Annals of Sri Lanka Department of Agriculture 2017. 19: 209 -221

FLORAL BIOLOGY, POLLINATION BEHAVIOR AND REPRODUCTION BIOLOGY OF BAEL FRUIT (*Aegle marmelos* L.) CULTIVARS GROWN IN LOW COUNTRY WET ZONE IN SRI LANKA

W.D. LESLY, D.D. GUNARATHNE AND J.A.C.D. JAYASINGHE

Fruit Research and Development Institute, Kananwila, Horana, Sri Lanka

ABSTRACT

The knowledge on reproduction biology of Bael fruit is important to improve the varieties and also to enhance the productivity. A study was conducted to study the floral biology of 17 germplasm which were collected from different locations of Low Country Wet zone. Inflorescences consist of 2-27 flowers and single flowers. Flowers possess five sepals, and 3- 5 petals within same accession. Number of anthers varied from 26.46 to 61.40. Length and width of flower buds varied from 4.03 to 9.53 mm and 3.73 to 6.40 mm respectively. Pistil height varied from 7.63 to 12.43 mm. Although flowers are hermaphroditic, self-pollinated fruit setting percentage was minimal ranging from 1.2 to 3.1 while, significantly higher fruit set was obtained through cross pollination. Apomictic fruiting was not observed. Flowers were protogynus and dichogamy. Stigma receptivity started one day before anthesis and continued till the following day after anthesis. Maximum receptivity was 99.12 at 6.00-12.00am and the maximum anthesis occurred at 7.00-8.00 am while receptivity and anther dehiscence synchronized. Maximum pollen viability observed was 95.22 followed by 90.68 in AMAC. 17, AMAC. 8 accessons respectively.

Key words: Anthesis, Bael, Floral biology, Reproduction biology, Stigma receptivity.

INTRODUCTION

Bael (*Aegle marmelos* (L.)) Correa is an important sub-tropical fruit tree indigenous to India. It belongs to the family Rutaceae (Stone, 1985) which is widely distributed in Sri Lanka. It has great mythological and religious significance in Indian history and culture. Further, each part of the tree has been traditionally identified to contain medicinal properties which have been described in the ancient medical treatise such as Sanskrit, Charaka Samhita

(Pawan *et al.*, 2015). The leaf extract considered as a potential hypoglycemic agent (Seema *et al.*, 1996) is effective in restoring blood glucose and body weight. Diabetes in pregnant and nursing mothers can be cured through herbal drugs prepared from Bael. (Alam *et al.*, 1990). Not only that the seeds of Bael fruit contain 62 % protein, 32 % oil, 3 % carbohydrate and 3 % ash (Banerji *et al.*, 1982) and the seed oil is recommended for its therapeutic value in treating inflammation associated with certain injuries and tumour (Pal *et al.*, 1993).

Bael grows well under subtropical climate thrives up to 1200 m, and has a reputation for surviving in conditions unsuitable for other plants. The crop has adapted to different agro climatic regions in Sri Lanka except in up country. Bael tree is very hardy and has capacity to adapt successfully to a wide range of habitat. Bael owing to its environment -friendly nature, is being placed among plant species group called "climate purifiers" as it emits a greater percentage of oxygen in sunlight as compared to other plants. (Anurag *et al.*, 2014). Bael shows a very high genetic variability having both 2n and 4n chromosome number and it was largely propagated through seeds until recently (Rai *et al.*, 2002).

Bael is self- fruitful (Jindal *et al.*, 1993) showing a very heavy initial fruit set however final retention is very low due to heavy fruit drop possibly due to climatic factors (Sharma *et al.*, 1996).

Bael has been categorized under the group of underutilized fruit crop in Sri Lanka and no studies have been conducted on Bael phenology, floral biology and reproduction biology. Therefore, following experiments were conducted to study the floral biology, flowering behaviour and reproduction biology of the crop using existing genetic variation.

MATERIALS AND METHODS

Seventeen accessions out of thirty accessions established in the field gene bank of the Fruit Crops Research and Development Institute, Kannawila were utilized for the study. All trees were established in a randomized complete block design with three replicates. Seven years old grafted trees were considered for the study after three consecutive bearings.

