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Research Article

Exploiting legume EST databases for the development of gene-derived SSR-markers in medicinal legume *Mucuna pruriens* L. (DC.)

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Abstract

This paper reports method for the development of microsatellite markers in *Mucuna pruriens* from a deluge of EST sequence data of related legume species available in public database. Totally, 2,86,488 EST sequences from four legume species *viz., Vigna unguiculata, Glycine max, Phaseolus vulgaris* and *Cicer arietinum* generated 22,457 SSRs comprising of 29.08% di-, 46.41% tri-, 7.29% tetra-, and 17.22% penta-nucleotide repeats. Based on 22,457 SSR containing sequences, 522 primer combinations were designed and 50 were screened against a diverse panel of 25 genotypes, of which 3 produced polymorphic profiles with an average PIC of 0.65. The work demonstrates low cost method for microsatellite development in resource poor medicinal plants and is first such report on sequence based marker development in *M. pruriens*.

Key words

EST- SSR, *M. pruriens*, orthologues, MISA, gene ontology.

Introduction

Mucuna pruriens L. (DC.) described as a self-pollinated species is a tropical legume classified within the phaseoloid clade of Leguminosae which also include soybean, common bean, mung bean and relatives. Reported to be native of Eastern India and Southern China (Wilmot Dear, 1987), it is now distributed in several parts of the World including tropics exhibiting tremendous diversity of morphological forms and biochemical variants. The plant commands rich history in Ayurvedic system of Indian medicine where it is used as an integral part of over 200 drug formulations (Oudhia, 2002). It is reported to possess anti-diabetic, anti-neoplastic, anti-microbial, aphrodisiac, learning and memory enhancing properties (Oudhia, 2002). Importantly, its efficacy in the treatment of Parkinson's disease is well recognized both in alternative and allopathic system of medicine. When used as drug, L-Dopa, which is copiously present in its seeds acts as a precursor for the synthesis of neurotransmitter drug dopamine, thus balancing its deficiency in the brain cells of Parkinson's patients (Farooqi et al. 1999; Kavitha and Thangamani, 2014). More recently it is also shown to promote male fertility in rats by recovering spermatogenic losses (Singh et al., 2013). Besides medicinal properties, M. pruriens also fixes nitrogen and is a well-known green manure cover crop (Bressani et al., 2002). It possesses high seed protein content and distinct nutritional benefits thus offering a promising candidate to bridge the gap between increasing demand for high protein and its limiting sources, especially in Asia and Sub-Saharan Africa (Leelambika et al., 2010).

Deployment of molecular markers can propel breeding efforts in resource poor medicinal plants. In *M. pruriens*, DNA markers such as Randomly Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphisms (AFLP) have been used previously for germplasm characterization (Padmesh et al., 2006; Sathyanarayana et al., 2010); genetic diversity analysis (Capo-chichi et al., 2001; and Leelambika et al., 2010) and development of frame-work linkage map (Capo-Chichi et al., 2004). However, owing to widely known limitations of these dominant markers (Jonah et al., 2011), there is a need to develop co-dominant markers particularly SSRs to support the breeding efforts in this speices for L-Dopa and other economic traits. Once developed, they can also be used for taxonomy and evolutionary studies in *Mucuna sp*.

Microsatellite development based on expressed sequence tags (ESTs) has emerged as promising alternative to cost intensive genomic-SSR based methods for research in medicinal plants. Mining SSRs from the public databases is now sufficiently streamlined to make it cheaper and more efficient (Cordeiro et al., 2001; Kantety et al., 2002; Chen et al., 2006). Processed ESTs are important sources to mine them and are now deposited in large numbers in public databases. EST-derived microsatellites are particularly popular because they are: (i) cost-effective, especially when developed using publicly available EST information; (ii) informative due to their multi-allelic and co-dominant nature (Powell, 1996) (iii) useful for studying functional diversity in natural

populations or germplasm collections as they are

derived from the transcription products (Varshney et al., 2005); and (iv) transferable to other species and can be used for comparative genomics as most are derived from the conserved coding sequences (Guo et al., 2006).

In this background, the present work was aimed to explore potential use of public legume EST databases for the development of gene-derived SSR-markers in medicinal legume *Mucuna pruriens* L. (DC.). For doing this, we hypothesized that the sequences which shared high percentage of homology between the species are most likely present in *M. pruriens*; and such sequences were used to generate EST-SSR markers and subsequently assessed for their ability to reveal polymorphism information and genetic diversity estimates.

