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JuJube floral biology and pollen germination for improvement in ber breeding under arid and semi-arid regions of South West Haryana Conditions

Anuradha Bishnoi* and **Mukesh Kumar**

CCSHAU RRS Bawal (Rewari) 123501, Haryana, India

ABSTRACT

Ber (Ziziphus mauritiana Lamk.) is a major fruit crop of arid and semi-arid regions. In this investigation, the jujube floral biology and pollen germination of nine cultivars were studied. Pollen quality is important for growers and breeders. The study was carried out at CCSHAU Regional research station Bawal to determine in vitro pollen germination in ber to remove the hurdle of breeding in ber. Pollen traits of varieties were studied using an in vitro medium containing 0 %, 5 %, 10 %, 15 %, and 20 % sucrose to determine the best sucrose concentrations for germination. In the second step, the germinated pollen was counted 1, 4, 6, 10, 12, and 24 hours later until there was no further germination. The best germination rates were found in 15 % sucrose concentration in most of the varieties. Pollen germination rates were recorded periodically from one hour to 24 hours in 15 % sucrose, and the results showed that pollen germination rates increased after 6 hours of being placed in culture media. The percentage of in vitro germination of pollen tubes among the varieties was also found to be different. Among the nine varieties, the highest pollen germination per cent (80%) was found in variety Chhuhara.

Keywords: *in vitro, media, pollen germination, jujube, floral biology, improvement, breeding.*

Introduction

The Indian jujube or ber (*Ziziphus mauritiana* Lamk.) is one of the ancient and indigenous fruits of India, which belongs to the family Rhamnaceae. The tree is an example of an extremely drought-hardy species, which can be grown in dry land areas and in degraded, eroded, gravelly, saline, and sodic wastelands. The jujube tree has commercial importance owing to the usefulness of almost all its parts. Berries are richer than apples in protein, phosphorus, calcium, carotene, and Vitamin C [15], and oranges in phosphorus, iron, vitamin C, and carbohydrates. It is also a good source of antioxidants. Most of the present-day commercial cultivars were developed through selection. Also, seedlings growing in nature were selected by the farmers based on economic characters and further propagated vegetatively to maintain their genetic identity. Major focus in ber breeding has been on clonal selection for early maturing clones. Most of the common cultivars are the result of selection in different regions [14].

Ber (*Ziziphus mauritiana* Lamk.) is a major fruit crop of arid and semi-arid regions. It belongs to the Rhamnaceae family, which has 600 species and about 50 genera. The tree is an example of an extremely drought-hardy species that can be grown in dry land areas and on degraded, eroded, gravelly, saline, and sodic wasteland. It is a dominant component of the natural vegetation in the Indian "Thar desert" and thrives well under a maximum annual temperature of 35-42°C and minimum temperature of 4-12°C. The plant can tolerate temperatures as high as 49-50°C and as low as -2°C. However, the growth and development of the plant are affected at both extremes. Most of the commercial cultivars are reported to be tetraploids. On the basis of cytological studies, [13] reported the chromosome number in 33 ber cultivars (Banarasi, Banarasi Pewandi, Chhuhara, Dandan, Desi Alwar, Golar, Gorva, Kaithali, Kala Gola, Katha Bombay, Kathaphal, Laddu, Mirchia, Nalgarhi, Narikeli, Nazuk, Noki, Pathani, Sandhura Narnaul, Sanaur, Sanaur-2, Sanaur-3, Sanaur-4, Sanaur-6, Seo, Safeda Selected, 28/1, Umran, Willyati, ZG-2, ZG-3, Hoshiarpur) was n=24. [17] confirmed that Illaich is an octaploid (2n=96) and Umran a tetraploid (2n=48), and *Ziziphus rotundifolia* is both tetraploid and hexaploid (n=24, n=36). Flowering time and duration of ber cultivars grown in India vary at different locations. In the subtropical climate of north India (latitude 24-30°N and longitude 72- 37 82°E), plants enter into a dormant phase during the extremely warm months (April-May). Flowers emerge mainly during the monsoon period (August-September) after resumption of growth. Flowering continues through early winter (November) with some sporadic flowering in late November and December [12 and 8]. In the tropical climate of southern India (latitude 9-20°N and longitude 77-78°E), growth continues throughout the mild winter and comparatively lower summer temperatures, with flowering continuing through the extended period from May to September. The flowers are borne on the current season's growth in the leaf axil, the inflorescence is cymose, each cyme containing 15-28 flowers. The anthesis takes place either in the morning (6-8 AM) or in the afternoon (12-2 PM), depending on the cultivars. For example, the anthesis in cultivar Seb is in the morning and in Gola in the afternoon. Flowering duration has been observed to be shortest (47 days) in cultivar Tikadi and the longest (61 days) in cultivar Illaichi [1].

