Release of *Stevia rebaudiana* cultivar

HIM STEVIA
(CSIR-IHBT-ST-01)

The cultivar 'Him Stevia' (CSIR-IHBT-ST-01; selection U-22-5-1) of *Stevia rebaudiana* Bertoni (Bertoni) has been developed by CSIR-Institute of Himalayan Bioresource Technology, Palampur through hybridization and selection approach. The cultivar was selected for higher proportion of rebaudioside-A content as compared to stevioside from advanced breeding lines developed from germplasm core collections through half-sib family selection followed by clonal selection. The cultivar has excellent nursery performance with respect to rooting and early establishment. It is vigorous in growth and has good adaptability.

Rebaudioside-A content in U-22-5-1 is in higher proportion (1.25) than stevioside based on steviol glycosides profiling as per JECFA* protocol. The selection performed consistently for rebaudioside-A/stevioside ratio over a period of 5 years under evaluation.

Steviol glycosides profile (using JECFA protocol) of improved cultivar 'Him Stevia' (U-22-5-1)

Compounds (dry weight basis)	U-22-5-1	Check	Improvement (%)
Reb-A/Stev. ratio	1.25	0.36	244.75
Stevioside content (%)	5.87	6.60	11.06
Rebaudioside-A (%)	7.34	2.40	205.83
Rebaudioside-B (%)	-	0.05	-
Rebaudioside-C (%)	0.96	0.70	37.14
Rebaudioside-F (%)	0.21	0.15	40.00
Rubusoside (%)	0.04	0.16	75.00
Steviol-bioside (%)	-	0.21	-
Dulcoside-A (%)	0.07	0.14	50.00
Total SGs content (%)	14.49	10.41	39.19

* The Joint FAO/WHO Expert Committee on Food Additives

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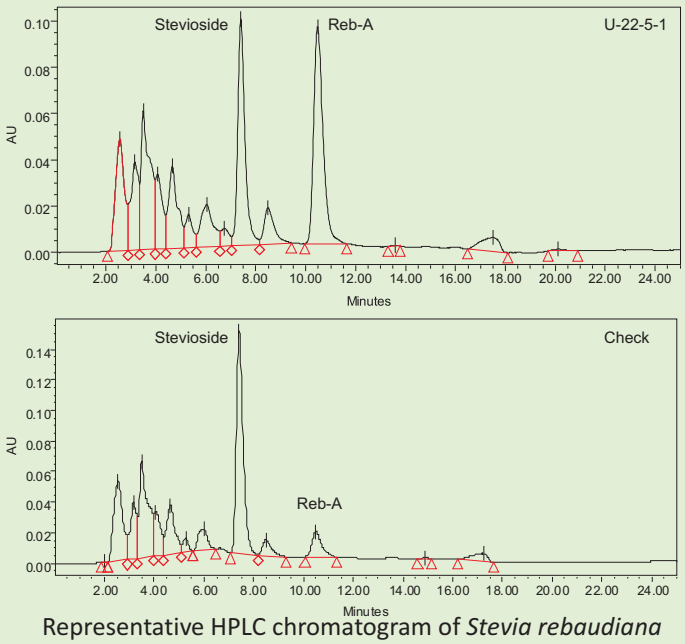
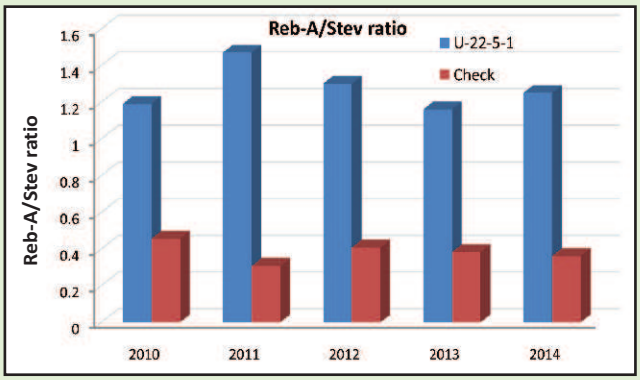
DNV

Stevia rebaudiana is a herbaceous perennial plant (2n=22) of genus Stevia, which consists of approximately 230 species of herbaceous, shrub and sub-shrub plants. Leaves of stevia produce diterpene glycosides, non-nutritive, non-toxic, high-potency sweeteners and may substitute sucrose as well as other synthetic sweeteners, being 300 times sweeter than sucrose. Rebaudioside-A is of particular interest among the glycosides produced in the leaf of stevia because of the most desirable taste profile, while stevioside is responsible for aftertaste bitterness. Development of new cultivar of stevia with higher content of rebaudioside-A and reduced content of stevioside is of prime importance for the improvement and utilization of this source as natural sweetener.

The steviol glycosides profile of plants was estimated through HPLC analysis from dried leaf samples. Stevioside and rebaudioside-A contents were estimated in the leaf samples on dry weight basis over five consecutive years from 2010 to 2014. The overall mean of rebaudioside-A/stevioside ratio in U-22-5-1 was 1.27 as compared to check (0.38).



