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Identification of Nucleotide Variants to Analyse Quantitative Trait Loci (QTL) in Drought Resistant Mulberry Cultivars.

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ABSTRACT: Mulberry (Morus Indica) leaves have gained commercial and economical importance in India by domesticated sericulture practices. Genetic engineering has paved way to enhance the breeding adroitness in developing transgenic plants. Multifarious approaches are being carrying out by researchers to increase yield of mulberry leaves throughout fluctuating climatic conditions followed by differing biotic and abiotic stress. Identification of genetic markers and mapping genetic markers within the genus for a target specific yield growth are challenging and so far they have substantiated the complications effectively. Significance of allelic variants distinguished by various Genetic markers like RADP, RFLP, AFLP, ISSR, SSR, and SNPs regulates in characterization of each genus and Genetic linkage mapping in relevance with Linkage disequilibrium score across GWA Study, Association mapping, QTL mapping are investigated with emphasis on crop yield amelioration. A concise account on applications of genetic markers in understanding morphological changes in accordance with genotypic and phenotypic characteristics.

KEYWORDS: Mulberry, Genetic markers, SNP, Abiotic Stress.

I. INTRODUCTION

Morus Indica is a flowering plant associated with the family Moraccea, habitually known as mulberry. Mulberry plants have potency to grow as a tree certainly decreases moisture content in leaf in-turn reduces the leaf yield. Mulberry leaves possessing vigorous moisture content are prerequisite for nutrified silkmoth
(Bombyx Mori) rearing to produce high quality natural silk. Morus Indica has a wide variety of species such as Kanva-2, Mysore local, Victory-1 (V1), S-36, RFS-175, Bilidevalaya, Bombay Piasbari, etc., (K Vijayan et al.,) adopted to different climatic conditions. Phenotypic characters of mulberry plant can be distinguished by size of the bark, number of branches, leaf lobation patterns, height of the plant, leaves per branch and so on. To analyse phenotypic characters with respect to genotype and their morphological changes genetic markers specific to mulberry plants have developed by researchers. Sustaining moisture content in mulberry leaves under different climatic conditions is being challenging for farmers to adopt irrigation plants with high water use efficiency (WUE). To enhance breeding adroitness of Morus Indica for high WUE researchers have developed genetic markers by evaluating available genetic databases within the genus Morus and few characteristics among the family of Moraccea. Moisture content of mulberry leaf suitable for silkmoth feeding is determined on the basis of dry weight. Moisture Content and retention capacity of mulberry leaf can be calculated by using the formulae (M Shivashankar et al.,):

$$\text{Percentage of Moisture Content} = \frac{(\text{Fresh leaf weight} - \text{Dry leaf weight})}{\text{Fresh leaf weight}} \times 100$$

$$\text{Percentage of Moisture retention} = \frac{(\text{Leaf weight at different intervals} - \text{Dry leaf weight})}{\text{Percentage of Moisture content at different level}} \times 100$$

Mulberry leaves moisture in Morus Indica varieties varies from 65 to 75% whereas Morus Alba, Morus notabilis, Morus Lavigata exhibit 75 to 85% of leaf moisture. According to Darwin theory of survival of the fittest, plants constantly experiencing stress either adopt to surrounding conditions or abandon themselves to despair, by altering genotypic and phenotypic characters through mutation. Mutation can effect both positively and negatively, positive mutations upregulate the gene expression and supportive

towards yield quality but most of the negative mutations downregulate the gene expression and have destructive impact on yield quality. Though leaf yield quality can be enhanced through conventional breeding within natural selection which demands many generations to procure succeeding generation adapted to resist stress, have least probability of acquiring qualitative breeds in defined specification. Identification of positive mutation and insertion in transgenic plants as well as identification of negative mutation and deletion in transgenic plants are achieved through genetic markers like RADPs, RFLPs, AFLPs, ISSRs, SSRs, and SNPs. Plant breeding based on markers can be broadly classified into Biochemical markers, Cytological markers, Morphological markers and Genetic markers. Biochemical markers reveal allelic variation information from diversification of plant proteins based on amino acid sequences. Cytological markers reveal information on chromosome from one plant species to another based on difference in chromosome number, size, shape and ploidy types. Genetic markers assist in identification of species based on the known chromosome location for a specified DNA sequence. Plant genetic markers can be categorized into Polymorphic markers, Tandem repeat markers, Single variant markers.

