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Existence of *Passiflora ligularis* Juss in North Eastern Himalayan Region of India

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ABSTRACT

North Eastern Himalayan region of India is considered as one of the rich sources of *Passiflora* species viz. purple type, yellow type and giant granadilla. Survey conducted during 2018-2020 for collection and characterization of *Passiflora* species found in North Eastern Himalayan region of India, existence of *Passiflora ligularis* Juss from Lunghar village of Ukhrul, Manipur and Sakhabama village of Kohima, Nagaland with altitude of 1633 m a.s.l. and 1077 a.s.l. m respectively was recorded. Quantitative and qualitative analysis revealed average fruit weight 52.73 g/fruit, juice content 16.78 mL/fruit, vitamin C content 13.12 mg/100 g, total soluble solids 15.15° Brix, vitamin A content 0.06 mg/100 g, total flavonoids 11.50 mg/100 g, antioxidant activity 7.35%, titratable acidity 0.64%, total carbohydrates 8.61% of fruit juice whereas, phenol content 311.09 mg/100 g vitamin C content 89.44 mg/100, anthocyanin content 1.20 mg/100 g, chlorophyll content 1.17 mg/g of leaves were recorded. SDS–PAGE seed protein profiling of *Passiflora ligularis* Juss shows molecular weights from 14.10 KD to 129.33 KD. However, monomorphic banding pattern recorded in both the genotypes which revealed that both the genotypes are belongs to same species. With these findings it can be concluded the existence of *Passiflora ligularis* Juss in NEH region of India.

***Key words*:** North Eastern Himalaya, PAGE electrophoresis, SDS, Sweet granadilla

Passiflora ligularis Juss commonly known as sweet granadilla in Spanish as mucus fruit for its pulp, belongs to Passifloraceae family which includes about 700 species and 16 genera in which only two genera, *Passiflora* L. and *Tetrapathaea* (DC.) Rchb., are cultivated [1]. About 520 species of the Genus *Passiflora* are distributed to Neotropics and Africa [2]. This species is native to the Southern Andes, where it grows between 1800 and 2400 meters above sea level in climates ranging from 16 to 24°C [3]. Sweet granadilla is a fast-growing climbing vine that is woody at the base. It can be found in western and northern parts of South America. The leaves are heart-shaped and the flowers are pale green and large. The sweet, ovoid, and orange-yellow fruits are eaten raw or made into drinks. Plant can be propagated through cuttings or grown from seeds. Due to its nutritional and commercial relevance, granadilla is classified as a "minor tropical fruit" [4]. In the leaves of *Passiflora ligularis* Juss Glucosyl-hydrolase inhibitors is found which catalyze the hydrolysis of glycosidic bonds in complex sugars [5].

During survey we have got *Passiflora ligularis* Juss from Lunghar village of Ukhrul, Manipur and Sakhabama village of Kohima, Nagaland. From the morphological, biochemical and seed protein profiling its identity was reported. In southern part of India, the existence of *Passiflora ligularis* Juss was reported by Saravanan and Parimelazhagan during 2014 from Western Ghats of Tamil Nadu [6]. Whereas, Kishore also collected it from western ghat during 2015 which indicates its existence in southern part of India [7], but none of the report indicates its existence in North Eastern Himalayan region of India earlier. Biochemical profiling of sweet granadilla revealed its potential to commercially in India also for commercial cultivation. Not only fruits but the leaves are also good source of vitamin C. The objective of this study was to validate the existence of *Passiflora ligularis* Juss via., morphological, biochemical and seed protein profiling which were collected from Lunghar village of Ukhrul district, Manipur and Sakhabama village of Kohima district, Nagaland, India.

MATERIALS AND METHODS

Survey conducted during 2018-2020 for collection and characterization of *Passiflora* species found in North Eastern Himalayan region of India, existence of *Passiflora ligularis* Juss from Lunghar village of Ukhrul, Manipur and

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Sakhabama village of Kohima, Nagaland were reported which is 1633 m a.s.l. and 1077 m a.s.l. respectively. Morphological observations were done at collection site and for physico-biochemical analysis, the fruits, leaves and tendrils were collected for both genotypes from the site into the laboratory. The identification and description of collected *Passiflora* genotypes were adopted from De Jesus [8].