Jujubes have small flowers with a diameter of 6–7 mm [32], which makes emasculation almost impossible.

*Corresponding Author: ***Anuradha Bishnoi***

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Even if emasculation could be done manually, it would still be difficult to have enough hybrid seeds with jujube's low fruit-set, one to several percent [9 and 32]. Also, jujube flowers are borne in an inflorescence in each leaf axil, so there are always flowers emerging after the emasculation, which can complicate any attempts at hybridization [32]. *Ziziphus* has hypanthium-type flowers (a cup or tube bearing the floral parts above the base of the ovary of the flower) with five membranous hood-like petals. The ovary has two chambers, each with a single ovule, and is broadly attached at the base. Each fruit can bear two viable embryos. The five stamens are epipetalous, each being surrounded by a petal. The pistil is central, terminating in two stigma lobes [7]. *Z. mauritiana* flower buds emerge from an axillary position in a cyme inflorescence. The flowers open only for 1 day, with flowers at the top of the plant being the first to open. Each cyme has 12-14 buds [31], depending on the cultivar. Ziziphus flowers exhibit synchronous protandrous dichogamy, i.e., flowers of an individual plant mature in synchrony and anther dehiscence precedes stigma receptivity with little or no overlap between the sexual stages [24]. Such temporal separation of sexual functions has been referred to as a ''temporal dioecism'' [6]. Genotypes of *Ziziphus* species are divided into two groups according to the timing of anthesis-morning or afternoon-designated groups A and B, respectively. In *Z. mauritiana*, anther dehiscence begins shortly after anthesis and terminates within 2 h to 4 h. Flowering phases of the two types overlap-; thus, utilizing complementary sexual morphs in orchard design is vital for successful crosspollination and optimum yields. *Z. mauritian*a is reported to be self-incompatible. The flowering period is prolonged; in Israel, the flowering season begins in early July and continues till the end of October. Late pruning may delay the flowering season. Flowers are visited by different species of insects, including wasps, flies, butterflies, and bees [16]. Earlier workers found that two processes take place rapidly in the pistil of *Z. spina-christi* following anthesis: elongation of the style and stigma development. Stigmas are considered receptive when they can support germination of compatible pollen grains. Based on the observations of a sticky, shiny secretion from the stigma and on-hand pollination tests, *Z. mauritiana* is reported to be receptive on the day of anthesis. Yet, detailed studies of style elongation and stigma development and the relationship of these two processes to stigma receptivity in *Ziziphus* are lacking [30].

Due to the phenomenon of cross-pollination along with the prevalence of self and cross incompatibility and pollen sterility, fruit set in the ber largely depends on timely pollination and its related factors like pollinators and environmental conditions, and on the success in fertilization. The life of an individual flower is very short, and many flowers apparently are not pollinated during their respective period. Consequently, in spite of a very profuse flowering, fruit set in the ber is very low even under open pollination. Hence, by studying the elaborated information's on various aspects, breeders can overcome the bottlenecks and hindrances in ber breeding. Various crosses were made among the cultivars, but the success rate was negligible. Some fruit sets were successful, but they dropped prematurely at pinhead stage.

