



Pollination Biology in Henna-Evidences from Semi-Arid Region of Rajasthan

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Abstract: A study was carried out to study the pollination system in henna at CAZRI, RRS, Pali marwar in hot semi-arid region of India. Two types of pollination systems viz., natural open pollination and pollination in controlled condition were studied. Total of 10 inflorescences borne on ten different 20-year old henna plants were selected and covered with muslin cloth bags and butter paper for controlled pollination. Simultaneously, 10 inflorescences of same plants were kept uncovered for open pollination. Out of three modes of pollination studied in henna, maximum fruit set (60.84%) was observed in natural open pollinated condition while minimum fruit set (11.78%) recorded in controlled pollination covered with butter paper followed by controlled pollination covered with muslin cloth (10.10%). The highest fruit flower ratio also registered in natural open pollinated condition (0.608) whereas lowest were recorded in controlled pollination covered with muslin cloth (0.117) followed by controlled pollination covered with butter paper (0.101). Besides, the common flower visitors also observed which may favour the cross pollination in henna flowers. This study confirms that henna is cross pollinated species.

Keywords: Henna (*Lawsonia inermis* L.), Pollination systems, Self-pollination, Cross pollination, Arid and semi-arid region

Lawsonia inermis L. belongs to the family Lythraceae commonly called as Henna or Mehndi has been commercially cultivated for promising dye yielding cash crop which is mainly used for dyeing hair, palm and feet since ancient times (Singh et al 2015). Henna cultivation is profitable under low rainfall conditions and give assured income returns at low cost investment in drought prone arid and semi-arid regions. Due to its drought hardiness, deep root system and perennial nature, it can be cultivated on lands that are drought prone, marginal or unsuitable for arable cropping. Economic production of leaves starts from the third year onwards that continues for the next 15-30 years (Chand and Jangid 2007). Globally, India has exported 2,383 tons of henna to several countries in the year 2002-03 which indicates high demand in international export market. The plants are glabrous, much branched shrub or small tree with greyish brown bark. Leaves are opposite, sub sessile, elliptical or lanceolate, entire and acute. Flowers are numerous, small white or pink coloured with fragrant and in terminal panicle cymes. Crop is propagated through seeds and vegetative propagation. Oil also extracted from leaves and flowers called "Otto of henna" and it is utilised as perfume (Jaimini et al 2005). Since leaf is economical part of henna, flowering is considered as undesirable trait. Generally from farmer's point of view flowering is an undesirable trait beyond its use in perfume industries and the fruiting have an impact on leaf yield and lawsone content. Therefore, pollination is very much essential for understanding the flowering and fruiting pattern in henna.

Pollination is an essential step in ensuring seed production and it is a critical stage in the sexual reproduction of plants. Transfer of pollen from the anther of the flower to the stigma of the same flower or of another flower is called as Pollination. It is a prerequisite for fertilization and fruit set. Some flowers develop fruits/seeds due to self-pollination (when pollen and pistils transfer from the same plant or often same flower) and some develops due to cross pollination (when pollen from one flower transfers to different plant). Many plants are self-incompatible and in this condition an animal or an insect that move pollen from the anthers to the stigmas of flowers, thus effecting pollination. This is usually as a result of their activities when visiting plants for feeding, breeding or shelter.

Plants have evolved diverse pollination strategies ranging from complete selfing to obligate outcrossing (Richards 1986). Except fully self-incompatible and dioecious species, most of the others show a mixed mating system permitting both self- and cross-pollinations. The proportion of each is highly variable between populations and species depending on the structure of the flower, breeding system and pollination environment (Shivanna 2015). Autogamy happens to be the most frequently evolved strategy in different groups of plants (Kalisz and Vogler 2003, Goodwillie et al 2005, Eckert et al 2006, Levin 2012, Wright et al 2013). Many of the species show flexibility in their pollination strategy and show a mixed mating system. Apart from self-pollination they permit cross pollination when the

pollinators are available (Goodwillie et al 2005). According to Miczak 2001, henna is a self-pollinating species; its seeds are so hardy that they must be soaked in water to facilitate germination. And also, Roy and Jindal (2009) mentioned that henna is self-pollinated woody shrub. No genetic improvement or biodiversity conservation programme can be success in absence of precise information on degree of selfing or out crossing. However, studies on mode of pollination of henna are still unclear and very limited. This study aims to study the mode of pollination of henna to improve the fruit set.