Table 1 List of collected *Passiflora ligularis* Juss in North Eastern Himalayan Region of India and their sources

Genotypes	Code	Sources	Latitude	Longitude	Altitude
Passilfora ligularis Juss	P_1	Lunghar Village, Ukhrul, Manipur	25º16'N	94º42'E	1633 m
Passilfora ligularis Juss	P_2	Sakhabama, Kohima, Nagaland	25º39'N	94º11'N	1077 m

The collection of fruits and leaves was done from different direction of single plant. 50 fruits were collected and each replication having 10 fruits with five replications. Recorded morphological traits viz., leaf length (cm), leaf breadth (cm), flower length (cm), number of flowers/node, peduncle length (cm), fruit length (cm), fruit breadth (cm), fruit weight (g), number of fruits/vine, peel weight (g), seed length (cm), seed breadth (cm), number of seeds weight/fruit and fruit yield (kg/vine). Biochemical traits viz., Juice content (mL/fruit), Total soluble solid content (°Brix), titratable acidity (%) [9], vit C content (mg/100 g) [10], total carbohydrate (%) [11], reducing sugar (%) [12], Vitamin A (mg/100 g) [13], total flavonoid (mg/100 g) [14] and antioxidant activity (%) [10]. Anthocyanin content (mg/100 g) of leaf, tendril and petiole [15], vit C content of leaf (mg/100 g) [10] and phenol content of leaf (mg/100 g) was determined using folin-ciocalteu reagent [10] and chlorophyll content of leaf (mg/g) [16] and shelf life (days) at room temperature.

Seed protein extraction

Seed protein extraction was done as by Lowry's method [17] in seven genotypes and protein banding pattern was estimated using SDS-PAGE. The variability of seed storage proteins was analyzed by SDS–PAGE as per procedure described by Laemmli [18]. 0.2 g of seeds were soaked overnight in phosphate buffer (pH 7.0) solution. Seeds were crushed with a solution of Tris-HCl 0.06 M (pH 7.4), 10 mM urea, 1 mM EDTA, 0.1% TCA, 2.5% glycerol, 0.5% SDS and 1.25% β-mercaptoethanol.

SDS-PAGE protein profiling

Electrophoresis was performed with a discontinuous buffer system in a vertical electrophoresis unit. The gel was run at 25 mA until the tracking dye approached the bottom of the gel. The system was disconnected and the gel was taken out from the slab. The gel was then subjected to silver staining as per procedure described by Mortz [19] through sensitizing with 0.02% sodium thiosulphate solution for 5 minutes and then washing twice with double distilled water for 1 minute. It was then transferred to staining solution and kept on gel rocker for 20 minutes in dark. The gel was then washed twice with distilled water for 45 seconds, transferred to developing solution and finally the reaction was stopped with 12% acetic solution. Gel was washed thoroughly but gently with double distilled water until protein bands became clearly visible for bands scoring**.** The electrophorograms were prepared on the basis of protein mobility and the density expressed in Rm values. The gels were scored as presence (+) or absence (-) of protein bands. Depending upon the presence (+) or absence (-) of bands, similarity index between the genotypes were calculated [20].

RESULTS AND DISCUSSION

Morphological characterization

Morphological characterization via., leaf is large, light green, base; cordate, margin; entire, nectarines; absent, heterophylly; absent. Flower have a sweet, musky odour sepal; acute, green outside, white inside, hypanthium; 0.5-0.9 cm long; peduncle; solitary or paired, 2-4 cm; bracts; 3. Anthers show the yellow-coloured pollen and the stigma is trifid. Fruit colour; greenish-yellow with white speckles, indehiscent capsule, ovoid to spherical to slightly flat at the poles with the tip pointing towards the stem, shape; ellipsoid, mesocarp; soft and spongy and the endocarp is a white film that separates from the mesocarp at maturity. The black elliptic and flat seeds are arranged on three longitudinal placentae and each seed is surrounded by a transparent jelly-like pulp that is very sweet and aromatic. Maximum leaf length (14.45 cm) and flower length (6.00 cm) was recorded in genotype P_1 [21-22].

