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FLORAL BIOLOGY OF INDIGENOUS PUMMELO GENOTYPES

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Abstract

Flower morphology and bud development of pummelo accessions CG-1, CG-18 and CG-151 were studied at the Pummelo Orchard of Regional Agricultural Research Station, BARI, Akbarpur, Moulvibazar and the Horticulture Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University during 2008-2009. Pummelo flowers were bisexual, bore singly on leaf axils or in clusters with or without leaf on stem in all accessions, and colour were white. Calyx diameter varied from 0.94 in CG-1 to 1.02 in CG-18. Number of petals per flower ranged from 4.0 to 4.5. Anthers were yellow in colour and only CG-151 produced few rudimentary styles. Diameter of stigma varied from 0.39 mm to 0.49 mm. Number of locules per ovary was in between 14.6 to16.0 and number of ovules per locules varied from 4.0 to 9.0. Stages of floral bud development from initiation to anthesis were divided into 9 distinct stages. In pummelo, a total of 27.7 to 31.2 days were required from a bud initiation to reach its fully developed stage. Suitable time for emasculation of pummelo flowers was found within 26 days from flower bud initiation. Between 3:00am to 5:00am, about 76% flowers were found to be opened and between 4:00pm to 5:00pm in all the three accessions, dehiscence of pollens was recorded. Abscission of stamen, petal and style started after 50.8, 76.4 and 162.3 hrs and completed after 128.4, 137.9 and 228.3 hrs of anthesis, respectively.

Keywords: Floral morphology, pummelo, bud development.

Introduction

Pummelo is an important fruit among the citrus fruits grown in our country. It is very rich in vitamin-C and is a good source of vitamin A and B. Pummelo can be grown easily and can tolerate drought and pest infestation is comparatively lower (Rashid *et al*., 1987). Fruits are palatable; rinds possess essential oils that are used in cosmetic industry. Fresh ripe fruits are eaten directly and fruits can also be used in preparing various kinds of recipes such as jam, jelly, pickles, cakes and drinks (Azmatullah *et al.*, 1987). In Bangladesh, pummelo is cultivated in an area of around 7460 ha with total production of 59198 metric tons and average yield per fruit bearing tree is around 38.0kg (BBS, 2011). It seems to be ranking first among the citrus fruits grown in Bangladesh (Azmatullah *et al.*, 2006). Pummelo can be grown successfully in all areas of Bangladesh. But unfortunately there was no released pummelo variety before 1997 except some

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local cultivars. In 1997 and onwards, four varieties of pummelo have been developed through selection from collected indigenous germplasm with high yield potential and good bearing ability.

Pummelo production in Bangladesh is not remarkably increasing as in the recent years a lot of problems regarding pest and diseases are being faced by the growers. So, there is an urgent need to develop variety(s) with high yielding potentials and resistant/tolerant to pest and diseases. For obtaining variety (ies) with desired traits, hybridization programme is very much essential. But in Bangladesh, so far, no hybridization programme has been undertaken for the improvement of pummelo. But in India and other pummelo growing countries, hybridization programme have been successfully undertaken for the improvement of this crop. The pre-requisites of effective hybridization programme included knowledge on floral morphology, biology, anthesis, dehiscence, pollen viability, stigma receptivity etc. (Ahmad, 1996). As pummelo is a cross pollinated crop, so, there is a tremendous amount of variability within the species with which the breeder can work that provide even a wider selection of characters (Janick and Moore, 1996).

Flower biology is the study of the science of flowers, which includes anthesis, dehiscence of anthers, pollen fertility and stigma receptivity (Kalloo, 1988). Detailed information regarding the floral biology of pummelo is not available in our country. Therefore, the present investigation was undertaken with the following objectives:

1. To know the morphological characters of pummelo flower.
2. To determine and characterize different stages of flower bud development in pummelo.
3. To determine the time of emasculation in pummelo.