Accessions used in the experiment were AMAC. 01, AMAC. 02, AMAC. 03, AMAC. 04, AMAC. 05, AMAC. 06, AMAC. 07, AMAC. 08, AMAC. 09, AMAC. 10, AMAC. 11, AMAC. 12 , AMAC. 13, AMAC. 14, AMAC. 15, AMAC. 16, and AMAC. 17. Studies were conducted during major flowering seasons in LCWZ; March - May and September – October during the period from 2014-2016. Basic floral biological data (sepals, petals, and flower colour, number of stamens, pistil height, flower bud height, and flower bud width) were collected from the selected 17 accessions. Flowering period, pollination method, flower opening time, anthesis, stigma sensitivity, viable and non- viable pollen count, fertilization, days taken to flower opening, stigma sensitivity, anther dehiscence and flower opening pattern were studied only in five selected accessions (AMAC.3, AMAC.7, AMAC.8, AMAC.11, and AMAC.17). The later five most promising accessions were identified after considering their fruit characters.

About 100 flowers from each accession were used and the data collection was continued in consecutive seasons of each year. Cross sections of flower buds one day before opening were used to obtain the petal and calyx arrangement. Data were recorded in one hour intervals starting from 5.00 am to 10.00 am to study the anthesis and anther dehiscence.

The two top flowers of the panicle was maintained while removing other flower buds and flowers were emasculated one day before anthesis and flowers were covered with paper bags to prevent undesirable pollination. Flowers were pollinated at the maximum anthesis time of 7.00-8.00 am in the following day. Pollens taken from the opened flowers covered with paper bags of selected paternal trees were used for the pollination. Pollination was performed manually by touching the anther containing pollen to stigma. After pollination of flowers were re-covered with paper bags. Reciprocal crossing experienced for all the accessions.

This manual pollination was made to confirm the geitonogamous and xenogamous mode and to determine any possibility of apomictic fruiting. Emasculated flower buds were bagged without pollination.

To evaluate the stigma receptivity, 90 floral buds were tagged and grouped into 9 groups per accession starting from 6.00 am of previous day of anthesis and continued in 6 hours interval until anthesis during the day time of the following day of. Petals were opened carefully using forceps to study the stigma receptivity. Stigma of receptive flower buds appears watery shiny after anthesis which could easily be observed after careful opening of the flower bud. To confirm the stigma receptivity 3% hydrogen peroxide solution was used. Receptive stigma emits oxygen bubbles when immersed in 3% hydrogen peroxide solution (Dafni, 1992). Pollen viability percentage was studied using 1% of acetocarmin stain test. If the pollen grains are viable they stained well and non- viable pollens are not stained. The data were statistically analyzed using SAS statistical package.

RESULTS AND DISCUSSION

Two major flowering seasons, March to April and August to September are observed in Low Country Wet Zone (LCWZ). Flowering period is lasting for 1.5- 2 months. However, irregular flowering could be observed in between major seasons after 2-3 weeks of dry spells.

Floral events

Size and shape of the flower buds varied in different accessions possibly owing to the genetic variation. (Figure 1, A1). Though the flowers showed a slight change in colour (Figure 1, A2) majority were dull greenish white with a good fragrance. Even in the same accession numbers of petals were different. (Figure 1, A2). Flower initiation could be observed 9- 12 days after the emergence of new flushes and flower bud appeared in axillary cymose panicles with solitary flowers (Figure 1, D). Flower buds appeared as minute swollen structures on inflorescence peduncles, developed into matured flowers within 20- 25 days after initiation. All the flowers in a panicle opened within one week period. Flowers opened from top to bottom (Figure 1, C and E). Anthers and peals turned brownish colour after 10-12 hours of anthesis, and exposure to direct sunlight advanced the colour changes while supporting pollen desiccation. (Figure 1, B and H). Stigma also turned into light brownish colour after 10-12 hours of flower opening though a wet surface observed.

Anthers attached longitudinally to the style (Figure 1, H) and after dehiscence of anthers they diverted to outside of the stigma (Fig. 01: A). This may be the one of avoidance mechanism of self- pollination. It was observed though the dehisced anthers and susceptible stigma were located close to each other the self- pollination is highly restricted. Anthers change to dull brownish in colour after 3-4 hours of direct sunlight and styles became more sidetracked outside the stigma.

