

Flower Characteristics and Pollination Behavior of *Euryale ferox* (Salisb.)

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Abstract

The *Euryale ferox* (Salisb.) or gorgon or makhana is one of the most important non cereal food crops of commerce from wetland ecosystem in India. Flower is cleistogamous (CLS) and predominantly self-pollinated. The variations in floral characters were observed in 10 types of germplasm viz., Manipur-2, Manipur-4, Manipur-7, Manipur-9, Selection-17, Selection-23, Selection-27, Selection-28, Superior Selection-1 and cv. Swarna Vaidehi. In our present study, the number of flowers varied from 8.33 (Manipur-9) to 16.33 (Superior Selection-1) per plant and flowering period was about 40 days. However, peak pollination was observed between 60 - 70 days after transplanting. The weather of August and September were ideal for pollination and fruit set. The temperature and humidity of this period were 29°C - 31°C and 79% - 81%, respectively. Besides cleistogamy (CL), chasmogamy (CH) is also observed after July flowering in *Euryale*, when crop gets matured, water level considerably goes down, and flowers are generally opened in air. There were rare chances for cross pollination by insect. In later stage, chasmogamous (CHS) flower increases up to 22.50% in October. Seed formation of the CHS flower was very less and seed number varies from July (11.25/fruit) to September (28.33/fruit). Artificial hybridization can be performed in CHS flower. The complete flower development was noticed within 72 - 96 hrs from floral initiation. Therefore, getting of CHS flower outside water is very less. There were strong correlations between number of embryos ($r = 0.762$), ovary area (longitudinal) ($r = 0.681$) with the yield of the *Euryale* plant.

Keywords

Euryale, Flower Characters, Yield and Correlation

1. Introduction

Gorgon nut, *Euryale ferox* (Salisb) or makhana is one of the most important aq-

uatic medicinal plants native to south east or eastern Asia, particularly India and China. However, wild forms of plants in wetland ecosystem were also found in Korea and Japan, as well as parts of eastern Russia [1]. During spring, it grows in water and produces bright purple flowers during May. It is a photo insensitive crop and can be grown successfully from March to September in economic way in India. Floating leaves are prickly on petioles and along veins and the underside of the leaf is purplish, while the upper surface is green. All the plant parts are thorny outside. It thrives best in ponds, wetlands, lakes and lowland in tropical and subtropical regions of India. Field cultivation of *Euryale* is also recommended where plenty of water is available. *Euryale* is famous for its [2] high valued seeds marketed in India, China, and Japan [3]. In India particularly in Bihar, more than 96,000 hectares were under cultivation in 1990-1991 [2]. But in present condition it is cultivated about 11,000 ha only [4]. According to [5] it is now becoming under extinction due to rapid urbanization and profitable fish production in wetland area instead of *Euryale*, which is labor intensive. In Mithila culture of Darbhanga, *Euryale* or *makhana* is known for its auspicious ingredient used in offerings to the Goddesses during “Durga Puja” festivals. Evidence from archaeobotany indicated that *Euryale* was a frequently collected as wild food source during the Neolithic period in the Yangtze region [6]. The earliest record showed that use of *Euryale ferox* to date was found in Gesher Benot Ya’aqov, Israel, among artifacts of the Acheulean culture 790 - 750,000 years ago [7]. Edible seeds of *Euryale* are used in traditional Chinese medicine, where they are often cooked in soups along with other ingredients [8]. Though it is an eastern and south Asian species, it is widespread and recorded from the Russian Federation, India (Assam, Bihar, Jammu and Kashmir, Manipur, Meghalaya, West Bengal), Japan (Honshu, Kyushu), Republic of Korea, Taiwan (Province of Thailand), Bangladesh and Myanmar [9] [10]. This plant has edible and medicinal uses. Makhana kernel powder is said to have control of cardiovascular diseases [11]. It is taken internally in the treatment of chronic diarrhoea, vaginal discharge and kidney weakness [12] [13]. The genera *Euryale* of east Asia, and *Victoria* from far South America, are closely related despite their geographic distance [14] [15] [16]. Flower is epigynous with more than 40 corolla [17], ovary 7 - 16 chambered with 6 - 8 seeds/locule [5].

