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Reproductive biology of *Gentiana kurroo* Royle

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Reproductive biology studies in *Gentiana kurroo*, an important endangered medicinal plant of temperate/subtemperate regions, revealed its flowers to be dichogamous due to protandry. The stigmatic lobes remain adpressed till almost complete anther dehiscence. The stigma becomes receptive to pollen germination about 6 days after initiation of anther dehiscence. The flowers are cross-pollinated and about 70–75% seeds germinated.

GENTIANA kurroo (family Gentianaceae), occurring in temperate regions, is valued as a bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier and carminative[1,2]. The drug plant, heavily extracted from its natural habitat is an endangered medicinal plant. Hence, the Ministry of Commerce, Government of India has put it in the negative list of exports vide Notification no. 2 (RE-98) 1997–2002 dated 13 April 1998. A perusal of the literature reveals that no information regarding its breeding system is available. The present study was undertaken to understand its breeding behaviour, which, to a large extent, determines the degree of variability expected in a population[3]. An understanding of the breeding system is fundamental to the establishment of cultivation, undertaking any genetic improvement for higher yield attributes, disease resistance, etc.

Plants of this species occur in some pockets in District Sirmour, Himachal Pradesh at an altitudinal range of 1700 to 2000 m at mean sea level. Morphological studies were carried out on both wild as well as field-grown plants, while pollination studies were conducted on field-grown plants only (in our university campus). Controlled pollination was done at stigma-receptive stage (stigma lobes in open condition) and between 8 and 10 am, using fresh pollen. In bagging and controlled selfing, buds that were about to open were covered with paper bags. While in the former the buds/flowers were left as such till fruit formation, in the latter, after flower-opening, the bag was removed, stigma hand-pollinated with pollen from the same flower and rebagged, repeating the process for 3–4 days. In open cross and controlled cross, buds about to open in next couple of days were carefully emasculated.

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While in the former such buds were left open, in the latter the emasculated buds were hand-pollinated with pollen from other plants, covered by paper bags and bagged. This process was also repeated for 3–4 days. In open pollination, unopened buds were tagged and left for natural pollination without any artificial intervention. Stigma receptivity was determined by placing fresh pollen on stigma and periodically observing for pollen germination under a microscope. Pollen viability was studied by hanging-drop method using 2% sucrose solution.

Flowers of *G. kurroo* are bracteate, pedicellate, complete, actinomorphic, hermaphrodite, hypogynous and pentamerous (Table 1, Figure 1 *a*). Flowers are large and entomophilous. Flowering starts from the third week of August and continues till the first week of November, with the peak between 15 September to 20 October. On average, a plant produces 20 flowers.

The corolla is gamopetalous and infundibuliform, deep blue from outside up to plicae, dotted white at the throat and white at the base from inside (Figure 1 *c*). The flowers close during night and under low light, and reopen when the sun shines brightly. The opening and closing of the corolla continues till fertilization is complete and stigma lobes become dry (Table 2). After fertilization, the corolla remains closed till it withers.

The five stamens are epipetalous, with their filaments flattened at the base and fused with the corolla up to half of their length. Anthers are grouped around the stigmatic tip in bud condition, but move away as anther dehiscence starts (Table 2). The gynoecium is bicarpellary and syncarpous. The ovary is unilocular and contains numerous anatropous ovules borne on parietal placentae. Initially, the two stigma lobes are adpressed and situated well below the anther level (Table 2). After anther dehiscence is complete, the stigmatic lobes open out and are lifted above the level of the anthers by elongation of the pistil. The type of dichogamy noted is caused by protandry (Table 1, Figure 1 *d*).

Table 1. Floral characters of *Gentiana kurroo*

Floral character	Observation
Flowering period	September–October
Flower type	Entomophilous
Flower colour	Plicae blue, throat dotted with white dots, base white from inside
Odour	Absent
Nectar	Absent
Time of flower opening	6–8 am
Time of anther dehiscence	7.30–10.00 am
Mode of anther dehiscence	Longitudinal
Type of dichogamy	Protandry
Pollen shape	Round to oval, triaperturate
Stigma type	Stigma bilobed much above the anther level with papillae only on the adaxial surface
Ovule type	Anatropous
Days taken for capsule maturity	25–29 days after anthesis

The anthers attain maturity and dehisce to discharge pollen 1–3 days after anthesis. The anthers are placed well above stigmatic region, with the stigmatic lobes in adpressed condition at dehiscence stage. Dehiscence continues for 3–4 days (Figure 1 *d* (left)). Although 94% of the pollen was viable at the beginning of dehiscence (Figure 1 *e*), there was a gradual reduction in pollen germination as the anther dehiscence progressed. However, substantial reduction in pollen viability was observed after 72 h (73.64%) up to 108 h (3.72%) on commencement of anther dehiscence (Table 3).

