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APPLICATION OF MOLECULAR MARKERS IN DUS TESTING OF NEW VARIETIES OF CHINESE CABBAGE

Document prepared by an expert from China

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The annex to this document contains a copy of a presentation on “Application of molecular markers in DUS testing of new varieties of Chinese cabbage”, prepared by an expert from China, to be made at the first session of the TWM.

[Annex follows]

Application of molecular markers in DUS testing of new varieties of Chinese cabbage

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Background

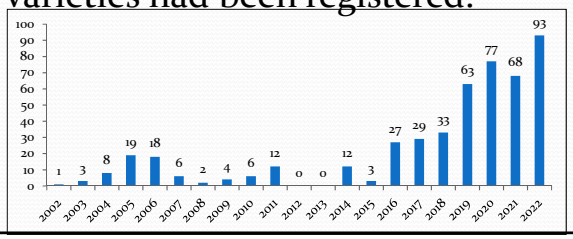
- Chinese cabbage is the largest vegetable crop grown in China, with an annual planting area of 1,500,000 hectares.
- Also grown in Japan, Republic of Korea and other countries in the world.

Background

- According to China's Seed Law, DUS test is required for Chinese cabbage variety right protection and variety registration.
- China started DUS testing of Chinese cabbage in 2000.

✓By July 2022, 485 Chinese cabbage varieties had been protected by variety rights.

✓ 2555 varieties had been registered.



Background

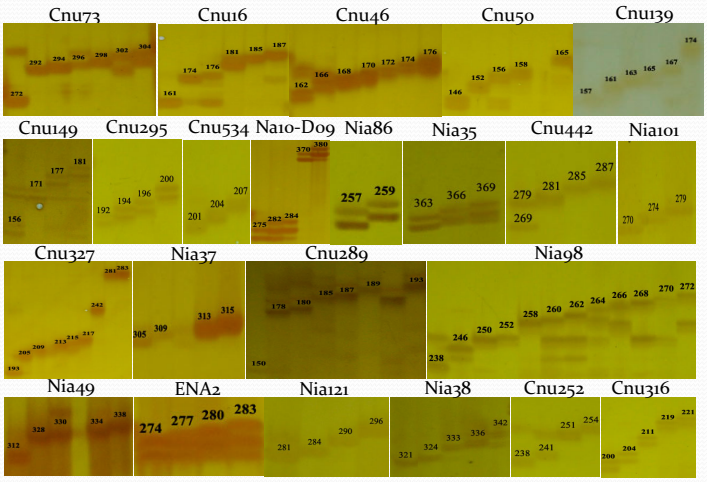
- The screening of the collection for similar varieties using characteristic-based method is time-consuming and laborious.
- In addition, the precision of the selection is often unsatisfactory with a large number of similar varieties to be planted
- In 2009, we started to work on SSR marker-aided management of reference varieties of Chinese cabbage.

DNA profiling protocol development

- Selection of SSR markers and detection platforms were according to the BMT guidelines(UPOV, 2010).
- 30 markers were chosen with 3 makers on each chromosome.
- alleles at each locus were named in the form of "size" in base pairs.
- Reference variety was determined for each allele.

DNA profiling protocol development

Alleles size determined

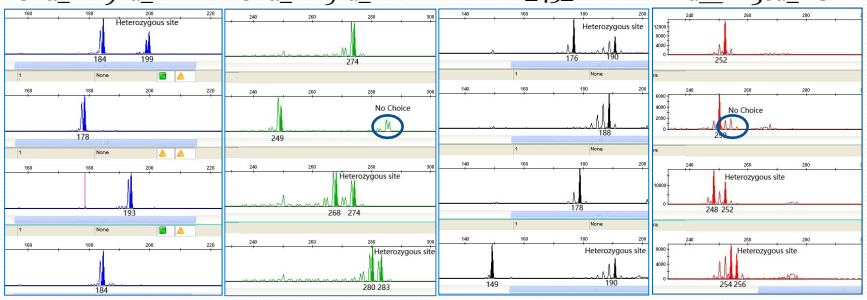


Polyacrylamide Gel Electrophoresis, PAGE

DNA profiling protocol development

Alleles size determined

Cnu_m090a_FAM | Cnu_m252a_HEX | CRIB1_43_TAM | nia__m138a_ROX



The allele will be choice as one real allele when the signal strength of the allele is more than half

