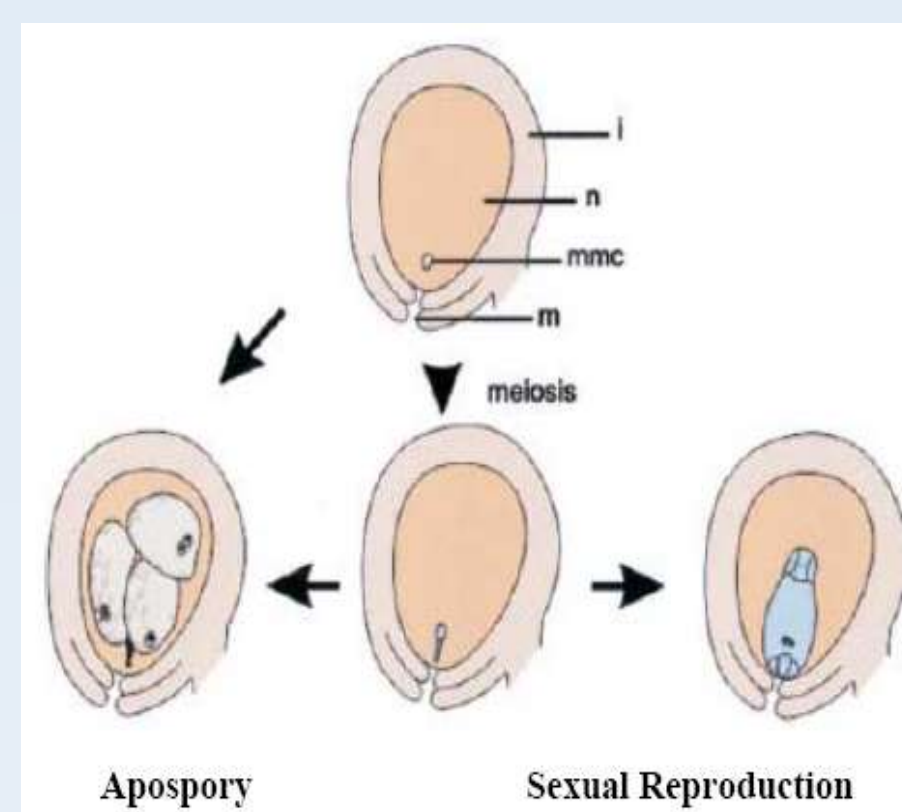
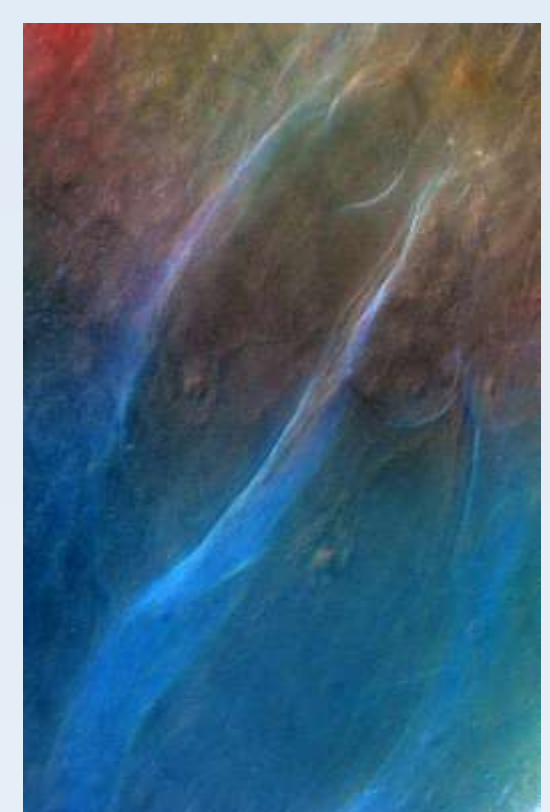