Materials and methods

Development of sequence resources:Sequence information obtained from four closely related legume species viz. *Vigna unguiculata*, *Glycine max* (L.) Merrill, *Phaseolus vulgaris* L. and *Cicer arietinum* L. via orthologues were used. The transcriptome sequences of all the species were downloaded from the TIGR plant transcript assemblies (http://plantta.jcvi.org) and TIGR gene indices (http://compbio.dfci.harvard.edu/tgi/plant.html). A total of 2,86,488 sequences across all the four species were downloaded and analyzed for the presence of SSRs (Table 1).

In silico SSR screening:Microsatellite detection was done by a modified version of MIcro SAtellite identification tool (MISA) (http://pgrc.ipk-gatersleben.de/misa/misa.html). It allows for identification and localization of perfect as well as compound microsatellites. MISA scripts, in this study, were modified to omit mononucleotide repeats by default; produce output for only those repeats equal to or greater than 15bp; include only SSRs with at least 200bp on either side and compute sequence length and display in an output file.

Identification of orthologues:To identify orthologues among the 22,457 microsatellites obtained, Blastn (Altschul et al., 1990) analysis was performed. The sequences containing SSR information of each species were separated into different files and blasted against each other. The result was parsed using the "BlastParser"(http://geneproject.altervista.org/) to identify sequences that are represented in more than one species (with 85 - 100% homology). The sequences thus obtained were divided into sequences common between three and two species respectively.

Primer design and in silico validation:The input sequences from 522 orthologues were fed into BatchPrimer3 (Frank et al., 2003) for primer design.The primers thus generated were validated for efficiency by running them along with corresponding sequences in In silico PCR tool available at http://insilico.ehu.es/userseqs allowing only a maximum of two mismatches at 3' end.

Amplification& SSR validation:From 522 primers, 50 were randomly selected (Table 2) for validation of EST-SSRs based on wet-lab experiments by screening against a panel of 25 diverse genotypes (Table 3). The amplification protocol used was as follows:

The reactions were carried out in 25µl reaction mixture containing 0.3mM dNTP's, 10mM Tris-HCl, 3mM $MgCl_2$, 50mM KCl, 0.1% Triton X-100, 1.0U Taq DNA polymerase, 0.2µM forward and reverse primers (all Eurofins Genomics India Pvt. Ltd.) and 50ng of genomic DNA. Amplification was performed in PTC-200 (MJ Research, USA) with an initial denaturation of 94°C for 3min followed by a touch-down cycle profile: 94°C for 30s, 60°C (−0.5°C/cycle) for 30s, and 72°C for 60s during 10 cycles until reaching the optimal annealing temperature of 55°C. At this temperature, 33 more cycles were achieved to complete the amplification. The products were held at 4°C. Reaction mixture in which template DNA was replaced by distilled water was used as negative control. Amplified products were resolved on 3% agarose gel (0.5X TBE) followed by ethidium bromide staining.

Genetic variability and data analysis:Each EST-SSR marker was treated as unit character and scored as a binary code (1/0). The results were analyzed using NTSYS-pc version 2.21c (Rohlf 2009) with SIMQUAL option based on Jaccard'scoefficient to generate similarity coefficients among all the possible pairs and ordered in similarity matrix (Jaccard 1908). The resulting matrices were subjected to clustering method by UPGMA (Sokal and Michener 1958). To find the robustness of the phenogram, bootstrapping was carried out (1000 replicates) with Winboot software (Yap and Nelson 1996). The goodness of fit of the clustering to the data matrix was calculated by the COPH and MXCOMP programs. Expected heterozygosity (H_e) was calculated as $H_e = 1 - \sum P_i^2$, where P_i is the frequency of the i^{th} allele. Observed heterozygosity (H_o) was calculated as the number of heterozygous genotypes divided by the total number of genotypes. Polymorphic information content (PIC) was calculated as $PIC_i = 1 - \sum P_{ij}^2$,

where P_{ij} is the frequency of j^{th} pattern for marker i and summation extends over n patterns (Anderson et al., 1993).

Gene ontology and domain analysis:For primer pairs which produced amplification, the corresponding amplified sequences from the source crops were retrieved and subjected to gene ontology and domain analysis. This exercise was carried out in 2 stages. (a) BLASTX analysis: First step of the domain analysis involved using the blastx tool. This tool converts all the amplified sequences into the 6 open reading frames and match protein sequences in the non-redundant database against it, giving the most probable translation of the amplified sequence. (b) Scanprosite analysis: Scan prosite is a web tool that gives us the various domains present with an amino acid chain. The presence of these domains will allow an easier understanding of the probable interactions that can take place between the genes and their putative functions.

