

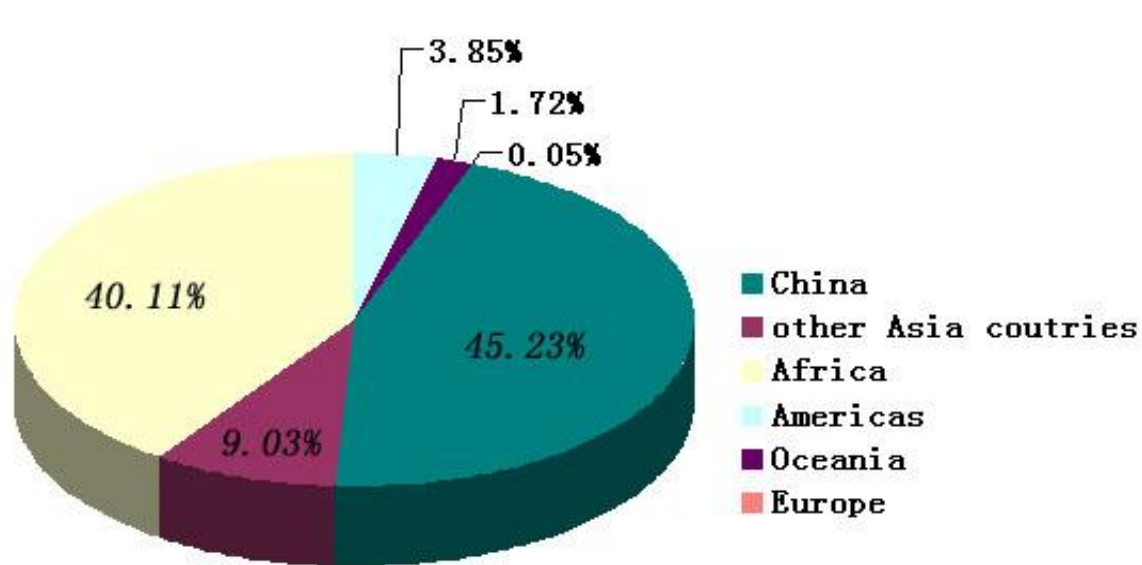
The 5th Korea-China-Japan Sweetpotato Workshop

**Characterization and development of EST-derived
SSR markers in sweetpotato**

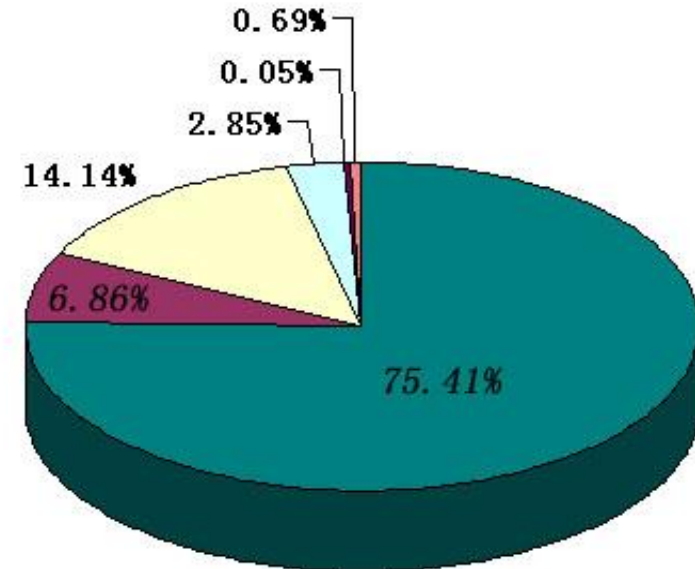
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Area and production of sweetpotato



Area harvested



Production

FAO, 2010

Multiple uses of sweet potato

Food

Animal feed

Processing as food, starch, and other industrial products



The Simple Sweet Potato Improves Health




Baby Food

Current status of sweetpotato genomic resources


9/2012

9/2010

634  PubMed: biomedical literature citations and abstracts

518  PubMed: biomedical literature citations and abstracts

463  PubMed Central: free, full text journal articles

281  PubMed Central: free, full text journal articles

none  Site Search: NCBI web and FTP sites

none  Site Search: NCBI web and FTP sites

58287  Nucleotide: Core subset of nucleotide sequence records

2378  Nucleotide: Core subset of nucleotide sequence records

23982  EST: Expressed Sequence Tag records

22726  EST: Expressed Sequence Tag records

1  GSS: Genome Survey Sequence records

1  GSS: Genome Survey Sequence records

1801  Protein: sequence database

1144  Protein: sequence database

1  Genome: whole genome sequences


10  Genome: whole genome sequences

10  Structure: three-dimensional macromolecular structures

6  Structure: three-dimensional macromolecular structures

1  Taxonomy: organisms in GenBank


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none  SNP: short genetic variations

