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In Silico Mining of SNPs for Drought Candidates Genes in Wheat (Triticum Aestivum L.)

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Abstract: Wheat (Triticum aestivum L.) is one of the most important staple crops worldwide, but its productivity is severely affected by drought stress, a major abiotic constraint intensified by climate change. Identification of genetic variations associated with drought tolerance is essential for developing resilient wheat varieties. In this context, in silico approaches provide a rapid, cost-effective, and high-throughput method for detecting molecular markers such as single nucleotide polymorphisms (SNPs).The present study focuses on the in silico mining of SNPs in drought-responsive candidate genes of wheat using publicly available genomic databases and bioinformatics tools. Candidate genes associated with drought tolerance—such as those involved in osmotic regulation, stress signaling, and transcriptional control—were selected and analyzed. Sequence alignment and comparative genomics approaches were employed to identify SNP variations across different wheat genotypes. Functional annotation and predictive analysis were further conducted to evaluate the potential impact of identified SNPs on gene expression and protein function.The results revealed a significant number of SNPs distributed across coding and non-coding regions, with several non-synonymous SNPs potentially influencing drought tolerance traits. These SNPs can serve as valuable molecular markers for marker-assisted selection (MAS) and genomic breeding programs. The study highlights the effectiveness of in silico strategies in accelerating genetic improvement and supports the development of drought-resilient wheat cultivars.

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I. INTRODUCTION

Wheat is a staple food for a large proportion of the world"s poor producers and consumers. The cultivation of wheat (*Triticum* spp.) reaches far back into history. Wheat production has made a major contribution to global food security. Bread wheat accounts for more than 90% of global wheat production and contributes substantially to the national food The demand for wheat, based on production and stock changes, is expected to increase from the current level of approximately 625 million tons to around 813 million tons in 2030 and more than 900 million tons in 2050 (FAO, 2006). In agriculture, the term „drought" refers to a condition in which the amount of water available through rainfall and/or irrigation is insufficient to meet the transpiration needs of the crop. Drought resistance is attained within three major physiological domains: (a) the maintenance of a high plant water status during stress; (b) the maintenance of plant function at low plant water status, and (c) the recovery of plant water status and plant function after stress. Drought is one of the main factors limiting yield in cereals. Plants respond to drought by altering the expression of a large number of genes, thereby modifying cellular, physiological and biochemical processes. Recently, a number of potential candidate genes (CGs) have been identified by transcriptome and transgenic approaches involved in the adaptive responses to drought in cereals including wheat However, the function of only very few CGs has been tested and no clear evidence exists today for the real contribution of these genes to drought tolerance. The exploration of genetic variation in genes involved in drought response can be an important prerequisite towards a better understanding of stress mechanisms. This knowledge is also of great relevance to plant breeders.

A SNP is defined as a single base change in a DNA sequence that occurs in a significant proportion (more than 1 percent) of a large population. SNPs can be found within a gene or may be found in its close proximity. When found within a gene, it may or may not be responsible for the mutant phenotype, but in either case, it can be used for positional cloning of the gene in question. Once a large collection of SNPs is available, their use will depend on whether or not genetic determinants for all traits are included in those SNPs.

II. REVIEW OF RELATED LITERATURE

Nahas et al. (2019) examined 6,717 ESTs from drought- and salinity-stressed wheat and organized them into contigs, identifying:Fourteen transcription factor families,Multiple enzyme classes.Genes in metabolism, biological activities, and genetic information processing. Sallam et al. (2019) and Farooq et al. (2014) highlighted that drought affects key growth stages such as seedling establishment and grain filling, making drought tolerance a major breeding objective.

Al-Husein et al. (2019) emphasized the role of bioinformatics in identifying gene ontology functions and stress-related pathways in wheat under drought conditions. Hui Liu et al. (2023) conducted a genome-wide association study (GWAS) using a 90K SNP array and identified genomic regions linked to drought tolerance traits such as root length and biomass .Their study identified:44 candidate genes associated with drought tolerance ,SNP markers located within or near functional genes ,Genes involved in signaling, metabolism, and stress response pathways. Guijun Yan et al. (2023) used bioinformatics tools such as Ensembl Plants, UniProt, and InterPro to functionally annotate SNP-associated genes and study their expression patterns under drought stress .The study demonstrated that integrating SNP data with gene expression analysis helps identify functionally relevant drought-responsive genes. Abdul Rauf et al. (2024) developed SNP-based genotyping assays targeting drought-related genes such as *SnRK2*. Their research showed that SNP polymorphisms in these genes are significantly associated with drought tolerance traits in wheat. Bioinformatics tools and databases play a crucial role in SNP mining and gene identification.Researchers have used:NCBI databases for EST and sequence retrieval,Ensembl Plants for genome annotation,KEGG for pathway analysis.

➢ *Objectives:*

- To mine the SNPs for candidate genes related to drought from Public Database and designing of primers.
- Screening of forty two lines of drought (tolerant and susceptible) with the designed SNPs.

III. MATERIALS AND METHODS

The present work was carried out at the Biochemistry and molecular biology lab of Directorate of Wheat Research, Karnal. Mining of SNPs have been done with the following approaches.

- *In-silico* Approach
- Molecular Approach

A. Materials for in-Silico SNPs Mining

Main objective of this project is to detect SNPs by *in-silico* in ESTs of *Triticum aestivum* using high-quality sequences and alignment parameters. Furthermore, we observed the predicted SNPs in drought candidates genes in wheat, validated putative SNPs in the gene like calmodulin (CaM), superoxide dismutase(SOD), cis (prolyl isomerase), Aquaporin, COR, RAB protein and LTP(lipid transfer protein). Firstly download UniGene sequence from NCBI and then VecScreen to remove contamination. The sequences were assembled into contigs using CAP3. To automate the process of SNP prediction, use AutoSNP to detect SNPs. AutoSNP reads the ACE file generated by CAP3 to identify candidate SNPs and primers were designed using Primer 3.0.**DNASTAR** is software for DNA and protein sequence assembly and analysis in which ***SeqMan Pro*** product used for mining and validation of SNP from EST data.

