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Characterization of Wild *Begonia* Species of Sikkim Himalaya: A Study for Morphological and Antioxidants Analysis

Bikash Bhattarai and Manju Rana*

Department of Horticulture, Sikkim University, Gangtok-737 102, India
**E-mail: mrana@cus.ac.in*

Abstract: Sikkim Himalaya harbors many flora and fauna diversity and is rich in wild ornamental and medicinal plants. To know its valuable resources, present paper emphasizes on three wild *Begonia* species found in Sikkim Himalaya. Survey and collection of samples was done with randomized sampling methods and collected specimens were evaluated at Sikkim University for its morphological variation and antioxidant potential. Three species viz. *Begonia picta*, *Begonia josephi* and *Begonia hatacoa* were selected for current experiment. All three species showed distinct phenotypic variation between and within the species level. All of them were found in two variations: red (dark) and green phenotypic variant. Dark variants showed superiority in phytochemical screening having higher anthocyanin, flavonoid, phenolic and antioxidant content as compared to green phenotypic variants. The study validates the folklore of these *Begonia* species having medicinal value and are edible in nature.

Keywords: Wild Begonia, *Begonia picta, Begonia hatacoa, Begonia josephi,* Antioxidant

Sikkim Himalayas falls in the eastern Himalayan region of India (27°4'46" - 28°7'48" N and 88°58"- 88°55'25" E), covers 7096 km^2 area, which harbor number of wild medicinally important plants which are edible as well as of ornamental importance. Sikkim is rich in flora and fauna due to its diverse agro-ecological variations. From this diverse region, *Begonia* under Begoniaceae is a widely distributed and morphologically diverse genus. Known for its showy foliage with different patterns and colour, *Begonia* flowers in diverse variations of white, yellow, pink to scarlet. *Begonia* is the sixth largest angiosperm genera of flowering plants (Hughes 2008, Moonlight et al 2018), with more than 1800 recorded species around the world (Averyanov et al 2019). *Begonia*s are native to moist subtropical to tropical climates, popular ornamental plant which is commonly grown ornamental houseplants in cold climates. Some of *Begonia* species are also known for their medicinal and edible properties. More than 10,000 registered artificial hybrids of *Begonia* are used for commercial purpose, many wild species are too taken into cultivation as parents for new hybrids development. *Begonia* traditionally used for medicine, vitamin C and as a source of food. Leaves of *Begonia* are eaten fried or in soups or salads and used to treat sore throats, also used for the treatment of diarrhea, skin diseases, blood cancer, respiratory tract infections, anti-HIV activity and anti-tumor activity (Girmansyah 2009, Rajbhandary 2013). Apart from the ornamental importance of *Begonia* species, the aim of present study is to validate the traditional knowledge known from literature cited by various researchers for its medicinal properties. Meanwhile, literature revealed that some species of *Begonia* were recognized as medicinal plants and may possess some amounts of phytochemical constituents in it (Karima et al 2017, Shrestha et al 2018). Bhattarai and Rana (2020) also study the wild *Begonia* of Sikkim Himalaya and reported varied morphological characters having potent antioxidant properties and anthocyanin in it. The phytochemical constituents present in the plant species such as flavonoids, alkaloids, phenols, anthocyanin and other compounds are responsible for antioxidant property and antimicrobial properties (Saffidine et al 2015, Awasthy et al 2016). The presence of these phytochemicals in the plants are responsible for their medicinal properties, as these phytochemicals involve in some biological activity in man and animals (Bagchi et al 2021). The present study investigated the morphological diversity, preliminary phytochemical analysis of *Begonia* plant extract in three different solvent *viz.* methanol, hexane and ethyl acetate for total phenolic, flavonoid and anthocyanin content along with DPPH test.