none  SNP: single nucleotide polymorphism

none  dbVar: Genomic structural variation

none  dbVar: Genomic structural variation

47  Gene: gene-centered information

35  Gene: gene-centered information


11  SRA: Sequence Read Archive

none  SRA: Sequence Read Archive

2  BioSystems: Pathways and systems of interacting molecules

1  BioSystems: Pathways and systems of interacting molecules

none  HomoloGene: eukaryotic homology groups

none  HomoloGene: eukaryotic homology groups

102  Probe: sequence-specific reagents

1  Probe: sequence-specific reagents

6  BioProject: aggregated biological research project data

none  Genome Project: genome project information

NGS references

1. Schafleitner, R., et al., *A sweet potato gene index established by de novo assembly of pyrosequencing and Sanger sequences and mining for gene-based microsatellite markers*. BMC Genomics, 2010. 11: p. 604.
2. Wang, Z., et al., *De novo assembly and characterization of root transcriptome using Illumina paired-end sequencing and development of cSSR markers in sweet potato (Ipomoea batatas)*. BMC Genomics, 2010. 11: p. 726.
3. Tao, X., et al., *Digital gene expression analysis based on integrated de novo transcriptome assembly of sweet potato [Ipomoea batatas (L.) Lam]*. PLoS One, 2012. 7(4): p. e36234.
4. Xie, F., et al., *De novo sequencing and a comprehensive analysis of purple sweet potato (Ipomoea batatas L.) transcriptome*. Planta, 2012. 236(1): p. 101-13.

