Development of SSR markers for identification and discrimination of tobacco cultivars

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Objective

To develop SSR markers for

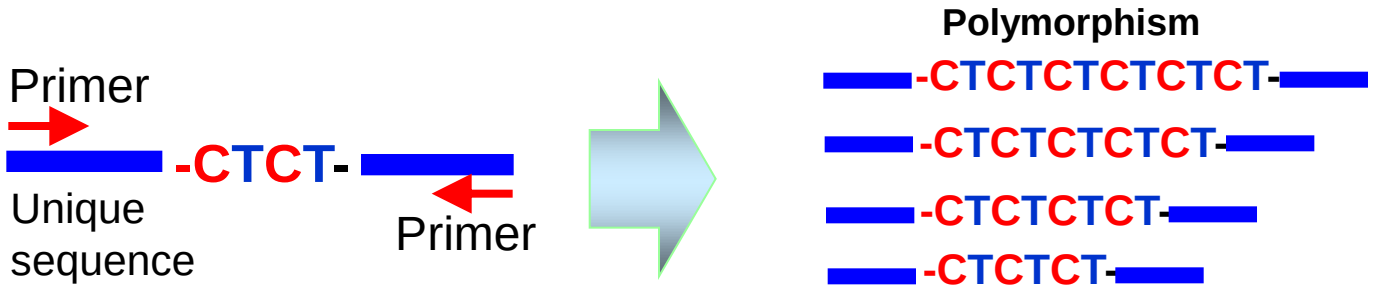
- **Cultivar identification of fresh leaves**
- **Cultivar identification of cured leaf tobacco**

SSR markers

➢**SSRs**: **S**imple **s**equence **r**epeats **(microsatellites)**

The repeated sequence is often simple, consisting of 2, 3, or 4 nucleotides, and can be repeated 3 to 100 times.

➢**SSR markers: SSRs and the flanking unique sequences**

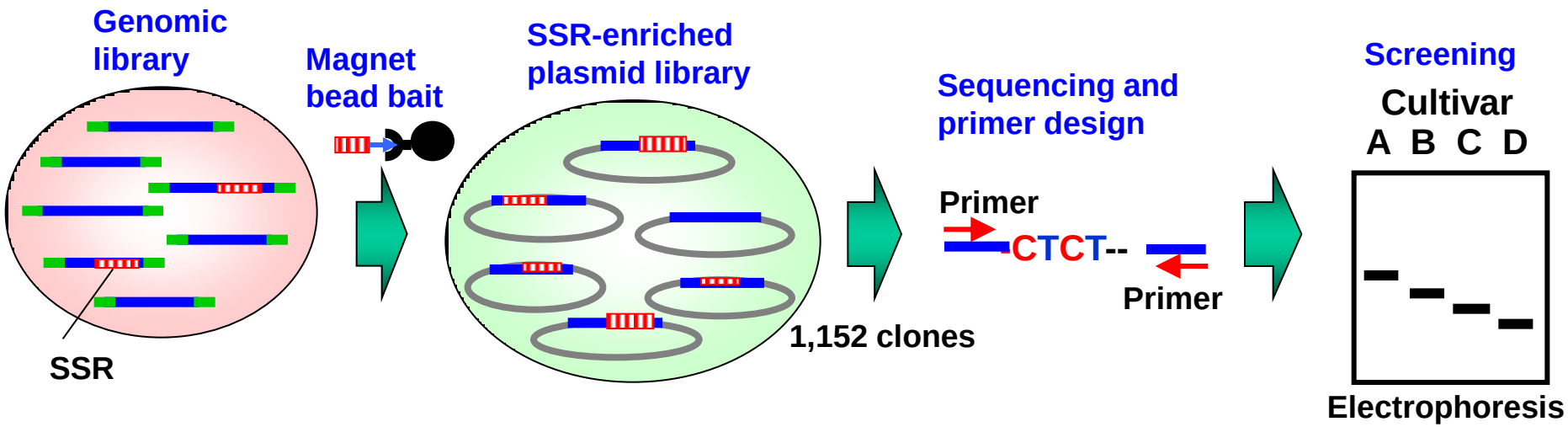


Advantages

1. **Highly abundant in plant genomes**
2. **Widely distributed throughout the genome**
3. **High level of polymorphism**

Screening of candidate SSR markers

Methods were referred from Kijas et al. (1994)

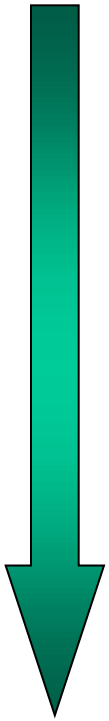


1. Construction of a genomic library from cv. BY4
2. Enrichment of DNA fragments including SSR motifs
   - Bait sequences: $(CT)_{12}$, $(AC)_{12}$, $(TAA)_8$
3. Cloning, sequencing, and design of PCR primer pairs
4. Screening of polymorphic SSR markers (fragment length)

1,152 clones ⇨ 46 candidate SSR markers

Development of SSR markers

46 candidate SSR markers



Selection

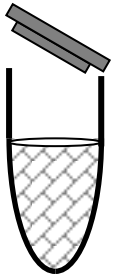
- ✓ **Suitable for use in multiplex reaction**

  (high throughput and less template DNA)
- ✓ **High reproducibility of PCR**
- ✓ **Different typing pattern from other markers**