MATERIAL AND METHODS

Whole plant of *B. picta, B. josephi* and *B. hatacoa* was collected from the wild and maintained in the experimental field of Department of Horticulture, Sikkim University. Detailed study of morphological characters was evaluated from the fresh plant including: plant height, plant form, no of leaves/plant, no of shoots/plant, nodes and internodes, shoot length, root length, stem diameter, length of petioles, leaf diameter, length of peduncles. Observation for various

taxonomic parameters was taken during the flowering stage of the plant as per the procedure of Dorrenbos et al (1998). For biochemical analysis extract prepared from fresh plant parts, which were allowed to shade dry, powdered and successively extracted with hexane, ethyl acetate and methanol. The solvents were removed completely under reduced pressure and a semi solid mass was obtained. The extracts were qualitatively analyzed (preliminary phytochemical screening) to find out the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, carbohydrates, glycosides and amino acid following standard methods (Harbone 1976, Kandelwal 2001, Kokatae 2002, Edeoga et al 2005, Gopinath et al 2012 and Yadav et al 2014).

Total phenolic content was determined by using Folin–Ciocalteu assay (Meda et al 2005). The absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer. Gallic acid was used as a standard and TPC of *Begonia* extracts was expressed in milligram gallic acid equivalents (mg GAE g^{-1} extract). Total flavonoid content was determined by the aluminum chloride colorimetric method (Lamaison and Carnet 1990), with some modifications. The absorbance of the solution was measured at 435 nm by using a spectrophotometer. The flavonoid content was expressed as milligram quercetin equivalent (mg QAE g^{-1}) extract and anthocyanin content (mg L^{-1}) was determined by following Sutharut and Sudarat (2012) methods. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test is widely used to determine antioxidant activity in plant extracts. Test was performed according to methodology described by (Shen et al 2010). 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of all the extracts in methanol at different concentrations (50, 100, 200 and 400 µg ml^{-1}). The mixture was vortex and kept in the dark for 30 minutes. The change in colour from dark blue to yellow was determined by measuring the absorbance at 517 nm using the UV-Vis Spectrophotometer. Ascorbic acid was used as standard and control was prepared using DPPH and methanol but without sample extract, whereas baseline correction was done using methanol. A lower absorbance value indicates the high radical scavenging activity. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC_{50} value indicates greater antioxidant activity.

RESULTS AND DISCUSSION

Morphological characters: The genus *Begonia* has distinct morphological variation among the species and within the species level. Morphological comparison of three *Begonia* species *viz. Begonia picta* Sm., *Begonia josephi* A.D.C and *Begonia hatacoa* Buch.-Ham. Ex D. Don was done during the flowering time. Morphological characterization was done by comparing the data with Flora of China (Gu Cuizh et al 2007), Flora of Sikkim (Hajra and Verma 1996), Flora of British India (Hooker 1854, Clarke 1879). Distinct character for each species was presented in Figure 1-3, which shows diverse species within the genus *Begonia* L.

***Begonia josephi* A.D.C.:** *B. josephi* is tuberous plant without stem, petioles directly arise from the tuber itself, monoecious herb having 32.60 to 37.54 cm high and 18.20 to 26.10 cm plant spread. Leaves variations showed distinct in each green and red phenotype, green phenotypes showed green colour of leaves in both surfaces (upper and lower) whereas, red phenotypic variation showed maroon red colour in underside of the leaves and dark green in upper side of the leaves. Leaf pubescent was present in both variants. Both variations having lamina oblong ovate to broadly ovate, peltate and rounded base, leaf length having (23.00 to 25.28 cm), leaf width (17.60 to 17.90 cm) and length of midrib (18.84 to 20.84 cm), leaf apex acute to acuminated. Number of leaves per plant were recorded 1.60 in both phenotypic variations. Petiole's length varies from 22.40 to 27.62 cm with petioles hair in both variations. Inflorescence cymose, terminal, peduncle length from 24.14 to 25.20 cm, number of female and male flowers/plant was found maximum in green phenotype (8.49 and 14.24, respectively), flowers white to pinkish in colour, male flowers; 4 tepals and female flowers; 5 tepals, capsule oblong to ellipsoid, one long triangular wing and two smaller wings. Species was distributed mostly on moist and shaded areas, altitudinal ranges from 1500 to 2500 amsl, peak flowering season June to September.