More EST sequences

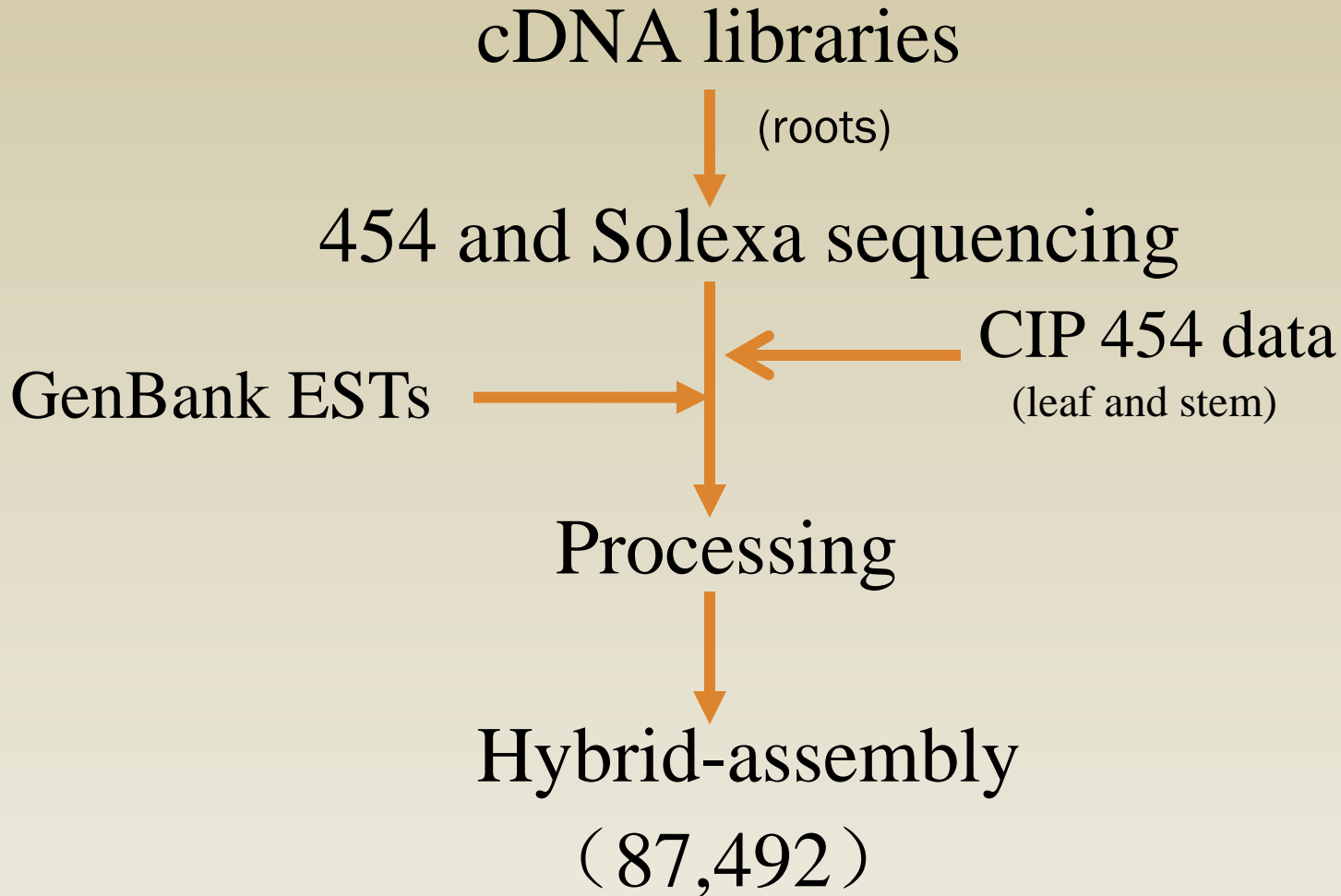
Few molecular markers

Low resolution genetic map

another bottleneck for genetic breeding

SNP or SSR ?

Big EST assembly

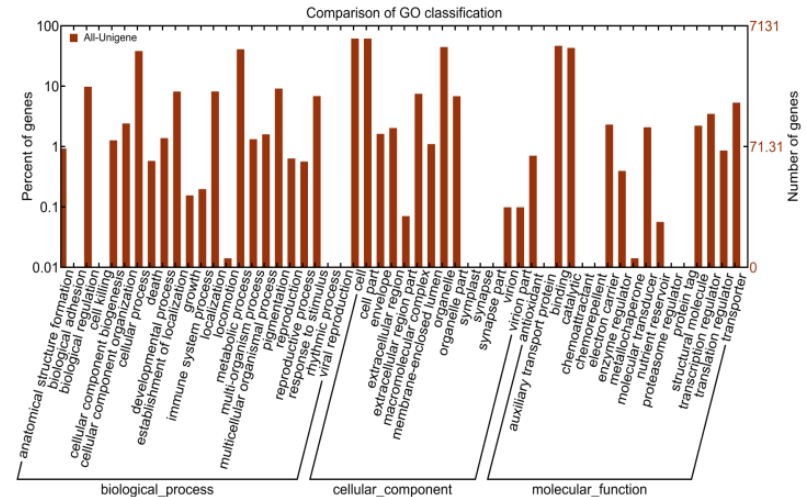
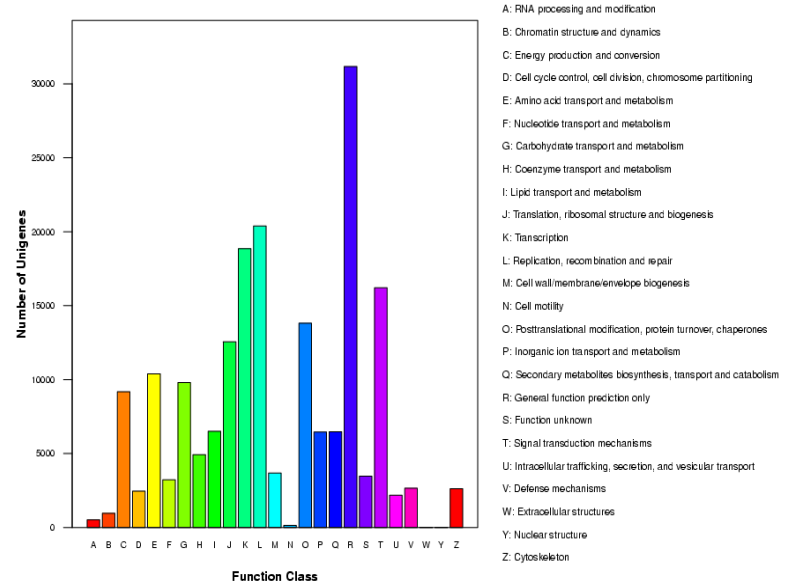


Functional annotation and pathway analysis

	Unigenes	
	Numbers	Percentage
Nr		
Matches to database	27,438	48.54%
Unique matches to proteins	18,496	32.73%
SwissProt		
Matches to database	26,287	46.21%
Unique matches to proteins	11,914	21.08%

KEGG pathway	Numbers
metabolic processes	10,631
Transcription	1162
Translation	559
Folding, Sorting ,Degradation	896
Replication and Repair	588
Transport	646
Signal transduction	352
Response to stress	333