II. MATERIALS AND METHODS

Plant material

The 22 mulberry germplasms adopted to different climatic conditions having variant level of resistant towards drought were analysed. *M.indica, M.serrata, M.laevigata, M.rotundiloba, M.multicaulis, M. multicaulis, M. alba, Assama Bola, Punjab Local, Thailand male, DD, S1, S13, S34, S145,Kanva-2, Mysore local, Victory-1 (V1), S-36, RFS-175, Bilidevalaya, Bombay Piasbari.* Both wild and cultivated mulberry species were collected for the analysis.

SNP analysis

Single Nucleotide Polymorphism (SNP) are the molecular markers which compares two DNA sequences on the basis of substitution in single nucleotides. The next generation sequencing approach to identify SNPs in mulberry cultivars subjected to drought condition, compares the drought resistant and drought susceptible DNA sequences. The raw sequences were downloaded from NCBI for the mulberry cultivar Morus alba (M. alba). The raw sequences were in SRA file format which were converted to fastq file format using the tool SRA toolkit. The converted fastq files were then checked the quality of DNA sequence using FASTQC. After checking the quality of the sequences, the low quality reads were trimmed using Trimmomatic. The trimmed sequences were aligned with reference genome using BWA. The aligned reads were sorted using Samtools. Finally, SNPs were extracted using SNPeff. The steps followed are as shown in Figure 1.

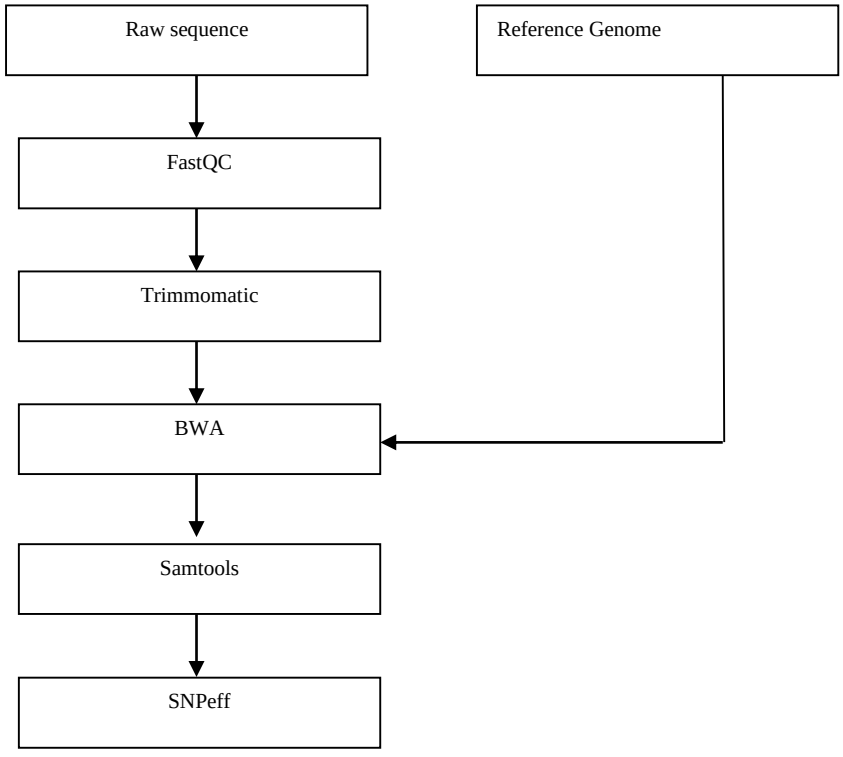

Figure 1. Steps Followed For Analysis

III. RESULTS

All the 22 mulberry germplasm varities were aligned to the reference genome M.alba . SNPs were identified for each genome sequences. The transitions (Ts) vs transversion (Tv) ratio for all sequences were found to be above 1.5.

