*), honeybee (*Apis sp*.) and the yellow wasp (*Polistes herbaracus*) are the most common pollinating agent for *Ziziphus* (Dhaliwal, 1975).

Size and shape of pollen grain: Nehra *et al*. (1984) observed the variability in pollen grains of *Z. mauritiana (*Illaichi, Umran) and four wild species. Hulwale *et al.* (1995) conducted pollen study in seven cultivars of ber, pollen grain size ranged from 20.05 to 32.04 mµ and stain ability ranged from 63.69 to 87.12 per cent. Perveen and Qaiser (2002) observed the morphology of pollen by light and scanning electron microscope of 5 genera and 11 species of *Rhamnaceae* collected from Pakistan and reported the variability in shape and size from oblate spheroidal and sub-prolate, tricolporate, exine surface sexine thicker than nexine, striate-rugulate and rarely reticulate-rugulate, often psilate in all the species. Pollen grains are monads, small in size with polar axis 22.65 µm and equatorial diameter 21.15 µm and P:E ratio 1.07, the shape is spheroidal-prolate, tricolporate, isopolar, polar view triangular, operculum irregular and interapertural area are sunken, exine surface regulate in the middle and smooth towards the colpi (Dinesh, 2018).

Fertilization

Chinese jujube has profuse flowering but less than 2 per cent of flowers developed into mature fruits (Qu and Wang, 1993). Heavy fruit drop just after the fruit set due to lack of fertilization and degeneration of ovule. Other biotic (insect-pest and disease) and abiotic reasons for fruit drops are moisture stress, low relative humidity, lack of sunlight and high winds during the initial phase of fruiting (Liu *et al*., 1997). In self-pollination or cross-pollination, it took at least 4 h to recognize pollen and stigma to each other, 6 h to germinate pollen on the stigma, 12 h to penetrate pollen tube into the mastoid cells and grow into the style, 24 h to reach pollen tube 1/4th of the style (Shao and Wang, 2020). Receptivity of the stigma and viability of pollen are important factors for fertilization. Fertilization was observed 10 days after pollination, proembryo formation after 20 days, globular embryo stage appeared after 30 days, and the embryo could be distinguished after 40 days. The embryo grew rapidly between 45 and 50 days post-anthesis. Two polar nuclei began to fuse to form the secondary nucleus in the embryo sac at 72 h after pollination. After 96 h of pollination, one sperm fused with the secondary nucleus in the embryo sac and formed the primary endosperm nucleus, and the other sperm moved to the vicinity of the egg cell. After pollination for 120 h, another sperm fused with the egg cell, forming a zygote. The zygote did not divide immediately; it began to divide after 4 to 5 days of dormancy (Shao and Wang, 2020). It first divided into a two-cell proembryo, then a three-cell proembryo, then a four-cell proembryo, and formed an early globular embryo after pollination for 10 days. Then 20 days after pollination the embryo body grew in length, width, thickness, and formed into a round shape (a globular embryo).

Hybridization

The flower size of *Ziziphus* is very small and flower emasculation is very difficult without damage to flower, so cross-pollination is difficult. In addition, to this the embryo abortion, self-incompatibility (Godara, 1981) and polyploidy are also serious problems. Better germplasm is identified and screened at different institute/ university because the performance of the germplasm/ cultivars is location-specific. Chundawat and Srivastava (1980) made attempts for inter varietal crosses but obtained seeds of Seb x Umran, Seb x Katha, and Umran x Seb. Vashistha and Pareek (1983) attempted several crosses of Gola, Seb, Sanaur 2, Katha and