MATERIAL AND METHODS

The effect of pollination control on fruit setting in henna was studied in henna experimental field, at ICAR-Central Arid Zone Research Institute (CAZRI), Regional Research Station (Pali-Marwar, Rajasthan) in hot semi-arid region of India during 2017-18 to 2020-21. The annual average rainfall of the experimental site is 460 mm with annual maximum mean temperature of 42°C and minimum 7°C. The experimental site of henna is located between 25°47'-25°49'N and 73°17'-73°18'E at 217-220 m msl. The soils were shallow in depth (30-45 cm) with sandy clay loam to sandy loam texture, 1.35-1.5 Mg m⁻³ bulk density, 7.7-8.4 pH, 0.15–0.55 dSm⁻¹ electrical conductivity and a dense underlying layer of murrum (highly calcareous weathered granite fragment coated with lime). The meteorological data for the study period has been given in Figure 1.

Uniform and healthy plants were selected randomly from the middle portion of the plantation. Two types of pollination systems were studied. These were i. natural open pollination, where buds were tagged and allowed to pollinate naturally; ii) controlled pollination, where whole inflorescence was

bagged as such with muslin cloth and butter paper. Total of 10 inflorescences borne on ten different 20-year old henna plants were selected and covered with muslin cloth bags and butter paper for controlled pollination. Simultaneously, 10 inflorescences of same plants were kept uncovered for open pollination. The inflorescences were tagged and already opened flowers occurring at the basal end and the young floral buds at the distal end were clipped off in all the selected inflorescences. The number of remaining floral buds and the fruits formed on the inflorescences were recorded and the fruit set calculated under both pollination conditions. And also, length of inflorescence (cm), number of flowers per inflorescence, length of fruits (mm), width of the fruits (mm) and flower to fruit ratio was recorded on ten well developed inflorescence on each of the ten plants. The observation was made to record the floral visitors for understanding of the mode of pollination in henna inflorescence.

RESULTS AND DISCUSSION

Inflorescence and fruit characteristics of henna:

Flowering in henna was observed with creamy white colour flowers during September in most of the plants. Flowering started in July after the onset of monsoon and continues till mid-September in Pali district of Rajasthan. The number flowers in inflorescence and length of inflorescence were recorded on ten well developed inflorescence on each of the ten henna plants. The length of inflorescence varied from 4.72 cm to 8.13 cm with mean value 6.42 cm. Maximum numbers of flowers per inflorescence was 218 and minimum was 22 with the mean 120. The berries are oval in shape and green colour berries turns into greyish black colour when it matures. The length of fruit ranged from 5 mm to 2.9mm. The maximum width recorded in berries was 8.3mm where

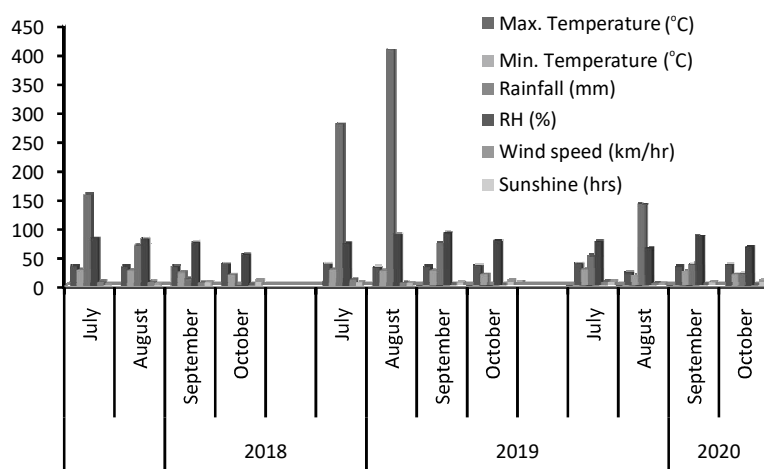


Fig. 1. Meteorological data for the study period at Pali marwar, Rajasthan

minimum was 3.8mm with the mean value 6.05mm. And the average fruit flower ratio recorded was 0.6. Maximum ratio of fruit flower recorded in the henna was 0.7 and minimum was 0.5 (Table 1).