The objectives of this study were to understand about the flower structure, pollination time and per cent pollination of *Euryale* in relation to weather. Different type of pollinations and the development of hybridization techniques in *Euryale* under field condition were other important objectives in this present study to initiate future breeding program of this crop in India.

2. Materials and Methods

The experiment was carried out at ICAR-RCER, Research Centre for Makhana, Darbhanga, Bihar, India, during 2017. Makhana was cultivated from April onwards to October. Flowering period covered from Mid May to November. In our

germplasm block, 28 germplasm of different morphological structures collected from different parts of India were the basic materials for study. We have selected 10 outstanding selections with differences in floral structures and pollination behaviors were studied. The floral characters like number of calyx, number of corolla, androecia, sessile ovary with different stigmatic color and ovaries with different chambers and their ovules were studied thoroughly. Complete Randomized Design (CRD) was followed to analyze the data statistically for flower characters. Fisher protected LSD ($P > 0.05$) was adapted to analyze the data on pollination % and duration of pollination and seed formation (number) in different types of pollination. Plant yield performance and yield correlation with flower morphological characters were delineated statistically by using Karl Pearson Correlation coefficient. Stigmatic color was recorded by UPOV color chart [18]. Favorite pollination periods with temperature range were recorded for better flowering and fruiting. To study the chasmogamy, air opened flowers before dehiscence of anthers were tagged from July to Nov and number of flowers to total numbers of flowers also calculated including their seed. Pollination % and different flower percent was calculated by the following sequence:

$$\text{Pollination (\%)} = \frac{\text{Number of Pollinated Embryos}}{\text{Total Number of Embryos}} \times 100$$

$$\text{CLS Flower (\%)} = \frac{\text{Number of Cleistogamous Flowers}}{\text{Total Number of Flowers}} \times 100$$

$$\text{CHS Flower (\%)} = \frac{\text{Number of Chasmogamous Flowers}}{\text{Total Number of Flowers}} \times 100$$

To obtain hybrid seeds from hybridization, a few precautions were taken as it was wetland aquatic crop and after artificial hybridization, hybrid fruit generally submerged again to mature the seeds after 7 - 10 days of pollination. Emasculation and hybridization were performed safely. Water level was maintained 10 - 15 cm during hybridization period so that pollinated flowers did not damage.

3. Results and Discussion

A close perusal of the **Table 1** revealed that the number of flower/plant varies from 8.33 (Manipur-9) to 16.33 (Superior Selection-1). These results were in conformity of the study conducted by [19]. The maximum floral parts were also observed in Superior Selection-1 (calyx + corolla + androecia = 4 + 42 + 127). The minimum floral parts were found in Selection 17 (calyx + corolla + androecia = 4 + 22 + 68). There were positive correlations with different floral parts to yield. On the contrary, Manipur-2 also produced little flower but its yield capacity was higher due to production of increased number of flower/plant. Flower size ranged from 23.16 cm² in Selection 17 to 33.79 cm² in Superior Selection-1. Flower stalk length varies significantly among the germplasm whereas as flower stalk girth did not. Zhuang [5] also reported that a flower of *Euryale* was about

Table 1. Physical characteristics of flowers of *Euryale* in wetland ecosystem in north Bihar-2017.

Germplasm	No. of flowers/plant	Flower Stalk Length (cm)	Flower Stalk Girth (cm)	No. of Calyx	No. of Corolla	No of Androecia	Flower size (Lx B) cm ²
Manipur-2	15.00	17.33	6.20	4.00	28.00	92.00	25.50
Manipur-4	10.66	14.33	6.30	4.00	32.00	102.00	29.40
Manipur-7	10.00	12.66	5.50	4.00	31.00	95.00	32.25
Manipur-9	8.33	14.00	5.00	4.00	39.00	85.00	28.75
Selection 17	9.80	15.33	6.40	4.00	22.00	68.00	23.16
Selection 23	11.50	18.00	6.10	4.00	40.00	120.00	26.46
Selection 27	11.33	16.33	6.30	4.00	34.00	101.00	32.11
Selection 28	11.00	15.33	5.80	4.00	40.00	105.00	26.05
Superior Selection-1	16.33	19.33	6.80	4.00	42.00	127.00	33.79
Swarna Vaidehi	10.66	20.00	6.50	4.00	40.00	110.00	29.62
CD (CRD) at 5%	1.68	1.75	NS	NS	2.34	6.51	1.36