The stigmatic lobes remain closed (in adpressed condition) till almost the completion of anther dehiscence. They start opening from the 4th to 6th day after anthesis and continue until 13th to 16th day after anthesis (Figure 1 *f*). This is the stage when the stigma is receptive to pollen germination (Figure 1 *b*). At this stage, the stigma lobes are well above the anther level (Figure 1 *d* (right)).

Response of *G. kurroo* to different pollination methods tested showed it to be strongly cross compatible, as was evident by significant fruit and seed set in controlled

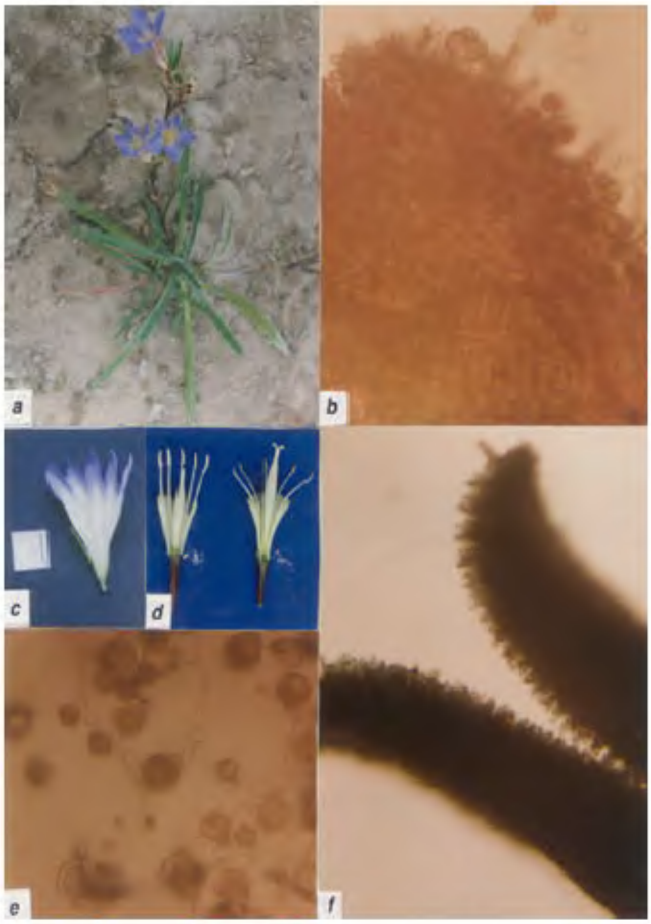

Figure 1. *Gentiana kurroo*. ***a***, Flowering plant; ***b***, Pollen germination on stigma; ***c***, Corolla opened showing whitish base inside; ***d***, dichogamy: *left*, anther dehiscence and adpressed stigma; *right*, withered anthers and stigma bilobed; ***e***, Pollen germination; ***f***, Stigma lobes opening and showing stigmatic papillae.

cross-pollination (Table 4). Absence of fruit and seed set in bagged flowers (T_2) may be due to the existence of protandry, which prevents self-pollination (Table 4). A similar phenomenon has also been reported in *Gentiana newberry* L., an alpine perennial species in which no fruit and seed set was reported in caged plants[4].

The fact that *G. kurroo* is cross-pollinated is further strengthened by the observation that open-pollinated (T_1) and open cross-pollinated (T_4) flowers performed statistically at par with each other with regard to all parameters studied (Table 4). The presence of anthers (in open-pollinated flowers) or their absence (due to emasculation in open cross-pollinated flowers) does not make any significant difference for seed and fruit set in open and open cross-pollinated flowers, respectively.

In flowers, which rely on insects for cross-pollination, the anthers and stigmas are clearly separated by at least a small gap to allow passage of insects[5]. In flowers of *G. kurroo*, it has been observed that anthers, which are grouped around the stigmatic region initially when the corolla is still closed, move towards the periphery when the corolla opens, creating a small gap between the anthers and the stigma. Although no nectariferous disc is present in its flowers, insect vectors are rewarded by

Table 2. Corresponding sequence of events in development of pollen and stigma receptivity

Stage of anthesis	Stage of anther/pollen development	Stage of ovary/stigma development
1–3 DBA	Anthers grouped in the centre of corolla tube	Stigmatic lobes in adpressed condition and below anther level
Anthesis	Anthers move towards corolla surface; flower opens	Ovary increases in size
1–3 DAA	Pollen dehiscence starts	Stigmatic region increases in size and is almost in level with anthers
4–6 DAA	Pollen dehiscence complete, with small amount of pollen adhering to the anther lobe	Stigma lobes start opening
7–9 DAA	Anthers become shrivelled	Stigma lobes continue bilobing and becoming reflexed; stigma receptive for pollen germination
10–13 DAA	Anthers start withering	Stigma lobes fully reflexed; stigma receptive for pollen germination
14–18 DAA	Anthers fully dried	Stigmatic lobes start curling backward and change colour due to drying and lose receptivity
19–21 DAA	Anthers fully dried	Stigma lobes completely dried up

DBA, Days before anthesis; DAA, Days after anthesis.