Results and discussion

Frequency and distribution of EST-SSR:A total of 2,86,488 sequences were analyzed that identified 22,457 SSRs from 19,854 (12.76%) SSR-containing unique ESTs (Figure 1) which comprised of 29.08% di, 46.41% tri, 7.29% tetra, and 17.22% penta nucleotide repeats. Among the dinucleotide repeats, motif AG/CT (64.57%) was the most frequent one followed by AT/AT (29.63%) and among trinucleotide repeats, AAG/CTT (25.88%) was most common followed by AAC/GTT (12.61%), AAT/ATT (12.02%) and ATC/ATG (11.84%).

Development of EST-SSR markers and their polymorphism: From the SSR-containing ESTs, a total of 522 sequences were further shortlisted based on their presence in more than one species of which 98 were represented in three, 474 in two and none in all the four species. After designing the primers for all of them, 50 primer pairs (Table 2) were randomly chosen for amplification and polymorphism studies by applying them against a panel of 25 morphologically and genetically diverse *M. pruriens* genotypes (Table 3). Out of these, 17 primers showed amplification and three exhibited polymorphic profiles in *M. pruriens* genotypes. A total of 14 polymorphic alleles were detected from these three primers (Table 4).

Genetic variability and data analysis:The number of alleles detected per locus for the three polymorphic primers ranged from 4 to 6, with an average of 4.66 alleles per locus. The H_e ranged from 0.71 to 0.73 (with an average of 0.72) and the H_o ranged from 0.58 to 0.74 (with an average of 0.67) (Table 4). The PIC ranged from 0.64 to 0.67, with an average of 0.65. The UPGMA dendrogram (Figure 2) grouped 25 *M. pruriens* accessions into four major clusters mainly based on varietal differences. Cluster-1 majorly grouped *M. pruriens var. pruriens* accessions along with two *M. pruriens var. utilis* accessions (500101KA, 500102KA). Cluster-2 comprised exclusively *M. pruriens* var. *utilis* accessions. Cluster-3 showed intermixing of *M. pruriens* var. *utilis* and *M. pruriens* var. *pruriens*; while cluster-4 contained all the *M. pruriens* var. *pruriens* accessions. Accession 500108KA belonging to *M. pruriens* var. *utilis* distinctly out grouped from the above clusters. Likewise, accession 500151AP which is *M. pruriens* var. *pruriens* outgrouped clusters-3 and 4 showing its distinctiveness in the *M. pruriens* var. *pruriens* collection.

Gene ontology and domain analysis:To find the putative functions of all the amplified sequences, gene ontology studies were done. The obtained results were divided into 3 groups (Figure 3): (a) Cellular component functions: 7 sequences showed similarity with cellular putative proteins. 33% of the sequences shared similarity with proteins integral to the membrane and other membrane proteins and 17% with intercellular proteins and plastids. (b) Molecular function: 34 sequences showed putative functions under this category. 13% sequences showed high similarity with transferase activity related proteins, 10% showed homology with protein kinase, protein serine/ threonine kinase activity and nucleotide binding activity. (c) Biological process functions: 13 sequences showed putative functions under metabolic functions category; 23% of the sequences were homologous with metabolic process related proteins and 15% with phosphorylation proteins, oxidation reduction related proteins and protein phosphorylation proteins. This kind of study would give us an in-depth understanding of the interactions of these genes at the protein level with the results obtained detailed in Table 5.

Discussion:*M. pruriens* is relatively isolated from current genetic research due to dearth of molecular markers and genomic resources. Earlier studies have suggested that the Expressed Sequence Tags (ESTs) from the closely related species offer time and cost-effective method for harvesting polymorphic SSRs. Transferability of such EST derived SSRs have been successfully demonstrated in several legume species such as peanut, Medicago, soybean etc. (Gutierrez et al., 2005; Garcia et al., 2010). In the absence of sequence information of its own, the present work explored the possibility of developing EST-SSR resources for aiding the genetic analysis in *M. pruriens*. The benefits of SSRs markers such as potential to

reveal high level of allelic polymorphisms, co-dominant mode of inheritance, transferability across species and high reproducibility are well recognized (Kuleung et al., 2004; Miah et al., 2013).

The frequency of EST-SSRs (12.76%) discovered in the present study is much higher compared to earlier reports in *A. hypogaea* (3.62%; Liang et al., 2009), *M. truncatula* (0.4%; Chandra, 2011) and P. sativum (3.2%; Gong et al., 2010). However, the redundancy in sequences obtained from dbEST was compensated by choosing the processed TIGR Plant Transcript assemblies and TIGR gene indices. Nonetheless, independent assembly using the TGICL package may also be used for this purpose.