5.0 cm diameter and outer skins of the flowers are densely pricked. Data pertaining to **Table 2** enable us to explain that the highest innerovary area was noticed in Superior Selection-1 (6.62 cm²) followed by Swarna Vaidehi (6.24 cm²). The germplasm Selection-17 had less number of colored corollas (Violet-10) whereas Superior Selection-1 had the maximum (Pink-22). According to [17], the total corolla was greater than 40, which was further confirmed by [7]. In the present study, we found that minimum locule 11 and embryos 4.8 which were found in Selection 17 whereas Superior Selection-1 had the maximum locule of 17 with 8 embryos/locule. [17] stated that multicarpellary, syncarpous inferior ovary which had 7 - 12 locules were present in *Euryale*. According to [5], in *Euryale ovary* was 7 - 16-loculed, each locule with 6 - 8 or more ovules. Flowers of *Euryale* were epigynous, petals outer purple, violet or pink fading to inner white and gradually smaller (oblong-lanceolate) and tapering large calyx kept the flower air tight in water. This result about flower structure was confirmed by [20] [21] [22]. After transplanting flowering needs and 45 days and flowering periods last for 40 days and peak flowering was observed between 60 - 70 days (**Figure 1** and **Plates 1-4**).

Arrangement of calyx and corolla was so nicely designed it paves the way to CL, assured self pollination before opening of the flower and in *Euryale* self pollination happened when flowers remain closed and with in water. By destructive method, we found that flower bud emerging out of the bract and after 3 - 4 days, self pollinated, *i.e.* CL occurred. CH *i.e.* self pollination in open flowers happened after July onwards when the water level of the field drastically reduced and in terminal 4th-5th flowers CH occurred. The terminal 1-3rd flowers were

Table 2. Dissected flowers of *Euryale* and their characters under wetland ecosystem of north Bihar-2017.

Germplasm	No. of Colored Corolla	No. of White Corolla	No of Locules/ Ovary	No. of Embryos/ Locule	Ovary area cm ² (inner)	Color of Stigma (Color Code)
Manipur-2	Reddish Violet-13	15.00	16.00	6.50	4.81	Orange Red [032A]
Manipur-4	Violet-16	16.00	12.00	4.50	4.87	Yellow Orange [016A]
Manipur-7	Violet-17	14.00	12.00	5.20	4.94	Brown Orange [032C]
Manipur-9	Violet-16	13.00	10.00	5.00	5.4	Light Yellow [004D]
Selection 17	Violet-10	12.00	11.00	4.80	4.68	Deep Orange [029A]
Selection 23	Violet 15	25.00	15.00	6.60	5.98	Light Yellow [004D]
Selection 27	Pink-18	16.00	11.00	6.00	4.84	Deep Orange [029A]
Selection 28	Violet-19	21.00	14.00	6.00	4.87	Yellow Orange [016A]
Superior Selection-1	Pink-22	21.00	17.00	8.00	6.63	Light Yellow [004D]
Swarna Vaidehi	Violet-20	18.00	16.00	6.40	6.24	Yellow Orange [016A]
CD (CRD) at 5%	2.05	2.12	1.82	0.68	0.99	...

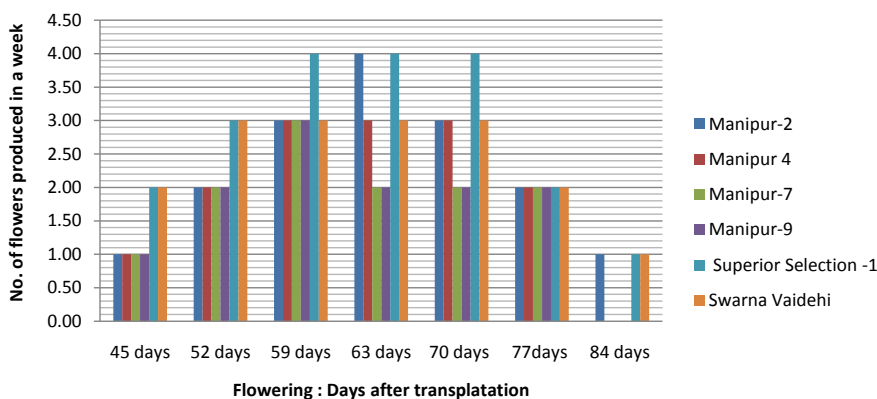


Figure 1. Pollination periods and number of flowers in some *Euryale* germplasm [CRD with 3 replications].