Umran and observed maximum fruit set in Umran x Katha and its reciprocal due to isogenic behaviour. The Illaichi had reported 95 per cent pollen sterility due to octaploids (2n = 96) genetic makeup (Khoshoo and Singh, 1963). Seb and Gola (tertaploid) are early and commercially accepted varieties included in the hybridization. Seb x Gola with Tikadi was crossed reciprocally, Gola with Tikadi were incompatible, but Seb x Tikadi was compatible and fruit set was hardly one per cent (Singh and Vashishtha, 1993). The successful seeds were raised in the pot and later on budded on rootstock; the plant has resembled with the Tikadi with higher TSS, and resistance to the fruit fly. Tissue culture can be opted to overcome the constraints and rapid multiplication (Sudherson *et al*., 2001). Singh and Vashishtha (1984) evaluated the ber germplasm at ICAR-CAZRI, Jodhpur to locate the fruit fly resistance germplasm. It was observed that the cultivar Illaichi performed moderately resistance while Tikadi performed resistant to the fruit fly. Thar Sevika (Seb x Katha Phal) hybrid of ber released by ICAR-CIAH, Bikaner.

Embryo abortion and embryo culture

Embryo abortion is common in *Ziziphus* large fruits show less embryo abortion as compared to small fruits. Embryo can be cultured in medium MS + BA 0.1-0.5 mg/l +IBA 0.5-0.1 mg/l +NAA 0.1 mg/l + sucrose 50g/l +agar 7g/l +LH (lactalbumin + hydrolysate) 500 mh/l, then transferred to growing medium MS + sucrose 30g/l +agar 5g/l. The plant can be obtained from the embryo using an immature embryo for direct development, callus formation from the immature embryo and keeping it development from the young embryo (less than 30 days old) (Qi and Liu, 2004). Qi and Liu 2004 also reported that the survival rate is 87-90 and 3.7 per cent for 55 days and 20 days embryo, respectively. For better embryo germination low level of BA and high level of IBA in the proper quantity of luteinizing hormone-induced adventurous bud. However, low temperature (2-4ºC) for 44 days favour plantlet formation from poor quality plantlet and darkness of 7-10 days favour rooting of the embryo. Embryo culture can be improved from earlier fruit set with the normal embryo, cutting the tip off main root stimulates the lateral root formation. Qi (2002) reported that the flesh of cultivars (seedless) contains high GA_3 and IAA level than cultivars having seeds. Similarly, high embryo abortion cultivars also having more GA_3 and IAA levels as compared to the flesh of seeded fruits. Wang (2004) reported that the high level of nutrients in flesh leads to low nutrients in the ovule, which cause embryo abortion at stone hardening stage. High ratio of Z/GA and zeatin (Z) caused the seed to shrink at the later stage of embryo development.

Male sterility

Wang (2004) reported two types of male sterility functional pollen sterility (high sterile pollen-pollen germination less than 3 %) and empty anther sacs (empty at yellow bud stage), it varies with the cultivars due to differing in cytological, physiological, and biochemical mechanisms. Pollen fertility is estimated by I_2-IK (Iodine- potassium iodide) staining. Based on the staining, cultivars were divided into 5 groups, *i.e.* high fertility (VPR *i.e.* viable pollen rate≥ 54%), medium-high fertility (43 % ≤VPR <54%), medium fertility (28 % ≤VPR <43%), medium sterility (17 % ≤VPR <28%) and high male sterility (VPR <17%). The nutrients, enzymes and hormone significantly differ in flowers bud of different cultivars cause variation of male sterility.