The frequency and type of SSRs obtained was consistent among the analyzed legume species. In all these cases, trinucleotide repeats were the most frequent which is in consensus with other legume species (Cardle et al., 2000; Gao et al., 2003; Varshney et al., 2004). However, in case of C. arietnium dinucleotide repeats were present in higher frequency than trinucleotides repeats.

It was observed that the highest percentage of SSR containing sequences was present in *Vigna unguicularis* (0.0798%) followed by *Cicer arietinum* (0.077%), *Phaseolus vulgaris* (0.061%) and *Glycine max* (0.068%). The contribution of G. *max* and *P. vulgaris* sequences to the final primer design was higher compared to *C. arietinum* which accounted lowest. Amplification and polymorphism percentage (17.65%) was higher for the primers designed from sequences that were common in 3 species as compared to two. This may be due to the amount of sequence information available and the extent of evolutionary distance between the species used.

Earlier reports indicate that the transferability of EST-SSRs from one species to another ranged from 40–89% (Thiel et al., 2003; Holton et al., 2002; Eujayl et al., 2004; Gao et al., 2003, Yu et al., 2004; Saha et al., 2004 & 2005; Varshney et al., 2005). In the present study, the percentage of transferability achieved for *M. pruriens* (34%) was lower than those reported for *M. truncatula* to *V. faba* (43%), *Cicer sp*. (39%) and *P. sativum* (40%) (Gutierrez et al., 2005), and higher than that reported for *P.vulgaris* to *G.max* (20%), and Dipterix (6%) (Garcia et al., 2010). The results thus demonstrate reasonable success of this technique for development of newer genomic resources.

The set of 3 EST-SSR primers used in this study revealed a total of 14 polymorphic alleles, with an average of 4.66 per locus. The H_e, H_o and PIC values were 0.72, 0.67 and 0.65, respectively. Earliest study using RAPD (Leelambika et al., 2010; Leelambika and Sathyanarayana, 2011) found heterozygosity in M. pruriens accessions to be 0.24 (estimating 18 accessions) and 0.14 (estimating 35 accessions), respectively. Higher heterozygosity estimates obtained in the present study reflect the ability of SSR markers to reveal unique alleles in the population. Similarly, the UPGMA tree based on similarity estimates grouped the 25 accessions into four major clusters based on varietal differences. The grouping of two *M. pruriens* var. *utilis* accessions (500101KA, 500102KA) along with *Mucuna* var. *pruriens* in cluster-I, as well as a few intermixed accessions found in cluster-II might be naturalized hybrids of *Mucuna* var. *pruriens* x *M. pruriens* var. *utilis*. This is not uncommon in M. pruriens natural population as these botanical varieties exhibit considerable inter-varietal compatibility. Such hybrids are also reported in earlier works of Padmesh et al. (2006). Likewise the out-grouping of accession 500108KA (*M. pruriens* var. *utilis*) might be due to unique morpho-agronomic characters displayed by the plant such as: white flower colour, non-itching pod, high seed pod and seed yield and longer life cycle. Also, the accession 500151AP which out grouped clusters 3 and 4 exhibited higher yields and other agronomic characters in comparison to other *M. pruriens* var. *pruriens* accessions in that group (data not shown).

Gene Ontology and domain data mining exercise allowed us to correlate the presence of the domains with their putative functions. In cases where the putative functions were not able to be assigned, using the domain information obtained, it was possible to understand the role of the gene. One reason why a few sequences showed no hits on scan prosite is due to the presence of low homologous sequences that were obtained after blastx analysis. A few sequences with as low as 17% coverage were obtained. This isn't conducive for the detection of domains.

Conclusion

In the absence of sequence information in M. pruriens, it has been demonstrated from the present study that transferability of EST-SSRs from a closely related species provides promising alternative for development of codominant SSR markers. However, limited number of primers and less robust genotyping method used restricts the relevance of the analysis for better scientific inferences. Therefore, testing large number of primers from an extended EST collection using high-throughput genotyping is recommended for ascertaining the actual percentage of transferable

EST markers that can be achieved through this method. Besides, dropping sequencing costs offers transcriptome analysis and genotyping by sequencing as some of the potential options for the development of species specific markers - the success of which might open up newer vistas for molecular breeding applications in medicinal legume species in general and *M. pruriens* in particular.