small and desiccated in air during pollination or hybridization periods. CHS flower increased up to 22.5% in October (Figure 2). For artificial cross pollination, terminal 4-5th were the vital flowers opened in air so emasculation and hybridization become feasible. Due to last season of growth, terminal last 2 flowers

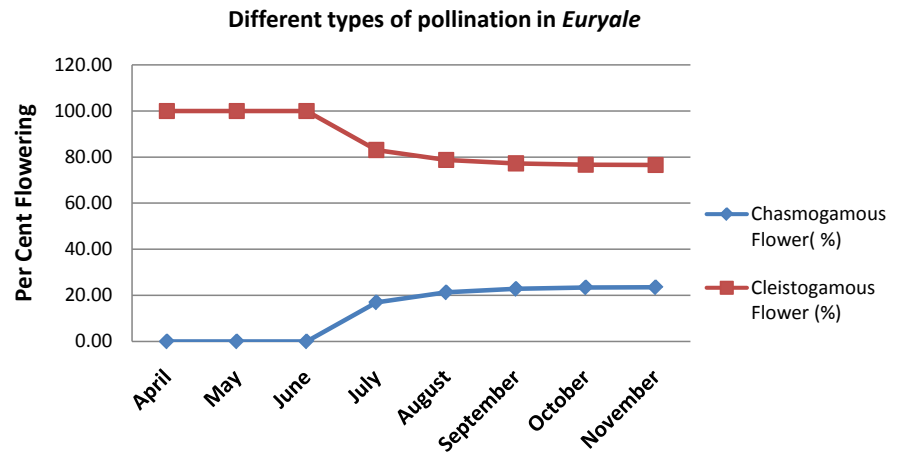


Figure 2. Percentage of different types of pollinated flowers of *Euryale* [Fisher protected LSD ($P \leq 0.05$)].



Plate 1. Manipur-2.



Plate 2. Selection-17.



Plate 3. Superior Selection-1.



Plate 4. Swarna Vaidehi.

get dried due to water stress. Although CH was happened in last 4-5th flower, the seed production was less. Number of seed ranged from 11.25/fruit in July to 28.33/fruit in September (**Figure 3**). The Japanese scientist Kadono [23] also reported that CH was gradually increased after July onwards in *Euryale* plant.

In our seasonal study, we grown *Euryale* from January to December and it was found that July transplanted and August transplanted gorgon nut thrives well under autumn atmosphere of India. Their flowering period was in August and September of the same season and gave highest plant growth and yield. It has been found that yield and growth and weather were correlated. The temperature 29°C - 31°C and humidity 79% - 81% were ideal for *Euryale* cultivation (**Figure 4**). This result was corroborated by Mandal *et al.* [24]. During October 80.00 per cent pollination was observed. Color of the stigma varied from Light Yellow (004D) in Manipur-9, Manipur 23 and Superior Selection-1 to Orange Red (032A) in Manipur-2 germplasm while pollen was very minute and white in all

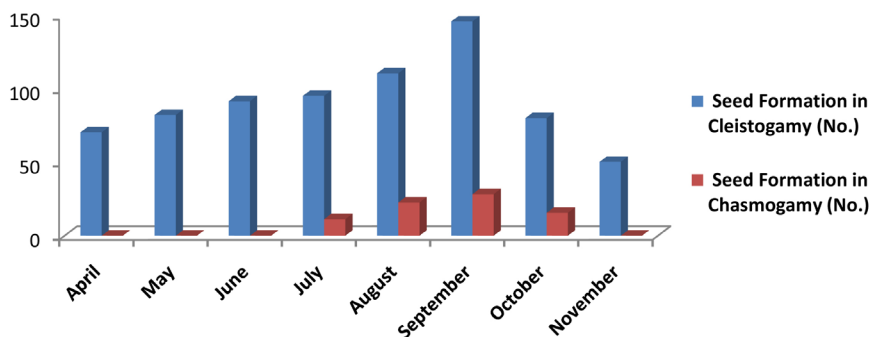


Figure 3. Seed formation of different types of pollinated flowers of *Euryale* [Fisher protected LSD ($P \leq 0.05$)].