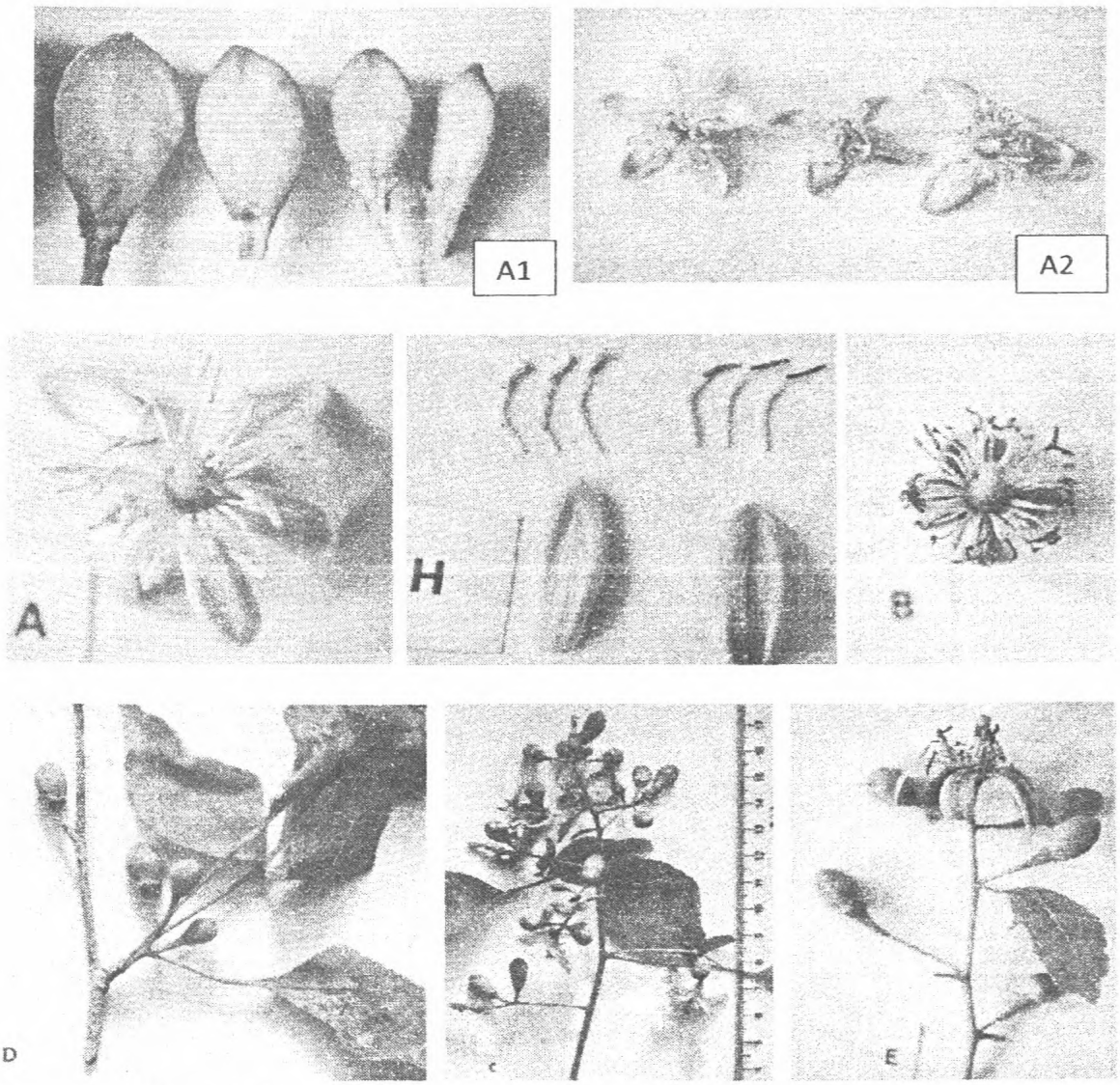

Figure 1. Different shapes and sizes of flower buds(A1) , variation of the open flowers (A2), Flower just after opening (A), anthers and petal colour changes(H), six hours after opening of flower (B), different inflorescence (C, D) , and anthesis pattern (E), of the Bael accessions.