Table 1 List of Online Tools and Software's Used in *in-Silico* SNPs Mining Online Tools

List of online tools and software's used in *in-silico* SNPs mining Online Tools	**Purpose**
NCBI-*UniGene*	For downloading sequences
VecScreen NCBI	Used for vector clipping
EST Trimmer	For removal of PolyA tail
Repeat Masker	Remove repetitive sequence
CAP3	For clustering
AutoSNP, SEAP	For SNPs mining
HaploSNPer	For detecting SNPs
DNA Star	For SNPs mining
Primer3	For primer designing

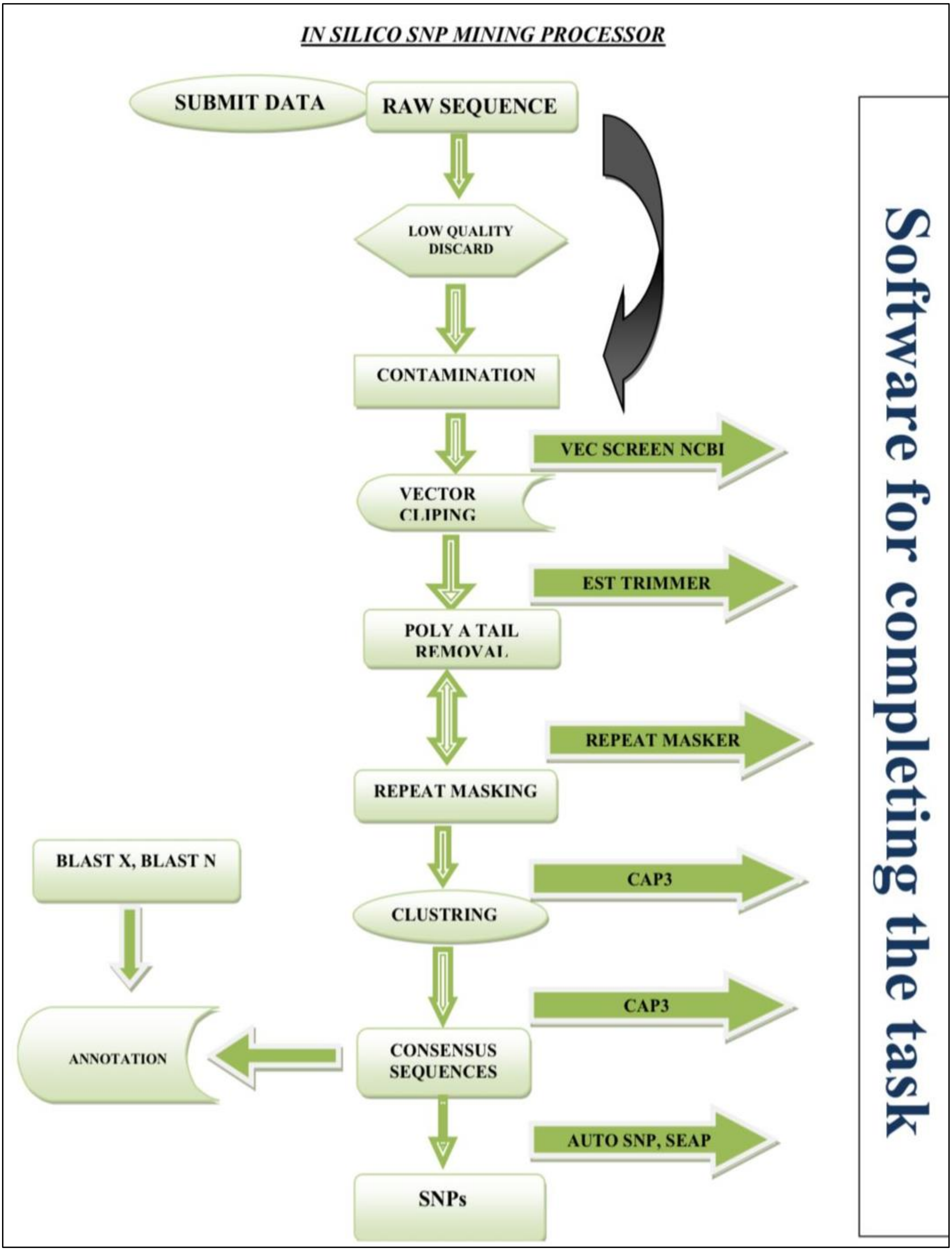
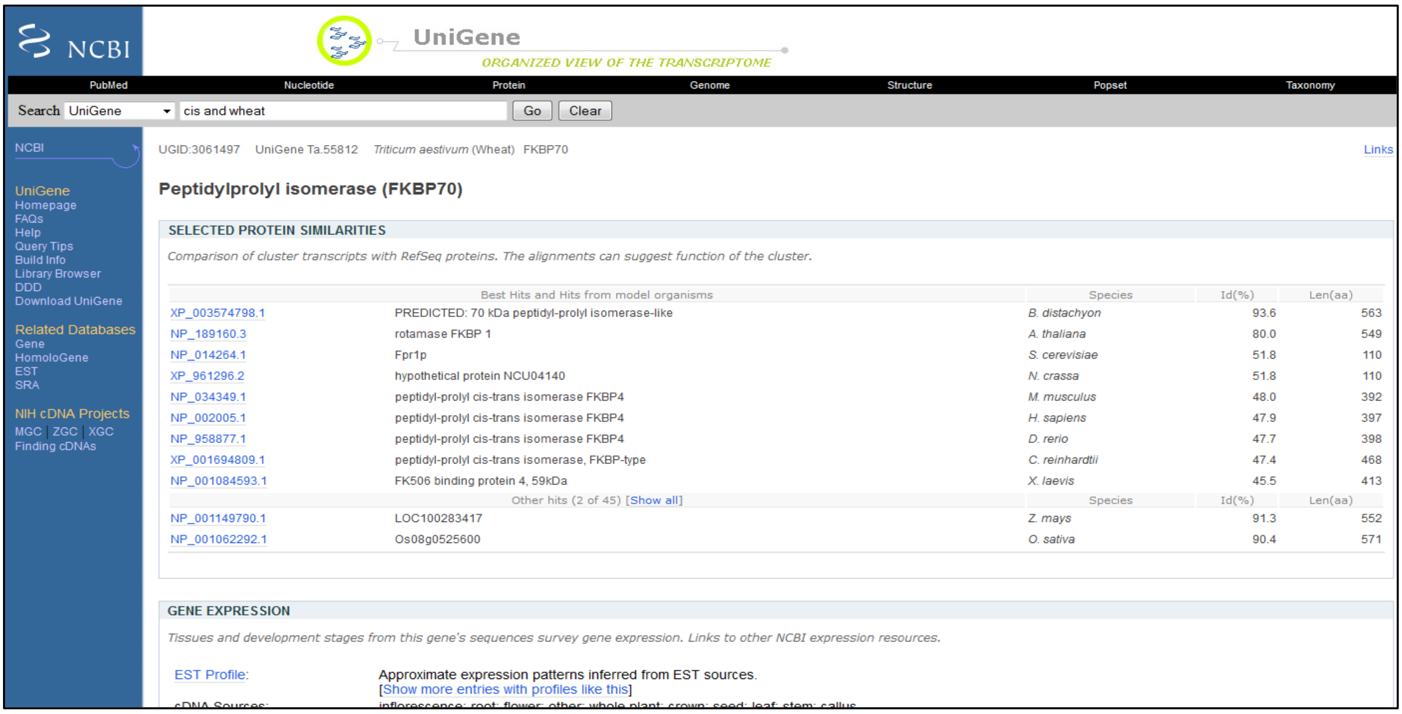