Materials and Method

The study was undertaken at the pummelo orchard of the Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Akbarpur, Moulvibazar during February 2008 to May 2009. Plant of the pummelo accessions CG-1, CG-18 and CG-151 was selected to study the different aspects of floral biology. Sapling of the accessions CG-1 and CG-18 were collected from Northern part of Bangladesh and then planted at RARS, Akbarpur, Moulvibazar in 1988, while CG-151 was collected from Sylhet region and planted at RARS, Akbarpur, Moulvibazar in 1998. The experiment was conducted following Randomized Complete Block Design (RCBD). Data were collected from three different plants of an accession where each plant was considered as a replication. During planting, fertilizer and manures were applied following the dose and method as described by Rashid *et al*. (1987). Irrigation, weeding, fertilizer

application and other crop management practices were followed as recommended by Ullah *et al*. (2006) to have a good healthy plant. Data on flowering habit of pummelo, chronology of bud development up to anthesis from the visible appearance of flower buds, time of anthesis and dehiscence of anthers, floral morphology and withering time of different floral parts were recorded.

Results and Discussion

Pedicel length, calyx diameter, number and characters of sepal, number of petals per flower and petal length and width in flowers of pummelo accessions are presented in Table 1. It is revealed from the table that pedicel length ranged from 1.38cm in CG-1 to 1.54cm in CG-151. Calyx diameter varied in the accessions from 0.98cm to 1.02cm. Number of petals per flower was 4.0 in the accessions CG-18 and CG-151, while in CG-1 it was 4.5. This implies that the accession CG-1 produced both tetramerous and pentamerous flowers. Petal length varied from 2.20-2.52cm; whereas, petal width varied from 0.88-1.20cm. Azmatullah (1987) found similar length (2.25cm) in petals while he was studying pummelo flowers. Number of sepal was 4 in the flowers of all accession and the four sepals were fused to one in the flowers. Azmatullah *et al.* (1987), Saha (2005) and Hossain (1983) recorded 4 to 5 petal and sepals in pummelo. This variation in number of petal and sepal might be attributed to genetic differences.

In the pummelo flowers, heterostyly was observed among the accessions (Table 2). It might be dis-adventitious for successful pollination and this condition favours cross pollination. All the accessions produced bisexual flowers that were white in colour and the anthers were yellow in colour. Schneider (1968) and Purseglove (1987) also reported the yellow colour of anthers in citrus. There was no rudimentary style observed in the flowers of CG-1 and CG-18 except some in the accession CG-151, which had a negative impact on successful fruit setting. Flowers were borne singly in the leaf axils or in clusters. This finding agreed with the findings of Webber and Batchelor (1948), Azmatullah (1987) and Hossain (1983).

Table 1. Pedicel length, calyx diameter, number of petal per flower, petal length and width and number of sepals per flower in pummel.

Accession	Pedicel length (cm)	Calyx diameter (cm)	Number of petal/ flower	Petal length (cm)	Petal width (cm)	Number of sepal/flower
CG-1	1.38	0.94	4.5	2.44	0.90	4.0
CG-18	1.42	1.02	4.0	2.20	0.88	4.0
CG-151	1.54	0.98	4.0	2.52	1.20	4.0
Mean	1.45	0.98	4.17	2.39	0.99	4.0
CV (%)	8.67	10.43	9.93	8.35	11.36	0.0
$LSD_{0.05}$	NS	NS	NS	NS	0.259	NS

Table 2. Length of anther related to stigma, flower type, colour of open flower, colour of anther, rudimentary style and flowers arrangement in pummel.

Accession	Length of anther related to stigma	Flower type	Colour of open flower	Colour of anther	Presence of rudimentary style	Arrangement of flower
CG-1	Shorter	Bisexual	White	Yellow	Nil	Single, cluster
CG-18	Shorter	Bisexual	White	Yellow	Nil	Single, Cluster
CG-151	Equal, longer	Bisexual	White	Yellow	Few	Single, cluster

Flowering time, number and length of stamen, and length and diameter of style in flowers of studied pummelo accessions are presented in Table 3. Flowering in the accessions varied from 2nd week of December (CG-151) to 2nd week of April (CG-1). Peak flowering time was observed in March, February and January for CG-1, CG-18 and CG-151, respectively. The starting of flowering in December indicated that the accession might be medium early, while the accession in which the flowering started in February might be late. This observation agreed with the findings of Hossain (1983) but varied slightly with the findings of Azmatullah *et al*. (1987), who found the flowering period from January to February. This variation in flowering time may be attributed climatic conditions that prevail during different years. Number and length of stamens in the flowers varied from 29.6 to 32.8 and 1.26-1.80cm, respectively. Schneider (1968) reported to have 20-40 stamens in citrus. The length and diameter of style in the accessions ranged from 0.84 to 0.87cm and 0.22 to 0.29cm, respectively. Hoque and Hossain (2012) reported a slightly varied result about stamen and style length; where they found a range of 0.85cm to 1.80cm length in stamen and a range of 1.02cm to 1.85cm length in style. The difference might be attributed to variations in genotype of the accessions.

