Genomic Resource Creation for Genetic Diversity Characterization and Implementation of Marker Assisted Selection in *Stevia Rebaudiana* Bertoni

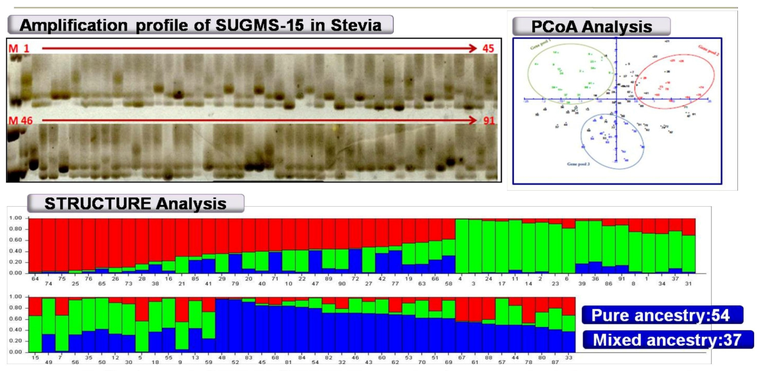
Background

- Stevia rebaudiana is a therapeutically important crop species in use since the times of Ayurveda.
- Being a zero calorie sweetener it is finding wide market as it is safer and healthier since calories can lead to obesity.
- The main aim of the current study was to generate a valid collection of genome wide screening markers that can be added to the genomic resource which is currently unavailable for this crop despite its multifarious importance.

Creation and characterization of genetic diversity

91 selections through open-pollination

Genetic variability of 91 selections based on SSR markers

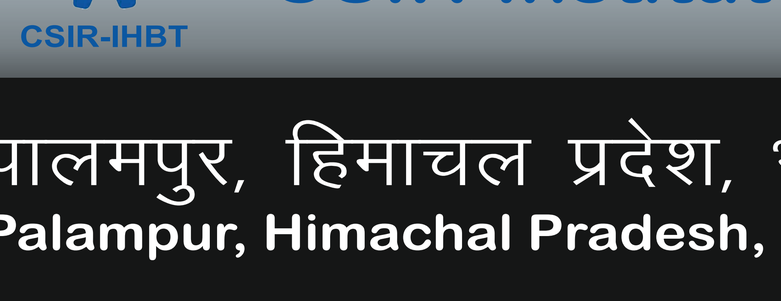


PCoA and STRUCTURE analysis revealed 3 gene pools

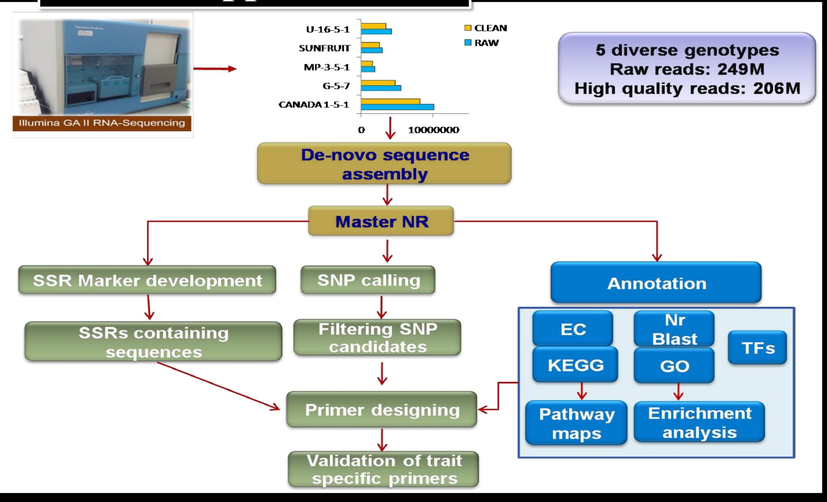
Creation of mapping population (12 reciprocal crosses; 1234 individuals)

Cross Plants	Individuals
U-24-4-12 X U-22-5-1 U-22-5-1 X U-24-4-12	96 88
U-24-4-12 X U-16-5-15 U-16-5-15 X U-24-4-12	77 23
U-22-5-1 X F-7-2-6 F-7-2-6 X U-22-5-1	14 16
U-24-4-11 X U-20-4-7 U-20-4-7 X U-24-4-11	25 50
U-24-4-11 X Canada-1-5-1 Canada-1-5-1 X U-24-4-11	100 15
E-1-2-1 X U-23-5-12 U-23-5-12 X E-1-2-1	120 10
U-22-5-1 X CIMAP CIMAP X U-22-5-1	345 255

Selection of 5 diverse genotypes based on SSR fingerprinting



NGS approaches



Genomic resources creation

- In total, 206162421 high quality reads were generated which were assembled into 48248 contigs (42.03 Mb) with an average length of 873 bp.
- Using this data, 290670 high quality SNPs and 3482 microsatellite markers were ascertained.
- The total SNP frequency came out to be 1/144.