Fig 1 In Silico Mining Processor

- *Method of in-Silico SNP Mining Retrieve EST Data: -*

Firstly, retrieve the UNIGENE sequence of all drought tolerance candidates genes in fasta format from ncbi.



UGID:3061497 UniGene Ta.55812 *Triticum aestivum* (Wheat) FKBP70

Peptidylprolyl isomerase (FKBP70)

SELECTED PROTEIN SIMILARITIES

Comparison of cluster transcripts with RefSeq proteins. The alignments can suggest function of the cluster.

Best Hits and Hits from model organisms		Species	Id(%)	Len(aa)
XP_003574798.1	PREDICTED: 70 kDa peptidyl-prolyl isomerase-like	*B. distachyon*	93.6	563
NP_189160.3	rotamase FKBP 1	*A. thaliana*	80.0	549
NP_014264.1	Fpr1p	*S. cerevisiae*	51.8	110
XP_961296.2	hypothetical protein NCU04140	*N. crassa*	51.8	110
NP_034349.1	peptidyl-prolyl cis-trans isomerase FKBP4	*M. musculus*	48.0	392
NP_002005.1	peptidyl-prolyl cis-trans isomerase FKBP4	*H. sapiens*	47.9	397
NP_958877.1	peptidyl-prolyl cis-trans isomerase FKBP4	*D. rerio*	47.7	398
XP_001694809.1	peptidyl-prolyl cis-trans isomerase, FKBP-type	*C. reinhardtii*	47.4	468
NP_001084593.1	FK506 binding protein 4, 59kDa	*X. laevis*	45.5	413
Other hits (2 of 45) [Show all]		Species	Id(%)	Len(aa)
NP_001149790.1	LOC100283417	*Z. mays*	91.3	552
NP_001062292.1	Os08g0525600	*O. sativa*	90.4	571

GENE EXPRESSION

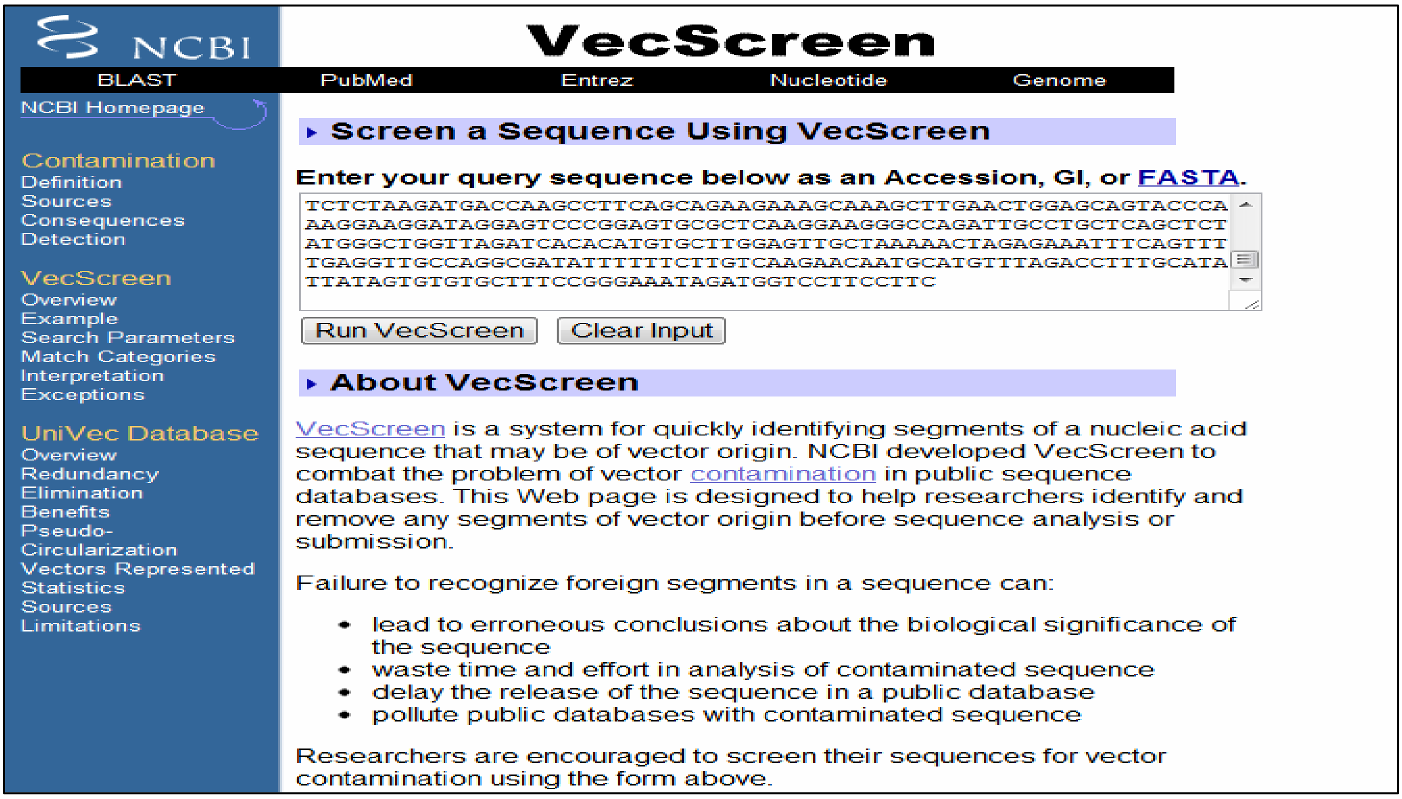
Tissues and development stages from this gene's sequences survey gene expression. Links to other NCBI expression resources.