Table 3. Flowering time, flowering month, number and length of stamen and style length and diameter in flowers of pummel.

Accession	Flowering time		Stamen		Style	
	Start	End	Number	Length (cm)	Length (cm)	Diameter (cm)
CG-1	1st week of February	2nd week of April	29.6	1.36	0.84	0.25
CG-18	1st week of January	4th week of March	30.4	1.26	0.86	0.22
CG-151	2nd week of December	4th week of February	32.8	1.80	0.87	0.29
Mean	-	-	30.9	1.47	0.86	0.25
CV (%)	-	-	8.26	15.67	11.63	9.93
$LSD_{0.05}$	-	-	NS	NS	NS	NS

Diameter of stigma in the flowers of studied accessions ranged from 0.39mm in CG-1 to 0.49mm in CG-18. Length of ovary ranged from 0.44cm to 0.47cm while, diameter of ovary ranged from 0.43cm to 0.54cm (Table 4). The similar length (0.44cm) and diameter (0.43cm) of ovary in CG-18 indicated that the fruit of the accession might be obloid; while, fruits of CG-151 and CG-1 might be semi-obloid and spheroid, respectively. Number of locules per ovary was 16.0 in CG-1 and CG-18, which indicated that the accessions had 16 segments while, CG-15 had 14 to 15 segments. This result partially agreed with the findings of Janick and Moore (1996), Purseglove (1987), Azmatullah (1987) and Ullah *et al*. (2001), who found 11-16 segments. Number of ovules per locules ranged from 4.0 to 9.0 in the accessions. As the ovules are the future seeds, therefore, it indicated the number of seeds in each segments.

The stages of floral bud development was divided into nine distinct stages (Fig.1) starting from the initiation of the detectable floral buds.

Table 4. Diameter of stigma, length and diameter of ovary, number of locules/ovary and number of ovules/ovary in flowers of pummel.

Accession	Diameter of stigma (mm)	Ovary		Number of locules/ ovary	Number of ovules/locules
		length (cm)	diameter (cm)		
CG-1	0.39	0.47	0.54	16.0	9.0
CG-18	0.49	0.44	0.43	16.0	4.0
CG-151	0.45	0.47	0.50	14.6	7.6
Mean	0.44	0.46	0.49	15.5	6.87
CV (%)	9.89	8.09	10.04	11.97	17.8
$LSD_{0.05}$	0.072	NS	0.02	0.691	2.77

The pedicel of buds at the first stage was deep green in colour with presence of hairs. At this stage the shape of bud was globose; colour of calyx and petal was deep green. Calyx length and diameter was very close to each other. Bud length was 12.19mm. Petals were about to enclosed with calyx. Among the accessions, bud took 6.3 to 7.5 days to reach the second stage (Table 5).

The colour of pedicel and petal turned to green and light green in the second stage. Shape of bud and colour of calyx remained same as observed in the first stage. Bud length was 15.99mm. On an average, 3.0-3.5 days required to reach in the third stage. Length of pedicel was about thrice to the length of petals.

At the third stage, the colour of calyx turned to light green and light separation line of splitting was observed in the petals. Bud length was 17.73mm. On an average, 3.4-3.7 days required to reach in the next stage (Table 5).

The colour of pedicel and calyx at the fourth stage were observed as green and light green respectively. Shape of buds were ovate and the petals were cream

coloured with some green spots on them (Fig. 1). Bud length was 20.18mm. Among the accessions, bud took 3.7 to 4.0 days to reach the next stage.

At the fifth stage of floral bud development in pummelo, no qualitative change observed in comparison to fourth stage except some quantitative changes. Length of buds increased to 27.46mm and the bud took 4.0-4.8 days to reach the sixth stage.