Inheritance

Detailed genetic study is yet to be conducted to find the relationship between quantitative and qualitative characters. Some attempts have been made to study the genetic correlation between desirable traits. The estimation of heritability is important for plant breeders to enable the selection programme based on the phenotypic performance of germplasm and predicting the results. Qualitative characters controlled by one or few genes are manipulated in genetic programme; quantitative characters are controlled by many genes (polygenes). The characters which are not affected by the environment are strongly affected as compared to the characters which are environmentally influenced. Genotypic coefficient of variation and phenotypic coefficient of variation was greatest for stomatal frequency while the lowest for relative water content. The high value of genetic gain and heritability was observed for the stomatal index, which is positively correlated with the yield (Praveen and Patil, 1998). High estimates of GCV, PCV, genetic advance and heritability were recorded for fruit weight, stone size, pulp stone ratio and yield indicating the improvement effectiveness through simple selection (Saran, 2005). The heritability values were observed highest for days from fruit set to ripening (99.2%); followed by fruit set (94.7%), days from pruning to sprouting (93.6%), fruit drop (85.0%) and shoot length (82.0%) (Panse, 1957). Heritability in 30 cultivars of ber was observed and the value for total soluble solids 54.2 per cent, for disease intensity 99.63 per cent, and the value for acidity was 91.61 per cent (Bisla and Daulta, 1988a), however, highest genetic gain (142.8%) was recorded for fruit set and lowest (3.5%) for days from pruning to flower initiation. Further, Bisla and Daulta (1988b) studied heritability in *Z. mauritiana,* it was 97.2 per cent for fruit weight, followed by fruit size (87.9%), pulp/stone ratio (87.5%) and seed weight (84.6%) at Hisar.

Anthesis

Anthesis in *Ziziphus* is cultivar specific it occurs in the morning, noon and evening hours. Anthesis in some cultivars is during morning hours (7.30-8.30 AM) like Seb, Jogiya, Illaichi, Ponda and Aliganj, while in Gola and Mundia it occurs between 12.00 noon to 1.00 PM. Vashishtha and Pareek (1983) reported the anthesis time from 7.30 to 8.00 AM in Seb and Sanaur-2, between 1.00 to 2.00 PM Gola, Katha and Umran. While Desai *et al*. (1986) reported anthesis time in Chhuhara between 5.30 to 7.30 AM; in Sanaur between 12.30 to 2.45 PM. Kim and Kim (1984) reported that the afternoon flower opening cultivars required a photoperiod of 12 h, while morning anthesis cultivars require a definite dark period for induction of flowering. After completion of anthesis, sepals turns outwards leaving anthers leaned on ovary (Qu *et al*., 1989a). Anthesis under south western Haryana (RRS, Bawal)

condition takes place from 6.30 to 7.30 AM in Narendra Selection 1, BS 1, BS 2; 6.30 to 8.00 AM in CIAH Selection 1 (Thar Bhuvraj) and CIAH Hybrid-1 (Thar Sevika); 12.00 noon to 1.00 PM in Chhuhara, Kaithali, Umran, Gola, Rohtak Safeda and Narendra Selection 2.

Dehiscence

In most of the cultivars dehiscence starts from the time of anthesis of flowering to 4 to 5 hours of anthesis, it varied with cultivars (Sharma *et al.*, 1990). Vashishtha and Pareek (1979) studied Gola, Seb, Mundia, Illaichi, Ponda, Jogiya and Aliganj cultivars and observed the completion of dehiscence in an hour after anthesis. Dehiscence starts just after the opening of the petal sometimes it may be before the emergence of anther from the sheath (Ackerman, 1961). Pollen dehiscence took place during the first day of flowering and it followed by anthesis from 0.5 h to 2 to 4 h (Dhaliwal and Bal, 1998).

Stigma receptivity

After the rapture of the flower bud/ opening of the flower, the moist and shining (exude nectar) stigma shows the sign of receptivity, it happens just after the opening of the flower (Dhaliwal and Bal, 1998). At the time of anthesis, the stigma look likes minute protuberance. Full development of the stigma takes place after 16-18 hours of anthesis, it indicted as bi or sometimes trifurcation. It remains receptive/ sticky for 13 to 24 h (Desai *et al*., 1986) and up to 48 h (Godara, 1980). Anthesis and receptivity of the stigma are a cultivar specific (Pareek, 2001). In some cultivars, anthesis and receptivity varied as the morning, noon and evening hours. However, the dehiscence of the anther (separation of petal stamen) occurs in the day time (Qu *et al.,* 1989). The time of anthesis influenced by temperature, rains and clouds (Wang *et al*., 1989). Ackerman (1961) observed the maximum pollination took place on the date of anthesis.