Table 3. Pollen germinability at different time intervals after anther dehiscence (by hanging-drop method)

Hours after anther dehiscence	24	36	48	60	72	84	96	102	108
Pollen germinability (%)	92.14	89.17	84.32	80.32	73.64	53.61	29.60	16.12	3.72
S.E. ±	0.76	1.07	1.40	0.61	0.93	1.61	2.62	1.91	0.92

Table 4. Effect of different pollination methods in *G. kurroo*

	Parameter						
Treatment	Fruit set (%)	Fruit length (cm)	Weight of fresh fruit (g)	Seed yield/pod (mg)	Seed yield/plant (mg)*	No. of seeds in a pod	No. of seeds per plant*
T_1 (open pollination)	80.57 (63.90)	5.47 (2.44)	2.03 (1.59)	8.87 (3.05)	142.32 (11.93)	69.32 (8.32)	1112.86 (33.36)
T_2 (bagging)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T_3 (controlled selfing-xenogamy)	7.25 (13.19)	4.97 (2.33)	1.66 (1.46)	5.20 (2.18)	7.54 (2.71)	40.62 (5.61)	58.89 (7.66)
T_4 (open cross)	81.32 (64.54)	5.77 (2.50)	2.06 (1.59)	10.81 (3.34)	175.80 (13.26)	84.45 (9.15)	1373.49 (37.06)
T_5 (controlled cross)	85.40 (67.69)	5.82 (2.57)	2.39 (1.70)	17.85 (4.27)	304.87 (17.46)	139.40 (11.80)	2380.90 (48.79)
$CD_{0.05}$	7.24 (8.62)	0.38 (0.08)	0.32 (0.01)	4.40 (0.92)	40.00 (2.16)	35.92 (0.85)	422.7 (6.52)

Figures in parenthesis are transformed values.
*Based on an average of 20 flowers/plant and fruit set percentage.

abundant pollen. The different insect vectors seen visiting flowers of *G. kurroo* are bumble bee (*Bombus* sp.), honeybee (*Apis mellifera*) and ladybird beetle (*Coccinella septempunctata*). Another feature that attracts the insects is the colour pattern of the corolla. The whitish interior of the corolla tube in contrast to the deep blue colour of the lobes makes the interior translucent, if held against light (Figure 1 *c*). This makes the interior look lighted to an insect, encouraging it to enter the flower and effect pollination. This is a common feature of many *Gentiana* species like *G. sino-ornata* and *G. acacilis*; also, the white dots at the throat region serve as guide marks for the visiting insects[5].

There is generally a time lag between pollen shedding and its transfer to the stigma. During this period pollen grains are exposed to a wide range of environmental stresses, particularly of temperature and humidity, which affect their competitive ability to sire vigorous progeny[6]. The factors affecting pollen viability, like the duration for which anthers continue shedding pollen and the range of environmental factors to which they are exposed, are critical for cross-pollinated species like *G. kurroo*. Availability of viable pollen over a longer period helps protandrous flowers in cross-pollination. This is more relevant in *G. kurroo*, where it has been observed (present investigation) that the stigma becomes receptive 4–6 days after anther dehiscence, and anthers continue shedding pollen for 3–4 days after initial anther dehiscence. Whether fresh pollen grains were being shed daily or those shed at the start of anther dehiscence remain adherent to the anther surface could not be ascertained. However, there was a gradual reduction in pollen germinability from 94.0% at the beginning of anther dehiscence to 29.6% after 96 h. Even after 108 h, a small amount of pollen (3.7%) retained germinability (Table 3).

Although *G. kurroo* seems to be chiefly cross-pollinated, as is indicated by no fruit and seed set in bagged flowers and statistically similar results obtained in open and open cross-pollinated flowers, 7.24% fruit set (Table 4) in controlled selfing (selfing effected manually at stigma receptive stage) indicates that some self-pollination is still possible. A small amount of pollen remaining viable even after 108 h may be responsible for fruit set.

The first fortnight of November is the ideal time for seed harvest after which the capsules open up, scattering the seed. 70–75% of seeds germinate and June is the ideal month for seed sowing. The seed has to be stored at low temperature (below 5°C) after harvesting. Otherwise, there is considerable reduction in germination percentage. Seeds more than one-year-old lose viability and do not germinate.

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