The development of reliable methods for determining the functional quality of pollen helps in monitoring pollen vigor during storage, genetics and pollen-stigma interaction studies, crop improvement and breeding, and incompatibility and fertility studies [28]. The quality of pollen is assessed on the basis of viability and vigor of the pollen grain.

Pollen vigor refers to the speed of germination of pollen grains and the rate of pollen tube growth [20]. Pollen that could not germinate usually shows poor tube growth which is likely to be ineffective in causing fertilization. In the breeding of fruit species, it is very important to use a suitable method for determining pollen viability [23]. In vitro germination tests have been used to indicate viability of pollen [26]. Therefore, the objectives in this study were to observe floral biology and check the viability and pollen germination of different ber cultivars cultivated at CCSHAU RRS Bawal for improvement in breeding program of ber. Assessment of pollen viability has direct relevance in hybridization as pollen of male parent takes part in the fertilization process.

Materials and Methods

The experiment was conducted at CCS Haryana Agricultural University, Regional Research Station, Rewari which is located at an altitude of 241.95 meters above sea level, at coordinates 28° 5' 44.1204" N latitude and 76° 35' 33.9036" E longitude, during the year 2022-23 and 2023-24. Bawal region is characterized by arid regions with hot and dry summer and cold winter. As trees were planted and grafted during the different years. A floral biology study was carried out at an experimental orchard in which plants were planted at a 7 ×7 m distance in a randomized block design. A randomized block design at a spacing of 7 m x 7 m. In the experiment, three plants from each cultivar were selected and maintained under regular agronomic procedures according to the package of practices.

From each plant, ten branches were randomly selected and tagged to determine the start of flowering. Five per cent opening of flower buds on the tagged branches was considered as flowering initiation, and the average date of initiation was noted. The flower position on the tagged branches was observed visually with the naked eyes at the flowering stage during regular visits in the orchard as axillary cyme, terminal, and axillary clusters based on groups or clusters of flowers arranged on a branch. A day before opening, 50 buds that were supposed to open the next day were marked to monitor the time of anthesis. The number of completely opened flowers was counted every half-hour interval starting from 5 a.m. the next morning. To stop recounting, fully opened flowers were labelled with delible ink. The observations on anthesis were carried out before 50 flower buds had fully opened in each cultivar during the flowering season. The findings were made in each cultivar on three successive days when the plants were in full bloom. Pedicel length (mm), petal length, and petal breadth (mm) were recorded with the help of a digital vernier calliper.

The pollen germination and pollen viability studies were conducted around the half bloom (flowering), when flowers were half opened. Pollen viability was tested using TTC (2,3,5 triphenyl tetrazolium chloride) [3]. 1% TTC (0.2g. TTC and 12g sucrose dissolved in 20_mL distilled water) was used in the first step, and a drop of the mixture was dropped on a microscope slide, and the pollen was spread with a slim brush, and covered with a coverslip. Pollen viability counts were made after two hours; then, pollen was placed in a TTC solution. Pollen grains stained that orange or bright red color were counted as viable [5,19 &18].

Stain tests are faster and easier than pollen germination tests, but in some cases, germination tests are necessary to observe the actual viability of pollen. For pollen germination, flowers were taken in a petri plate and culture media having various concentrations of sugar (5 & 15%), boric acid (5, 10 &15mg),

and calcium nitrate (25,30 & 35 mg) in 100 ml double distilled water were prepared. pH was adjusted to 5.8 [10] For pollen germination tests, 0.35-0.4 ml of media was used per slide and smeared with a glass rod. With jujube's small flowers, direct inoculation was used in this study [10] Each cultivar had three slides as replications with four to five open flowers on each slide. Each slide was put into a petri dish with a piece of wet paper towel to buffer the humidity. Petri dishes were sorted in a covered tub. After 4-5 h of incubation at 22 ^{0}C, pollen germination was observed and photographed under an Advanced Research microscope with a digital imaging system. Around 200 pollen grains were counted for pollen germination rate and abortion rate. For pollen germination data, the germination rate of each cultivar per slide was the average of three slides. Pollen grains were considered germinated when the pollen tube length reached pollen diameter [4]. The experiment was a completely randomized design. The statistical analysis of the data obtained in the experiment was conducted using software OP STAT [27], and treatment means were compared at a 5% level of significance. Correlation analysis was performed to determine the relationship between pollen viability and pollen germination, and evaluated using M.S Excel.