Capillary Electrophoresis, CE

DNA profiling protocol development

SSR	alleles	SSR	alleles	SSR	alleles	SSR	alleles	SSR	alleles	SSR	alleles
nia121	281	**nia98**	238	**cnu289**	150	**cnu295**	192	**cnu90**	169	**nia138**	249
	284		246		178		194		184		251
	290		250		180		196		193		253
	296		252		187		198		199		255
	299		258		189		200		201		257
cnu149	156		260		191	**cnu252**	238		204		259
	171		262		193		241	**cnu316**	200		261
	177		264	**nia35**	363		251		204		263
	181		266		366		254		211		267
cnu537	180		268		369	**cnu50**	146		219	**Cnu288**	145
	187		270	**cnu46**	161		152		221		147
	189		272		174		156	**cnu327**	193		149
	191	**cnu182**	268		176		158		205		151
	193		271		181		160		209		155
	195		274		185		165		211	**Nia22**	309
	197		277		187	**nia37**	305		213		313
	199		280	**cnu534**	201		309		215		316
	201		283		204		311		217		318
	203		286		207		313		242		325
nia101	270	**nia86**	257		210		315		281	**ENA2**	274
	274		259		261		319		283		277
	279	**Cnu73**	272	**Na10-D09**	275	**Cnu16**	162	**nia49**	312		280
cnu442	269		292		282		166		372		283
	279		294		284		168		381	**Cnu139**	157
	281		296		370		170		332		161
	285		298		378		172		334		163
	287		302	**nia38**	321		174		338		165
cnu530	285		304		324		176				167
	287				330						174
	291				336						
					342						

DNA profiling protocol development

NO.	SSR	Chr.	Sequence of SSR	Fluorescence	Alleles size	Reference variety
1	CRIB1-43	A1	F: TGAAATGCTGTGAGTTGTTGTGAT R: TTCAACCTTTTCTTTGCCTTCT	5′ HEX	269 272 275 278 281 284 287 290 293	
2	CRIB1-54	A1	F: TCCGAGTAAGCGAATTGGTAAAG R: GAAGCACTATCCCCACAAGAAAC	5′ HEX	221 224 227 238	
3	nia_m138a	A1	F:GTTTTAAATGCCGCGTTG R:GGGATCAAGAGATGTGGGA	5′ ROX	245 247 249 251 253 255 257 259 261	
4	CRIB2-23	A2	F:AGGGAGAGATGTGGCAGATTGTTTT R: ATGTGGTGGAATAACTTCTTGGCG	5′ ROX	201 204 207 210 218	
5	cnu_m046a	A2	F:GCTAAAGGTTTAGTCCAAATAGGATTC R:GCAAAATGATGCCCCATAAA	5′ TAMRA	161 173 181 185 187	

DNA database construction

- A DNA database of Chinese cabbage reference varieties was constructed using 30 markers
- A software can be developed and used

✓ to calculate the genetic similarity between a candidate variety and any other variety in the database.

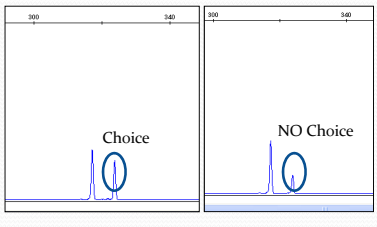
✓ to display the similar varieties at any set threshold.

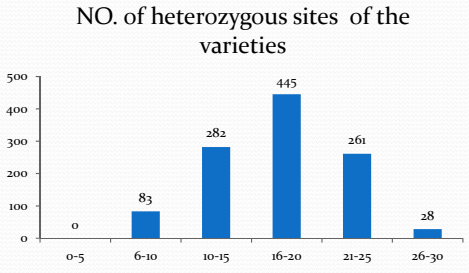
✓ the allele data collected according to the protocol can be used directly with no need of conversion.

A software was used to Select Similar the Varieties according to the genetic distance

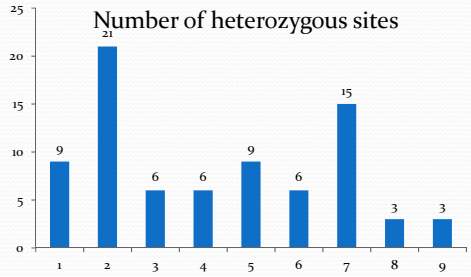
Genetic similarity threshold determined

- As a hybrid, there are a lot of heterozygous loci in Chinese cabbage
- Technical duplication and biological duplication (like the preference of PCR amplification) results in a smaller true molecular distance between varieties





- The results of different biological replicates of 78 cultivars were analyzed
- The error due to heterozygosity (1-9) results in the Genetic similarity is between 0.03 and 0.15



DETERMINATION OF THE THRESHOLD

➢ Our results

- 75% was determined as the threshold for Chinese Cabbage distinctness test

Table3 Relation between genetic similarity and percentage of non-distinct varieties

Genetic similarity	GS≥95	90≤GS<95	85≤GS<90	85≤GS<80	GS<75
Number of variety pairs	13	9	5	3	45
Number of non-distinct variety pairs	8	3	1	1	0

DUS test for Application varieties and similar varieties

- The genetic similarity was 92%
- non-distinct variety

- 90% was chosen as the threshold for in Molecular safety threshold.
- Genetic similarity value (Dice) of 75% was chosen as the threshold for similar varieties selection.

Application

- The approach was first applied in 2015

✓ the number of similar varieties to be planted is greatly reduced

✓ the precision of similar varieties selection is improved.

[End of Annex and of document]