COG Function Classification of Dioscorea_totalRNA-Unigene.fa Sequence

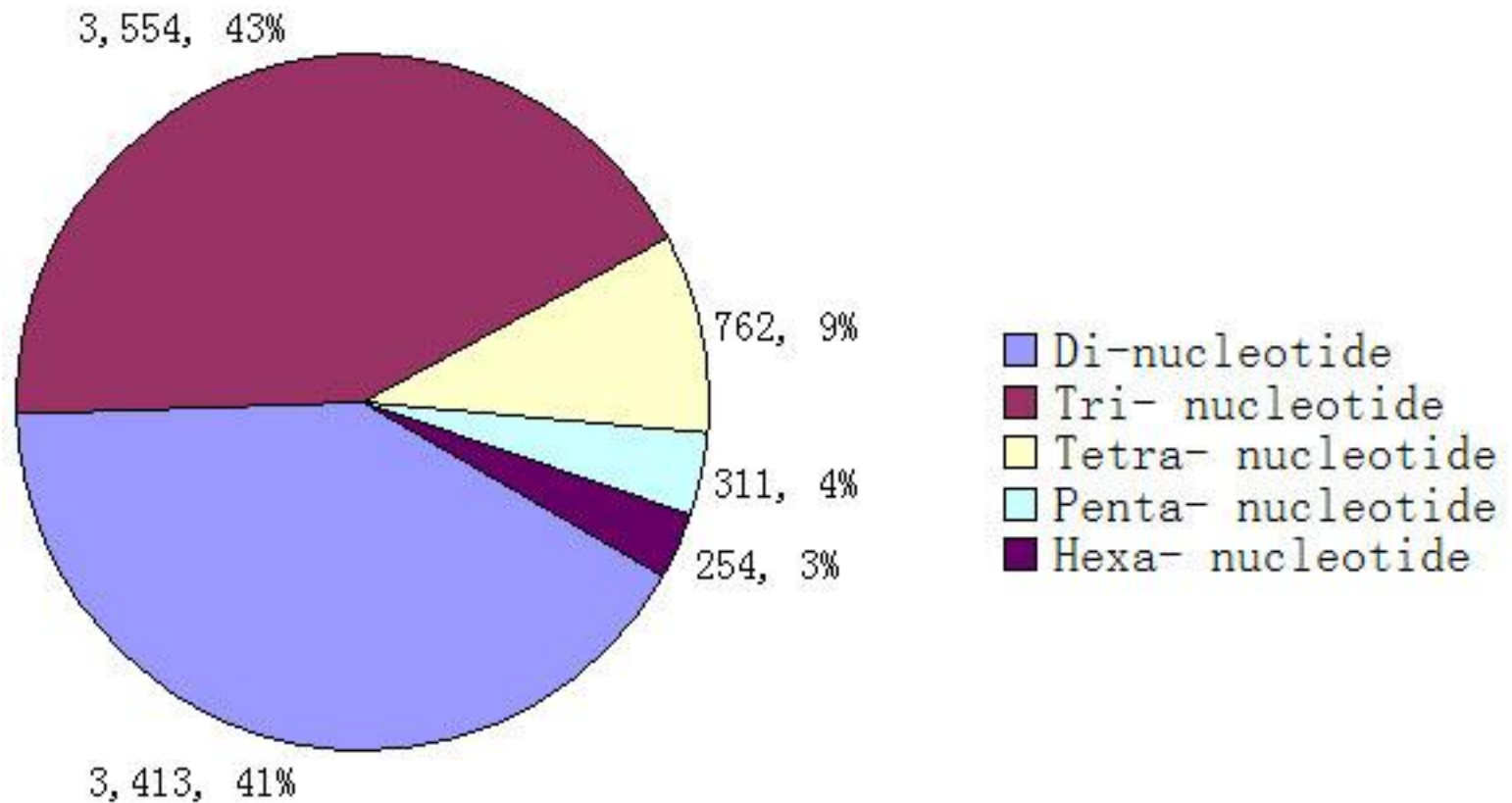


Summary of EST-SSR searching results

Searching Items	Numbers
Total number of sequences examined	87,492
Total size of examined sequences (bp)	58,678,639
Total number of identified SSRs	8,294
Number of SSR containing sequences	7,163
Number of sequences containing more than 1 SSR	949
Number of SSRs present in compound formation	539