EST Profile:	Approximate expression patterns inferred from EST sources. [Show more entries with profiles like this]
cDNA Sources:	inflorescence; root; flower; other; whole plant; crown; seed; leaf; stem; callus

Fig 2 NCBI

- *Used Online Tools:*

- VecScreen: Submit the sequence in VecScreen for purification of the data. VecScreen remove the vector sequences from that contaminated sequences with the help of one additional tool Bioedit. Bioedit tools give the output in fasta format without vector sequence data.



Screen a Sequence Using VecScreen

Enter your query sequence below as an Accession, GI, or FASTA.

```
TCTCTAAGATGACCAAGCCTTCAGCAGAAGAAAGCAAAGCTTGAACTGGAGCAGTACCCA
AAGGAAGGATAGGAGTCCCGGAGTGCGCTCAAGGAAGGGCCAGATTGCCTGCTCAGCTCT
ATGGGCTGGTTAGATCACACATGTGCTTGGAGTTGCTAAAAACTAGAGAAATTTCAGTTT
TGAGGTTGCCAGGCGATATTTTTTTCTTGTCAAGAACAATGCATGTTTAGACCTTTGCATA
TTATAGTGTGTGCTTTCCGGGAAATAGATGGTCCTTCCTTC
```

About VecScreen

VecScreen is a system for quickly identifying segments of a nucleic acid sequence that may be of vector origin. NCBI developed VecScreen to combat the problem of vector contamination in public sequence databases. This Web page is designed to help researchers identify and remove any segments of vector origin before sequence analysis or submission.

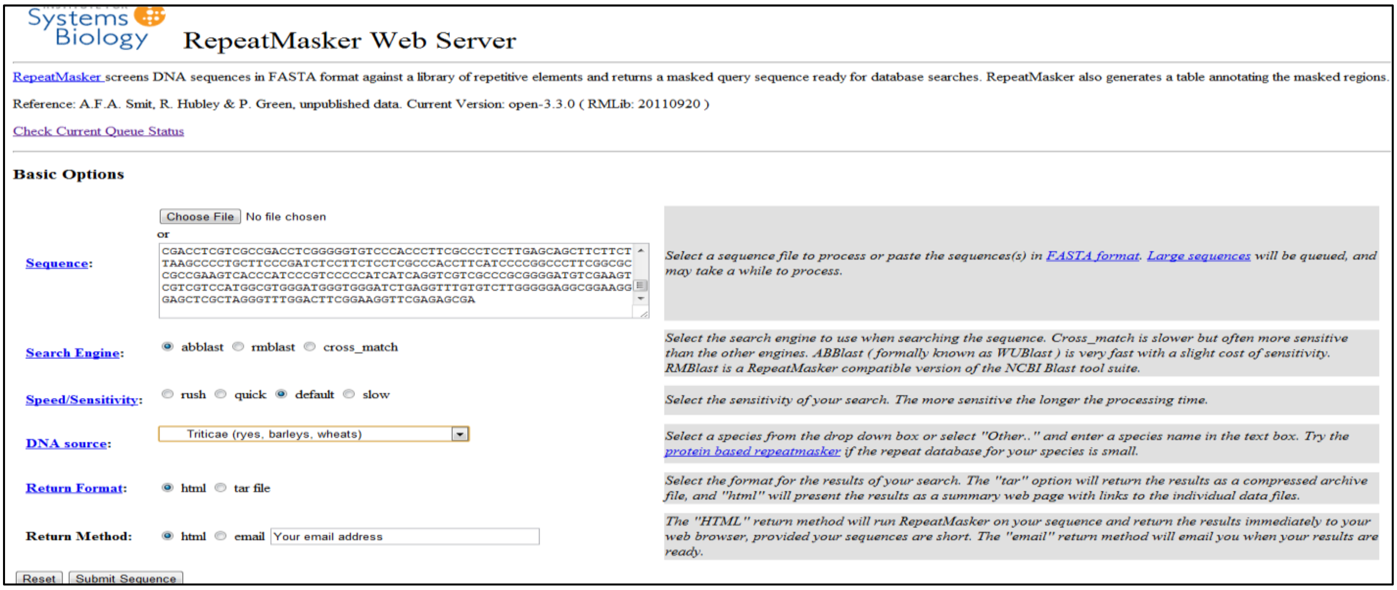
Failure to recognize foreign segments in a sequence can:

- lead to erroneous conclusions about the biological significance of the sequence
- waste time and effort in analysis of contaminated sequence
- delay the release of the sequence in a public database
- pollute public databases with contaminated sequence

Researchers are encouraged to screen their sequences for vector contamination using the form above.

