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Studies on stages of flower to select parents for breeding in hibiscus (*Hibiscus rosa sinensis*)

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ABSTRACT

Hibiscus rosa sinensis has a good response from people due to its medicinal and aesthetic values. Since it is a bisexual and complete flower, it still needs deep observation of flowering stages to identify the best stage for breeding purposes and genetic improvement of the crop. This experiment was carried out by three male and female parents in BCKV, Mohanpur, West Bengal. Flowers from selected varieties were plucked at five different stages in the morning hours, and each part of the flower was carefully separated for further observation. The report of this research reveals that the Versicolor Pin Wheel variety shows good pollen parent features in each stage, especially in the after-senescence stage (stamen length: 1.385cm, anther amount: 108.13 cm, and pollen viability: 95.11%), while in the brilliant variety it was less. Regarding seed parent, the variety that has less style and style arm length (Agni: 7.43cm and 3.93mm), more ovary length and diameter (Cinnamon Girl: 11.817 mm and Double Peach: 8.7 mm), a higher percentage of ovule development and stigma diameter (Double Peach: 91.33% and 3.157 mm), and more stigma receptivity (Cinnamon Girl: +++++) at the end of the flower is considered a suitable one. From this report, breeders can select a variety at the perfect stage of flowering for their breeding program.

Keywords: *Hibiscus, stages of flower, stamen, anther, pollen viability, stigma, style, ovary, ovule*

1. INTRODUCTION

Hibiscus is known as China rose; this genus belongs to the family Malvaceae. Hibiscus originated in the tropics; many of its species are found in India, which is considered the oldest hybrid in the world [1]. It bears a complete bisexual flower with calyx, corolla, androecium & gynoecium [16]. The flower has diverse types of sizes (4–20 cm dia.) and shapes with various petal color like white, cream, yellow, red, pink, blue, purple, orange, brown, and multicolour shades; some varieties and species also have double flowers.

According to species diversity, the hibiscus flower has both male and female parts in different sizes. The superior ovary and pistil of the flower lie in the main structure of the flower. The Pistil has five "style arms" at the top, which hold the stigma. The stamen consists of stem-like filaments and anthers. Each filament ends with the pollen-producing anther. Each anther may contain around 250 to 500 pollen [17], which are deep yellow to orange. Some varieties or species produce pollen with spines on them [11], [5] and mucilaginous substances that help to attached to the surface of the stigma. Pollens of some varieties were without the spines (like Double Peach).

To increase the genetic potency of this plant, some efforts through hybridization are necessary. Hybridization aims to obtain the desired genetic combinations by crossing two or more different plant genotypes [4]. Information about the generative reproduction aspects of *Hibiscus rosa-sinensis* is still limited, so research on specific stages of flower pollination is necessary. The research aims to find out the best stage of flowers within a selected 6 genotypes for the morphological study to improve breeding progress and phenological development of flowers for generative reproduction success. The information obtained is expected to be a basic reference for further research related to this species, especially for genetic improvement programs or hybridization.

2. MATERIAL AND METHODS

The present investigation of an experiment was conducted from November 2022 to June 2022 at Mondouri Farm BCKV, Mohanpur, West Bengal. The evaluation was carried out in five distinctive stages (premature bud, mature bud, before anthesis, after anthesis and senescence) of each male and female parent to find out the most suitable stage for pollination. The harvesting time was restricted to early morning when they are fully turgid. The experiment was carried out in the Quality Control Laboratory; Faculty of Horticulture. The statistical analysis was factorial randomized block design at a 5 percent difference in three replications.

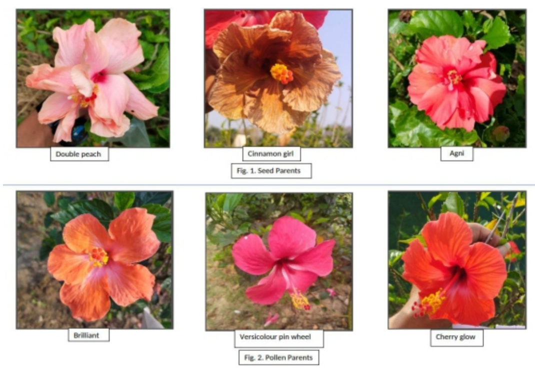

Fig. 1. Varieties used for morphological study

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Table 1: list of stage of flower and varieties

Parents	Varieties	Stage of flower
Pollen parent	Brilliant	Premature bud
	Versicolour Pin Wheels	Mature bud
	Cherry Glow	Before anthesis
Seed parent	Agni	After anthesis
	Cinnamon girl	Senescence
	Double peach	

2.1 Observation for male parent

The flowers were plucked from 3 selected varieties (Table No. 1), (Fig.1) in 5 different stages and have been studied for continuous development of the androecium for accessing the best stage of pollen collection.