Colour of pedicel changed to light green from green at this stage. The shape of bud changed to obovate. Length of pedicel was about twice to the length of petals. Petal length in this stage was greater than width, which implies that the shape of corolla was changing to obovate from ovate. Buds were 30.40mm long that took 3.0-3.2 days to reach in the next stage (Table 5 and Fig 1).

At the seventh stage, the colour of pedicel and calyx were same as observed in Stage-VI. Bud shape was obovate and cream coloured petals were without any spot. A prominent constriction of splitting was observed in the petals. Length of bud was 36.74mm and the bud took 2.0-3.0 days to reach the next stage.

Qualitative characters at Stage-VIII were similar to Stage-VII. Length of pedicel and petals were about equal. Buds were 38.51mm long that took 1.8-2.0 days to reach the next stage (Table 5 and Fig. 1).

At ninth stage, all the qualitative characters were same as observed in Stage-VII and Stage-VIII, except one or two petals were observed to be splitted in Stage-IX (Fig. 1). The buds reached to its fully developed stage with an average length of 43.56mm (Table 5).

Table 5. Days required for flower bud development up to anthesis in pummelo and description of stages.

Accession	Days required for passing from one stage to another								
	I-II	II-III	III-IV	IV-V	V-VI	VI-VII	VII-VIII	VIII-IX	Total time required from I-IX
CG-1	7.5	3.5	3.5	4.0	4.5	3.2	3.0	2.0	31.2
CG-18	6.3	3.3	3.4	3.9	4.8	3.2	2.1	1.9	28.9
CG-151	6.5	3.0	3.7	3.7	4.0	3.0	2.0	1.8	27.7
Mean	6.8	3.3	3.5	3.9	4.4	3.1	2.4	1.9	29.3
CV (%)	9.5	10.7	14.3	13.4	11.1	13.7	13.2	12.3	12.1
$LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS
Stage-I	= Hairy and deep green pedicel; calyx and petals are also deep green; buds are globose shaped and 12.19mm long. Petals are about to enclosed with calyx.								

Stage-II	= Hairy, green pedicel; deep green calyx and light green petals, buds are globose, 15.99mm long. Length of pedicel is about thrice than the length of petals.
Stage-III	= Green and hairy pedicel, light green calyx and petals, buds are globose and 17.73mm long with light separation line on petals. Length of pedicel is about thrice than the length of petals.
Stage-IV	= Green, hairy pedicel; light green calyx, creamy petals with green spots, buds are ovate, 20.18mm long with light separation line on petals.
Stage-V	= Green, hairy pedicel, light green calyx, creamy petals with green spots, buds are ovate, 27.46mm long with light separation line on petals.
Stage-VI	= Light green and hairy pedicel, buds are creamy, obovate and 30.40mm long. Petal length becomes greater than diameter. Length of pedicel is about twice than the length of petals.
Stage-VII	= Light green, hairy pedicel; light green calyx, creamy petals with no spots, buds are obovate, 36.74mm long with prominent constriction of splitting on petals. Length of pedicel and petals are about equal.
Stage-VIII	= Light green pedicel, buds are cream coloured, obovate shaped and 38.51mm long with prominent constriction of splitting on petals. Length of pedicel and petals are about equal.
Stage-IX	= Cream coloured petals, buds are obovate shaped and 43.56mm long with one or two splitting petals.

Cumulative time required from a bud initiation to reach at its successive stages among the accessions are presented in Fig. 2. It is revealed from the table that the accession CG-1 took more time to reach in the next stages than other two accessions. To reach in the last stage of floral bud development, the accession CG-1 took 31.2 days followed by CG-18 (28.9 days) and CG-151 (27.7 days). Therefore, it can be said that in pummelo, a total of 27.7-31.2 days are required from a bud initiation to reach its fully developed stage (Fig. 2).