Results and Discussion

Flowering is an important trait in horticulture; evaluation of this complex trait is a long process because of the prolonged juvenile period of trees and the influence of environmental conditions affecting gene expression year by year. Consequently, flowering time has to be studied for several years to have statistically significant results. The flowering was observed in ten ber cultivars; different ber cultivars showed variation in it. Flowering started first in Gola, followed by Kaithali and Umran. Most of the cultivars were in peak bloom in the first and second week of October however, Chhuharha was first to come into full bloom (5-9 October), followed by Reshmi (5-10 October). These results are in line with the finding, of [2] and [21].

The data on anthesis are presented in Table 1, which shows that variations were found regarding the time of anthesis among the different cultivars. Anthesis was completed on the same day. The data revealed that anthesis was observed earliest in the Cultivar Apple Ber and BS2. The differences in the time of anthesis among the varieties might be due to their genetic factor and environmental effects. Flowering occurred in the leaf axil in all the cultivars, and the number of flowers per cyme lies between 19 to 35. Calyx color and apex of petals were found to be light green and acute, respectively, among all the cultivars. The highest pedicel length was found in cultivar Reshmi (9.08 mm), and petal size (length and breadth) were in cultivar Apple Ber (1.98 mm * 1.04 mm), followed by Gola (1.85 mm * 1.04 mm). Flowering time variations in ber may be connected to environmental factors like temperature, humidity, and rainfall, as well as the genetic composition of the variety/ germplasm [25]**.** In ber, time of anthesis differed from cultivar to cultivar. It happened in the morning in some cultivars while in the afternoon in others (Table 1).

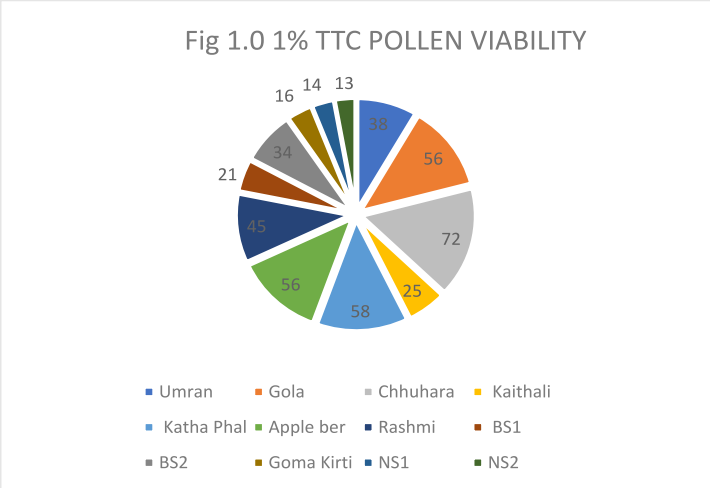
Table 01 Flowering parameters of ber (Ziziphus mauritiana L.) cultivars under south west Haryana conditions