The only stamen part of the flowers was separated from each stage with the help of a needle and forceps. They were kept on a graph sheet (0.5 cm x 0.5 cm) for the measurement of stamen length. After that, the stamen part was kept under magnifying lens glass to count the number of anthers present at each different stage with the help of a needle.

2.1.1 Pollen viability

The procedure followed for the estimation of pollen viability was more or less similar to that adopted by Erdtman's method [7]. Pollen grains of flowers at two stages i.e., after the anthesis and senescence stage of each variety mounted in acetocarmine were examined after 30 minutes. The pollen grains, which appeared normal, plump and well stained, were taken as viable and the unstained and shriveled ones as non-viable. Observations were made in 10 different microscopic fields and the mean percentage of viable grains was calculated.

2.2 Observation for female parent

The flowers were collected from 3 selected varieties (Table No. 1), (Fig.1) in five different stages and have been studied for continuous development of gynoecium for accessing the best stage of pollen pollination.

To examine the length of style, style arm length, ovary length, ovary diameter and stigma diameter in five stages of flowers, each part of the flowers was separated carefully with the help of a needle, forcipes and blade. The measurement was done by Vernier Caliper (India Tools and Instruments co.) and recorded for further analysis. The percentage of ovule development in each stage of flowers is calculated by visual record of seed locule development and number of ovule development in each stage of flower.

The studies of receptivity of stigma in percentage at different stages of flower were done by the following method which is given by Zeisler [22]. The stigma samples were collected from flowers and kept under observation. A 6% solution of hydrogen peroxide was placed on collected stigma of flowers and the appearance of bubbles was observed.

3. RESULT AND DISCUSSION

3.1 Observation of male parent

3.1.1 Stamen length

The perusal data of stamen length at different stages of the flower are presented in Table 2. Significant differences were observed in each stage of the flower; out of the senescence stage of the flower shows maximum stamen length (2.17cm) and the lowest in the premature bud stage (0.26cm). During the comparison, the variety Versicolour Pin Wheel shows the significantly highest stamen value (1.385cm) followed by Cherry Glow (1.345cm) and the lowest in the Brilliant variety (0.955cm).

The interaction effect of stamen length is highest in Cherry Glow at the senescence stage of the flower (2.48cm) followed by Versicolour Pin Wheel at the senescence stage (2.4cm) and lowest in Cherry Glow at the premature bud stage (0.203cm).

We can observe significant variation in stamen length among the selected varieties for stages of the flower due to increasing stamen length continuously. That means growth of the stamen continues from the premature bud stage to the senescence stage of the flower. Also, variation among the varieties is by season, organic matter of soil, and environmental effect on flowering plants, which is supported by Bell and Thomas [1] in *Hibiscus tiliaceus,* and also it can be ascertained that the number of stamens and stamen-petal intermediates in crested single-like flowers is generally always more than the number of staminodium petaloids [19] in *Hibiscus rosa-sinensis*.

3.1.2 Amount of anther present in pollen parent

Significant differences were observed among the pollen parent between the variety and flower stages, which are presented in Table 2. The amount of anther count is significantly highest in the senescence stage of the flower (111.22) and lowest in the premature bud stage (96.55). As in varieties, the significantly maximum amount of anther count is recorded in Versicolour Pin Wheel (108.13), which is the lowest in Brilliant (91.13). In the interaction effect of anther count, the highest was recorded in the Brilliant variety at the senescence stage of the flower (115.35), and the lowest was recorded in the same variety at the premature bud stage (83.33).

The amount of anther present in stages of the flower concerning varieties shows significant variation in the hibiscus genus because of stamen length, which is affected by environmental conditions and nutrients available for plants during the flowering time. This is the statement of Ibrahim et al. [9] during his research on Hibiscus rosa-sinensis. The anther contains pollen; if the anther count is higher, that means that the plant has more potential as the pollen parent. The significant variation in anther number is due to genetic variation and climatic effects on flowers [2].

3.1.3 Pollen viability percentage

The data in Table 2 revealed significant variations in pollen viability of pollen variety at each five stages of flower. As in after the anthesis stage, the flower has maximum pollen viability (76.06%) and the lowest in premature bud, mature bud, and before anthesis stages of the flower (0%). In pollen parent, the Versicolour Pin Wheel variety shows the highest amount of pollen viability (37.49%) and the lowest in the Brilliant variety (22.43%). The interaction effect ranges from 0 to 95.11%, and this effect is more in Versicolour Pin Wheel variety at the senescence stage (95.11%) and lowest in the first three stages of flower in every variety (0%).