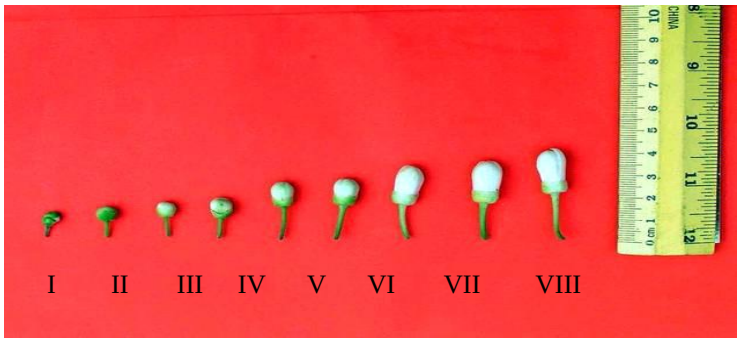

Fig. 1. Different stages of flower bud development in pummelo.

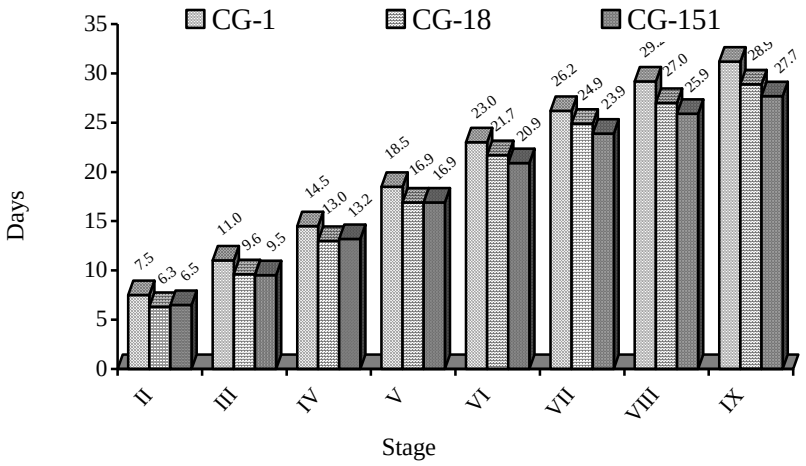

Fig. 2. Cumulative days required for passing from stage-I to another during flower bud development in pummelo.

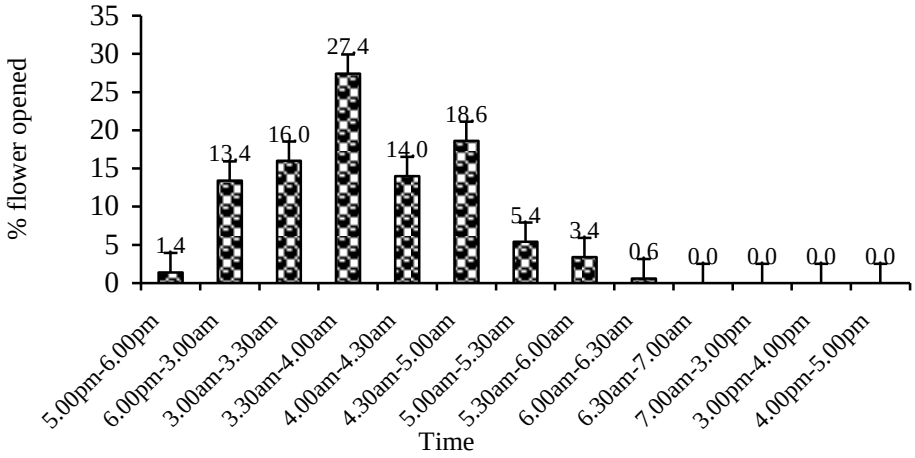

Fig. 3. Percentage of flower opening at different time of the day in pummelo.

Time of anthesis and dehiscence of anthers in flowers of studied pummelo accessions were observed and are presented in Table 6. Time of anthesis is very much important to a breeder, as selfing must be done before anthesis. On the other hand, bagging and emasculation for crossing must be done before anthesis and starting of receptivity of stigma. It was observed in the accessions that opening of pummelo flowers started between 5:00pm to 6:00pm and continued up to 6:30am. The maximum flowers were opened between 3:30am to 4:00am in