# Retrotransposon-Based Molecular Marker Development for Apomixis in *Pennisetum squamulatum*

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## Introduction

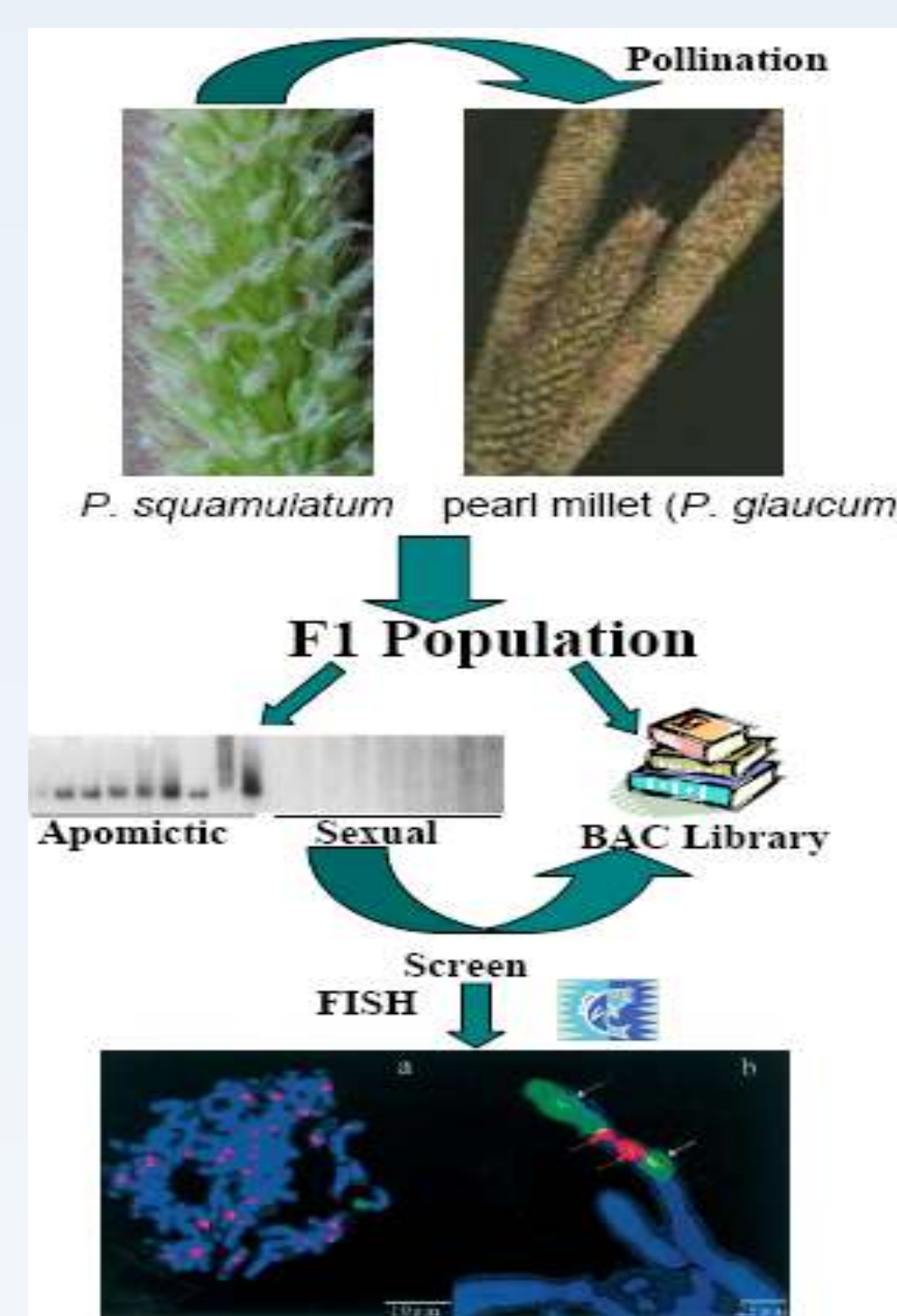
Although sexual reproduction is ubiquitous in the plant kingdom, asexual reproduction through seeds or apomixis predominates in some species of flowering plants. One of these is *Pennisetum squamulatum* which reproduces by apospory, a type of apomictic reproduction where non-generative nucellar cells develop into unreduced embryo sacs and the embryos further form without fertilization of the egg cell. The biological significance and potential economic value of apomixis has been discussed in many reports<sup>[1-2]</sup>. The application of molecular markers to genetic studies of apomixis is proving invaluable for mapping regions of genomes associated with components of apomixis<sup>[2]</sup>. The goal of this study was to develop apomixis-linked markers for *P. squamulatum* based on a particularly abundant retrotransposon.