Marker assisted cross breeding

In a conventional cross-breeding, the varieties/plants are propagated in the same rootstock and cover it with the net house to avoid the pollination from the outside. A box of the honey bee is placed inside the net house for proper cross-pollination. In addition to this embryo rescue, marker-assisted cross breeding has also been carried out by different researchers. Different marker studies such as RAPDs and AFLPs are used for identification of seed obtained as a cross-pollination (hybridization) or self-pollination.

Ploidy manipulation

Cross-pollination in the *Ziziphus* is very difficult, polyploidy breeding is the easy way for breeding in for large fruits and high resistance. Diversity in chromosome number of Chinese ber is very low. Qu *et al*. (1986) studied 117 cultivars and found only one triploid and others were diploid. Jiang (2003) treated the ber shoots cultivars of Dongzao, Linyilizao, and Lijiaozao with colchicine 0.15%/18hr, 0.1%/30 hr and 0.15%/18hr, and showed induction rate 50, 43.3 and 43.3 per cent, respectively and produced tetraploid and showed chromosome 2n = 24 (normal diploid number) and 48. The plants with induced tetraploid showed double chromosome number, large and longer cells and guard cells, more chloroplast and lower density of stomata. The pollen grain is large, deformed and had 4 germination pores. Internodes are shorter but leaves are rounded and thick. Wang, 2004 also treated cluster bud of sour ber and Dongzao with colchicine 50 mg/l for 40 days with a doubling rate of 36.7 and 26.3 per cent, respectively. He also studied the 4 days pre-culture, 15 days dark treatment and addition of 0.5 per cent DMSO and 1.5 mg/l activated carbon improve the doubling effect. Cells of tetraploid shoots were larger than diploid. Tetraploid parts can be purified effectively after 3 or 4 times (at most 8 times) of transplanting.

Biotechnology and mutation

Zhou (2004) observed the most efficient regeneration medium for cultivar Dongzao was MS + TDZ 1.0 mg/l +IBA 1.0 ~ 0.5 mg/l (pH 5.8-6.4) during the first 4 weeks followed by medium with CTK (MS +IBA 0.1 mg/l+ GA_3 0.05 mg/l), it achieved 91.3 per cent regeneration. However, for cultivar Lajiaozao was WPM +TDZ 0.5 mg/l +IBA 0.1 mg/l (pH 5.8 -6.4) and MS +IBA 0.1 mg/l +GA_3 0.05 mg/l, respectively was most suitable medium, achieved 78.9 per cent regeneration rate. Supplementation of 0.1 mg/l, $AgNO_3$ to the medium was beneficial and dark treatment for 14 days increased regeneration rate. Sin'ko and Chemarin (1979) tried gamma irradiation on growth and development of jujube breeding and Sin'ko and Karakhanova (1982) obtained some mutants.

Techniques for improving fruit set

Fruit of jujube is solitary with stony seed and fruit set during November-December. The flowering is very abundant in Chinese jujube but the fruit set is very low (<1.0%) under natural conditions. Improvement in fruit set is mandatory for better fruit production. The marked variation in fruit set was observed among different cultivars. Gupta and Minhas (1991) reported the fruit set under bagging was 8 per cent in Illaichi, followed by Umran (6.0%), whereas fruit set was observed nil in Sanaur-2, Sanaur-3, Sanaur-4, Sanaur-5, Kaithali and Chhuhara. Vashishtha and Pareek (1979) observed self-incompatibility in jujube varieties (Gola, Seb, Jogia, Aliganj, Ponda, Illaichi and Mundia); Godara (1980) observed in Banarasi Karaka, Illaichi, Karkrola Gola, Kaithli, Kathaphal, Mundia Murhara, Reshmi, Sandhura Narnaul, Safeda Selected and Umran; Mehrotra and Gupta (1985) in ZG-2 and Sanaur-2; Neeraja *et al*. (1995) in Gola, Umran and Seb. Josan *et al*. (1981) did not found any fruit on self-pollinated varieties. However, Illaichi and Umran were observed as self-fruitfulness varieties (Mehrotra and Gupta, 1985). Singh and Vashishtha (1993) also reported cross-incompatible varieties. The crossing of ZG-4, Kala Gola and Sanaur-2 with Umran (male parent) observed no fruit set (Gupta and Minhas, 1991). However, Umran (as female) is cross-compatible with Umran (male parent). But Godara (1980) reported that the Umran is less compatible with other varieties as male as well as the female parent. Mehrotra and Gupta (1985) reported Sanaur-2