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Table 1. Details on the the sequences analyzed from different legume species. for identifying EST-SSRs

Species	Total number of sequences examined	Total number of identified SSRs	Number of SSR containing sequences	Largest incidence of SSR	
				Unit size	Number of SSRs
Glycine max	231658	18873	16559	3	8010
Phaseolus vulgaris	20629	1851	1578	3	736
Cicer arietinum	1272	112	106	2	56
Vigna anguicularis	32929	3708	3148	3	1646

Table 2. Sequences of 50 EST-SSR primers used for validation

GeneID	Primer name	Primer Sequence 5' to 3'
gmTA46747_(F)	MUC_1	GGAAGAGACCCTCAATTCTT
gmTA46747_(R)	MUC_1	CAGGGAGAACACTCTCTTTGT
pvTA255_(F)	MUC_4	AATTCAGTGACCAGTTGAGG
pvTA255_(R)	MUC_4	AGATTCCTCCCTTGTTGAAG
gmTA40944_(F)	MUC_8	CTGGTGGTGGTTTCTTCTAGT
gmTA40944_(R)	MUC_8	AATTCCGACCACAACCTC
gmTA43454__(F)	MUC_11	GTAAAGAGATGCTTCCACCA
gmTA43454__(R)	MUC_11	CTCCAAGTCTTCCCTGATCTA
vuTC7090_(F)	MUC_14	CTGTGGTTAACAGGAAGAGAC
vuTC7090_(R)	MUC_14	CCTTGTATTCCTCTGATTGC
vuTC7068_(F)	MUC_15	CCCAAAGAAAGGTGAAGAAG
vuTC7068_(R)	MUC_15	GAGCAATGGACTACCTTTTG
vuTC17148_(F)	MUC_21	CCTAGCTTCACATCCTCTTG
vuTC17148_(R)	MUC_21	ATCCAAGATCAGGCACAGTA
vuTC15897_(F)	MUC_22	GTACTAGCGCATTACTTCCAC
vuTC15897_(R)	MUC_22	CCCACTTTCCTTTACTGAAC
vuFG839710_(F)	MUC_24	GGCTGAATGAACTAATGGAG
vuFG839710_(R)	MUC_24	GGAGTTTGAAGGAGAGTAGTGA
gmAW348838_(F)	MUC_28	GAGCGATGAATGGACAGAA
gmAW348838_(R)	MUC_28	ACTCGAACTCAAACCACAAC
gmBE473390_(F)	MUC_29	GAAGCCAAAGTTCCATTACC
gmBE473390_(R)	MUC_29	CCCTATCCATTCCTCTATTCC
gmCA783351_(F)	MUC_31	CTGGAACAAGAGTCGAAAGAG
gmCA783351_(R)	MUC_31	TTTCGGGATCGGAGAAAGA
gmCO979073_(F)	MUC_34	TGTTGAACTCCATGACAGACC
gmCO979073_(R)	MUC_34	CACAACAAGTGGAGAGCATAAC
gmCO983158_(F)	MUC_36	ACATTGTCGCATGGGTTAG
gmCO983158_(R)	MUC_36	GAACTGCTGCTTTTGGATTG
pvCV535219_(F)	MUC_40	GCAAAGACAGCTTCAACCAA
pvCV535219_(R)	MUC_40	TTCTCCCAAACAGACCAAG
pvTA263_(F)	MUC_48	CTGAGAATCCTACACCTGCTT
pvTA263_(R)	MUC_48	GGAGCACAAGAACACAACATC

