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Exploration, collection and conservation of genetic resources of *Bergenia ciliata* (Haw.) Sternb. in darjeeling and Sikkim Himalayas

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Abstract

Bergenia ciliata also known as Pakhanbed is an important medicinal plant used in various indigenous systems of Medicine in India. The whole plant is used for medicinal purpose but the rhizome is the main economic part. Developing cultivars with high rhizome yield and quality are the important breeding objectives in *B. ciliata*. There has been no effort made for germplasm collections and to study the diversity which hindered the pathway for developing high yielding varieties focused on medicinal plants and its improvement. The main objective of the study is to explore the natural population and to conserve its variability. An exploration and collection mission for wild populations of was carried out during 2017 -2021 in the Sikkim and Darjeeling Himalayas. Eight explorations were undertaken for collection and understanding the distribution patterns and population status in a natural habitat and twenty-five accessions were collected and regenerated *ex situ* for conservation and diversity studies. Morphologically accessions showed distinct variability for Rhizome Yield/Plant (gm), Plant height (cm), Inflorescence/Plant (cm), Leaf length (cm), Leaf width (cm), and Rhizome length (cm). The germplasms will serve as a baseline for increasing yield and quality of *B. ciliata*.

Keywords: Pakhanbed, bergenia ciliata, exploration, germplasm

1. Introduction

For their primary health care needs, about 80% of the population of developing countries rely on traditional medicines, usually plant remedies (WHO, 2008). According to an ethnobiological survey conducted by the Ministry of Environment, Forests and Climate Change of the Government of India, Indians use over 8,000 different plant species (Tewari, 2000) [2]. There are 247 vulnerable plant species in India, out of a total of 560, according to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Sharma and Thokchom, 2014) [3]. The therapeutic or medicinal use of plants' leaves, flowers, stems, berries, and roots was recognized in ancient times. These medications were first available in the form of tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1996; Samuelsson, 2004) [4, 5].

Bergenia ciliata also known as Pakhanbed locally is one of the highly valued medicinal plant species of the Himalayan regions of Sikkim and Darjeeling. Rhizomes and roots are bitter in taste, astringent, diuretic, and demulcent, aphrodisiac. Extract of rhizome and root is used to cure fever, diarrhea, pulmonary affection and renal and muscular calculus (Rai and Sharma, 1994; IUCN Nepal, 2004) [8, 7]. It is also considered as an important drug for dissolving kidney and bladder stones (Walter *et al.* 2013) [6]. It is used in many formulations in Ayurveda like Ashmarihara Kashaya, Vastyamayantaka Ghrita, Mutravirechaniya Kashaya, tablets like Cystone, Calcury, Diureston, Nephrolex etc. This species has been included in the vulnerable category by a Conservation Assessment and Management Plan Workshop Process, WWF, India, ZOO/CBSG, India. All efforts must be concentrated on conservation and utilization efforts for overall development and application in medicinal and pharmaceutical science and its cultivation. It has also been listed amongst prioritized plants for cultivation under scheme of National Medicinal Plant Board, Ministry of AYUSH with eligibility for 30% subsidy under cultivation as its high in demand by AYUSH industry and for exports. Despite its significant demand and use in traditional medicine and Ayurveda in India, *B. ciliata* is still not cultivated. The foremost challenge in breeding is the requisite to identify elite germplasm and locations representing the best environments for the production, as for medicinal plants the efficacy of

formulation is directly related to the location of plants despite all other characters it may possess.

As a result, in order to boost production and productivity, high yielding cultivars with desirable quality must be developed. The fundamental raw materials required for the improvement of *B. ciliata* are its diverse germplasm. The current exploration was designed with the goal of collecting diverse germplasms for conservation and breeding purposes from the Himalayan region of Sikkim and Darjeeling hills.

2. Materials and Methods

The study was conducted at Regional Research Station, Hill Zone, UBKV, Kalimpong during the year 2017 to 2021. Exploration trips were conducted at East, West and South districts of Sikkim and Darjeeling and Kalimpong districts of West Bengal. An exploration conducted during January to April as per the availability of plants as well as seeds. The whole plant as well as seeds was collected for *ex situ* regeneration and multiplication, maintaining them in form of seed bank and field gene bank at the Research station for further study and use.