Table 1 shows the data on number of sepals, number of petals, number of stamens, pistil height, flower bud height and flower bud width of the 17 Bael accessions. It was observed that there was no difference in number of sepals.

Table 1. Some floral characters of selected seventeen Bael fruit cultivars.

Accession Number	Number of petals / flower	Number of anthers/ flower	Pistil height (mm)	Length of flower bud (mm)	Width of flower bud (mm)
AMAC. 1	4.46±0.50	26.46±0.80	12.42±0.15	7.43±0.25	5.40±0.20
AMAC.2	3.73±0.64	52.00±0.72	10.36±0.20	9.36±0.15	6.23±0.15
AMAC.3	3.77±0.69	53.06±0.61	10.56±0.20	9.53±0.30	6.40±0.20
AMAC.4	4.11±0.19	33.60±0.91	7.63±0.15	6.53±0.32	4.63±0.45
AMAC.5	4.33±0.61	33.13±0.50	9.43±0.15	8.80±0.26	5.46±0.30
AMAC.6	4.63±0.33	41.20±0.72	8.43±0.15	7.66±0.50	4.43±0.28
AMAC.7	3.66±0.57	38.86±0.30	9.63±0.30	6.46±0.37	4.46±0.32
AMAC.8	3.55±0.50	43.40±0.91	10.43±0.15	7.56±0.20	5.43±0.35
AMAC.9	4.22±0.38	61.40±0.72	8.43±0.15	4.80±0.26	3.90±0.20
AMAC.10	4.16±0.20	56.86±0.50	9.33±0.25	5.53±0.40	4.36±0.20
AMAC.11	4.44±0.50	54.10±0.75	10.46±0.40	7.70±0.45	5.43±0.32
AMAC.12	4.86±0.23	56.86±0.50	8.46±0.20	5.53±-.32	4.43±0.25
AMAC.13	3.6±0.52	48.66±0.50	9.70±0.36	6.53±0.20	5.3±0.26
AMAC.14	4.55±0.50	28.1±0.79	7.70±0.26	4.03±0.32	3.73±0.35
AMAC.15	3.93±0.30	56.8±0.40	12.43±0.35	8.56±0.15	5.5±0.36
AMAC.16	4.53±0.50	28.36±1.00	8.56±0.15	6.43±0.32	4.36±0.30
AMAC.17	4.27±0.15	52.33±1.00	12.43±0.25	9.46±0.30	6.13±0.30

Number of petals varied from 3.55.±0.050 to 4.86± 0.23 (Table 1). However, it was observed that different number of petals found within same genotype was not attached each other. Numerous free stamens found in flowers showed higher variation among the genotypes. The highest average number of anthers per flower (61.40±0.72) was in AMAC 8 and the lowest average number (26.46±0.80) was in AMAC. 1. Changes in the number of anthers per flower were observed in the same accession. Height of pistil, length of flower bud and width of flower bud also showed a variation among accessions. The highest pistil height was observed in AMAC.17 followed by AMAC.01 and the lowest pistil height (7.63±0.15) was observed in AMAC.4 followed by AMAC.14 (7.70±0.26). The highest flower bud length (9.53±0.30 mm) was in accession AMAC.3 while the lowest bud length was in AMAC14.

Accession AMAC. 3 showed the highest flower bud width (6.40±0.20 mm) and the lowest bud width (3.73±0.35 mm) was in AMAC.14.

Table 2. Viable and non-viable pollen percentage in different times of different Beal accessions.