Fig 3 VecScreen

- EST Trimmer: - removes the polyA tail from those sequences. 27
- Repeat Masker: - Remove the repeated sequence from the result file obtain from EST Trimmer.



RepeatMasker screens DNA sequences in FASTA format against a library of repetitive elements and returns a masked query sequence ready for database searches. RepeatMasker also generates a table annotating the masked regions.

Reference: A.F.A. Smit, R. Hubley & P. Green, unpublished data. Current Version: open-3.3.0 (RMLib: 20110920)

Sequence:

```
CGACCTCGTCGCCGACCTCGGGGGTGTCCCACCCTTCGCCCTCCTTGAGCAGCTTCTTCT
TAAGCCCCTGCTTCCCGATCTCCTTCTCCTCGCCCACCTTCATCCCCGGCCCTTCGGCGC
CGCCGAAGTCACCCATCCCGTCCCCCATCATCAGGTCGTCGCCCGCGGGGATGTCGAAGT
CGTCGTCCATGGCGTGGGATGGGTGGGATCTGAGGTTTGTGTCTTGGGGGAGGCGGAAGG
GAGCTCGCTAGGGTTTGGACTTCGGAAGGTTCGAGAGCGA
```

Select a sequence file to process or paste the sequences(s) in FASTA format. Large sequences will be queued, and may take a while to process.

Search Engine: ● abblast ○ rmblast ○ cross_match

Select the search engine to use when searching the sequence. Cross_match is slower but often more sensitive than the other engines. ABBlast (formally known as WUBlast) is very fast with a slight cost of sensitivity. RMBlast is a RepeatMasker compatible version of the NCBI Blast tool suite.

Speed/Sensitivity: ○ rush ○ quick ● default ○ slow

Select the sensitivity of your search. The more sensitive the longer the processing time.

DNA source: Triticae (ryes, barleys, wheats)

Select a species from the drop down box or select "Other.." and enter a species name in the text box. Try the protein based repeatmasker if the repeat database for your species is small.

Return Format: ● html ○ tar file

Select the format for the results of your search. The "tar" option will return the results as a compressed archive file, and "html" will present the results as a summary web page with links to the individual data files.

Return Method: ● html ○ email Your email address

The "HTML" return method will run RepeatMasker on your sequence and return the results immediately to your web browser, provided your sequences are short. The "email" return method will email you when your results are ready.

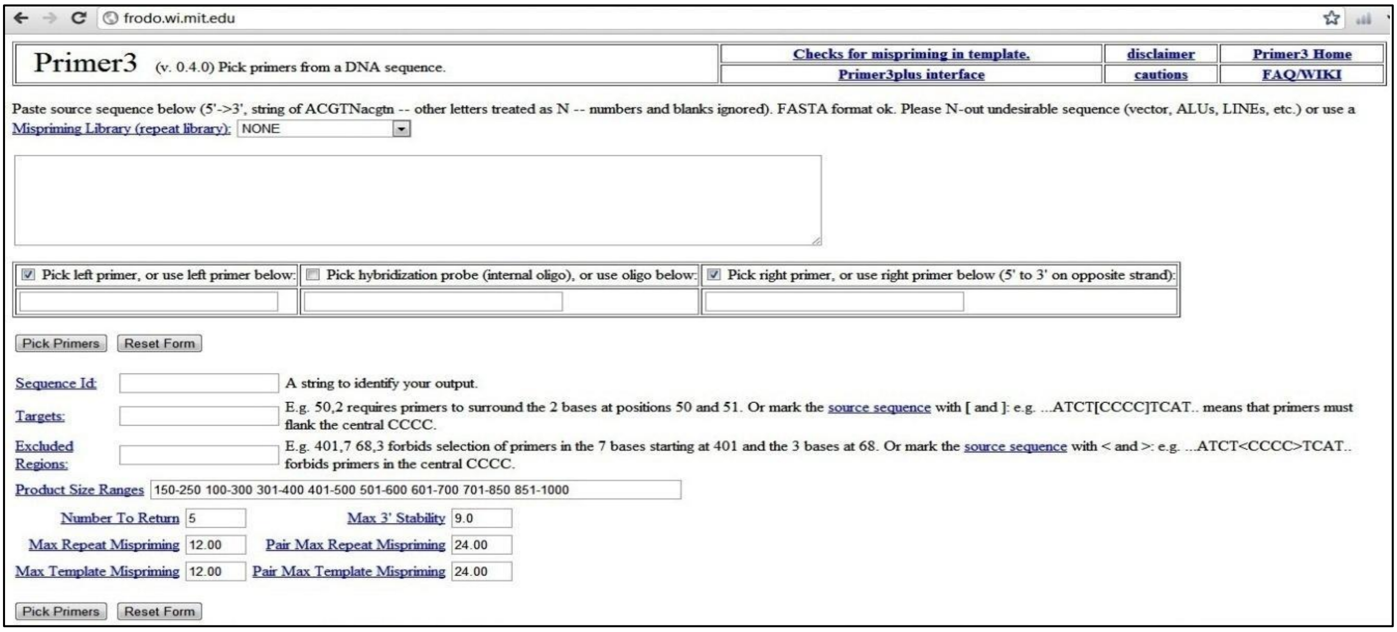
Fig 4 Repeat Masker

- CAP3: - It‟s also an offline tool run on Linux window. Its mainly used for clustering the sequences and output in ace file which is directly used by the software AutoSNP.
- AutoSNP: - AutoSNP is a program to detect SNPs and insertion/deletion polymorphisms (indels) in expressed sequence tag (EST) data. The program uses cap3 to cluster and align EST sequences, and uses redundancy to differentiate between candidate SNPs and sequence errors. AutoSNP gives 3 types of result file. 1. SNP summary 2. SNP table 3. SNP result file.
- Primer3:- Primer3 use to design primers for all consensus sequences. Primer3 is an online tool that follows specific criteria for designing of primers for PCR.