During the observation, we can see pollens in the flower only after the anthesis stage of the flower. In this stage and the senescence stage, we can check the pollen viability percentage in different selected pollen parents. This result is in agreement with the views of Ostapenko [13] that the staining methods are only of relative value in determining pollen viability, for lower values were obtained by germinating pollen grains in sugar-agar medium than by acetocarmine. The pollen grains of the different varieties show great variation in germination in nutrient media, and the acetocarmine test gives either poor or no germination of pollens [20].

Table 2: Morphological parameter of pollen parents

	Seamen length in (cm)				Anther count				Pollen viability (%)			
Treatments	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**
S1	0.253	0.323	0.203	0.260	83.333	108.4.77	97.533	96.556	0.000	0.000	0.000	0.000
S2	0.500	0.720	0.510	0.577	86.333	105.667	104.333	98.778	0.000	0.000	0.000	0.000
S3	1.067	1.417	1.290	1.258	85.317	105.333	115.35	102.000	0.000	0.000	0.000	0.000
S4	1.333	2.067	2.233	1.878	86.333	109.667	107.333	101.111	58.833	95.117	74.250	76.067
S5	1.623	2.400	2.487	2.170	114.35	111.20	108.117	111.222	53.333	92.333	69.333	71.667
Mean	0.955	1.385	1.345		91.133	108.133	106.533		22.433	37.490	28.717	
	S	**V**	**(S x V)**		**S**	**V**	**(S x V)**		**S**	**V**	**(S x V)**	
C.D (5%)	0.120	0.120	0.120		2.808	2.808	2.808		1.545	1.545	1.545	
SEm (±)	0.041	0.041	0.041		0.964	0.964	0.964		0.531	0.531	0.531	

S- stage of flower - S1- pre-mature bud, S2-mature bud, S3-before anthesis, S4-after anthesis, S5-senescence stage
V-variety – V1-Brilliant, V2- Versicolor Pin Wheel, V3-Cherry Glow.

3.2 Observation of female parent

3.2.1 Style length

The data represented in Table, 3. shows significant difference among the stages, among the varieties and interaction between the stages of flower and different selected varieties.

As shown in Table, 3, the senescence stage of the flower shows the significantly highest style length (8.107cm), followed by the after-anthesis stage (8.071cm) and the lowest in the premature stage (1.306cm). Among the varieties, the Double Peach variety has the maximum style length (5.503cm), and the Agni variety shows lowest(4.686cm). The interaction ranges from 1.083cm to 9.13cm, which is highest in the Double Peach variety at the senescence stage (9.13cm) and lowest in the Agni variety at the premature bud stage of the flower (1.083cm).

The style of flowers grows continuously from premature bud stage to senescence stage, and this will vary for different selected varieties. We can observe that significant variation is due to the wide diversity among the genotypes in *Hibiscus rosa-sinensis* [19], and the style length variation could be influenced by photosensitivity and climate type [12].

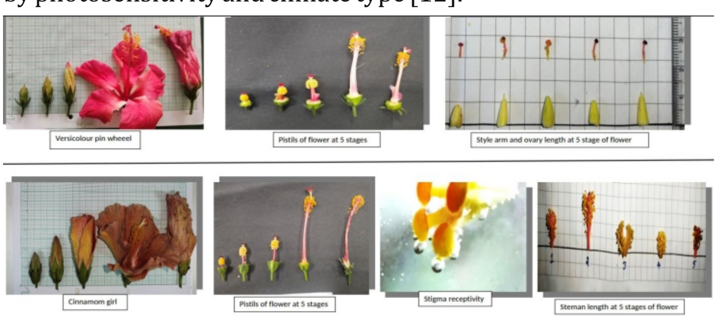

Fig.2. Parts of the Pollen and Seed parent

3.2.2 Style arm length

The perusal data of style arm length is presented in Table, 3. Significant differences were observed in the style arm length of selected seed parents in each stage of the flower.

The senescence stage shows maximum style arm length in each variety (5.778 mm) followed by the after-anthesis stage (5.7mm) and the lowest in the premature bud stage(1.08mm). In between the varieties, Double Peach shows the significantly highest style arm length (4.88mm) and Agni the lowest (2.65 mm). The interaction effect is highest in Double Peach after the anthesis stage of the flower (7.067mm), followed by the senescence stage of the same variety (6.833mm), and lowest in Agni at the premature bud stage (1.03mm). According to [19] in Hibiscus rosa-sinensis and [12], the variation among the varieties for the style arm length of the flower could be attributed to inherent genetic and environmental factors under study, which concluded that style arm length in each stage of flower will vary due to continuous growth in each stage of flower.