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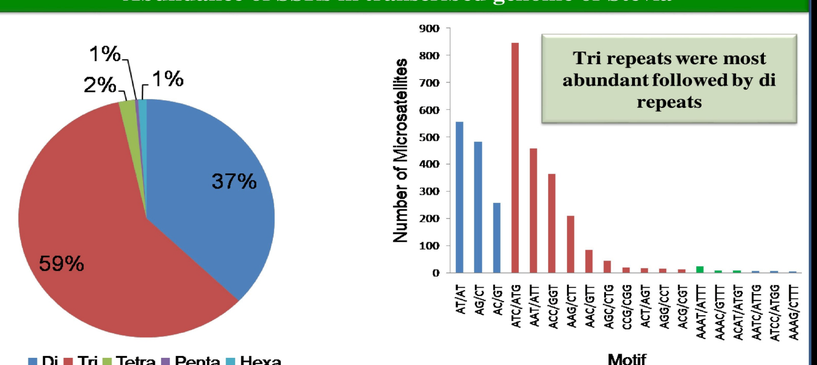
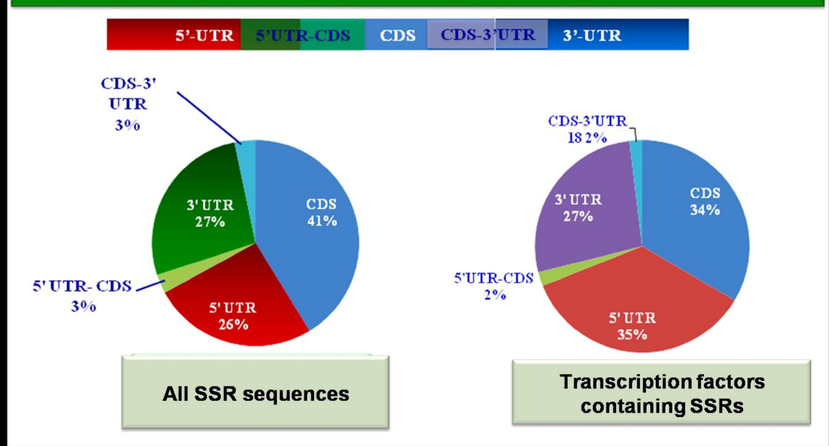
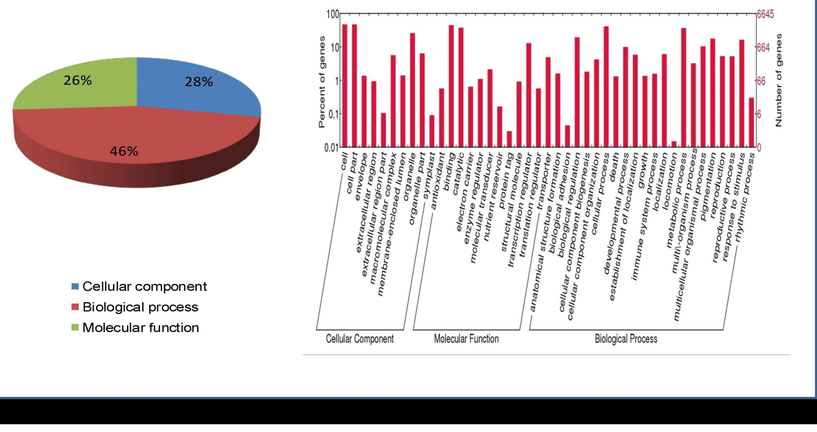
Abundance of SSRs in transcribed genome of Stevia