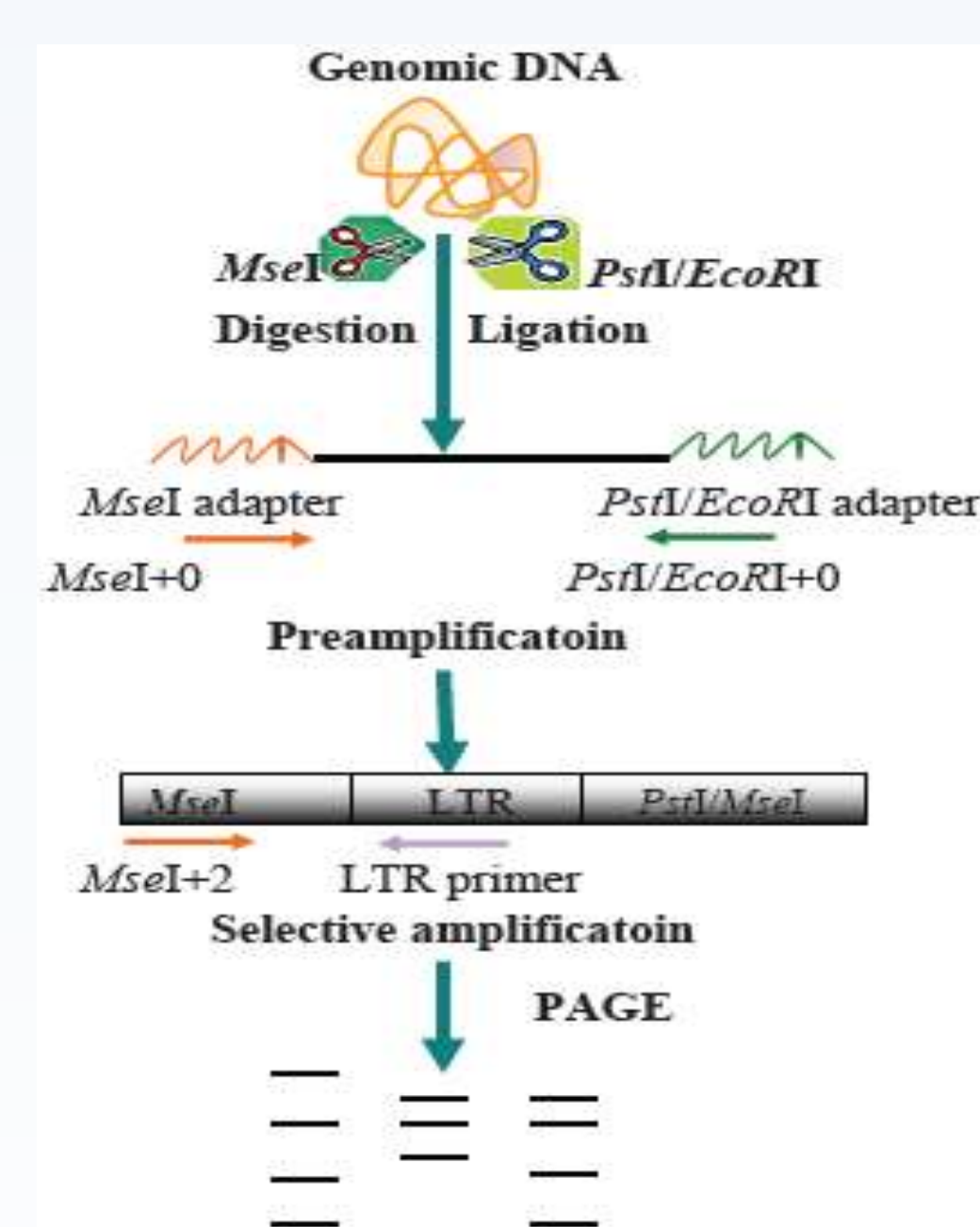
◀ Ovule development during apospory or sexual reproduction<sup>[2]</sup>. In sexual reproduction, a single megaspore survives after meiosis of the megaspore mother cell (*mmc*) and divides to form an eight-nucleate, seven-celled embryo sac. In apospory, sexual reproduction usually is arrested in favor of one or more unreduced embryo sacs that develop from somatic cells of the nucellus (*n*). *m*: micropyle; *l*: integument.