Significant differences were observed among the seed parent in between the variety and flower stages, which are presented in Table 3. As in stages of flower, the ovary length is highest in the after-anthesis stage (10.914mm) and lowest in the premature bud stage(5.012mm). As in varieties, the maximum ovary length is recorded in Cinnamon Girl (8.797mm), which is the lowest in Double Peach (7.46mm). The interaction effect ranges from 4.12mm to 11.817mm. The highest was recorded in the Agni and Cinnamon Girl varieties at the after-anthesis stage of flower (11.817mm), followed by the senescence stage of Cinnamon Girl (11.73mm), and the lowest in the Agni variety at the premature bud stage (4.12 mm). The ovary is having less length at the premature bud stage of the flower, and its growth continues up to the senescence stage of the flower; that's why it shows significant variation with respect to the stages of the flower in different varieties. The variation in ovary length is due to genotypic and phenotypic variation among the varieties [14].

3.2.4 Ovary diameter (mm)

The data represented in Table 4 shows a significant difference in ovary diameter among the stages, among the varieties, and in the interaction between the stages of flower and varieties.

Among the stages of flower, the senescence stage of flower shows the significantly highest ovary diameter (7.53mm) and is lowest in the premature stage (2.25mm). Among the varieties, the Double Peach variety has the maximum ovary diameter at every stage of the flower (6.527mm), and the Cinnamon Girl variety has the lowest (3.58mm). The interaction ranges from 1.5mm to 9.28 mm, which is highest in the Double Peach variety after the anthesis stage (9.28mm) and lowest in the Cinnamon Girl variety at the premature stage (1.5mm). The variation in diameter of the ovary at five stages of the flower for different selected seed parents shows significant variation. The ovary diameter of a flower is influenced by genetic factors, floral character, correlation effects, and phenotypic character of the flower [10].

Table 3: Morphological parameter of seed parents

	Style length in (cm)				Style arm length (mm)				Ovary length (mm)			
Treatments	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**
S1	1.083	1.55	1.283	1.306	1.033	1.100	1.133	1.089	4.12	5.183	5.733	5.012
S2	2.75	2.783	2.633	2.722	1.933	2.640	3.717	2.763	6.05	6.5	6.7	6.417
S3	5.067	5.483	5.75	5.433	2.567	5.063	5.657	4.429	9.5	8.75	7.25	8.50
S4	7.43	8.067	8.717	8.071	3.817	6.233	7.067	5.706	11.81	11.817	9.117	10.914
S5	7.1	8.087	9.133	8.107	3.933	6.567	6.833	5.778	11.7	11.733	8.5	10.644
Mean	4.686	5.194	5.503		2.657	4.321	4.881		8.636	8.797	7.46	
	S	**V**	**(S x V)**		**S**	**V**	**(S x V)**		**S**	**V**	**(S x V)**	
C.D (5%)	0.339	0.263	0.587		0.187	0.145	0.324		0.217	0.168	0.376	
SEm (±)	0.116	0.090	0.202		0.064	0.050	0.111		0.075	0.058	0.129	

S- stage of flower - S1- pre-mature bud, S2-mature bud, S3-before anthesis, S4-after anthesis, S5-senescence stage
V-variety – V1-Agni, V2- Cinnamon Girl, V3-Double Peach.

3.2.5 Percentage of ovule development (%)

The different selected varieties in each five stages of flowers show significant variation along with interaction between them; this is represented in Table 4. In stages of flower as a factor one, the percentage of ovule development is significantly higher in the senescence stage (89.44%) and less in the premature bud stage (0). As in different variety as a factor two, significantly more ovule development is observed in Double Peach (56.93%), which is lowest in Agni (51.53%). During the observation of ovule development, the interaction ranges from 0 to 91.33%. The ovule development percentage is higher in the senescence stage of the Double Peach variety (91.33%), which is followed by the senescence stage of the Agni variety (89.6%), and is less in the premature bud stage of all varieties. The shape and number of ovules from the early stage are not different from the later stage. The difference is only the size of the ovules, in which the ovules in the later stage are completely developed [14].

3.2.6 Stigmatic receptivity

The stigmatic receptivity of individual flowers is represented by '+' marks. If a selected variety shows more stigmatic receptivity, then for that variety we indicate more '+' marks to that respected variety (Table 4). Regarding this, Cinnamon Girl has more stigmatic receptivity (+++++), followed by Double Peach and Agni (++++). These reports indicated that we could primarily judge the stigma receptivity by the stigmatic morphological traits, which would provide some reference for the judgment of the effective pollination period [21]. The peroxidase test was the only procedure that showed correspondence with all of the other tests when they indicated receptivity of selected varieties [3].