Base changes (SNPs)

	A	C	G	T
A	0	2,662	8,377	3,440
C	2,719	0	2,386	9,481
G	9,353	2,444	0	2,761
T	3,331	8,479	2,639	0

Ts/Tv (transitions / transversions)

Transitions	55,425
Transversions	35,327
Ts/Tv ratio	1.5689

Number variants by type

Type	Total
SNP	58,072
MNP	0
INS	0
DEL	0
MIXED	0
INV	0
DUP	0
BND	0
INTERVAL	0
Total	58,072

Number of effects by impact

Type (alphabetical order)	Count	Percent
HIGH	42	0.013%
LOW	7,381	2.255%
MODERATE	2,264	0.698%
MODIFIER	317,933	97.033%

Number of effects by type and region

Type

Type (alphabetical order)	Count	Percent
3_prime_UTR_variant	1,974	0.602%
5_prime_UTR_premature_start_codon_gain_variant	78	0.024%
5_prime_UTR_variant	575	0.175%
downstream_gene_variant	34,944	10.649%
initiator_codon_variant	1	0%
intergenic_region	19,255	5.868%
intragenic_variant	1,083	0.33%
intron_variant	108,066	32.933%
missense_variant	2,284	0.696%
non_coding_transcript_exon_variant	547	0.167%
non_coding_transcript_variant	117,478	35.802%
splice_acceptor_variant	7	0.002%
splice_donor_variant	9	0.003%
splice_region_variant	790	0.241%
start_lost	1	0%
stop_gained	25	0.008%
stop_retained_variant	1	0%
synonymous_variant	6,623	2.018%
upstream_gene_variant	34,395	10.482%

Region

Type (alphabetical order)	Count	Percent
DOWNSTREAM	34,944	10.675%
EXON	9,377	2.865%
INTERGENIC	19,255	5.882%
INTRON	107,386	32.806%
SPLICE_SITE_ACCEPTOR	7	0.002%
SPLICE_SITE_DONOR	9	0.003%
SPLICE_SITE_REGION	779	0.238%
TRANSCRIPT	118,581	36.22%
UPSTREAM	34,395	10.507%
UTR_3_PRIME	1,974	0.603%
UTR_5_PRIME	653	0.199%

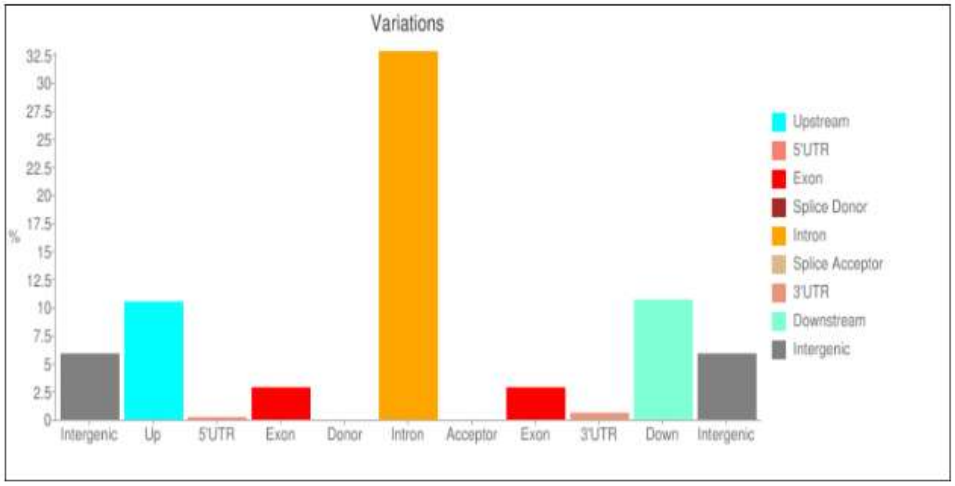

Figure 2. SNPeff results

IV. CONCLUSION

The 22 mulberry germplasms namely *M.indica, M.serrata, M.laevigata, M.rotundiloba, M.multicaulis, M. multicaulis, M. alba, Assama Bola, Punjab Local, Thailand male, DD, S1, S13, S34, S145,Kanva-2, Mysore local, Victory-1 (V1), S-36, RFS-175, Bilidevalaya, Bombay Piasbari,* were analysed and identified SNPs for each sequneces. Base change, Transition vs Transversion, Variants effect by impact and variants effect by type and region are identified for each sequences. These SNPs identified plays an important role in QTL identification in drought resistant mulberry.

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