Maximum fruit length (7.29 cm), fruit weight (53.73 g), peel weight (31.39 g) and peduncle length (7.23 cm) were recorded in genotype P_1 which was accordance with Ramaiya [23]. Maximum seed length (0.59 cm), seed breadth (0.17) and seed weight (5.27) recorded in genotype P1 whereas, leaf breadth (11.58 cm) number of fruits/vine (87.33) and fruit yield (4.67 kg/vine) was recorded maximum in genotype P_2 [24]. In genotypes P_2 yield is maximum because of a greater number of fruits/vine also due to maximum leaf width which impart more photosynthate accumulation in the leaves.

Table 2a Morphological characters of *Passiflora ligularis* Juss in North Eastern Himalayan region of India

Genotypes	Leaf length (cm)	Leaf breadth (cm)	Flower length (cm)	Peduncle length (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)
P_1	14.45	10.51	6.00	7.23	7.29	5.12	53.73
P_2	14.38	11.58	5.97	6.43	7.18	5.38	51.73
Mean	14.42	11.04	5.98	6.83	7.23	5.25	52.73
CV (%)	2.02	3.13	2.46	2.74	1.85	1.39	3.55
SE(m)±	0.67	0.65	0.08	0.11	0.08	0.10	1.08
C.D (5%)	1.43	1.35	0.31	0.39	0.28	0.37	1.39

Table 2b Morphological characters of *Passiflora ligularis* Juss in North Eastern Himalayan region of India

Genotypes	No. of Fruits/vine	Peel weight (g)	Seed length (cm)	Seed breadth (cm)	Seed weight/ Fruit (g)	Fruit yield (Kg/vine)
P_1	86.67	31.39	0.59	0.17	5.27	4.62
P_2	87.33	29.94	0.58	0.16	5.25	4.67
Mean	87.00	30.67	0.58	0.17	5.26	4.65
CV (%)	5.58	2.63	0.12	0.14	1.96	2.24
SE(m)±	0.37	0.47	0.01	0.01	0.06	0.28
C.D (5%)	1.35	1.69	0.01	0.02	0.22	1.03

Biochemical characterization

Among the horticultural characters, significant result was recorded. Maximum juice content (mL/fruit) was recorded in P_1 (17.11 mL/fruits) genotype. Maximum total soluble solid content was recorded for genotype P_2 (15.53°Brix). The higher TSS may be due to the fact that since the fruit tree is grown under natural water scarce condition without care and management it tends to accumulate more dry matter eventually increasing the TSS content [25]. Vitamin C content was recorded maximum in P1 (13.45 mg/100 g) [26]. Concentration of ascorbic acid in *Passiflora* genotypes says the potential use of the fruit as a good source of ascorbic acid. The recommended daily intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children. Therefore, these collected genotypes could be regarded as potential source of ascorbic acid with respective of human nutrition.