The location of the exploration was chosen based on the flora of the area and a survey of the literature. (Rai and Sharma, 1994., Hussain and Hore, 2007; Yonzon *et al.*, 2012(a,b)) [8, 11, 9, 10]. The data on passport information containing ecological conditions (*viz.* altitude, latitude, longitude) and morphological observation *viz.*, plant height (PH), Flowers/Inflorescence, Inflorescence/plant, Leaf length, Leaf width, Rhizome Length, Rhizome Diameter, Rhizome yield/Plant, and Flower color were collected at the collection site and was recorded later in the field gene bank and experimental trials conducted after regeneration. All the location was marked with the help of GPS (Garmin etrex Vista HCx) for recording the data on altitude, latitude and longitude and distribution. Each population were marked with the help of GPS and to avoid population depletion from the wild and the seeds will be collected from the wild during their fruiting stage if needed in future or germplasm gets damaged. Agronomic package and practices were followed

recommended by National Medicinal Plants board according to Agro-techniques of selected medicinal plants after collection and *ex-situ* regeneration was done to grow a successful uniform crop. From each treatment five plants were selected at random for recording data on the above mentioned morphological traits. The collections were multiplied and maintained at RRS, HZ, UBKV, Kalimpong, India for further study and use.

3. Results and Discussion

The goal of the exploration was to acquire germplasm accessions for conservation and breeding purposes. As *Bergenia ciliata* being a mandate of Kalimpong AICRP on MAP&B Centre, very few accessions were maintained at the Research station. From 2017 to 2021, eight exploration trips were conducted, yielding a total of twenty-five accessions from various parts of Darjeeling, Kalimpong, and four districts of Sikkim, which were classified into five strata based on elevation: 1000-1500m (5), 1500-2000m (12), 2000m – 2500m (6), and >2500m (2). Table 1 showed the list of collections together with their passports, which were illustrated in Figure 1. Considerable variation was observed for morphological characters among the collections (Table 2). Rhizome yield per plant ranged from 11.87gm (KBC-12) to 25.09gm (KBC-2) in the collection indicating wide variability. Other traits such as Plant height (cm), Inflorescence/Plant (cm), Leaf length (cm), Leaf width (cm), and Rhizome length (cm) also highlighted diversity in collections by exhibiting variability. The accessions were regenerated at Regional Research Station, Hill Zone, Kalimpong after exploration and collection and distinct morphotypes were identified *viz.* Fleshy leaves (KBC-10), Less ciliated leaf (KBC-2), White Flowers (all other collections), pink flower (KBC-3,19) (Figure 2). These qualitative indicators (morphotypes) were discovered for the first time in *Bergenia ciliata* and will be used to characterise and utilize germplasm for further breeding program (Manivel *et al.*, 2019) [14].

Table 1: Passport information of the germplasm accessions of Pakhanbed *Bergenia ciliata*(Haw.) Sternb.