Localization of SSRs

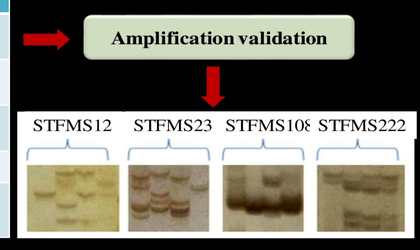


Gene Ontology annotation for SSR containing sequences

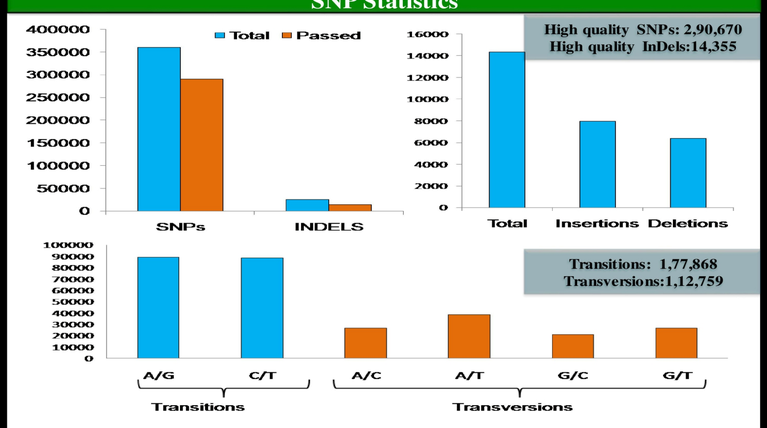


e-PCR and screening of SSRs

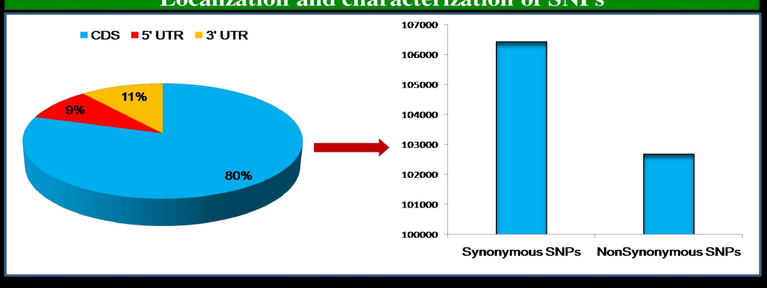
Characteristic	Number
Total number of primers checked	2387
Number of polymorphic primers	1362
Number of monomorphic primers	368
Not working	657
Number of ploymorphic primers with length variation	284



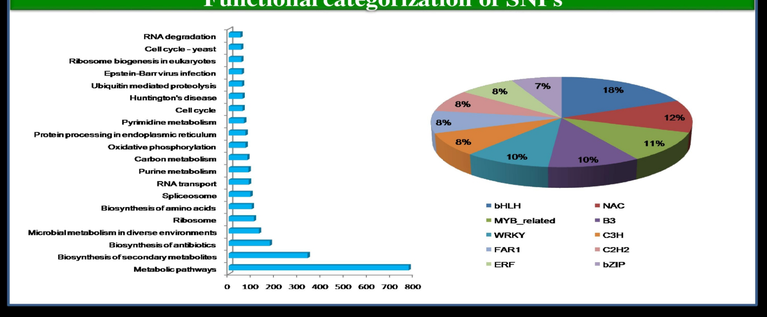
SNP Statistics



Localization and characterization of SNPs



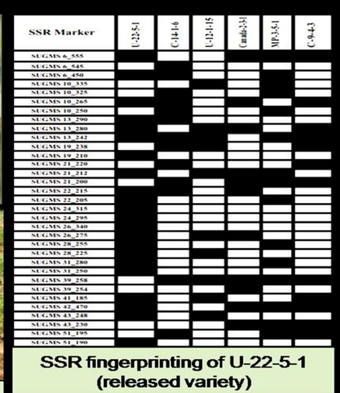
Functional categorization of SNPs



Field view after 3 weeks of plantation

Biochemical properties Him-Stevia

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



Way forwards

- It is the first time that such a vast marker resource has been provided for this crop.
- Phylogenetic, disease related or genome wide association studies can be performed on this resource.
- These markers can also aid in the selection or generation of an elite genotype.
- High through put biotechnological approaches coupled with breeding efforts will expedite genetic improvement, potential selection process with higher steviol glycoside and attract farmers/ entrepreneurs for larger cultivation

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CSIR-IHBT

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