Note: The polymorphic primers highlighted in bold. (F)- Forward and (R)- Reverse

Table 3. *Mucuna pruriens* accessions analyzed through EST-SSR markers

Sl. No.	Accession Name	Accession Number	Place of collection	Latitude and longitude
1.	*M. pruriens* var. *utilis*	500102KA	Karnataka	-
2.	*M. pruriens* var. *utilis*	500101KA	Karnataka	-
3.	*M. pruriens* var. *utilis*	500108KA	Karnataka	13°14' N, 77°62' E
4.	*M. pruriens* var. *utilis*	IC385925	Jharkand	24°26' N, 87°24' E
5.	*M. pruriens* var. *utilis*	IC385928	NBPGR	-
6.	*M. pruriens* var. *utilis*	IC392850	NBPGR, Orissa	22°06' N, 86°40' E
7.	*M. pruriens* var. *utilis*	IC385841	NBPGR, Jharkand	24°62' N, 87°84' E
8.	*M. pruriens* var. *utilis*	IC392241	NBPGR, Jharkand	23°78' N, 86°43' E
9.	*M. pruriens* var. *utilis*	IC385926	NBPGR, Jharkand	24°26' N, 87°24' E
10.	*M. pruriens* var. *utilis*	IC385842	NBPGR, Jharkand	24°48' N, 86°69' E
11.	*M. pruriens* var. *utilis*	IC326953	Himachal Pradesh	30°90' N, 77°09' E
12.	*M. pruriens* var. *utilis*	IC471870	NBPGR, Delhi	28°62' N, 77°23' E
13.	*M. pruriens* var. *utilis*	500155AP	Andhra Pradesh	-
14.	*M. pruriens* var. *pruriens*	500112KA	Karnataka	12°18' N, 76°42' E
15.	*M. pruriens* var. *pruriens*	500113MH	Maharashtra	20°00' N, 73°77' E
16.	*M. pruriens* var. *pruriens*	500110KA	Karnataka	13°33' N, 77°10'E
17.	*M. pruriens* var. *pruriens*	IC265577	NBPGR, Kerala	9°58' N, 76°52' E
18.	*M. pruriens* var. *pruriens*	IC391941	NBPGR, Orissa	21°65' N, 85°63' E
19.	*M. pruriens* var. *pruriens*	500138TN	Tamil Nadu	10°38' N, 78°82' E
20.	*M. pruriens* var. *pruriens*	500123KL	Kerala	-
21.	*M. pruriens* var. *pruriens*	500109KA	Karnataka	13°56' N, 75°38' E
22.	*M. pruriens* var. *pruriens*	500115TN	Tamil Nadu	-
23.	*M. pruriens* var. *pruriens*	500144AP	Andhra Pradesh	18°03' N, 78°18' E
24.	*M. pruriens* var. *pruriens*	500146AP	Andhra Pradesh	18°39' N, 78°10' E
25.	*M. pruriens* var. *pruriens*	500151AP	Andhra Pradesh	19°37' N, 78°30' E

Table 4. Diversity statistics for the 3 EST-SSR loci described by the number of alleles (N_a) per locus, expected (H_e) and observed (H_o) heterozygosities, and polymorphism information content (PIC) observed in the 25 *M.pruriens* genotypes.

LocusName	N_a	H_e	H_o	PIC
MUC_4	4	0.72	0.71	0.64
MUC_31	6	0.73	0.74	0.67
MUC_48	4	0.71	0.58	0.64
Total	14	2.16	2.03	1.95
Mean	4.66	0.72	0.67	0.65

*, **. Significant at P= 0.05 and P=0.0 1

Table 5. Details of Gene ontology and domain analyses conducted

GI Id	Putative Functions	Domain Found
pvTA255	-	DCD Domain
pvTA263	Nucleotide binding, Kinase activity, Protein phosphorylation, Signal transduction, Transferase activity	Protein kinase, NAF, Protein kinase serine thronine and ATP domains
pvCV535219	DNA binding, Zinc ion binding activity	ZF_DOF 1 and 2 domain
pvTA2970	Catalytic activity, Transferase activity	Transketolase Signatures
gmTA40944		No Hits
gmAW348838	Nucleic Acid binding activity	Eukaryotic RNA recognition motif
gmBE473390	Integral to membrane, transport activity	No Hits
gmCO983158	Membrane protein, Nucleotide binding, Catalytic activity, Receptor Activity, Kinase activity, Transeferase activity, Oxidoreductase activity	Leucine rich domain
gmCA783351	Metal ion binding activity, Metal ion transport	Heavy metal associated domain
vuFG839710	-	No Hits
vuTC7090	Catalytic activity	Transketolase Signatures
vuTC7068		No Hits
vuTC17148	Intracellular proteins, Zinc ion binding activity	Zinc finger box profile
gmTA46747	Catalytic activity, Transferase activity	Transketolase Signatures
gmTA43454	-	Ankyrin repeat profile, Zinc finger C3H1 type profile
vuTC15897	-	No Hits
gmCO979073	Cysteamine dioxygenase activity	No Hits