Accession number	Percentage at anthesis		Percentage after 6 hours		Percentage after 12 hours	
	Viable	Non viable	Viable	Non viable	Viable	Non viable
AMAC.3	81.42±0.44	18.52±0.04	64.52±0.77	35.48±0.82	44.68±0.92	55.32±0.76
AMAC.7	78.53±0.71	21.47±0.15	62.31±0.78	37.69±0.99	36.54±0.52	63.46±0.42
AMAC.8	90.68±0.67	9.32±0.62	68.44±0.84	31.56±0.75	48.56±0.45	51.44±0.16
AMAC.11	89.44±0.85	10.56±0.53	71.31±0.49	28.69±0.95	38.63±0.43	61.37±0.5
AMAC.17	95.22±0.32	4.78±0.17	78.16±0.81	21.84±0.28	52.59±0.37	47.41±0.47

There was no anther dehiscence observed before anthesis. Viable pollen percentage was higher at anthesis in all accessions. Singh and Misra (2012) revealed that pollen viability was recorded high in each genotype ranged from 92.02% (PB-16) to 98.87 % (PB-4). However, Vije kumar *et al.* (2011) suggested that the actual pollen viability was 47 % to 55%. Among the studied accessions the highest viable pollen percentage of 95.22±0.32 was in AMAC. 17. (Table 02). Pollen viability at the anthesis was also found to be high in AMAC. 17, while the lowest percentage was observed in AMAC. 7 (78.53±0.71). These findings are comparable with the results of Kanchan and Singh (2000) and Singh (1989). It was observed that a prevailing of similar trend of viable pollen percentage after 12 hours of anthesis , though the percentage of viable pollen count was lower than that at anthesis. After 12 hours of anthesis, the highest viable pollen percentage 52.59±0.37 was observed in AMAC 17 followed by AMAC. 8. Most of the flowers on tip of the branches exposed to the direct sunlight which could be one of the reasons for recording of high percentage of non-viable pollen within 12 hours period. However, it was observed that direct sunlight and rainy situation adversely affected the pollen viability.

Fruit set and fruit retention under self- pollination and cross-pollination in different Bael accessions are shown in Table 3. Results of this study are in agreement with the observation that the self-pollination of AMAC.3, AMAC. 7, AMAC. 8, AMAC.11 and AMAC. 17 results significantly lower yield than

crossing among the accessions (Table 3). However, 12.5 % was the highest fruit retention percentage observed in a cross between AMAC. 7 x AMAC. These results showed that the Bael restricted the self-fertilization. Mahendra Pal and Misra (2005) stated that crossing between 'Pant Shivani' x 'Pant Sujata' gave a fruit set of 67.36% and final fruit retention of27.04% reducing fruit drop by 59 86%. Though the reciprocal crossings were practiced here no differences was observed in fruit setting. Therefore, the data were not considered for analysis. And it was also observed that no fruits were observed in emasculated flowers covered with paper bags without pollination. Therefore, Bael doesn't have an apomictic fruit formation. Bael normally grown as a back yard crop and most of the cases only one tree exists per large area. Therefore, it has low chances to cross pollinate. That may be the reason for low yields in backyard Bael trees.

Table 3. Effect of cross pollination and self -pollination on fruit setting percentage of different Bael accessions.

	% of fruit setting				
	AMAC.3	**AMAC.7**	**AMAC.8**	**AMAC.11**	**AMAC.17**
AMAC.3	2.1 b	11.6 a	12.2 a	10.4 a	11.4 a
AMAC.7	9.3 a	1.3 b	10.2 a	12.5 a	9.3 a
AMAC.8	12.1 a	9.3 a	3.1 b	8.4 a	12.2 a
AMAC.11	8.2 a	11.1 a	9.3 a	1.2 b	10.4 a
AMAC. 17	9.3 a	8.2 a	6.4 a	7.1 a	2.5 b

Note: Mean in each column followed by the same letters are not significantly different at $p \leq 0.05$

Anthesis in Bael started from 5.00 a.m. and continued up to 10.00 a.m., however the maximum number of flowers anthesis was observed between 7.00 to 8.00 a.m. in all genotypes followed by 6.00 to 7.00 a.m (Figure 2).