***Begonia picta* Sm.:** *B. picta* belongs to tuberous rootstock and plant height was recorded between 18.66 to 19.18 cm, and plant spread was recorded about 12.67 to 16.14 cm, leaf base was unequally cordate to equally cordate, margin dentate to denticulate, leaf apex was acute to acuminate and leaf lamina was ovate to orbicular in both the phenotypic variants, length of leaf lamina was found 13.34 to 14.80 cm in both variants. Leaf length (15.02 to 16.94 cm), leaf width (11.32 to 14.12 cm), midrib length (12.70 to 14.80 cm) and densely leaf pubescent was recorded in both the variants. In the dark phenotypic variant, the upper surface of leaves was found dark green to maroon red leaf surface and reddish purple and green blotches in lower surface, whereas, green phenotypic variant showed greenish colour in upper side and light green in lower surface of the leaves. The number of leaves/plants was varied from 2.60 to 3.20 in both the variants. Length of petioles was around 4.94 to 6.20 cm and densely puberulous in both the variants. Inflorescence cymose, terminal, peduncles length was ranges from 16.02

to 16.28 cm), pink to white flower colour, capsule oblong, ellipsoid, one longest and two short wings, the number of female flowers per plant were ranges from 5.45 to 6.40 and male flowers per plant ranges from 6.46 to 8.20 in both variants, distributed on shady and moist places along with the low growing areas having fertile soil. Plants are mostly found around altitude-800-1500 amsl, with peak flowering season during July to September.

***Begonia hatacoa* Buch.-Ham. Ex D. Don:** *B. hatacoa* is rhizomatous, shade loving, monoecious herbs, upto a height of 31.04 to 35.02 cm and plant spread was around 18.54 to 22.34 cm, varied in their phenotypic appearances, dark or red, green and variegated phenotypic variant. Other characters of the plant remain the same, leaf lamina is ovate to lanceolate with cuneate leaf base and sub entire to denticulate leaf margin, apex acute to acuminate, leaf length varies from 12.82 to 15.08 cm, leaf width 5.52 to 5.70 cm, midrib length 10.94 to 12.24 cm in both dark and green

Table 1. Description of habit and leaf characteristics of wild *Begonia* species of Sikkim Himalaya

Species	Plant habit	Leaf lamina	Leaf margin	Leaf base	Leaf apex	Leaf surface upper side	Leaf surface lower side
B. josephi (Red)	Tuberous	Oblong ovate to broadly ovate, peltate	Acute lobes with denticulate	Rounded	Acute to acuminate	Dark green	Maroon red
B. josephi (Green)	Tuberous	Oblong ovate to broadly ovate, peltate	Acute lobes with denticulate	Rounded	Acute to acuminate	Green	Light Green
B. picta (Red)	Tuberous	Ovate to orbicular	Dentate to denticulate	Cordate	Acute to acuminate	Dark green to maroon red	Reddish purple and green blotches
B. picta (Green)	Tuberous	Ovate to orbicular	Dentate to denticulate	Cordate	Acute to acuminate	Green	Light Green
B. hatacoa (Red)	Rhizomatous	Ovate to lanceolate	Subentire to denticulate	Cuneate	Acuminate	Dark green	Maroon red
B. hatacoa (Green)	Rhizomatous	Ovate to lanceolate	Subentire to denticulate	Cuneate	Acuminate	Green	Light Green

Table 2. Description morphological characters of wild *Begonia* species of Sikkim Himalaya

Species	Leaf Pubescent	Stipules' colour	Petioles colour	Petioles hair	Flower colour	Flower pubescent	Capsule colour	Capsule hair	Peduncle colour	Peduncle hair
B. josephi (Red)	Present	Brown	Greenish brown	Rarely present	Pinkish white	Present	Dark brown	Absent	Red	Absent
B. josephi (Green)	Present	Brown	Green	Rarely present	Pinkish white	Present	Green	Absent	Reddish Green	Absent
B. picta (Red)	Dense	Green	Reddish	Dense	Pink	Present	Green	Present	Green	Present
B. picta (Green)	Dense	Green	Green	Dense	Pink	Present	Green	Present	Green	Present
B. hatacoa (Red)	Rare	Brownish green	Greenish brown	Rarely present	Pinkish white	Absent	Reddish green	Absent	Reddish green	Absent
B. hatacoa (Green)	Rare	Brownish green	Greenish brown to green	Rarely present	Pinkish white	Absent	Greenish brown	Absent	Greenish brown	Absent