Table 4: Morphological parameter of seed parents

	Ovary diameter (mm)				Percentage of ovule development (%)				Stigma diameter (mm)				Stigma receptivity		
Treatments	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**	V1	V2	V3	**Mean**	V1	V2	**V3**
S1	2.750	1.500	2.500	2.250	0.000	0.000	0.000	0.000	0.253	0.357	0.583	0.398	-	-	-
S2	4.500	2.467	4.583	3.850	20.000	28.333	31.667	26.667	0.900	0.977	1.117	0.998	-	-	-
S3	6.257	3.750	7.567	5.858	68.333	60.000	75.000	67.778	1.860	2.180	2.173	2.071	+++	++++	+++
S4	7.817	4.727	9.283	7.276	79.667	87.000	86.667	84.444	2.387	2.420	2.767	2.524	++++	+++++	++++
S5	8.417	5.500	8.700	7.539	89.667	87.333	91.333	89.444	2.897	3.063	3.157	3.039	++--	++--	++--
Mean	5.948	3.589	6.527		51.533	52.533	56.933		1.659	1.799	1.959				
	S	**V**	**(S x V)**		**S**	**V**	**(S x V)**		**S**	**V**	**(S x V)**				
C.D (5%)	0.261	0.202	0.452		3.496	2.708	6.055		0.059	0.046	0.102				
SEm (±)	0.090	0.069	0.155		1.201	0.930	2.07		0.020	0.016	0.035				

S- stage of flower - S1- pre-mature bud, S2-mature bud, S3-before anthesis, S4-after anthesis, S5-senescence stage
V-variety – V1-Agni, V2- Cinnamon Girl, V3-Double Peach.

3.2.7 Stigma diameter (mm)

The data represented in Table 4 shows significant differences in stigma diameter among the stages, among the varieties, and in the interaction between the stages of the flower and different selected varieties.

As shown in Table 4, among the stages of the flower, the senescence stage shows the significantly highest stigma diameter (3.157mm), which is lowest in the premature stage of the flower (0.253mm). Among the varieties, the Double Peach variety has the maximum stigma diameter at the senescence stage of the flower (3.157mm), and the Cinnamon Girl variety has the significantly lowest stigma diameter (0.253mm). The interaction of stigma diameter between the stages of the flower and selected varieties ranges from 0.253mm to 3.157 mm, which is highest in the Double Peach variety after the senescence stage of the flower (3.157mm) and lowest in the Agni variety at the premature stage of the flower (0.253mm). Various flowers show significant differences in the diameter of the stigma, which is due to the genotypic and phenotypic characters of selected varieties [19].

4. CONCLUSION

This morphological study across five developmental stages of six *Hibiscus rosa-sinensis* genotypes revealed significant variations in both male and female reproductive structures. For the male parent, the senescence stage consistently exhibited the longest stamen length and the highest anther count across the varieties 'Brilliant', 'Versicolour Pin Wheel', and 'Cherry Glow'. Notably, pollen viability was only observed after anthesis, peaking at the senescence stage, with 'Versicolour Pin Wheel' showing the highest viability. For the female parent ('Agni', 'Cinnamon Girl', and 'Double Peach'), the senescence and after anthesis stages generally displayed the most developed gynoecial characteristics, including the longest style, style arm length, ovary length and diameter, and the highest percentage of ovule development. 'Cinnamon Girl' exhibited the highest stigmatic receptivity based on the hydrogen peroxide test. In essence, the after-anthesis and senescence stages appear to be the most suitable for both pollen collection and pollination in the studied *Hibiscus rosa-sinensis* genotypes to maximize the chances of successful hybridization. However, the optimal stage may vary slightly depending on the specific genotype and the trait of interest.

FUTURE SCOPE

This research lays the foundation for understanding *Hibiscus rosa-sinensis* reproductive phenology. Future studies should assess pollen viability and compatibility using electron microscopy, staining techniques, and in vitro germination assays. Detailed stigma analysis will clarify receptivity, while controlled cross-pollination experiments will determine optimal pollination stages. Molecular markers and genetic analysis will enhance parent selection. Investigating environmental factors and using time-lapse imaging will refine breeding strategies. Understanding compatibility mechanisms is vital for overcoming hybridization barriers and advancing *Hibiscus* breeding.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for the publication of the manuscript.

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