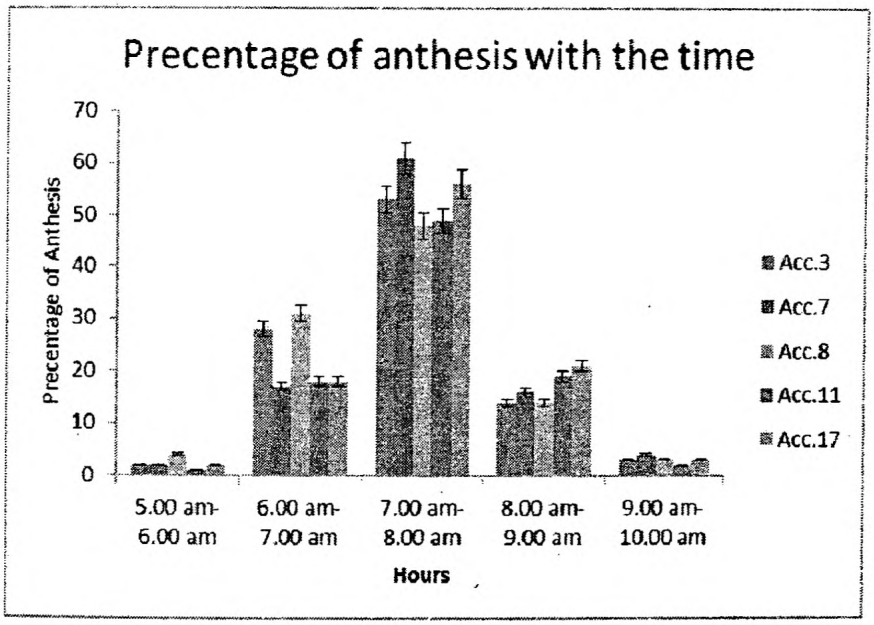

Figure 2. Percentage of anthesis with the time of the day in different Bael accessions.

Similar pattern of anthesis was observed in all accessions. However, Kumar *et al.* (2010) stated that the time of anthesis in bael varied from 3.00 am to 8.00 am with a peak period between 4.00 – 5.00 am followed by 5.00 to 6.00 am and 6.00 -7.00 am. It was observed that, with the increase in the sunshine, anthesis was also increased. Early anthesis was observed in eastward branches compared to rest of the branches owing to early falling of sun light. Anther dehiscence was synchronous with anthesis and all the anthers in a flower dehisced synchronously presenting the pollen mass.

Stigma receptivity commenced 24 hours before anthesis and it continued up to 48 hours of anthesis making a total receptive period of 72 hours. All the accessions evaluated showed similar pattern of receptivity and maximum receptivity showed on the day of anthesis between 6.00 am to 6.00 pm. The highest percentage of anthesis, 99.24±0.16 was observed from 6.00-12.00 am period on the day of anthesis in AMAC 7 followed by AMAC 11 (99.12±0.70). However, stigma receptivity showed gradual reduction after anthesis.

CONCLUSIONS

Based on the results, we concluded that Bael showed protogyny flowers. Though the Bael possesses perfect flowers and synchronous maturity of both sexual parts, the crop showed a significant increase in yield when cross pollination is taken place. Maximum viable pollen percentage, maximum anthesis and maximum stigma receptivity coincided together. According to observations, sunlight and dry environmental conditions advanced the anthesis and anther dehiscence.

Table 4. Percentage of stigma receptivity and time of anthesis in different Bael accessions.

	Percentage of stigma receptivity								
	One day before anthesis			On the day of anthesis			One day after anthesis		
	6.00 am	12.00 am	6.00 pm	6.00 am	12.00 am	6.00 pm	6.00 am	12.00 am	6.00 pm
AMAC.3	16.34±0.14	24.58±2.64	64.16±1.13	84.41±1.20	98.76±0.45	97.42±0.41	55.34±1.61	32.24±0.97	18.24±1.40
AMAC.7	12.14±0.70	38.42±1.28	59.21±2.41	86.17±1.46	99.24±0.16	97.44±0.84	43.24±3.53	26.64±0.49	15.33±0.79
AMAC. 8	18.62±0.41	30.46±1.37	48.22±1.44	90.33±0.62	97.68±0.89	97.02±0.91	36.41±2.67	28.92±0.61	12.24±2.12
AMAC.11	19.71±0.87	28.74±1.11	57.26±1.47	89.73±1.05	99.12±0.70	98.14±0.60	42.24±1.40	27.22±1.45	14.44±0.84
AMAC.17	22.34±1.34	32.54±1.47	62.74±1.13	91.22±1.55	96.48±0.53	95.32±0.77	39.41±1.27	21.42±0.67	10.23±2.77

REFERENCES

Alam, M. M., M. B. Siddiqui and W. Hussain. 1990. Treatment of diabetes through herbal drugs in rural India. Fitoterapia. 61 (8): 240-242.