Representative HPLC chromatogram of *Stevia rebaudiana* leaf samples of U-22-5-1 and Check



Year-wise variations for rebaudioside-A/stevioside ratio

Field view of cv. 'Him Stevia' 3 weeks after plantation

Morphological features of 'Him Stevia' (U-22-5-1) in comparison to check

Character	U-22-5-1		Check		Mean improvement (%)
	1st year	2nd year	1st year	2nd year	
Morphological trait					
Plant height (cm)	95.90	104.20	105.50	112.30	8.13
Branches/plant (no.)	12.00	22.00	8.00	18.00	30.77
Stem thickness (mm)	6.38	5.26	6.41	5.42	1.61
Internode length (cm)	2.90	3.40	3.10	3.80	8.70
Max. leaf length (cm)	12.20	9.54	8.80	7.84	30.65
Max. leaf width (cm)	4.83	4.24	3.80	3.52	23.91
Agronomic trait					
Fresh leaf yield (t/ha)	7.14	12.95	6.23	9.28	29.53
Dry leaf yield (t/ha)	2.04	3.68	1.78	2.65	29.12
Harvest index (HI)	0.42	0.43	0.39	0.42	4.94

Conventional plant breeding approaches, such as selection and inter-crossing among various desirable genotypes along with chemical profiling for high rebaudioside-A content, is the best method for improving quality traits in a highly cross-pollinated crop like stevia. The hybridization and selection programme was carried out at the experimental farm of the Institute, from 2008 onwards. The selections were evaluated for field performance during 2012-13 and 2013-14. The field evaluation experiment was laid out in randomized block design (RBD) with five genotypes including U-22-5-1 (each replicated thrice). Standard agronomic practices were followed for raising the crop.

The cultivar recorded 2.04 t/ha of dry leaf yield during the first year of production which was 29% higher than the check. The cultivar has a potential of yielding 3.68 t/ha of dry leaf yield during the second year of production.

Statement of distinction

Cultivar 'Him Stevia' is medium in height with compact plant stature and green stems which are erect, upright and multiple branched. The leaf is large, dark green with pubescence having deep serrations on the upper margins.

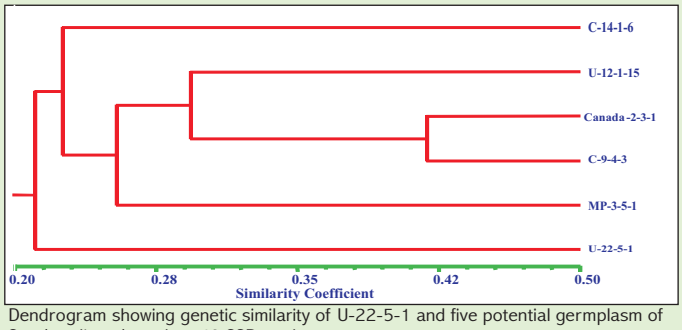
The cv. 'Him Stevia' exhibiting a) mature leaves, b) root biomass after second cropping season and c) mature plant

Use of biotechnological approaches, such as tissue culture for the mass propagation of elite genotypes and molecular marker technology for identification of elite genotypes based on DNA fingerprints have created new opportunities for plant breeders with respect to production of large scale planting material and maintaining its fidelity. *In vitro* multiplication has successfully been used to multiply the selected clone (U-22-5-1) with a high response to propagation, rooting and hardening as well as good field performance of the tissue culture raised plants.

Tissue culture raised plantlets a) root induction and b) hardening of plantlets under greenhouse condition

DNA Fingerprinting of improved cultivar 'Him Stevia' using SSR markers

Genetic distinctness of cultivar 'Him Stevia' (U-22-5-1) was established using 40 SRR markers recently reported by our group. Five potential germplasm collections namely, Canada-2-3-1, C-14-1-6, U-12-1-15, MP-3-5-1 and C-9-4-3 of *Stevia rebaudiana* were used for comparison purpose. In total, 216 alleles were detected ranging from 2 to 15 with an average of 3.6 alleles per SSR locus. Fourteen SSR markers evincing reproducible polymorphic loci among the improved selection U-22-5-1 and germplasm collections were used for development of fingerprints. Based on the SSR data, consolidated DNA fingerprints were developed with rare or unique marker loci. Cluster analysis of six genotypes based on 216 polymorphic loci grouped in two major groups. Improved selection U-22-5-1 captured maximum diversity and clustered as single solitary genotype. Pair-wise genetic similarity (GS) of U-22-5-1 varied from a minimum of 17% (C-14-1-6, MP-3-5-1) to maximum of 27% (U-12-1-15), followed by Canada-2-3-1 and C-9-4-3 (22%). In conclusion, genetic similarity data based on 216 polymorphic loci suggested that improved selection U-22-5-1 has captured high level of genetic diversity and can be potentially used as promising parental group for future genetic improvement programme of stevia.



Dendrogram showing genetic similarity of U-22-5-1 and five potential germplasm of S. rebaudiana based on 40 SSR markers

SSR Marker	U-22-5-1	C-14-1-6	U-12-1-15	Canada-2-3-1	MP-3-5-1	C-9-4-3
SUGMS 6_555						
SUGMS 6_545						
SUGMS 6_450						
SUGMS 10_335						
SUGMS 10_325						
SUGMS 10_265						
SUGMS 10_250						
SUGMS 13_290						
SUGMS 13_280						
SUGMS 13_242						
SUGMS 19_238						
SUGMS 19_210						
SUGMS 21_220						
SUGMS 21_212						
SUGMS 21_200						
SUGMS 22_215						
SUGMS 22_205						
SUGMS 24_315						
SUGMS 24_295						
SUGMS 26_340						
SUGMS 26_275						
SUGMS 28_255						
SUGMS 28_225						
SUGMS 31_280						
SUGMS 31_250						
SUGMS 39_258						
SUGMS 39_254						
SUGMS 41_185						
SUGMS 42_470						
SUGMS 43_248						
SUGMS 43_230						
SUGMS 51_195						
SUGMS 51_190						

Diagrammatic representation of DNA fingerprints of U-22-5-1 and five potential germplasm revealed by SSR markers amplicons

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