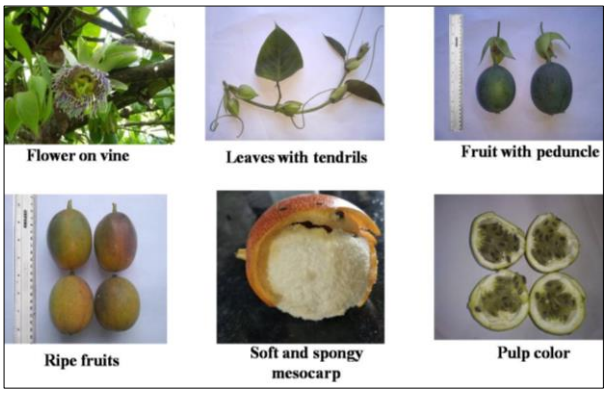

Fig 1 Glimpses of flower, leaf and fruit of *Passiflora ligularis* Juss

The titratable acidity of a fruit is an important component of its organoleptic quality [27]. Maximum titratable acidity was recorded in P_1 genotype with 0.65% [28]. The high acidity may be due to prevalent primary organic acids, such as malic and citric acid, in mature fruits, which accumulate in the mesocarp cells during the fruit development process, which is controlled by genetic and environmental factors. Maximum antioxidant activity was recorded in P_1 genotype with 7.52% [26]. The reason for this is that fruits are known to contain a variety of antioxidant compounds, and ascorbic acid (vitamin C) which implying that fruits high in vitamin C are powerful antioxidants [29]. The increasing sugar is due to the hydrolysis of starch to sucrose as fruit approach to ripening [30]. P_2 genotype with maximum reducing sugar that is 4.03%. It may be due to the breakdown of carbohydrates into polysaccharides and hydrolysis processes at ripening process, leads to increase reducing sugar [31].

Total flavonoid content was recorded maximum in genotype P_2 (11.97 mg/100 g) [32] and it may be due to the diverse climatic and nutritional factors. Maximum vitamin A was recorded in genotype P_2 (0.07 mg/100 g) [33] and may be due to biosynthesis genes controls its accumulation and composition in fruit during maturity. The expression of upstream and downstream genes in the pathway are critical variables for fruit colour variation and vitamin A content in fruit [34].

Maximum total carbohydrate was recorded in genotype P_2 (8.80%) followed by P_1 (8.41%). Carbohydrates are one of the most important nutrients in fruits, as they are the cell's principal energy source and the simplest macromolecules to be generated naturally [35]. Shelf life was maximum for genotype P_2 with 13.33 days at room temperature. The storage of sweet granadilla is due to papery mesocarp which prevent to easy decay under stress conditions.

Significantly higher phenol content in the leaves were recorded for P_2 genotype (311.34 mg/100 g) followed by P_1 (310.85) and similar finding for phenol content in the leaves [36]. Significantly higher total chlorophyll content of leaves was recorded for P_1 (1.23 mg/g), P_1 [37]. Maximum anthocyanin content was recorded in tendril (2.64 mg/100 g) in genotype P_1 followed by petioles (2.16 mg/100 g) in P_1 genotype whereas in leaves its concentration was less 1.31 mg/100 g in genotype P_2. The anthocyanin, phenol and chlorophyll concentration of petioles, tendrils and leaves might differ according to a variety of external and internal factors such as genetic, agronomic and climatic factors [38].

The maximum vitamin C content of leaves was recorded for P_1 (90.62 mg/100 g) followed by P_2 (88.27 mg/100 g). Vitamin C protects against the damaging effects of light during photosynthesis, the process of converting carbon dioxide into plant matter using light energy may be higher at maximum altitude [29].

Table 3 Biochemical parameters in fruits of *Passiflora ligularis* Juss in North Eastern Himalayan region of India

Genotype	Juice content (mL/fruit)	Vit. C (mg/100g)	TSS (°Brix)	Vit. A (mg/100g)	Total flavonoids (mg/100 g)	Antioxidant activity (%)	Titratable acidity (%)	Total carbohydrates (%)	Reducing sugar (%)	Shelf-life (days)
P_1	17.11	13.45	14.77	0.05	11.02	7.52	0.65	8.41	3.51	13.00
P_2	16.44	12.79	15.53	0.07	11.97	7.18	0.64	8.80	4.03	13.33
Mean	16.78	13.12	15.15	0.06	11.50	7.35	0.64	8.61	3.77	13.17
CV (%)	3.89	5.02	3.11	0.04	1.76	3.50	0.42	1.59	1.89	1.75
SE(m)±	0.47	0.38	0.27	0.01	0.12	0.09	0.02	0.31	0.29	0.09
C.D(5%)	1.71	1.38	0.99	0.02	0.42	1.25	0.14	0.68	0.52	0.98