Table 3. Morphological characters of wild *Begonia* species of Sikkim Himalaya

Species	PH (cm)	PS (cm)	LM (cm)	LL (cm)	LW (cm)	PTL (cm)	PL (cm)	NL/P	NFF	NMF
B. josephi (Red)	37.54±2.83	26.10±2.79	20.84±1.78	25.28±2.08	17.90±2.14	27.62±2.00	25.20±0.76	1.60±0.24	5.80±1.69	12.60±0.60
B. josephi (Green)	32.60±3.50	18.20±1.11	18.84±2.62	23.00±2.57	17.60±2.25	22.40±2.25	24.14±0.87	1.60±0.24	8.49±2.21	14.24±0.78
B. picta (Red)	18.66±0.79	12.67±0.65	13.34±0.97	15.02±1.02	11.32±0.88	4.94±0.38	16.28±0.41	2.60±0.51	6.40±0.75	8.20±0.66
B. picta (Green)	19.18±1.06	16.14±0.65	14.80±2.53	16.94±2.53	14.12±2.03	6.20±0.72	16.02±0.98	3.20±0.37	5.45±1.18	6.46±0.72
B. hatacoa(Red)	35.02±1.93	18.54±2.26	12.24±1.13	15.08±1.70	5.70±0.39	6.06±0.62	13.60±0.73	10.40±1.17	12.40±1.17	14.60±0.81
B. hatacoa (Green)	31.04±2.70	22.34±1.37	10.94±0.99	12.82±0.74	5.52±0.29	5.72±0.37	11.02±1.00	9.00±1.73	9.76±1.31	12.64±0.94

PH- Plant height, PS- plant spread, LM- length of midrib, LL- Leaf length, LW- Leaf width, PTL- Petiole length, PL- Peduncle length, NL/P- Number of leaves per plant, NFF- Number of female flowers per plant, NMF- Number of male flowers per plant

variants. Upper surface of the leaves of dark phenotypic variants showed dark green coloration and maroon red in the lower surface of leaves whereas, the green phenotypic variant showed green colour in the upper side and light green in the lower surface of the leaves. Petiole length ranged from 5.72 to 6.06 cm and sparsely pubescent in both variants. Peduncle length were ranges from 11.02 to 13.60 cm, inflorescence cymose, terminal, male flowers; (2+2) outer tepals, female flowers (2+3) 2 outer and 3 inner tepals, colour pinkish white, having pinkish streaked in outer tepals, capsule ellipsoid, glabrous, one long triangular and two short wings, number of female flowers per plant were ranges from 9.76 to 12.40 and male flowers per plant were ranges from 12.64 to 14.60 in both variants. Peak flowering season was recorded during June-September, and found mostly between altitude 500-1500 m.

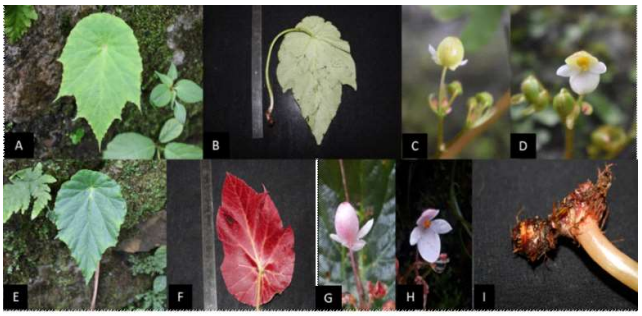

Fig. 1. *Begonia josephi* A.D.C: (A-D) Plant habit, Abaxial leaf surface, back side of flower petals and front view of flower petals of *B. josephi* Green Phenotype, (E-H) Plant habit, Abaxial leaf surface, back side of flower petals and front view of flower petals of *B. josephi* Red Phenotype and (I) tuber

Fig. 2. *Begonia picta* Sm.:(A-C) Plant habit, Adaxial and Abaxial leaf surface of *B. picta* Green phenotype, (D-F) Plant habit, Adaxial and Abaxial leaf surface of *B. picta* Red phenotype