Table 6. Time of anthesis and dehiscence of anther in pummelo

Accession	Number of flower opening at different hours											
	5:00pm to 6:00pm	6:00pm to 3:00am	3:00am to 3:30am	3:30am to 4:00am	4:00am to 4:30am	4:30am to 5:00am	5:00am to 5:30am	5:30am to 6:00am	6:00am to 6:30am	6:30am to 7:00am	7:00am to 4:00pm	4:00pm to 5:00pm
CG-1	0	5	9	13	8	10	3	2	0	0	0	0+
CG-18	0+	7	8	13	5	9	5	2	1	0	0	0+
CG-151	2	8	7	15	8	9	0	1	0	0	0	0+
Total	2	20	24	41	21	28	8	5	1	0	0	0
Mean	0.667	6.67	8.00	13.7	7.00	9.33	2.67	1.67	0.33	-	-	-
CV (%)	25.0	18.4	8.84	8.45	20.2	25.9	18.4	14.8	20.0	-	-	-
$LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	-	-

+ = dehiscence of pollen

Table 7. Abscission time (h) of different floral parts in pummelo after anthesis.

Accession	Stamen		Petal		Pistil	
	Start	Complete	Start	Complete	Start	Complete
CG-1	54.8	123.6	80.1	131.8	172.8	228.3
CG-18	54.9	128.4	77.2	137.9	167.3	224.4
CG-151	50.8	119.2	76.4	128.3	162.3	226.2
Mean	53.5	123.7	77.9	132.7	167.5	226.3
CV (%)	12.40	11.83	12.21	11.24	10.86	10.6
$LSD_{0.05}$	2.912	5.15	NS	3.73	3.26	3.052

all the accessions. A total of 41 flowers among 150 flowers were opened within this time in the accessions, which is about 27.3% of total flower. Between 3:00am to 5:00am, a total of 114 out of 150 flowers, which is about 76% of total flower were found to be opened in three observed accessions. There was no flower observed to be opened from 6:30am to 5:00pm (Table 6 and Fig. 3). This was in agreement with the findings of Shinde and Dhuria (1960). Like anthesis, it is also important to know the time of dehiscence of anthers for successful breeding work. In flowers of studied pummelo accessions, dehiscence of pollens was observed between 4:00pm to 6:00pm. Between 4:00pm to 5:00pm in all the three accessions, dehiscence of pollens were recorded which indicated that pollen dehisced in pummelo flowers mainly in this time (Table 6), which is about 10 hours after anthesis. Practically, it was observed that in a flower cluster some flowers were in bud stage, some other at anthesis and some other in a stage when floral parts were withering or already withered.

Among the floral parts, stamen withered and abscised first followed by petal and pistil. Abscission of stamen started after 50.8 hrs of anthesis and completed after 128.4 hrs of anthesis. Petals started to be abscised after 76.4 hrs of anthesis and completed after 137.9 hrs of anthesis (Table 7). Practically, it was observed that when petals started to wither and abscise, some stamens were still attached with the flowers. It was also seen in some flowers that petals abscised after completion of stamen withering. Pistil took a long time to start abscission and it was 162.3 hrs after anthesis. Abscission of pistil completed after 228.3 hrs of anthesis (Table 7). This result of abscission of different floral parts differed slightly with the findings reported by Frost and Soost (1968) in *C. lemon*. They reported the abscission time of petals and stamens after six days of pollination and abscission of style occurs after 10-15 days of anthesis. This variation might be attributed to genetic and environmental differences, because environmental factors such as, temperature, light, humidity, wind speed etc. are important for abscission of floral parts. Eti and Stosser (1992) reported the abscission time of style in mandarin was after 12 days of anthesis.

Conclusion

Pummelo flowers are bisexual, white coloured, borne singly or in clusters, having 4 sepals and petals, 27 to 34 stamens with 1.0 to 2.0cm long. Stages of floral bud development from initiation to anthesis may be divided into 9 distinct stages. In pummelo, a total of 27.7 to 31.2 days are required from a bud initiation to reach its fully developed stage. Emasculation of pummelo flowers should be done within 26 days from flower bud initiation. Starting from initiation, a floral bud of pummelo took 28 to 32 days up to anthesis. About 76% pummelo flower were found

to be opened between 3:00am to 5:00am, while dehiscence of anthers occurred between 4:00pm to 5:00pm. Therefore, selfing should be done in pummelo in the early morning and preferably before 3:00am to avoid cross pollination.

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