Fruit set in henna under different pollination conditions:

Ten inflorescences were selected and recorded the observations for the fruit set under open pollinated condition and controlled pollination condition (covered with muslin and butter paper). Maximum number of fruits (618 fruits) was

recorded in open pollinated condition from 1015.6 flowers (Table 2). Fruit produced under controlled pollination, where the inflorescence covered with muslin cloth produced 136.6 fruits out of 1160 buds (Table 3). Minimum number of fruit set (105.6 fruits) out of 1045.6 buds was registered in controlled pollination, where the inflorescence covered with butter paper (Table 4). The same trend was observed throughout the study period in all types of pollination in henna. Similar study of the mixed pollination biology of *Oreocereus*

Table 1. Flower and fruit characteristics of Henna

	Minimum	Maximum	Mean	SD
Length of inflorescence (cm)	4.72	8.13	6.42	2.41
No. of flowers per inflorescence	22.0	218.0	120.0	138.5
Length of fruits (mm)	2.90	5.00	3.95	1.48
Width of the fruits (mm)	3.80	8.30	6.05	3.18
Flower to fruit ratio	0.50	0.70	0.60	0.14

Table 2. Fruit set in henna under open pollination conditions

No. of Inflorescence	2018		2019		2020		Mean	
	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set
1	99.00	46.00	180.0	99.00	118.0	77.00	132.3	74.00
2	28.00	21.00	73.00	57.00	179.0	124.0	93.33	67.33
3	60.00	42.00	80.00	62.00	41.00	26.00	60.33	43.33
4	22.00	13.00	147.0	80.00	127.0	86.00	98.66	59.66
5	96.00	75.00	94.00	47.00	67.00	39.00	85.66	53.66
6	47.00	20.00	77.00	51.00	122.0	79.00	82.00	50.00
7	50.00	21.00	343.0	181.0	97.00	66.00	163.3	89.33
8	56.00	18.00	63.00	36.00	66.00	39.00	61.66	31.00
9	38.00	17.00	136.0	87.00	119.0	78.00	97.66	60.66
10	107.0	67.00	150.0	84.00	165.0	116.0	140.6	89.00
Total	603.0	340.0	1343.0	784.0	1101.0	730.0	1015.6	618.0

Table 3. Fruit set in henna under controlled pollination (covered with muslin cloth) conditions

No. of Inflorescence	2018		2019		2020		Mean	
	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set
1	88.00	29.00	213.0	13.00	97.00	4.000	132.6	15.30
2	28.00	16.00	108.0	24.00	179.0	18.00	105.0	19.30
3	93.00	22.00	194.0	5.000	93.00	25.00	126.6	17.30
4	64.00	22.00	161.0	9.000	75.00	12.00	100.0	14.30
5	132.0	7.000	143.0	0.000	61.00	2.000	112.0	3.000
6	26.00	16.00	85.00	2.000	85.00	3.000	65.33	7.000
7	95.00	4.000	81.00	4.000	173.0	11.00	116.3	6.300
8	36.00	3.000	208.0	16.00	210.0	18.00	151.3	12.30
9	78.00	4.000	294.0	36.00	101.0	20.00	157.6	20.00
10	42.00	3.000	159.0	52.00	78.00	10.00	93.00	21.60
Total	682.0	126.0	1646.0	161.0	1152.0	123.0	1160.0	136.6

celsianus (Cactaceae) mating breeding system has been reported for globose cactus species in northern tropics (Nassar and Ramirez 2004, Nassar et al 2007).

Fruit set (%) and fruit flower ration in henna under different pollination condition: Pollination and fertilization is the two important process of annual cycle for fruit setting in henna. In henna, significant differences were observed between the different pollination condition in fruit set (%) and fruit flower ratio. Out of three modes of pollination studied in henna, maximum fruit set (60.84%) was observed in natural open pollinated condition. Minimum fruit set (11.78%) was recorded in controlled pollination covered with butter paper followed by controlled pollination covered with muslin cloth (10.10%) (Table 5). Similarly, a decrease in the percentage of fruit from the controlled crossing over time was observed in this argane tree orchard (Ait Aabd et al 2022).