➢ *Specific Criteria for Designing of Primers for PCR.*

Table 2 Specific Criteria for Designing of Primers for PCR.

Criteria	Default
Length	18-23
Tm range (°C)a	55 – 65
GC (%)	45 – 65



Primer3 (v. 0.4.0) Pick primers from a DNA sequence.

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library): NONE

☑ Pick left primer, or use left primer below: ☐ Pick hybridization probe (internal oligo), or use oligo below: ☑ Pick right primer, or use right primer below (5' to 3' on opposite strand):

Sequence Id: A string to identify your output.

Targets: E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

Excluded Regions: E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

Product Size Ranges 150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000

Number To Return 5
Max 3' Stability 9.0
Max Repeat Mispriming 12.00
Pair Max Repeat Mispriming 24.00
Max Template Mispriming 12.00
Pair Max Template Mispriming 24.00

Fig 5 Primer 3

- *Working with DNA Star:*

Use of additional online softwares for SNPs detection. With the help of DNA Star mine SNPs directly by setting some parameters like match size=40, sequence length 100 and match 45%.Minimum match % should be 95%. In this firstly EST data retrieve from NCBI and directly submit to DNA Star which will mine SNPs in many different ways. DNA Star gives the output in the form of many files like consensus sequence, contigs information, and SNPs positions in the sequences.

- *Steps Involved in DNA Star*

- Retrieve the UniGene EST data from NCBI in fasta format.
- Than this data submitted to DNA Star for its further processing.
- Different software will be work on this data with different accuracy.
- Firstly remove vector sequences from the data.
- In next step remove PolyA tails.
- Then set some parameters like match size, match % and sequence length etc. For further work.
- In last step we will retrieve contigs formation. On the basic of which we find SNPs.

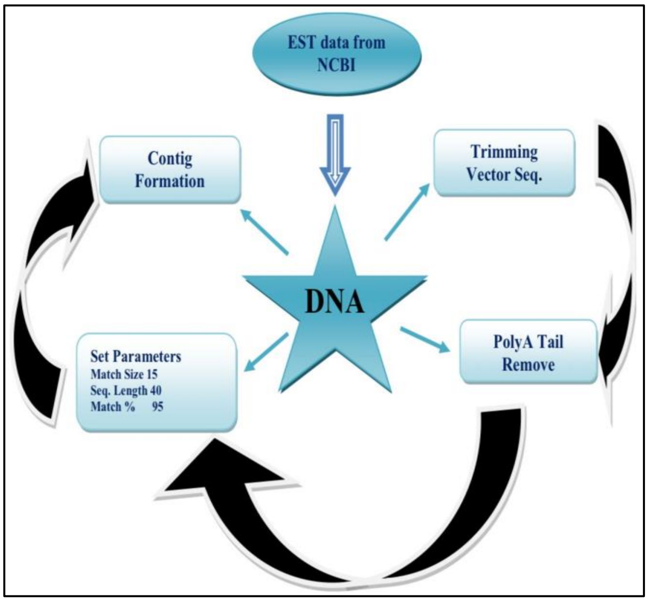


Fig 6 DNA Star

B. *Molecular Approach*

- *Materials*

- *Plant Material*

In total 42 Lines (Drought resistant and susceptible) from the wheat breeding programme grown in Directorate of Wheat Research, Karnal. During 2011-2012 crop season were used for the biochemical evaluation and molecular marker characterization.

- *Chemicals*

Taq DNA polymerase and dNTPs used for polymerase reaction were purchased from NEB, USA. Rest of the chemicals used in the investigation was of molecular biology grade or analytical grade and were procured from Sigma Chemicals Co., USA; Promega Inc., USA; Gibco BRL Inc., USA and E. Merck, India.

- *Molecular Marker*

SNPs primers were designed for candidate genes like Rab, Cis, LTP, COR, Aquaporin, CaM, SOD to screen allelic variation in 42 cultivars of Bread wheat.

- *Isolation by CTAB Method*

✓ *Genomic DNA Preparation*

Genomic DNA was isolated using modified CTAB method of Saghai-Maroof et al. (1984) from young leaf samples of durum wheat genotypes. Leaf samples were taken from 2-3 week old seedlings and grinding into fine powder using liquid nitrogen in a sterile pestle and mortar. Approximately five gram of the ground leaf tissue powder was mixed with 700 µl of preheated (65˚C) CTAB buffer and incubated at 65˚C for 90 minutes with regular gentle mixing of samples. After incubation, samples were cooled to room temperature and 750 µl of Chloroform: Isoamylalcohol (24:1) mixture was added and mixed well by inverting the tubes gently for 10-15 min. Tubes were then centrifuged at 10,000 rpm for 10 min. and upper aqueous layer was mixed with equal volume of ice-cold isopropanol to precipitate DNA. The DNA was then spooled out and centrifuged at 12,000 rpm for 5 min to pellet down. The DNA pellet was given 70% ethanol washing and left overnight for drying. DNA was subsequently dissolved in appropriate volume of T.E. Samples were stored at -20˚ C till further use.

- *Polymerase Chain Reaction (PCR)*

PCR is an in vitro method for enzymatic DNA synthesis that employs two oligonucleotide primers that hybridize to opposite strands and flank the desired area of the target DNA. The method amplifies small amounts of certain DNA fragments between 106 and 1012 times. Gradient Thermocycler PTC-100TM (Biorad, USA) was used to do PCR amplification of template DNA. The total volume of the PCR reaction mixture was 25 ml, which contained 1 x PCR buffer, 200 mM dNTPs, 300 mM of primer (both), 1.5 mM MgCl2, 1 unit Taq DNA polymerase, and 40-50 mg template DNA. PCR amplifications were conducted out at the optimal annealing temperatures for each marker. PCR amplification involved denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 1 minute, annealing at 55-60˚C for 1 minute, and extension at 72˚C for 1 minute. The last extension was done at 72˚C for 5 minutes, followed by chilling at 40˚C. Amplification products were stored at -20˚C until further usage. 1. Initial denaturation at 94˚C for 4 minutes. 2. Denaturation at 94˚C for 1 minute. 3. Anneal at 60˚C for 1 minute. 4. Extend at 72˚C for 2 minutes. 5. Final extension at 72˚C for 10 minutes. Cycles were created by repeating stages II through IV 35 times. Following the final extension (polymerization) stage, the samples were moved to 4 °C or -20 °C.