## Research Background

- ▶ Apospory, a single dominant trait in *P. squamulatum*
- ▶ Twelve PCR-based markers strictly cosegregate with aposporous embryo sac development<sup>[3]</sup>.
- ▶ This region has been defined as the Apospory-Specific Genomic Region (ASGR)
- ▶ An apomictic polyhaploid BAC library (derived from *P. squamulatum*) was created<sup>[4]</sup>.
- ▶ Physical location of ASGR - a single chromosome in *P. squamulatum*<sup>[5]</sup>.
- ▶ Abundant Opie-2-like retrotransposon in ASGR<sup>[5]</sup>.
- ▶ Large physical size of the ASGR (> 50Mb)<sup>[5]</sup>.
- ▶ Problem to be solved: more markers for high resolution mapping and further characterization of the ASGR.



## Materials and Methods



### Plant materials

*P. squamulatum* (PI319196) and its F1 population

### BAC clones

Nineteen ASGR-linked BAC clones were included in this study.

### Sequences analysis tools

Clustal X, Blast, Artemis Comparison Tool (ACT), Vector NTI

### Sequence-Specific Amplified Polymorphism (S-SAP)

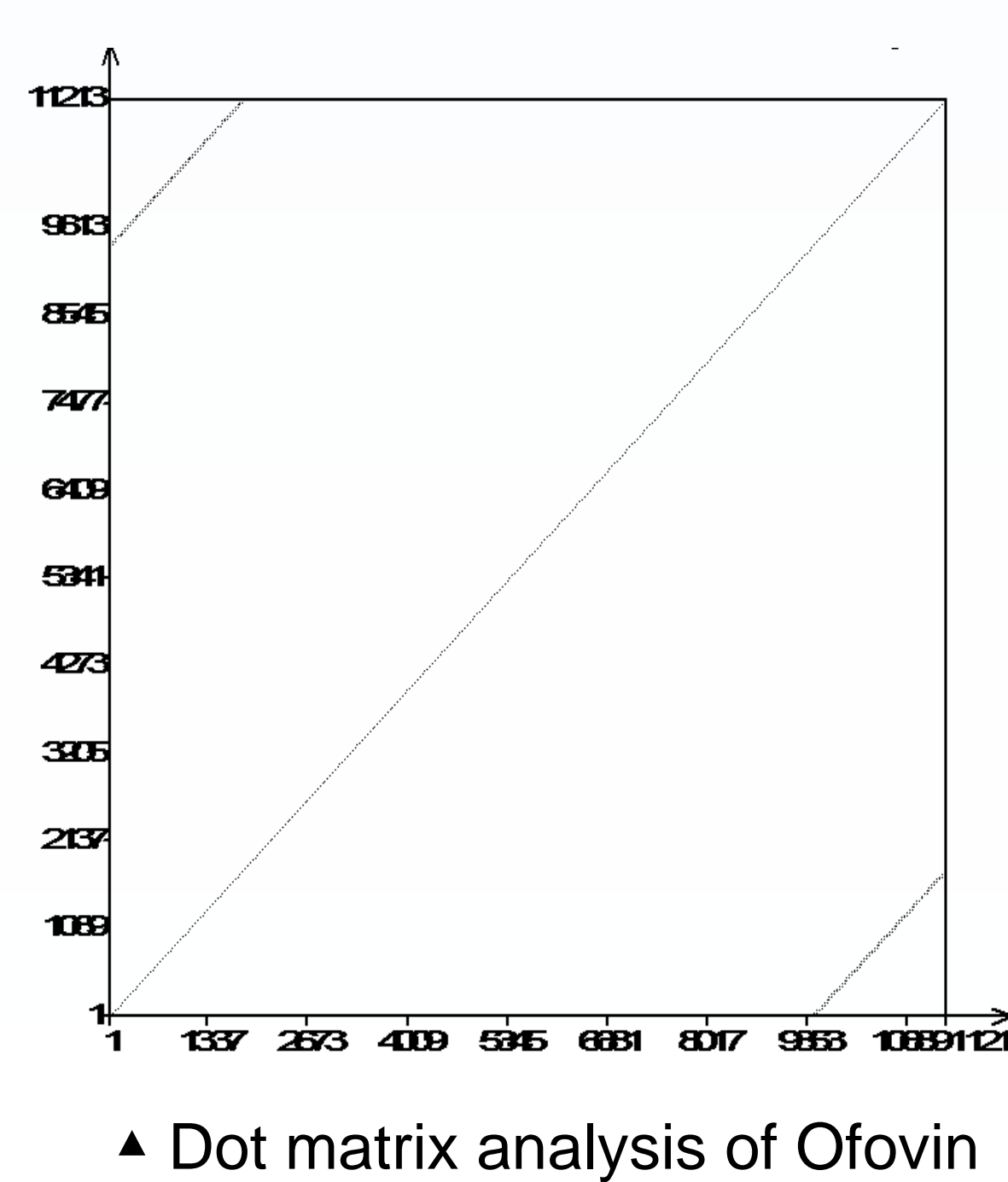
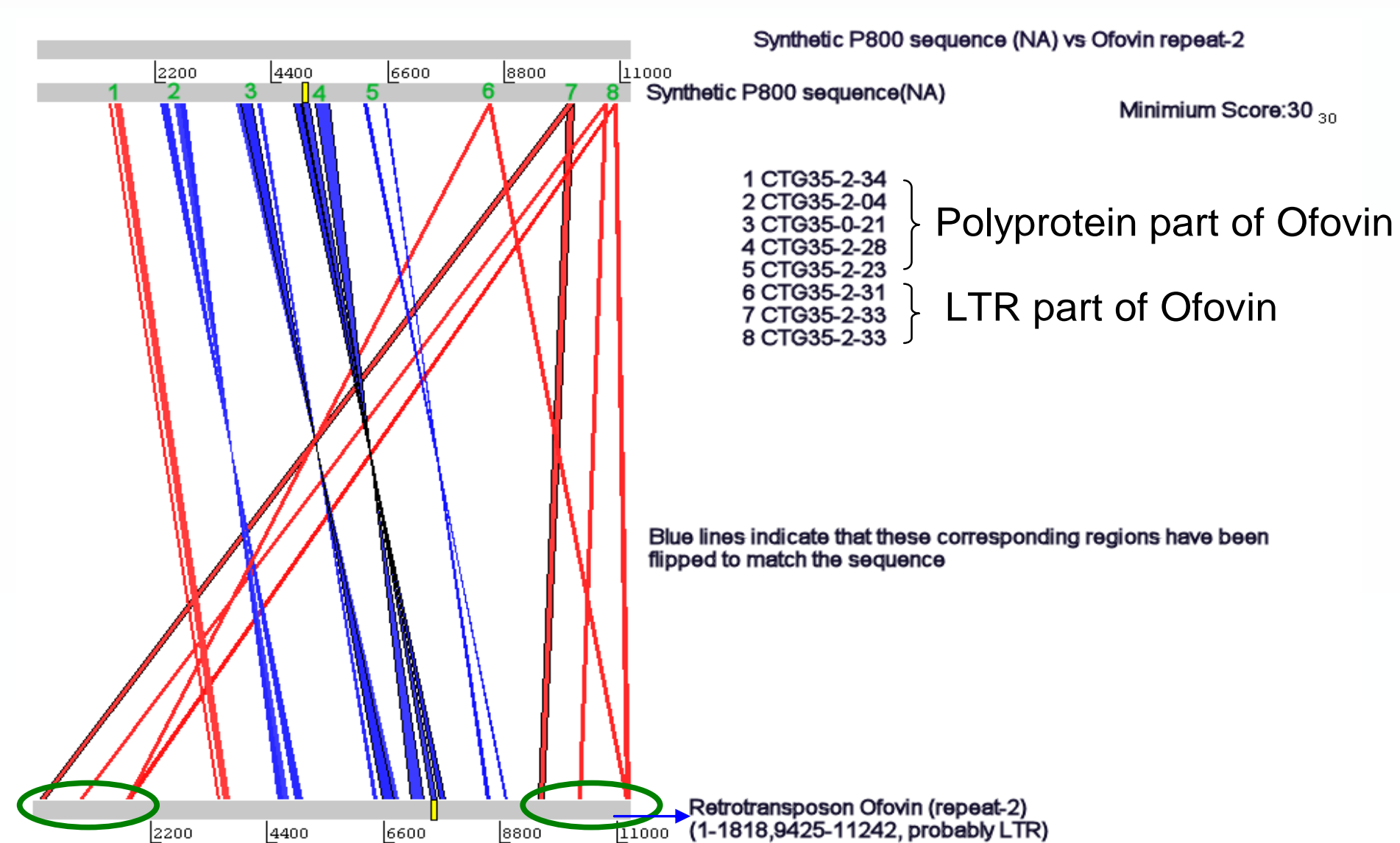
The protocol for S-SAP is shown in the left figure<sup>[6]</sup>.