Variety	Period of bloom	Peak of bloom	Time of anthesis	Number of flowers per cyme	Pedicel length (mm)	Calyx color	Apex of petal	Petal length (mm)	Petal breadth (mm)
Gola	1 Sept. - 28 Oct	11-15 Oct.	11:20 am to 1:15 pm	31	8.1	Light green	Acute	1.85	1.04
Umran	02 Sept.-25 Oct.	12-13 Oct.	11:20 am to 1:15 pm	25	8.26	Light green	Acute	1.58	0.96
Chhuharha	4 Sept. to 20 Oct.	5-9 Oct.	11:20 am to 1:15 pm	20	6.83	Light green	Acute	1.5	0.97
Kaithali	2 Sept. to 27 Oct.	15-19 Oct.	11:45 am to 1:30 pm	31	7.21	Light green	Acute	1.61	0.84
Katha Bombay	6 Sept. to 27 Oct.	12-15 Oct.	11:45 am to 1:15 pm	25	8.36	Light green	Acute	1.53	0.96
Reshmi	05 Sept. to 30 Oct.	5-10 Oct.	12:00 pm to 1:30 pm	35	9.08	Light green	Acute	1.53	0.94
Apple ber	01Sept. - 28 Oct	12-17 Oct.	6:00 am to 8:00 am	23	8.5	Light green	Acute	1.98	1.02
BS1	12 Sept. 15 Oct	10-13 Oct.	6:15 am to 8:00 am	21	6.23	Light green	Acute	1.58	0.96
BS2	10 Sept. - 28 Oct	11-15 Oct.	6:00 am to 8:00 am	19	7.5	Light green	Acute	1.55	0.95

Table 02 Fruiting parameters of ber (Ziziphus mauritiana L.) cultivars under South west Haryana conditions

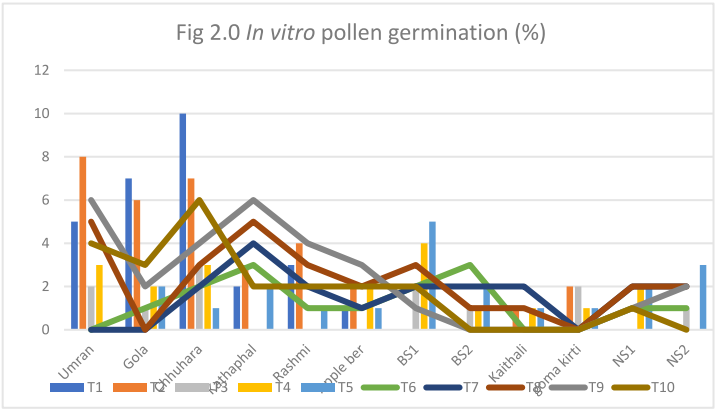
Variety	Time of fruit set	Peak time of fruit set	Number of picking per season	Time of harvest	Peak time of harvest	Number of fruits per cluster	Yield per tree (kg)	Cultivar class
Gola	7 Oct to 22 Nov	29 Oct.	3-4	05 Feb -10 March	28 Feb.	2-3	45	Early
Umran	05 Oct. to 25 Nov.	05 Nov.	3-4	20 March to 30 March	20 March	2-3	100	Late
Chhuharha	10 Oct to 8 Oct	29 Oct.	3-4	3 Feb -20 March	05 March	2-3	55	Early
Kaithali	12 Oct. -08 Nov.	06 Nov.	3-4	22 Feb.- 15 March	15 March	2-3	42	Medium
Katha Bombay	08 Oct. 11 Nov.	10 Nov.	3-4	17 Feb. -20 March	17 March	2-3	36	Late
Reshmi	09 Oct. -19 Nov.	27 Oct.	3-4	01-16 March	12 March	2-3	30	Medium
Apple ber	01 Oct. 10 Nov.	01Nov.	3-4	02 Feb. -20 March	03 March	2-3	45	Early
BS1	10 Oct. -15 Nov.	06 Nov.	3-4	10-26 March	22 March	2-3	75	Late
BS2	12 Oct.- 18 Nov.	10 Nov.	3-4	05-25 March	18 March	2-3	80	Late
CD	-	-	-	-	-	--	6.3	-