Table 4 Biochemical parameters in leaves, petioles and tendrils of *Passiflora ligularis* Juss in North Eastern Himalayan region of India

Genotypes	Leaf				Petiole	Tendril
	Anthocyanin (mg/100 g)	Vit. C (mg/100 g)	Phenol (mg/100 g)	Chlorophyll (mg/g)	Anthocyanin (mg/100 g)	Anthocyanin (mg/100 g)
P_1	1.21	90.62	310.85	1.23	2.16	2.64
P_2	1.31	88.27	311.34	1.11	2.05	1.94
Mean	1.26	89.44	311.09	1.17	2.10	2.29
CV (%)	1.61	6.75	1.79	1.17	1.03	1.79
SE(m)±	0.20	0.73	0.83	0.11	0.32	0.54
C.D(5%)	0.74	2.03	0.69	0.39	0.28	0.92

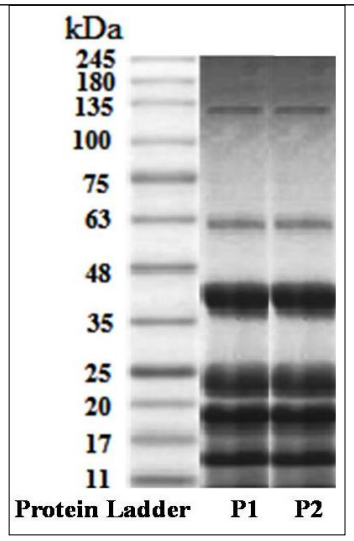

Fig 2 SDS–PAGE protein profiling of *Passiflora ligularis* Juss in North Eastern Himalayan region of India

Protein banding pattern by SDS–PAGE

The variability of seed storage proteins was analyzed by SDS–PAGE as per procedure described by Laemmli [18]. 0.2 g of seeds were soaked overnight in phosphate buffer (pH 7.0) solution. Seeds were crushed with a solution of Tris-HCl 0.06 M (PH 7.4), 10 mM urea, 1 mM EDTA, 0.1% TCA, 2.5% glycerol, 0.5% SDS and 1.25% β-mercaptoethanol. Electrophoresis was performed with a discontinuous buffer system in a vertical electrophoresis unit. The gel was run at 25 mA until the tracking dye approached the bottom of the gel. The system was disconnected and the gel was taken out from the slab. The gel was then subjected to silver staining as per procedure described by Mortz [19] through sensitizing with 0.02% sodium thiosulphate solution for 5 minutes and then washing twice with double distilled water for 1 minute. It was then transferred to staining solution and kept on gel rocker for 20 minutes in dark. The gel was then washed twice with distilled water for 45 seconds, transferred to developing solution and finally the reaction was stopped with 12% acetic solution. Gel was washed thoroughly but gently with double distilled water until protein bands became clearly visible for bands scoring**.** The electrophorograms were prepared on the basis of protein mobility and the density expressed in Rm values. The gels were scored as presence (+) or absence (-) of protein bands. Depending upon the presence (+) or absence (-) of bands, similarity index between the genotypes were calculated [20]. Total of 12 protein bands were identified by silver staining in both the genotypes and molecular weights ranging from 14.10 KD to 129.33 KD. However, monomorphic banding pattern shown among sweet granadilla genotypes which indicates both the genotypes belongs to the same species.

CONCLUSION

Under present investigation morphological, biochemical and seed protein profiling of both the genotypes it can be concluded that the both genotypes belong to *Passiflora ligularis* Juss which were collected from from Lunghar village of Ukhrul, Manipur and Sakhabama village of Kohima, Nagaland. With the above study we can say that the existence of *Passiflora ligularis* Juss in North Eastern Hill region of India. Because of its higher fruit yielding capability, the fruits of this plant have a lot of potential in the food processing industry. However, just a little amount of research has been done yet. This could be due to people's lack of understanding of the sweet granadilla potential in NEH region. As a result, in order to use the plant for future improvement initiatives, extensive studies and suitable exploration are required for long-term genetic conservation.

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