### Genetic linkage analysis software

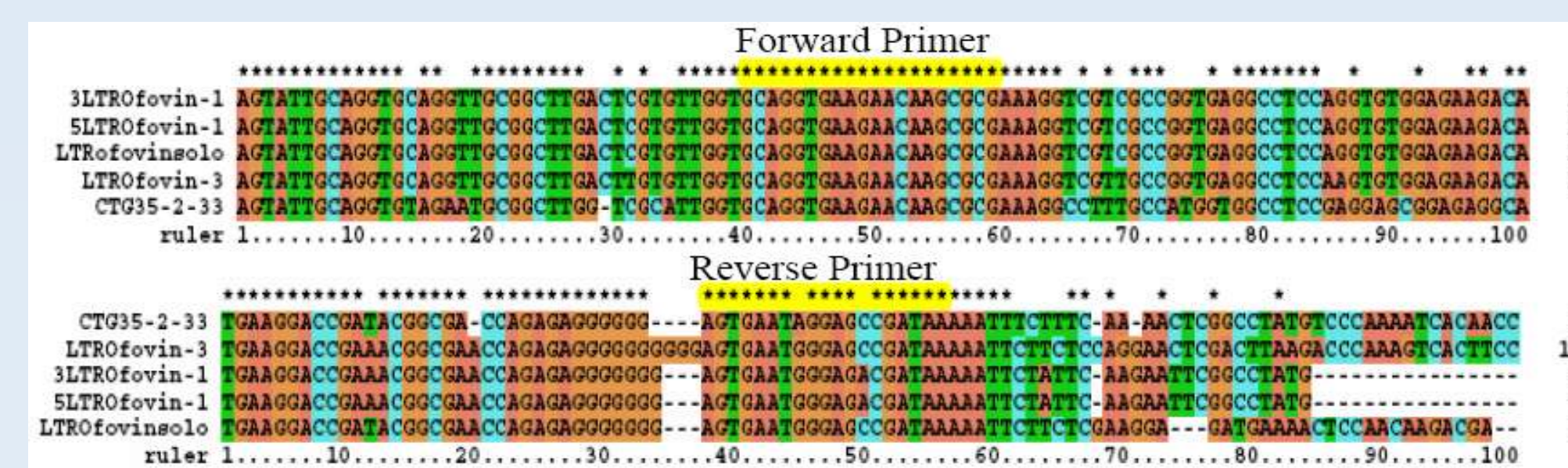
Join Map 2.0

## Results

Sequence contig 35-2-33 of P800 vs. the Ofovin retrotransposon from a pearl millet BAC (AF488414). ▼