Anurag S, H.K.Sharma, K. pragati and U. Ashtosh. 2014. Bael (*Aegle marmelos* (L.) Correa) products and processiong : Areview. African Journal of Food Science. 8 (5) : 204-215.

Banerji M, M. Maiti, S. Sem and P. C. Datta. 1982. Pharmacognosy of *Aegle marmelos* (L.) Correa seed. A new protein source, Acta Pharmaceutica Hungarica. 52 (3): 97-101.

Dafni, A. 1992. Polination ecology:a practical approach University press, New York.

Jindal, K. K., D. R. Gautam and B. K. Karkara. 1993. Pollination and pollinizers in fruits. In: Advances in Horticulture. K. L. Chadha and O. P. Pareek (edition). Malhotra Publishing House, New Delhi, India. 01.

Kanchan, K.S., and H.K. Singh. 2000. Floral biology of Bael (*Aegle marmelos* (L.)) cultivars, Indian Journal of Agricultural Sciences. 70 (11): 797-8.

Kumar K., S. Sharma and R. Niwas. 2010. Studies on relationship of weather with floral biology and fruit drop in bael (*Aegle marmelos* (L.) Correa). Haryana Journal of Horticultural Sciences. 39 (¾): 205-207.

Mahendra Pal and K.K. Misra. 2005. Effect of pollen on fruit set, fruit drop and final fruit retention in self and cross pollination in Bael (*Aegle marmelos* (L.)). Indian journal of Agricultural Sciences. 75 (12): 800-804.

Pal, P. K., D. K. Bhattacharya and S. Ghosh. 1993. Chemical examination of the seed of *Aegle marmelos* and its medicinal importance. Journal of Oil Technological Association of India. 25 (3): 55-59.

Pawan S.G., L. Narayan, K.G. Alok, S.M. Evening. 2015. A review of medicinal values and commercial utility of bael, International Journal of Life-Sciences Scientific Research (ILLSSR). 01(01): 5-7.

Rai, D., K. K. Misra and V.P. Singh. 2002. Analysis of genetic divergence in Bael (*Aegle marmelos* Correa) germplasm. Progressive Horticulture. 34 (1): 35-38.

Seema, P. U., B. Sudha, P. S. Padayatti, A. Abraham, K. G. Raghu and C. S. Paulose. 1996. Kinetic studies of purified malate dehydrogenase in liver of steptozotocin-diabetic rates and the effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. Indian Journal of Experimental Biology. 34: 600-602.

Singh,V.P. and K.K. Misra. 2012. Pollengrain studies in Bael (*Aegle marmelos* Correa), Department of Horticulture, G.B. Pant University of Agriculture and technology, Pantnagar- 263145. http://www.ijour.net/ijor.aspx?target=ijor:pho&volume=42&issue=1&article=011 (Accessed on 05 04. 2017).

Singh.R.D.1989. Studies on bael (*Aegle marmelos* Correa) morphology of flower, anthesis, dehiscence and receptivity of stigma . Progressive Horticulture. 32:15-24.

Sharma, S. R., S. K. Dwivedi, V. P. Varshney and D. Swaroop. 1996. Antihyperglycaemic and insulin release effects of Aegle marmelos leaves in streptozotocin diabetic rat. Phytotherapy Research. 10: 426-428.

Stone , B. 1985. Rutaceae. In Sassanayake, M.D. and Fosberg, F.R. (edition). A revised Handbook to the Flora of Ceylon. Amerind, New Delhi. 5: 414.

Vije Kumar, S., S. Athula, P. Kumar and J. Kaur. 2011. Phenology, Pollination and breeding system of *Agle marmelos*(Linn.) correa (Rutaceae) from India. New Forests. 42: 85- 100.