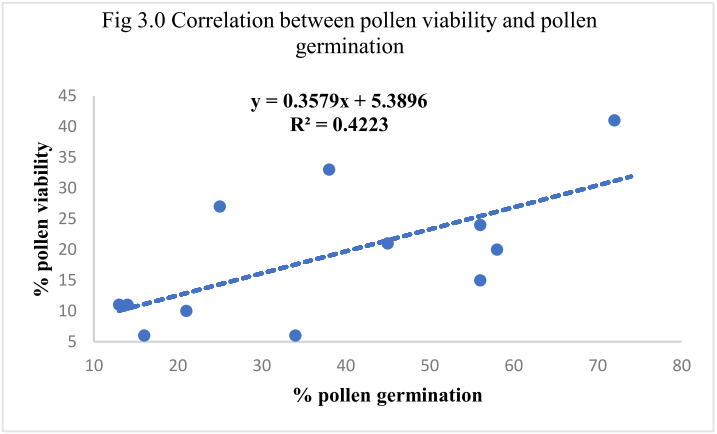
The data presented in Table 2.0 depicts the fruiting of ber cultivars. Fruit set started in the first week of October and ends in the Last week of November in the ber cultivars. However, the peak time of fruit set was the last week of October to the first week of November. This might be due to the combination of the genetic makeup of the plant and the environmental factors. If the environmental conditions are favorable, then fruit set may be early. Harvesting of fruits starts from February and ends in March. Cultivars are categorized into Early, Mid, and Late on the basis of maturity. The highest yield was recorded in the Umran (100 Kg/plant), followed by the Bs2.



In this study, the pollen viability of the cultivars was high in the staining test. The ber cultivars exhibited differences in pollen viability. In the TTC test, cultivar Chhuhara had the highest pollen viability (72%) followed by the Katha Phal, Apple Ber, and Gola (Fig. 1.0). Determining the viability of pollen grains is an easy method to increase the efficiency of breeding programs and for the selection of a suitable pollinizer.

A total of ten media combinations were used for *in vitro* pollen germination. It was observed that pollen of different cultivars responded differently to variation in the concentration of sugar and boric acid in the media. The data presented in Fig. 2.0 indicated that there was significant variation in pollen germination of the varieties and also between the types of media used. Among different media combinations, media used with 5 % sucrose, 15 mg boric acid, and 30 g calcium nitrate in 100 ml double-distilled water were found best for *in vitro* pollen germination in all twelve cultivars of *jujube*. Among the cultivars highest per cent germination was recorded in Chhuharha, followed by Umran and Gola (Fig. 2.0). The germination of pollen is comparatively lower in all the varieties, which might be due to the genetic makeup of the crop. The differences in germination of the pollens among the varieties were due to their varietal character, while the differences between the two media might be due to the addition of boron, which helps in the better growth of pollen tubes. Additionally, the pollen germination in a crop is not high and uniform, as a number of factors, viz., genotype, constituents of pollen germination medium, floret and anther stage, moisture content of the pollen, incubation period, and the purity during pollen germination would have an effect on pollen germination. The reason for the non-germination of the pollens might be due to the loss of viability or decrease in vigour [11].





The correlations between pollen viability and germination tests were also determined and were found poorly significant in the TTC test (2 = 0.4223) (Figure 3.0). Parfitt and Ganeshan (1989) have established that the pollen stain tests (including TTC) are not reliable or consistent and are not positively correlated with in vitro germination tests. Similar results were reported by [29] in cherry laurel, that the correlation between pollen germination and pollen viability was not significant, and reported that stains might be used to determine pollen viability in fruit species to provide only a rough estimate of viability. These results are in agreement with our findings.

Conclusion

This study, results showed pollen viability and germination rates in various conditions for cultivars of Ber. Results of this study indicated that TTC and the in vitro germination test could be used to determine the viability results close to real germination rates. The results presented here are the first observation on the *Ziziphus species* pollen viability and germination rate that will help in a ber breeding improvement program.

Future Thrust

Assessment of pollen viability has direct relevance in hybridization, as the pollen of the male parent takes part in the fertilization process. Therefore, pollen germination study is an important activity in order to determine the potential of the male parent for fertilization and seed setting after crossing.

Declaration of competing interest: Author declares that they have no conflict of interest.

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