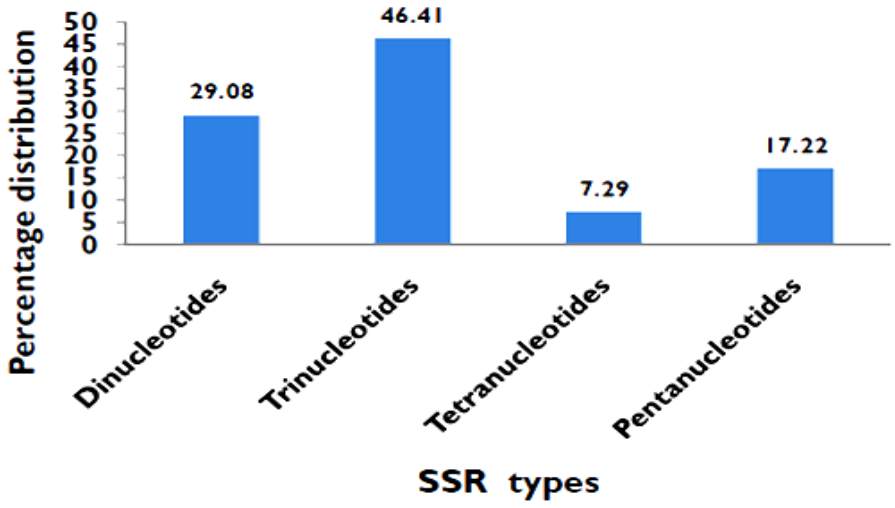


Figure 1. Frequency distribution of different nucleotide repeats in identified SSR sequences.

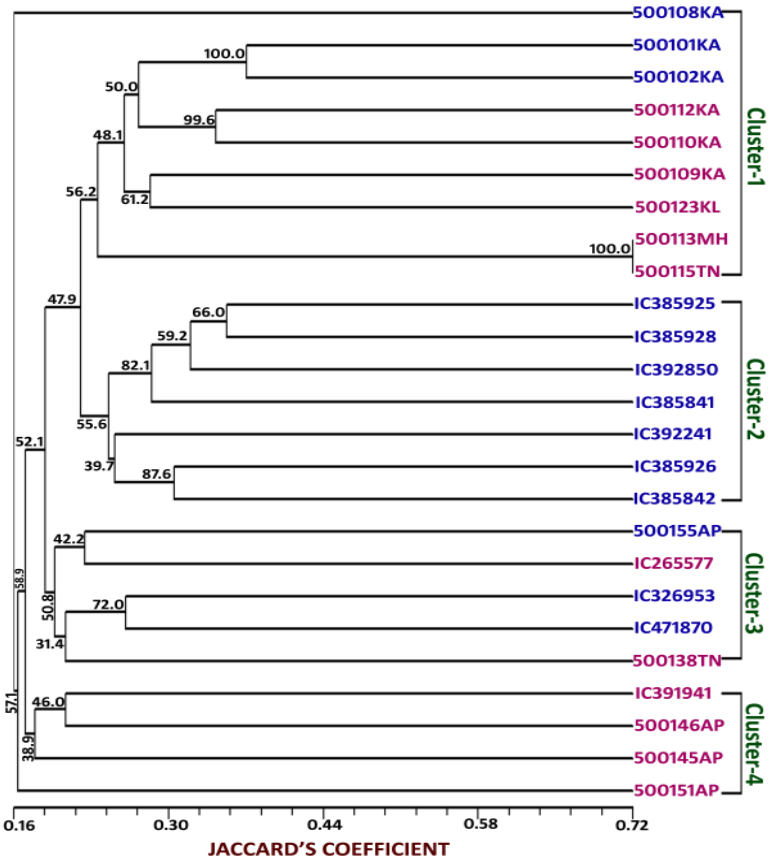
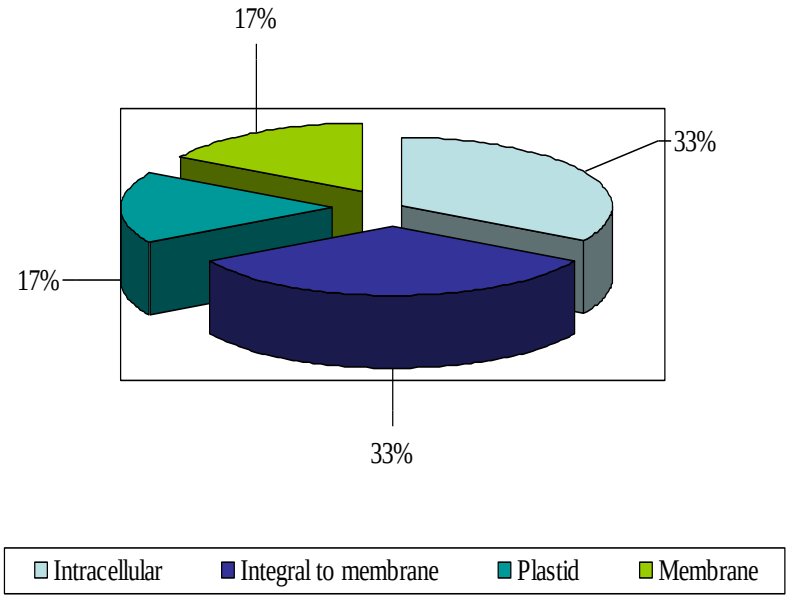
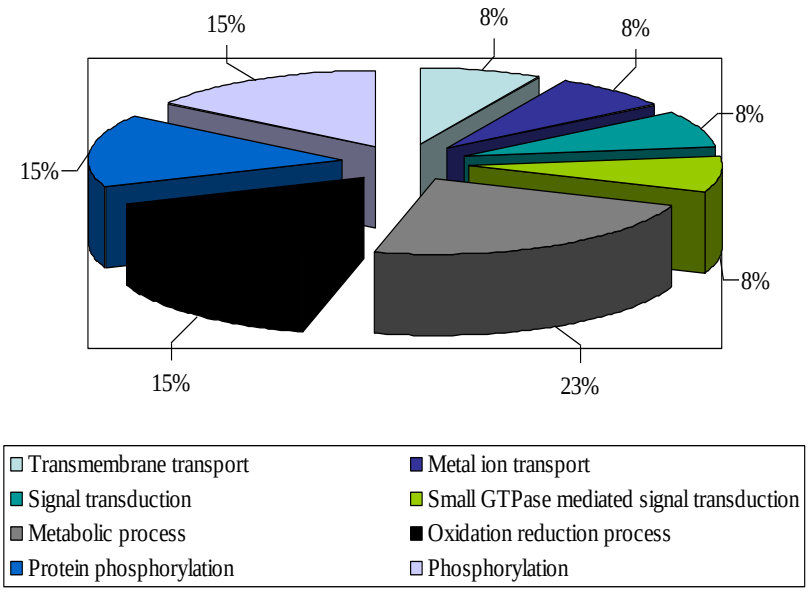