MISA <http://pgrc.ipk-gatersleben.de/misa/>

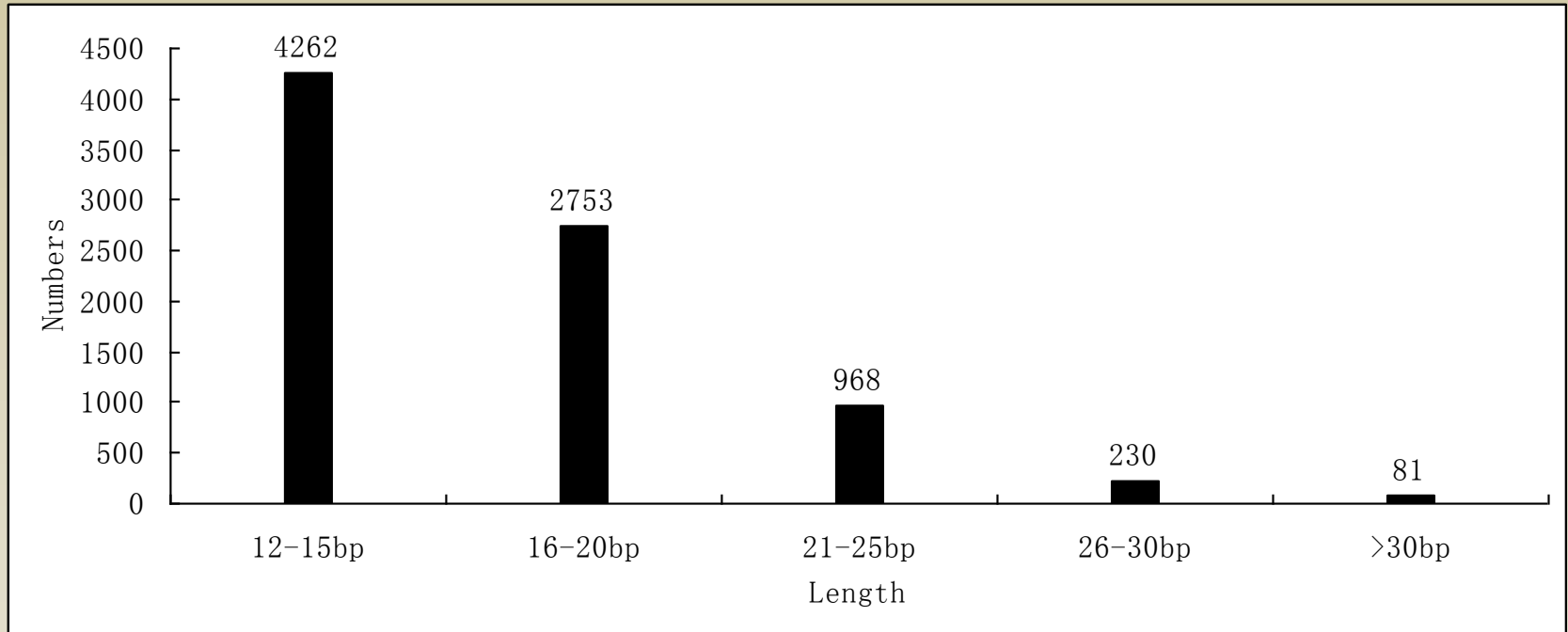
Frequency distribution of EST-SSRs based on nucleotide types