as self-incompatible as well as cross incompatible with Umran (female parent). Highest fruit set were observed in Illaichi x Kakrola Gola, while the combined ability of Umran showed best (as male as well as female) (Godara, 1980). Fruit set in open pollination was observed highest in Umran (23.1%); followed by Seb (18.6%) and Gola (13.7%). Highest fruit setting in hand pollination (60%) was recorded in Umran x Seb as compared to open pollination (Neeraja *et al*., 1995).

Creating optimal pollination conditions

Fruit set can be improved by keeping honey bee in the orchard during flowering. The pollen does not germinate in humidity below 60 per cent. High temperature and drought conditions cause poor pollination and fruit set, spraying of water helps improve the pollination. Physiological and environment conditions are responsible for fruit setting. Fruit drop was observed highest (68.6%) in Illaichi and lowest (24.1%) in Ponda (Vashishtha and Pareek, 1979). Panwar (1980) reported the highest fruit drop (80.6%) in Kakrol Gola than Umran (7.2%) and Kaithali (12.1%). The smaller fruit (<1.0 cm diameter) observed >50 per cent drop and it decreased with increase in size of the fruits.

Spraying growth regulators and chemicals

It is better to improve the fruit regulation and prevent the pre-harvest fruit drop. Fruit setting can be improved by spraying 10-20 ppm GA_3 3-4 times at 5-6 days interval at full bloom stage or two sprays of borate 0.2 per cent during full bloom stage for rapid fruit growth (Liu, 2004). 10.4 to 12.2 per cent fruit set and 22.1 to 28.8 per cent seed set obtained in pollination during morning hours, followed by the spray of boric acid 100 or 200 ppm in the afternoon (Yun *et al*., 1995). However, pollination during evening hours followed by a spray of boric acid 200 ppm along with NAA 1 ppm observed 2.0 per cent fruit set and seed set 11.8 per cent. The premature fruit drop can be controlled by the application of NAA 10-20 ppm before the expected fruit drop and 4 weeks before harvest (Yung and Liu, 1990).

Rootstock genetic resources

In addition to the above facts the potential use of rootstock and maintenance in the collection is required. Not much emphasis has been given to the rootstocks only wild species are being used and tested as rootstocks. Bal *et al.* (1997) has reported the rootstocks compatibility species for Indian jujube. They reported that *Z. mauritiana, Z. mauritiana* var. *rotundifolia* (wild/naturalised), *Z. Abyssinia* as most successful and widely used rootstocks. Less successful but mostly compatible and often cultivar specific were *Z. nummularia, Z. xylopyrus, Z. spina-christi, Z. mucronata, Z. oenoplia* and *Z. jujube.* Chinese jujube rootstocks are most frequently wild materials (especially var. *spinosa)* related to the cultivars, but several other wild species have been tried in areas of China with more extreme climates (Ming and Sun, 1986).

Exploitable attributes of wild species in ber improvement

Z. nummularia and *Z. lotus* are drought tolerance, having dwarf tree stature and extensive root system and early fruit maturity. *Z. jujuba* found *r*esistance to low-temperature damage with excellent dehydration quality of fruits and high vitamin C and P contents in fruits. *Z. mistol is r*esistance to low-temperature damage while *Z. mauritiana* var. *rotundifolia* had *v*igorous tree frame, wood of marginal timber value (Pareek, 2001).

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