- *Agarose Gel Electrophoresis*

The PCR products were resolved on a 2% (w/v) Agarose gel using submerged horizontal electrophoresis. The washed and dried gel casting plate was taped at both ends. To make a gel with a thickness of 0.5 cm, melt agarose in 1xTAE and add 5µg/ml ethidium bromide. Pour into a plate with comb. After Agarose polymerization, the sealing tapes were removed, and the gel casting plate was inserted into an electrophoretic chamber containing 1xTAE. Adding 6x loading dye to prepared samples. Samples were placed into the wells, and electrophoresis was performed at a constant voltage of 70–80 volts. Following electrophoresis, bands were inspected under UV light and saved in the gel documentation system.

- *Reagents and Solutions*

- ✓ Extraction Buffer
- ✓ Chloroform: isoamylealcohol (24:1)
- ✓ RnaseA (10mg/ml)
- ✓ Isopropanol
- ✓ Wash 1:76% ethanol with 0.2 SODIUM ACETATE:
- ✓ 76ml 99% Ethanols
- ✓ 6.67ml 3M Sodium acetate
- ✓ Add to 100ml with autoclaved ddH2O
- ✓ Wash 2:76%Ethanol with 10Mm Ammonium acetate
- ✓ 76ml 99%Ethanol
- ✓ 77.0mg Ammonium acetate
- ✓ Add to 100ml with autoclaved ddH2O
- ✓ TE BUFFER (10Mm Tris pH 8.0 and 1Mm EDTA pH8) 3.12 buffers and reagents
- ✓ Tris-acetate EDTA buffer (50X)
- ✓ 242g Tris base
- ✓ 57.1ml glacial acetic acid100ml 0.5%
- ✓ EDTA (Ph8.0)10mg/ml
- ✓ Etidium bromide solution
- ✓ 6X loading dye:
- ✓ Sucrose 4 g
- ✓ Bromophenol blue 0.025 g
- ✓ Xylene cyanol 0.025 g
- ✓ Final vol. 10ml.

IV. RESULTS AND DISCUSSION

Genes for specific features or disorders are identified using a genome-wide candidate gene technique, which can then be used to uncover SNP. Different online and offline approaches are utilized to identify SNPs in drought tolerance candidate genes in wheat. Sequences were downloaded in fasta format from UniGene at NCBI and then submitted to VecScreen, an online program that uses Bioedit to remove vector sequences from tainted data. After that, EST Trimmer deleted the PolyA tail from the result file obtained from Bioedit, and the EST result file was given to the repetition masker, which eliminated the repeating sequences. Running CAP3 in a Linux window yielded the result in the form of an ace file. CAP3 produces clustering results. From the final result obtained by AutoSNP which detect SNPs primers were designed using Primer3. Along with this, a paid software name DNA Star was also used for mining SNPs in candidate genes of drought. *In silico approach*- DNA STAR:- By using this software consensus sequence or contigs of candidates genes related to drought tolerance were retrieved and found the SNPs present in that consensus sequences of all contigs. Primers were designed by using that SNPs present in consensus sequence. DNA Star also provides SNPs report on the bases of some selected criteria like SNPs of criteria >40%.

- Result of Primer 3:- Primer 3 results showed start position, bp lengths, product size, GC% tm and the end stability of the primers designed. After considering all these parameters value, primers were synthesized for validation in the 42 genotypes of the wheat.

Table 3 SNP Detection in Candidate Genes: Gene

SNP detection in candidate genes: Gene	No. of sequence	SNP	Single tone anomalies
Aquaporin 1:2	978	56	No
Aquaporin 7 (AQP7	211	17	No
Peptidylprolylisomerase	175	22	No
Rab protein	132	32	No
Rab 15B	355	21	No
SOD 3.1	245	22	No
Lipid transfer protein	237	3	No
Calmodulin 3	379	21	No

Four primers of SNPs were created from wheat for the aquaporin candidate gene, however only three of them were amplified in the selected cultivars, indicating the presence or absence of the SNP in that genotype. Similarly, Cam, Rab, SOD, cis, and LTP amplified in drought-selected genotypes, but no one amplified for the discovered SNP using COR primers. A SNP marker derived from the candidate gene for red flesh and foliage color in apples was recently developed (Chagne et al., 2007). The detected allelic variation in SNPs of different candidate genes will be correlated with phenotypic data gathered from various centers for 42 wheat cultivars in two sets of settings, namely rainfed and irrigated, using statistical software.

PCR Amplification Result:

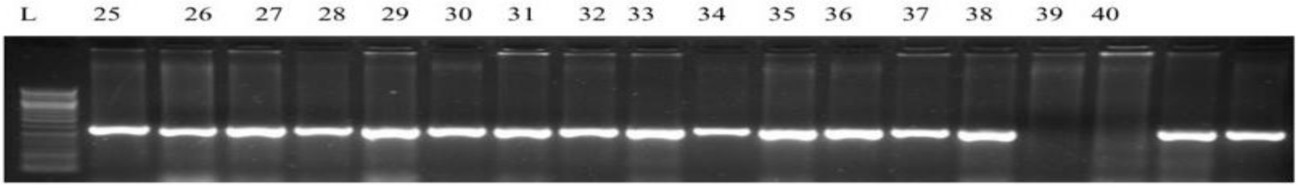


Fig 4.3:- Primer CIS-750

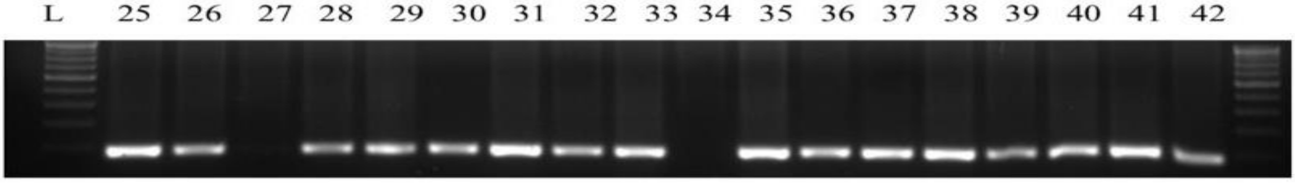


Fig 4.4:- Primer LTP-837

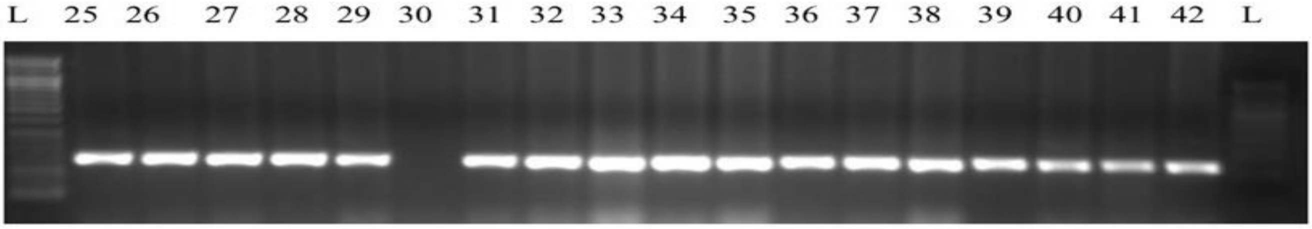


Fig 4.5:- Primer RAB-39

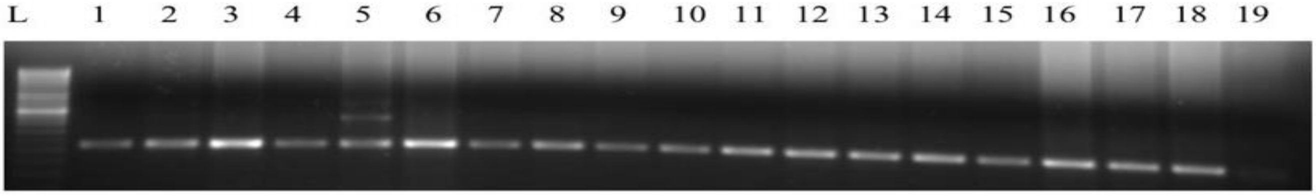


Fig 4.6:- Primer COR-761

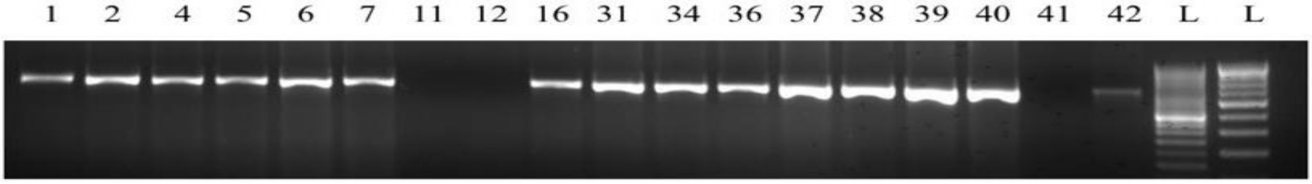


Fig 4.7:- Primer SOD-395

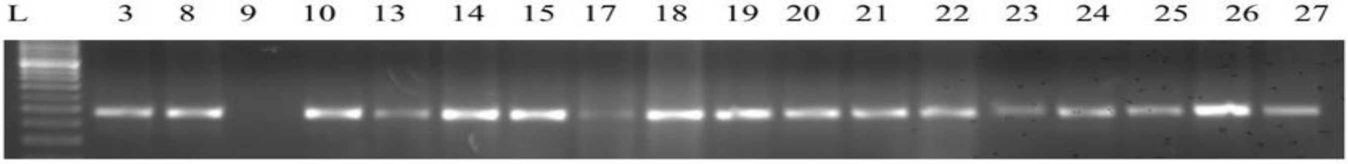


Fig 4.8:-Primer CAM-396

L lanes represent Ladder while Nos. 1 to 42 – wheat cultivars listed in table 1.

Fig 7 PCR Amplification Result

V. CONCLUSION

The availability of vast sequencing databases for a variety of plant species allows for the identification of DNA variants associated with SNPs. SNPs have emerged as the new generation of molecular markers, and they will be discovered in a variety of complex crops such as wheat. In crop plants, however, only a start has been made in terms of SNP finding and detection. A variety of gel-based and non-gel-based approaches will be employed to detect previously identified SNPs and genotype populations at these SNP sites. The outcome of the current investigation: SNPs were mined from drought-related genes in the NCBI database using AutoSNP and DNA Star. Primer 3.0 software was used to create primers. The 42 wheat genotypes (drought tolerant and susceptible) were screened with the designed primers for the candidate genes, and some of the varieties showed amplification (positive and negative), which will be correlated with the phenotypic data collected from the field using statistical software. We found that PCR primers based on SNPs in sequences can reliably amplify simple DNA products and detect specific alleles in the multicopy gene family.Future, SNPs will certainly be used in a number of crops, not only for studies involving associations with a number of traits of economic value, but also for the study of genetic diversity and variety identification. Enormous genomic and cDNA sequence data that are accumulating in the databases will be extremely useful in future for discovery of new SNPs. In the coming decade, those working in the area of molecular markers in crops will remain busy with the discovery and use of SNPs in a number of crops for a variety of purposes relevant to crop improvement. This is certainly true about the laboratories in the developed world. One can only hope that in India also, facilities will be created at least in some select laboratories, to make its share of contribution in this exciting area of research involving SNP discovery and detection, particularly in plant systems.

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