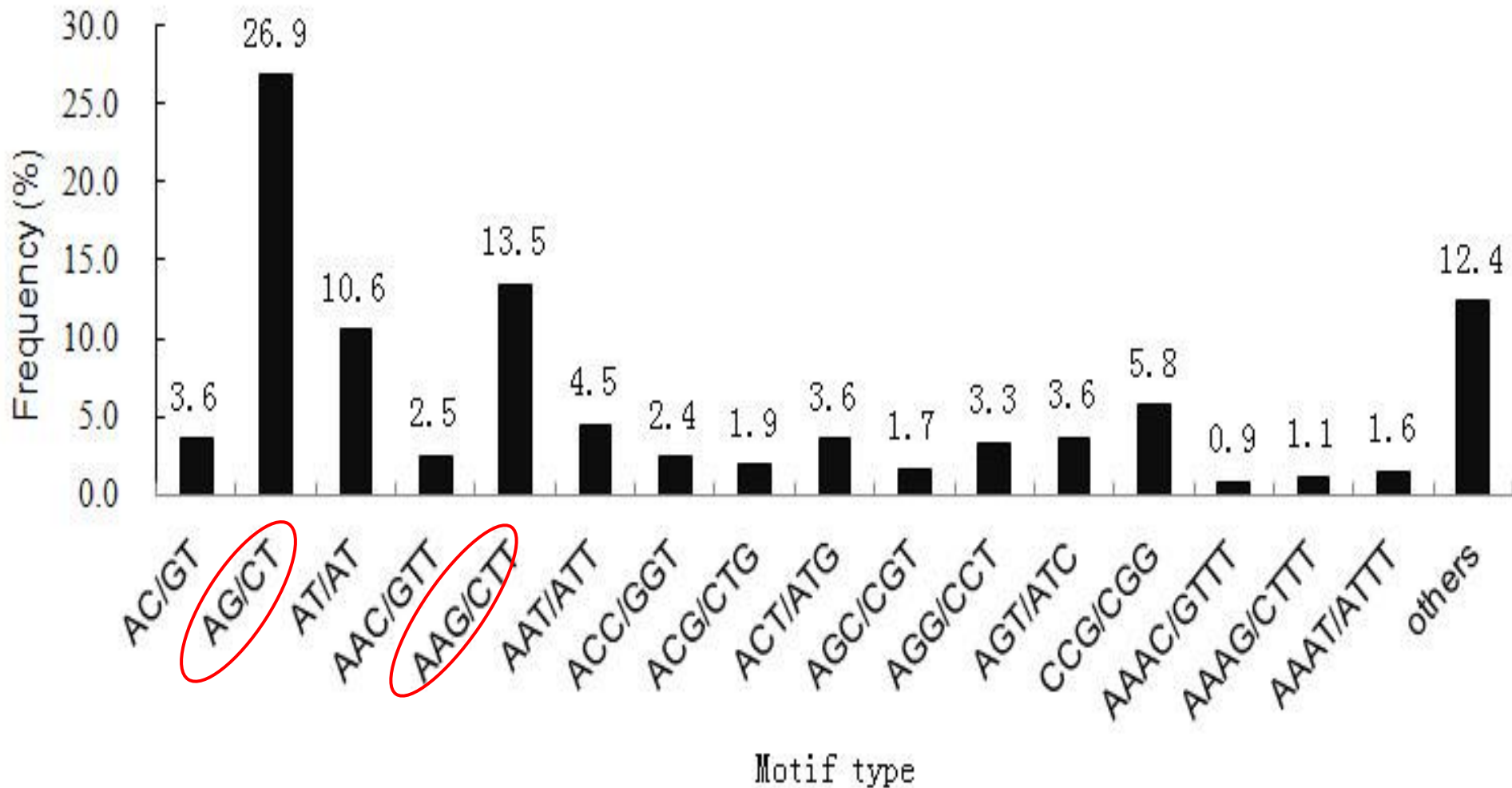
Repeat unit distribution of EST-SSRs

# of repeat unit	Di-	Tri-	Tetra-	Penta-	Hexa-	Total
4	0	0	527	248	207	982
5	0	2188	158	50	34	2430
6	1286	840	48	11	9	2194
7	788	306	18	1	2	1115
8	468	126	6	1	0	601
9	319	50	2	0	1	372
10	193	15	0	0	1	209
11	121	12	1	0	0	134
12	110	7	1	0	0	118
13	49	5	1	0	0	55
14	17	1	0	0	0	18
≥15	62	4	0	0	0	66

Length distribution of EST-SSRs