26 SSR markers

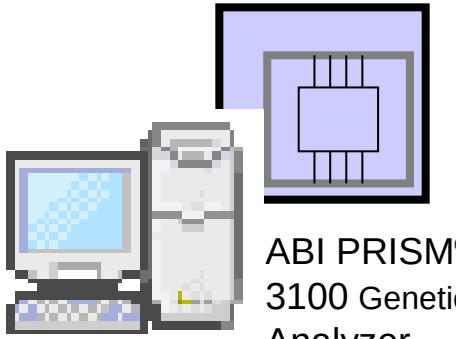
Flow chart of DNA typing

Fresh leaf



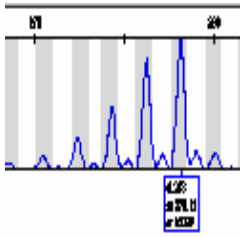
DNA extraction

PCR of SSR



ABI PRISM® 3100 Genetic Analyzer

Electrophoresis by a DNA sequencer



GeneMapper® software

Fragment analysis

SSR	Peak Size	
SSR A	175	bp
SSR B	98	bp
SSR C	151	bp
SSR D	192	bp
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SSR N	132	bp

DNA typing data

DNA typing of fresh leaves

Tobacco cultivars

- ✓ 22 burley
- ✓ 28 flue-cured
- ✓ 12 oriental
- ✓ 22 other types

1. DNA extraction from a fresh leaf
2. Multiplex PCR
3. Electrophoresis by a DNA sequencer

DNA typing data of 84 cultivars were obtained.

DNA typing data (fresh leaf)

Cultivars	Group1					Group2							Group3					Group4				Group5				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
B21	153	170	151	219	274	155	183	154	92	88	141	213	163	125	187	117	140	159	175	139	99	187	128	189	203	203
KY10	153	168	155	219	272	155	181	154	96	88	143	213	163	125	187	117	140	159	175	139	99	177	130	189	213	203
White Burley	153	170	155	219	274	155	183	154	92	88	141	199	163	125	187	117	138	157	175	141	111	185	128	189	201	203
Michinoku 1	153	170	153	219	274	155	183	154	92	88	173	213	163	125	187	121	140	159	175	139	111	185	128	191	203	203
TN86	153	170	153	219	274	155	183	154	92	88	141	213	163	125	187	117	140	167	175	139	99	185	128	189	203	203
TN90	153	170	151	219	270	153	183	154	92	0	141	213	163	125	187	117	140	159	175	139	99	185	128	189	203	203
KY14	153	170	155	219	274	157	181	154	92	88	143	213	163	127	187	117	140	159	175	137	99	173	130	189	203	203
BANKET A1	153	170	153	219	274	155	183	154	92	88	165	205	163	123	187	121	140	159	175	139	99	183	128	189	201	203
KY17	153	170	157	219	274	157	183	154	94	88	139	213	163	125	187	117	140	167	175	139	99	185	130	189	213	203
MBN2	153	170	153	219	274	157	181	154	92	88	141	213	171	125	187	117	140	159	175	139	99	177	128	189	203	203
KY907	153	170	153	219	274	157	179	154	92	0	141	213	163	125	187	117	140	167	175	139	99	185	130	189	215	203
Taihei	153	170	153	219	274	157	183	154	92	88	141	213														
WWH155	153	170	153	219	274	155	183	154	92	88	141	213														
LAB21	153	170	151	219	274	155	183	154	92	88	141	213														
Alta	149	172	155	219	274	157	191	164	94	88	143	205														
L8	149	168	153	219	274	155	183	154	92	88	141	205														
Michinoku 2	153	170	151	219	274	155	183	154	92	88	141	213														

Cultivars	Group1				
	1	2	3	4	5
B21	153	170	151	219	274
KY10	153	168	155	219	272
White Burley	153	170	155	219	274

- **84 cultivars were discriminated by 26 SSR markers.**
- **Application to purity management of germplasms will be possible.**

DNA typing of cured leaf

Degree of fragmentation and modification of DNA

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Purpose

- **DNA typing of fragmented DNA may be difficult.**
- **Cultivar identification of cured leaf was examined.**

Samples

- **Samples of each cultivar were collected from different harvesting areas in Japan.**
  - **Flue-cured (Coker 319, Virginia 115)**
  - **Air-cured (Michinoku 1, Taihei)**

DNA typing of cured leaf

Cured leaf samples			Estimated cultivar			
			The best match		The 2nd-best match	
Cultivar		No. of samples	Cultivar	No. of 100% matches (% of average)	Cultivar	No. of 100% matches (% of average)
Flue-cured	Coker 319	12	Coker 319	12/12 (100)	MC1	0/12 (85)
	Virginia 115	12	Virginia 115	9/12 (98)	Delgold	0/12 (88)
Air-cured	Michionoku 1	11	Michionoku 1	7/11 (98)	Taihei	0/11 (83)
	Taihei	10	Taihei	9/10 (99)	B21	0/10 (95)

- **In any case, the best matching cultivar was the expected one.**
- **These SSR markers are useful for cultivar identification of cured leaf.**

Conclusion

Twenty-six SSR markers were developed.

1. **Application to fresh leaf**

   - **Eighty-four cultivars were discriminated.**
   - **It is suggested that these markers are useful in managing the purity of germplasms.**

2. **Application to cured leaf**

   - **These SSR markers are useful for cultivar identification of cured samples such as commercial leaf tobacco.**