Fruit flower ratio was also calculated in henna under different pollination conditions. The highest fruit flower ratio was registered in natural open pollinated condition (0.608) whereas lowest were recorded in controlled pollination covered with muslin cloth (0.117) followed by controlled pollination covered with butter paper (0.101) (Table 6). The data showed that one fruit is produced out of two flowers when it is pollinated under natural open condition while one

fruit is produced out of ten flowers when it is pollinated under controlled condition. The exploitation of knowledge on self-incompatibility mechanisms in flowering plants is very useful. Since Darwin's (1876) studies, considerable knowledge has been acquired about these mechanisms of self-incompatibility. Recently, for other species, incompatibility systems create barriers to avoid self-fertilization and promote cross-pollination (Dutta et al 2013, Pereza et al 2016). In this study, minimum fruit set during controlled pollination suggested that the lack of self-incompatibility and pollination even in the absence of flying insects in controlled pollination. As cross pollination by insect or air-borne pollen is checked by muslin cloth and butter paper cover, the fruiting observed under the controlled condition is apparently due to self-pollination. The fruiting observed under open conditions is considered to be the result of cross pollination. Similar study was also conducted by Shivanna 2015 on pollination strategies of perennial weeds particularly of Indian species (*Cassia auriculata*, *Ipomoea obscura*, *Oxalis corniculata*, *Plumbago zeylanica* and *Dodonaea viscosa*).

Flower visitors in henna: Even though there was no separate study conducted for flower visitors, some flower visitors were observed through direct observations on several flowers during the experiment. A large numbers of insects

Table 4. Fruit set in henna under controlled pollination (covered with butter paper) conditions

No. of Inflorescence	2018		2019		2020		Mean	
	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set	No. of buds	No. of Fruit set
1	97.00	14.00	140.0	7.000	260.0	12.00	165.6	11.00
2	36.00	11.00	146.0	4.000	112.0	29.00	98.00	14.60
3	58.00	9.000	82.00	11.00	127.0	12.00	89.00	10.60
4	63.00	11.00	145.0	10.00	165.0	13.00	124.3	11.30
5	25.00	4.000	93.00	8.000	117.0	6.000	78.30	6.000
6	37.00	3.000	123.0	6.000	60.00	4.000	73.30	4.330
7	46.00	7.000	87.00	3.000	78.00	6.000	70.30	5.330
8	87.00	11.00	202.0	22.00	65.00	7.000	118.0	13.30
9	46.00	12.00	107.0	15.00	81.00	7.000	78.00	11.30
10	68.00	9.000	229.0	39.00	155.0	5.000	150.6	17.60
Total	563.0	91.00	1354.0	125.0	1220.0	101.0	1045.6	105.6

Table 5. Fruit set (%) in henna under different pollination conditions

Type of pollination	2018	2019	2020	Mean
Open pollination (Uncovered)	56.38	58.37	66.30	60.84
Controlled pollination (Covered with muslin cloth)	18.47	9.781	10.67	11.78
Controlled Pollination (Covered with butter paper)	16.16	9.231	8.278	10.10
S.Em±	1.24	1.20	1.35	1.25
CD (p=0.05)	4.30	4.14	4.66	4.34
CV(%)	9.45	10.72	10.95	10.50

Table 6. Fruit Flower ratio in henna under different pollination conditions

Type of pollination	2018	2019	2020	Mean
Open pollination (Uncovered)	0.563	0.583	0.663	0.608
Controlled Pollination (Covered with muslin cloth)	0.184	0.097	0.106	0.117
Controlled pollination (Covered with butter paper)	0.161	0.092	0.082	0.101
S.Em±	0.01	0.01	0.01	0.01
CD (p=0.05)	0.04	0.04	0.05	0.04
CV (%)	9.45	10.73	10.96	10.51

were seen around the inflorescence and plants. The insects such as honey bee, blister beetle, ants and butterflies were observed during the crossing study. The inflorescence and flowers which produces the fragrance may attracted the insects and favours the cross pollination in henna. Cross-pollination in the henna can be performed by the wind or by pollinators as reported by several studies (Benlahbil et al 2003, Nerd et al 1998, Ajerrar 2020). These studies confirm that insects play an essential role in the pollination of the henna. However, the role of vectors in pollination (wind and insects) was not well understood during our study of crosses in henna.

CONCLUSIONS

The study reveals that maximum fruit set was observed in natural open pollinated condition while minimum fruit set in controlled pollination covered with butter paper followed by controlled pollination covered with muslin cloth. And also the highest fruit flower ratio registered in natural open pollinated condition whereas lowest were recorded in controlled pollination covered with muslin cloth followed by controlled pollination covered with butter paper. Besides, the common flower visitors also observed in henna flowers. The study concluded that minimum number of fruit set in henna under controlled pollination confirms the lack of self-incompatibility and maximum number of fruits set under open pollinated condition confirms the result of cross pollination. So, the study confirms that henna is cross pollinated species.

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