SSR length was mostly distributed from 12 to 20 bp, accounting for 84.6%, followed by 21-30 bp length range (14.4%).



Frequency distribution of EST-derived SSRs based on motif sequence types

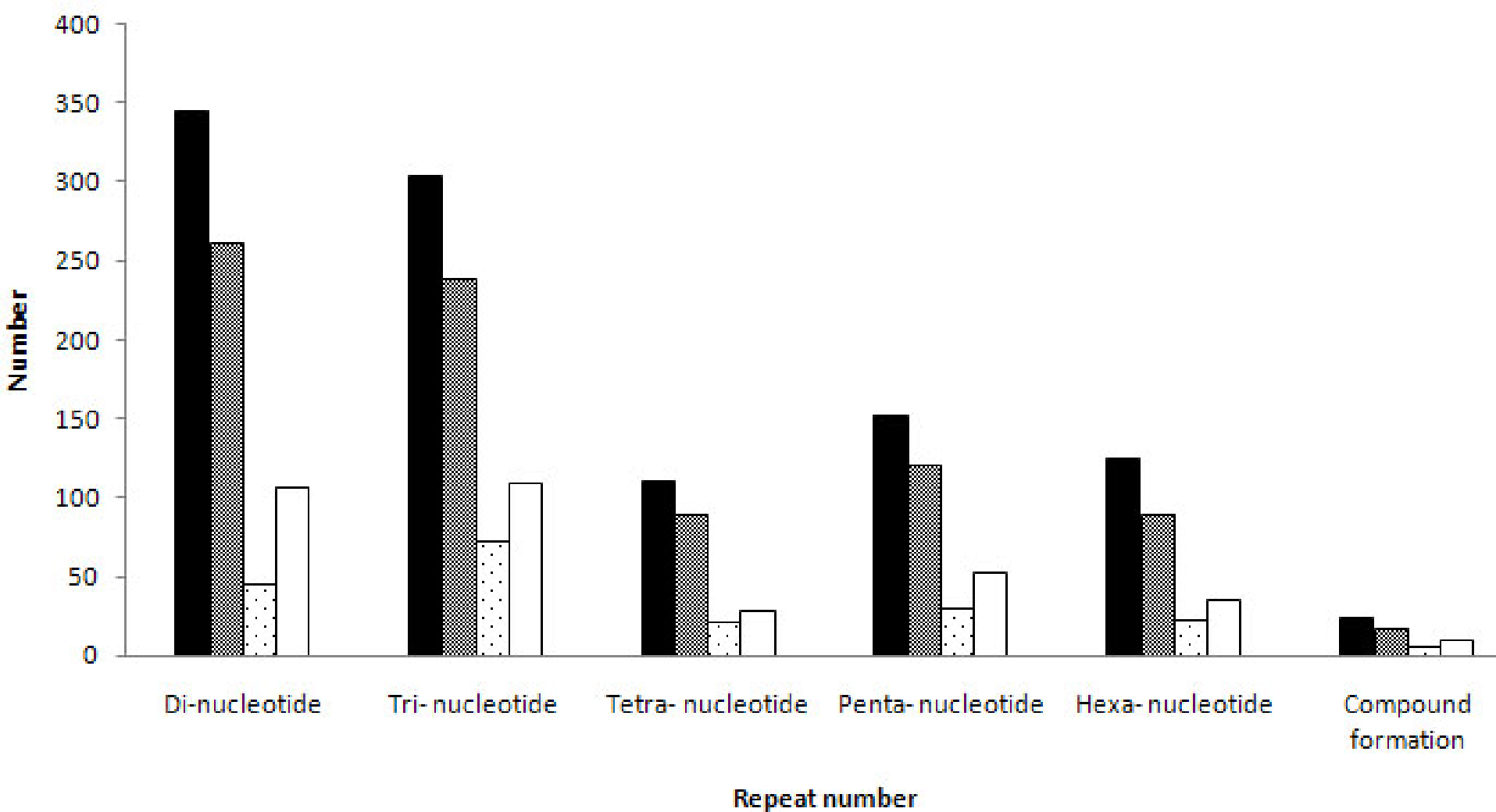
A total of 224 SSR motifs were identified. The AG/CT was the most abundant motif, followed by AAG/CTT, AT/TA, CCG/CGG, AAT/ATT, AGT/ATC, AC/GT, ACT/ATG, AGG/CCT and AAC/GTT. The frequency of remaining 214 types of motifs accounted for 22.0%.