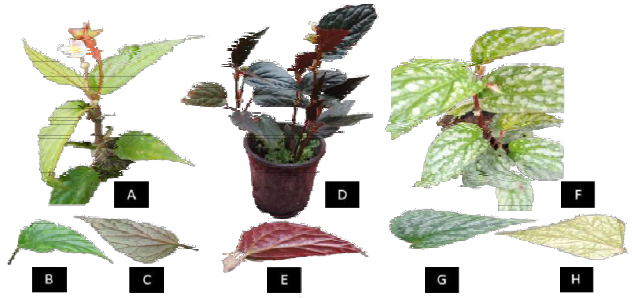

Fig. 3. *Begonia hatacoa* Buch.-Ham. Ex D.Don: (A-C) Plant habit, Adaxial and Abaxial leaf surface of *B. hatacoa* Green Phenotype, (D-E) Whole plant and Abaxial leaf surface of *B. hatacoa* Red Phenotype and (F-H) Plant habit, Adaxial and Abaxial leaf surface of *B. hatacoa* variegated Phenotype

Phytochemical screening of *Begonia* species: Phytochemical screening of different phenotypes does not vary within the species but great variation was found between the species level. The significant number of phytochemicals were present in each species level (Table 4).

Total phenolic, flavonoid and anthocyanin content: The total phenolic content and total flavonoid content was maximum in methanolic extract of *B. picta* (red) 55.03 QAE g^{-1} and 35.19 QAE g^{-1}, respectively while lowest amount was in hexane extract of *B. hatacoa* (green) 2.73 GAE g^{-1} and 0.92 QAE g^{-1} respectively. The result clearly indicates that phytochemical concentration varies from one species to another in different tested solvents, as well as between and within the species level. The red colour leaf variants showed highest phenolic and flavonoid content as compared to green leaves in each species tested. Anthocyanin content of tested *Begonia* species was in range from 31.83 to 185.33 mg L^{-1}, red phenotypes of *B. picta* showed the highest anthocyanin content. Similar kind of variation was also noted by Wang et al (2016) in *B. fimristupula* and Jose et al., (2016) in *B. trichocarpa*. Awasthy et al (2016) and Bhattarai and Rana (2020) found anthocyanin was one of the important phytochemicals present in *Begonia* species and is a potential source of natural antioxidant. Phytochemical constituents present in the plant species such as flavonoids, alkaloids, phenols, and other compounds are also responsible for antioxidant property and antimicrobial properties (Saffidine et al 2015, Bemmansor et al 2021). Phenolic compounds are the important class of antioxidants which have the ability to donate hydrogen atoms to free radical scavenging activity properties.

Difference in morphological and physiological traits of different plants of the same species or in a single plant exhibits phenotypic or ecotypic differentiation may be induced by environmental change (Deng et al 2012, Kurepin et al 2012). Alterations in phenotypic characters of same species allow the plant to modify its physiological characteristics and pigment content when habitat conditions

change. It may also be due to the gene expression or hormonal regulation when the plant receives appropriate environmental signals (Schlichting and Smith 2002). Anthocyanins were commonly produced in the abaxial surface of leaves of understory plants and are responsible for red colour of the leaf blade (Lee and Collins, 2001). Previous studies on anthocyanins demonstrated that anthocyanin can significantly affect plant responses to environmental stress, protect organs and substances involved in photosynthesis processes, and relieve photo-oxidation damage to leaves. Therefore, anthocyanins can greatly improve viability and resistance of plants (Pourcel et al 2013).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) test: Methanolic extract of *B. picta, B. josephi, B. hatacoa* and Ascorbic acid showed antioxidant activity in dose dependent manner in the rage of 50-400 µg ml^{-1} and produced maximum scavenging activity at a dose of 400 ug ml^{-1}. The mean IC_{50} values of three *Begonia* species and ascorbic acid were in the range of 376.50 to 17.89 µg ml^{-1}. Among all the tested samples of *Begonia* species, methanolic extract of *B. picta* (66.72 µg ml^{-1}) showed the high radical scavenging activity. The antioxidant potential of *Begonia* species between the various publications may vary both due to the methods used, as well as the method of bioactive substances extraction. The determination of antioxidant activity of plant extracts is an unresolved problem. The results from different antioxidant assays are even difficult to compare because of the difference in substrates, probes, reaction conditions and quantification methods. Loizzo et al (2016) observed that the different edible flowers which are largely consumed in Italy serve as natural source of antioxidants. Perez et al (2007) found that the methanolic extract of *Rosmarinus officinalis* L. had higher antioxidant properties than its ethanol and aqueous extract. Also, the methanolic extract of *R. officinalis* L. shows antibacterial properties (Bemmansor et al 2021). Wang et al (2016) studied antioxidant properties of 12