Accession No.	Village	District	State	Type of Material	Alt. (m)	Latitude	Longitude
KBC -1	Pokhriabong	Darjeeling	West Bengal	Seeds /Rhizomes	1655	27.9500 °N	88.18000°E
KBC -2	Sitong	Darjeeling	West Bengal	Seeds /Rhizomes	1219	26.8713° N	88.3616° E
KBC -3	St Mary's hill	Darjeeling	West Bengal	Seeds /Rhizomes	1895	26.8947 ° N	88.2820 ° E
KBC -4	Tiger Hill	Darjeeling	West Bengal	Whole plant/Rhizomes	2590	29.9967°N	88.2944°E
KBC -5	Maneybhangyang	Darjeeling	West Bengal	Whole plant/Seeds/Rhizomes	2310	27.0159° N	88.25734°E
KBC -6	Alubari	Darjeeling	West Bengal	Seeds /Rhizomes	2262	27.0196° N	88.25731°E
KBC -7	Palmajua	Darjeeling	West Bengal	Seeds /Rhizomes	1910	27.0705°N	88.09060°E
KBC -8	Lamahatta	Darjeeling	West Bengal	Seeds /Rhizomes	1737	27.0535° N	88.3516° E
KBC -9	Mirik	Darjeeling	West Bengal	Seeds /Rhizomes	1495	26.8853° N	88.1828° E
KBC -10	Lava	Kalimpong	West Bengal	Whole plant/Rhizomes/ Seeds	2200	27.0863° N	88.6615° E
KBC -11	Algarah	Kalimpong	West Bengal	Whole plant/Rhizomes/ Seeds	1780	27.1172° N	88.5837° E
KBC -12	Gitdablang	Kalimpong	West Bengal	Whole plant/Rhizomes	1877	27.0504° N	88.5988° E
KBC -13	Kafer	Kalimpong	West Bengal	Whole plant/ / Seeds	1675	27.0042° N	88.59029° E
KBC -14	Pedong	Kalimpong	West Bengal	Seeds /Rhizomes	1240	27.1594° N	88.6157° E
KBC -15	Yuksom	West	Sikkim	Seeds /Rhizomes	1780	27.3724° N	88.2230° E
KBC -16	Pitamchen	East	Sikkim	Seeds /Rhizomes	2438	27.2124° N	88.6205° E
KBC -17	Zuluk	East	Sikkim	Seeds /Rhizomes	2865	27.2518° N	88.7775° E
KBC -18	Namchi	South	Sikkim	Whole plant/Seeds	1315	27.1670° N	88.3652° E
KBC -19	Pangthang	East	Sikkim	Whole plant/Seeds	1760	27.3849° N	88.6582° E
KBC -20	Rabongla	South	Sikkim	Whole plant/Rhizomes	2300	27.3066° N	88.3640° E
KBC -21	Dzongu	North	Sikkim	Whole plant/Rhizomes	1700	27.5098° N	88.4375° E
KBC -22	Tathangchen	East	Sikkim	Seeds /Rhizomes	1619	27.3348° N	88.6290° E

KBC -23	Samdruptse	South	Sikkim	Whole plant/Rhizomes/ Seeds	1676	27.1854° N	88.3808° E
KBC -24	Pakyong	East	Sikkim	Seeds /Rhizomes	1188	27.1466 ° N	88.3582° E
KBC -25	Chemchey	South	Sikkim	Seeds /Rhizomes	2006	27.2198° N	88.3656° E

Table 2: Variability among the accessions for various morphological characters of Pakhanbed *Bergeniaciliata* (Haw.) Sternb

Accession no.	Plant height (cm)	Inflorescence/ Plant	Flowers/ Inflorescence	Leaf Length (cm)	Leaf Width (cm)	Rhizome diameter (cm)	Rhizome length (cm)	Rhizome yield per plant (g)
KBC -1	13.44	2.67	32.00	11.95	9.19	1.96	5.29	18.75
KBC -2	9.90	2.67	35.33	8.39	6.15	1.61	7.76	25.09
KBC -3	11.63	2.67	21.33	8.40	6.32	1.48	5.05	14.71
KBC -4	11.97	2.67	23.33	8.86	9.00	1.22	4.19	17.29
KBC -5	9.43	2.67	28.67	10.89	7.97	1.49	4.86	17.63
KBC -6	7.10	2.67	26.33	11.08	7.96	1.98	5.59	18.22
KBC -7	8.26	3.33	27.33	10.96	8.74	1.54	5.67	22.82
KBC -8	9.44	2.67	25.33	7.78	5.46	1.96	5.33	16.31
KBC -9	15.12	2.33	35.00	10.77	7.78	2.03	6.29	22.50
KBC -10	10.67	2.33	25.33	9.06	7.85	2.07	6.26	24.25
KBC -11	8.70	3.00	30.00	9.82	7.57	1.67	5.58	17.37
KBC -12	10.11	2.33	33.33	9.15	6.74	1.21	4.32	11.87
KBC -13	9.99	2.67	30.00	9.92	8.13	1.73	4.07	17.66
KBC -14	14.52	2.67	32.67	11.99	9.81	1.86	4.82	19.81
KBC -15	11.05	3.33	29.67	7.76	5.99	1.13	4.47	14.39
KBC -16	10.08	3.67	25.33	9.74	8.14	1.92	5.91	19.72
KBC -17	10.09	2.67	44.00	9.28	9.90	1.43	4.04	17.64
KBC -18	9.52	3.00	26.67	9.95	7.11	1.60	5.85	18.08
KBC -19	11.00	2.67	20.67	8.89	6.73	1.92	6.76	23.66
KBC -20	10.86	2.67	26.67	6.81	5.06	2.02	6.39	23.54
KBC -21	9.46	2.33	24.33	8.18	6.59	1.68	4.78	16.71
KBC -22	9.80	3.00	25.33	10.59	8.32	2.11	5.50	21.78
KBC -23	8.90	3.33	31.67	8.26	6.78	1.37	5.47	22.61
KBC -24	9.84	3.00	28.00	8.43	6.46	2.26	4.61	13.39
KBC -25	6.99	3.33	23.00	9.71	8.41	2.04	6.27	18.52
Range	6.99-15.12	2.33-3.67	20.67- 44	6.81-11.99	5.06 - 9.90	1.13 - 2.26	4.04-7.76	11.87- 25.09
Mean	10.31	2.81	28.45	9.46	7.53	1.73	5.41	18.97
SD	1.94	0.36	5.16	1.34	1.29	0.31	0.92	3.55