Sweetpotato accessions used for EST-SSR validation and evaluation

Nursery No	Cultivar name	Origin	Description
GN1284	E Shu 3 Hao	China	Improved variety, mapping parent
GN1337	Guang 2K-30	China	Improved variety, mapping parent
GN0442	Nancy Hall	USA	Introduced variety
GN0520	Sheng Li Bai Hao	Japan	Introduced variety
GN1245	AB940078-1	Peru	Improved variety
GN0815	Nortnnigo	Philippines	Introduced variety
GN1256	Tai Nong 57	Taiwan	Improved variety
GN0386	Hua Bei 553	China	Improved variety
GN0069	Yu Bei Bai	China	Landrace
GN0010	Bao Ting Zhong	China	Landrace

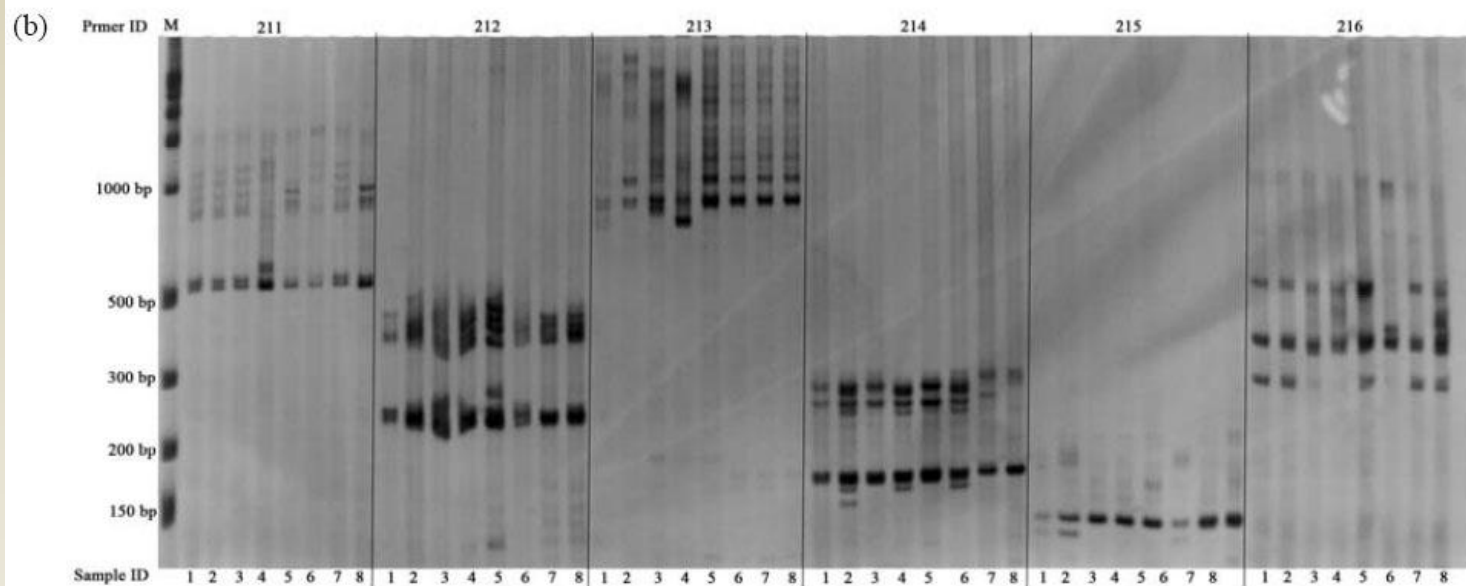
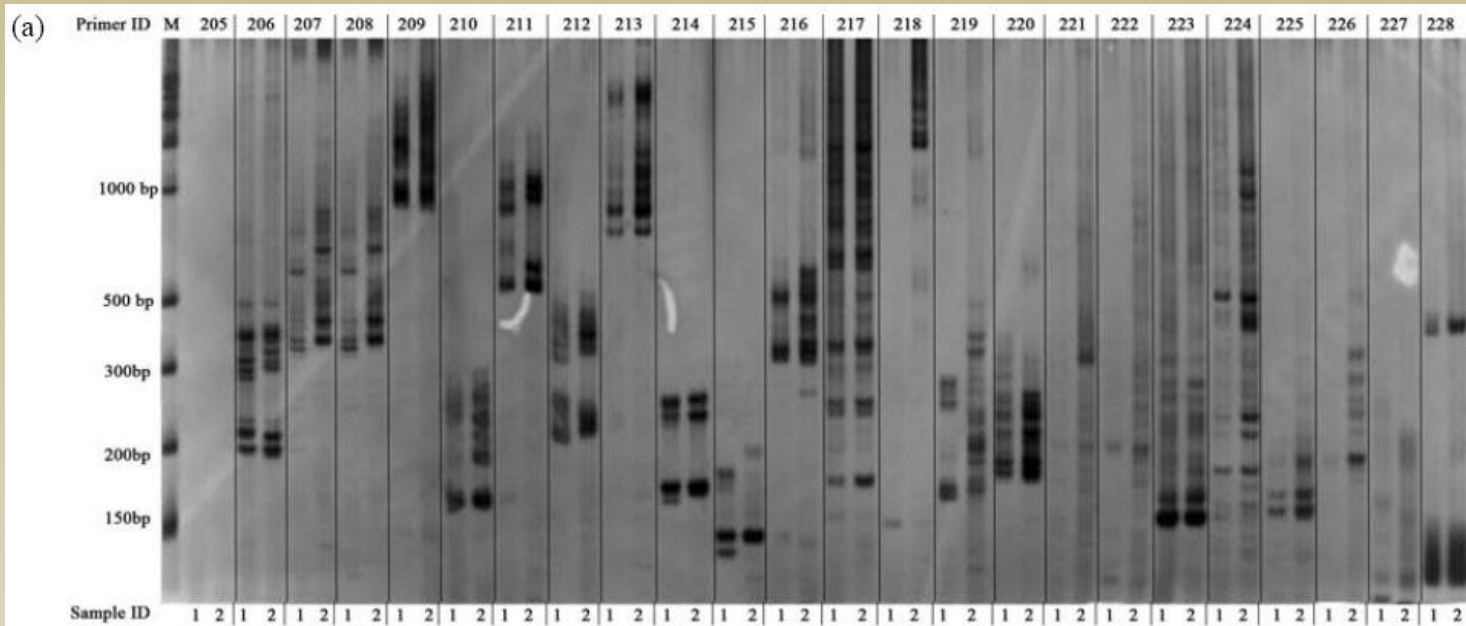
Number of designed and polymorphic primer pairs

	Numbers	Percentage (%)
primer pairs designed	1060	
primer pairs amplified	897	84.6
polymorphic loci in two parents	195	21.7
polymorphic loci in eight accessions	342	38.2



Number of designed primer pairs and polymorphic primer pairs.

Number of primer pairs designed (black columns), primer pairs amplified (gray columns), polymorphic loci in two parents of our mapping population (dotted white columns) and polymorphic loci in the eight cultivars (white columns).



Examples of PCR products amplified by SSR primer pairs. (a) PCR products amplified by 24 primer pairs (GDAAS 205-228 listed on the top of the gel image) in E Shu 3 Hao and Guang. (b) PCR products in eight sweetpotato cultivars amplified by six effective SSR primer pairs selected from figure 3a.

Conclusion

- (1) gives an insight into the frequency, type and distribution of sweetpotato EST-SSRs
- (2) demonstrates successful development of EST-SSR markers in cultivated sweetpotato.
- (3) could enrich the current resource of molecular markers for the sweetpotato community
- (4) would be useful for trait mapping, marker-assisted selection , evolution and genetic diversity studies.

BMC Plant Biology, 2011, 11:139

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