Table 4. Phytochemical screening of wild *Begonia* species of Sikkim Himalaya.

Species	*Begonia josephi*			*Begonia picta*			*Begonia hatacoa*		
Phytochemicals screened	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane
Flavonoid	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	+	-	-	-	-	-
Tannins	+	-		+	+	-	+	-	-
Phenol	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Glycosides	+	-	-	+	-	-	+	-	-
Saponins	+	-	-	+	-	-	-	-	-
Amino acids	+	+	-	+	-	-	+	+	+
Terpenoids	+	-	-	+	+	-	+	-	-

Phytochemical present + ; Phytochemical absent -

Table 5. Total phenolic content, total flavonoid content and anthocyanin content of wild *Begonia* species of Sikkim Himalaya

Species/Solvent	*Begonia josephi*		*Begonia picta*		*Begonia hatacoa*	
	Red	Green	Red	Green	Red	Green
Total phenolic content (mg GAE g^{-1})						
Hexane	17.79±0.663	7.48±0.180	21.99±0.340	12.85±0.504	8.85±0.321	2.73±0.255
Ethyl acetate	20.79±0.387	8.48±0.351	36.34±0.326	16.22±0.509	13.61±0.866	5.44±0.403
Methanol	32.36±0.358	13.76±1.248	55.03±0.455	23.82±0.855	19.20±0.847	9.35±0.488
Total flavonoid content (mg QE g^{-1})						
Hexane	6.10±0.590	2.14±0.400	15.72±0.380	8.63±0.983	1.90±0.391	0.92±0.199
Ethyl acetate	5.82±0.258	4.18±0.543	17.33±0.225	12.91±1.098	4.12±0.538	2.17±0.973
Methanol	9.76±0.0.401	5.23±0.907	35.19±0.247	16.58±0.777	8.47±0.746	3.44±0.293
Anthocyanin content (mg L^{-1})						
	163.89±0.87	31.83±0.72	185.33±1.45	36.12±0.44	152.97±0.91	33.50±1.71

Chinese edible flowers, results show that the aqueous extract of *R. officinalis* L. and *Chrysanthemum morifolium* Ramat. possess good antioxidant *in vitro* and *in-vivo*. Previous researchers (Joshi et al 2015, Jose et al 2016) confirmed that *Begonia* species showed DPPH radical scavenging ability. In this experiment, the methanolic extract of *Begonia* species had significant scavenging effects on the DPPH which was increasing with the decreasing concentration of the sample from 50-400 µg ml^{-1}. Methanolic extract of *B. picta* showed best scavenging activity as compared to *B. josephi* and *B. hatacoa*. This might be due to the presence of flavonoid, phenol and anthocyanin content, the most required bio-compounds for scavenging activity in this extract.

CONCLUSION

This morphological and phytochemical study of *Begonia* gives an important insight into the diversity of the genus in the region. The study validated the medicinal potential of the three wild *Begonia* species of Sikkim Himalaya which are commonly used as folklore medicine as well as confirms its edibility by conducting phytochemical analysis. All three species show marked morphological and phytochemical variations. In all three species under study, *B. picta* red variant having higher antioxidant properties as compare to *B. josephi* and *B. hatacoa*. When comparing for various phytochemicals *viz*. total phenol, total flavonoid, anthocyanin and antioxidant activity of red and green variant of *Begonia*, red variant showed higher value against the green. Similarly red variant had more anthocyanin content as compared to green leaves, thus having better antioxidant properties. Ornamentally morphologically red variant species will be useful in breeding purposes for the development of new commercial varieties of Begonia.

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