Figure 2. Dendrogram of the 25 *M.pruriens* genotypes obtained after UPMGA cluster analysis. Blue- *M. pruriens* var. *utilis*; Pink- *M. pruriens* var. *pruriens*

a) Cellular Putative Functions



b) Biological Putative Functions



c) Molecular Putative Functions

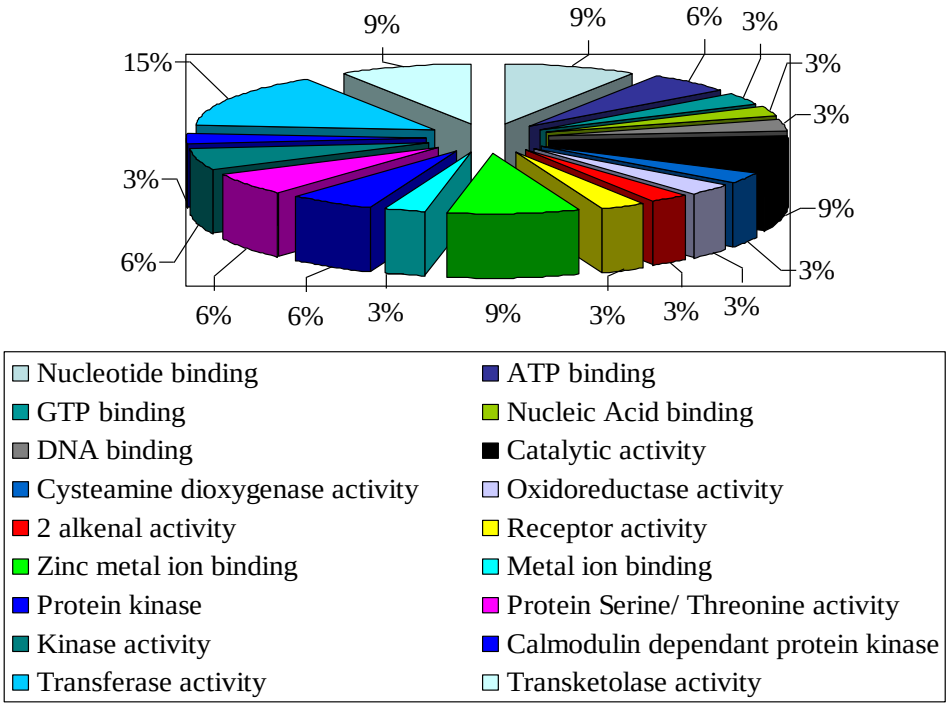


Figure 3. Putative functions of the 17 amplified sequences based on Gene Ontology analysis