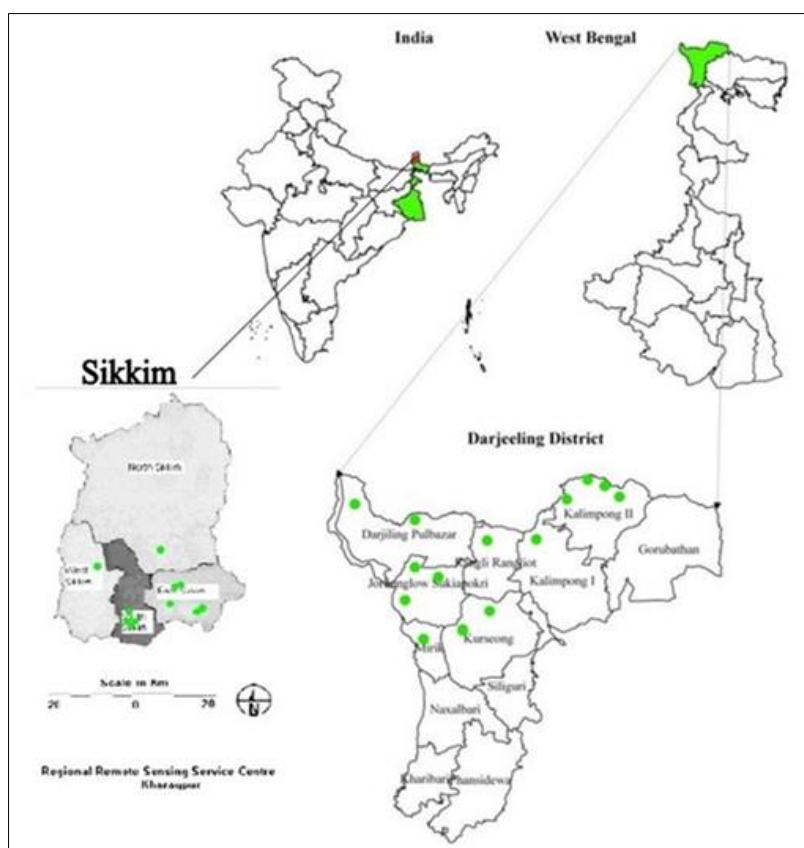


Fig 1: Exploration trips undertaken to collect germplasm accessions of Pakhanbed *Bergeniaciliata* (Haw.) Sternb from Sikkim and Darjeeling