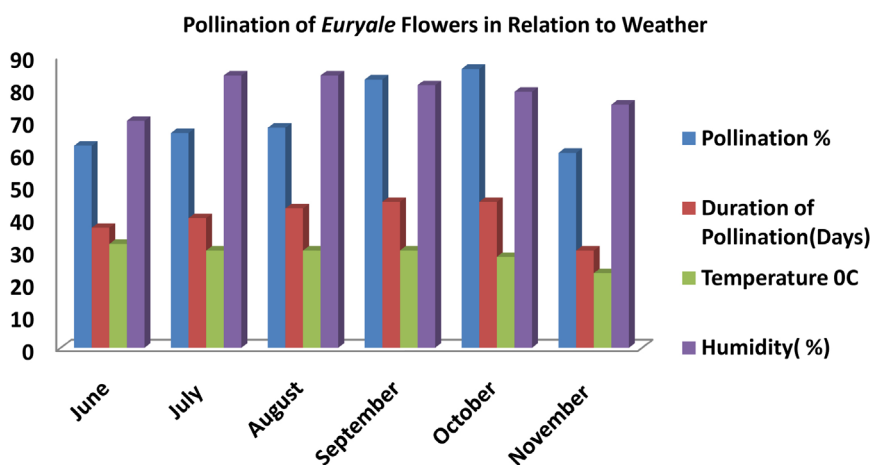


Figure 4. *Euryale* flowers and pollination in relation to a particular weather [CRD with 3 replications for characters pollination % and duration of pollination].

Euryale germplasm.

All floral characters were correlated with yield, which is calculated from number of fruit, number of seed and average weight of seed. It has been found that besides flower stalk, girth of stalk and number of calyx, all the quantitative characters were highly correlated with yield. There was a strong correlation between number of embryos ($r = 0.762$) and ovary area (longitudinal) ($r = 0.681$) with yield (Figure 5). According to [25] among 14 traits studied, 100-seed weight was observed to have highest contribution (57.30%) towards genetic divergence followed by seeds/fruit (18.25%) and fruit diameter (17.46%). Stigma was sessile and stigmatic color varied from light yellow (004D) to orange red (032A). The flowers having deeply colored stigma produced less yield as compared to light colored once.

4. Conclusion

From the present study, we concluded that CLS flowers are appropriate for commercial cultivation. Hybridization can be possible through CHS flower as these flowers are produced at later stage of plant growth and development and opened in air before dehiscence of anther. These flowers are small in size and

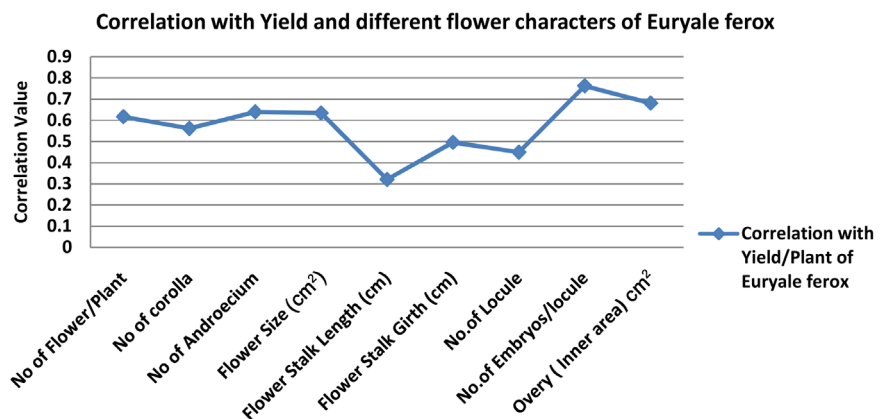


Figure 5. Correlation of different floral characters with yield of *Euryale*. [Karl Pearson correlation, *Significant $r = 3.61$].

produce less seeds in their fruits. For hybridization program water level of the field must be maintained 10 - 15 cm. After hybridization flowers were tagged and kept in air for 7 - 10 days for healing after emasculation injury. After that again water level was increased to submerge the fruits to get mature hybrid seeds. Flowers having a greater number of floral parts *i.e.* calyx, corolla and androecia produced the higher yield. The flower size and inner ovary area had the positive correlation to yield.

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