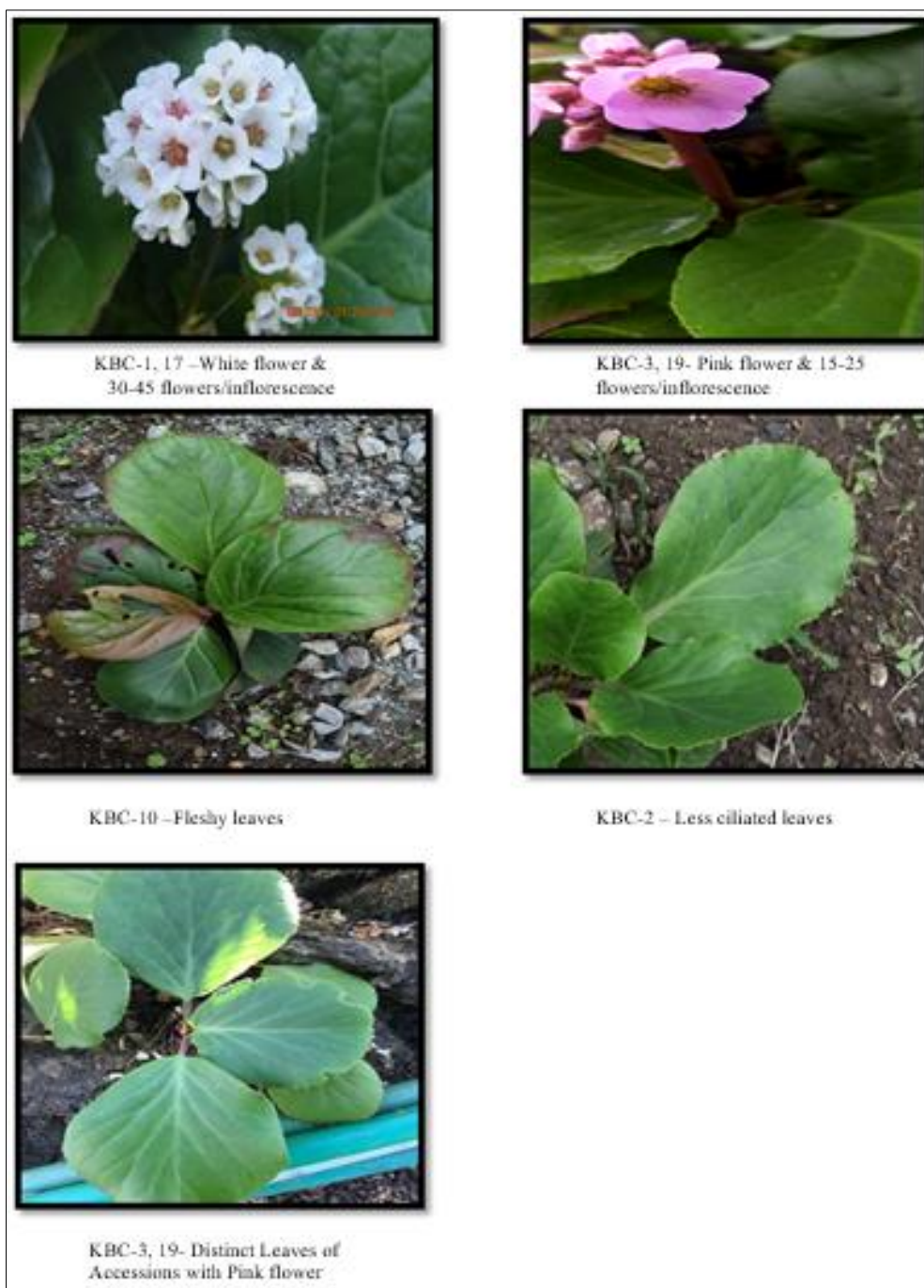


Fig 2: Distinct morphotypes of *Bergenia ciliata* (Haw.) Sternb identified

4. Conclusion

Twenty-five accessions were collected and regenerated *ex situ* for conservation during eight explorations. Accessions differed morphologically in terms of rhizome diameter, length, yield/plant, and floral colour. The distinct morphotypes reported will serve as a foundation for improving *Bergenia ciliata* yield and quality, and can be used in future study to breed varieties to promote cultivation and lessen reliance on wild stock.

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