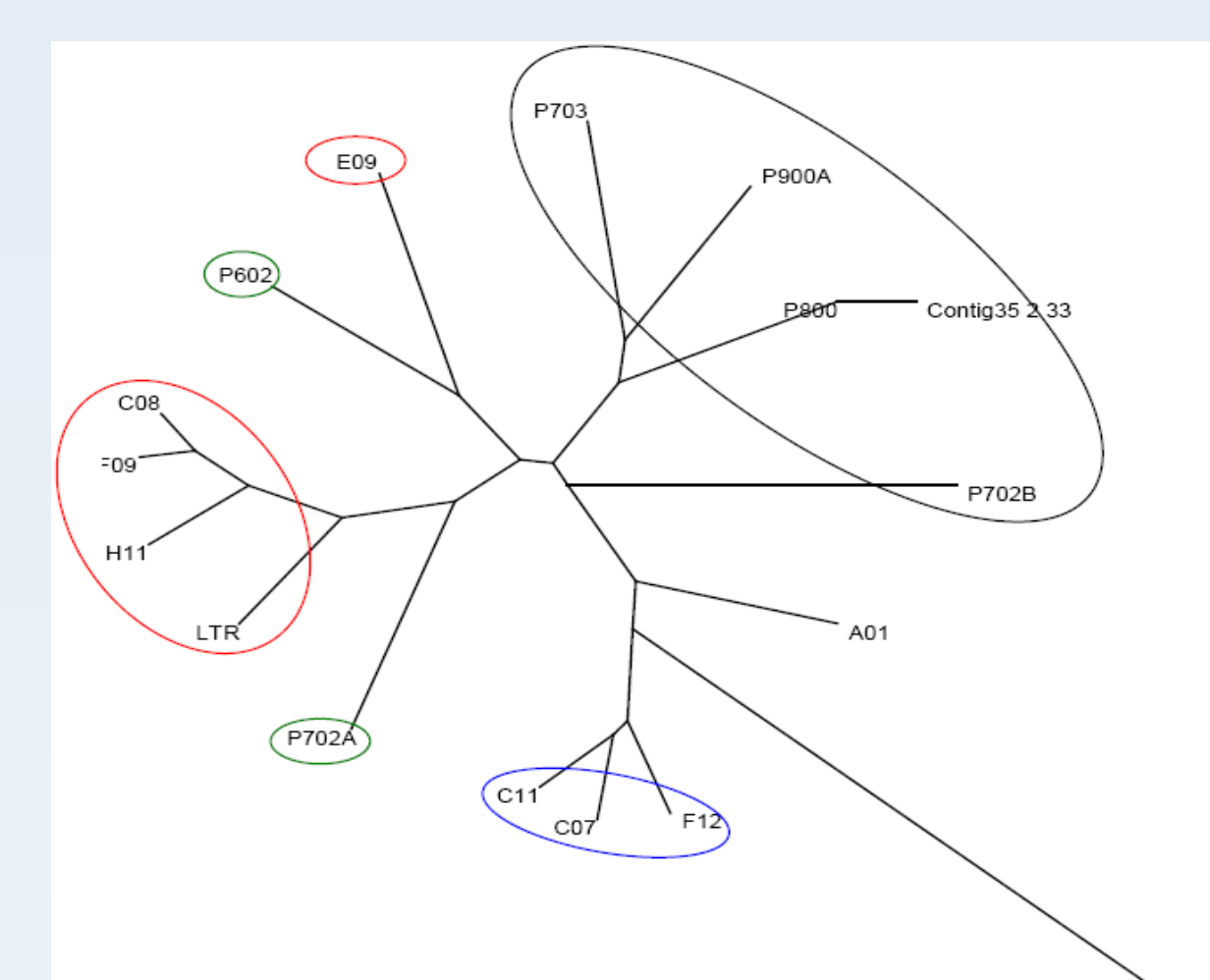
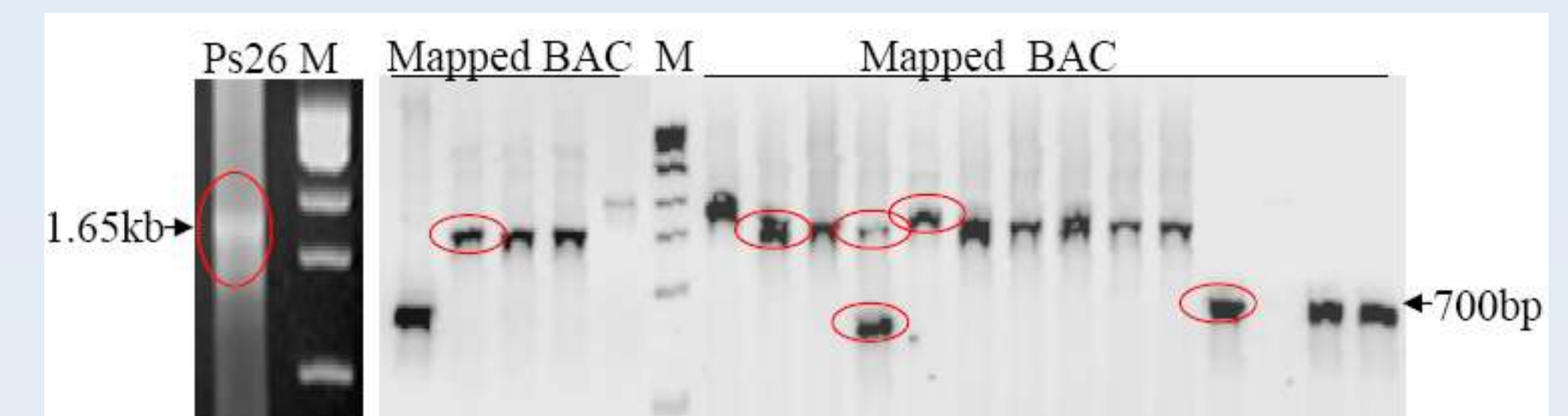


## Results (Cont'd)

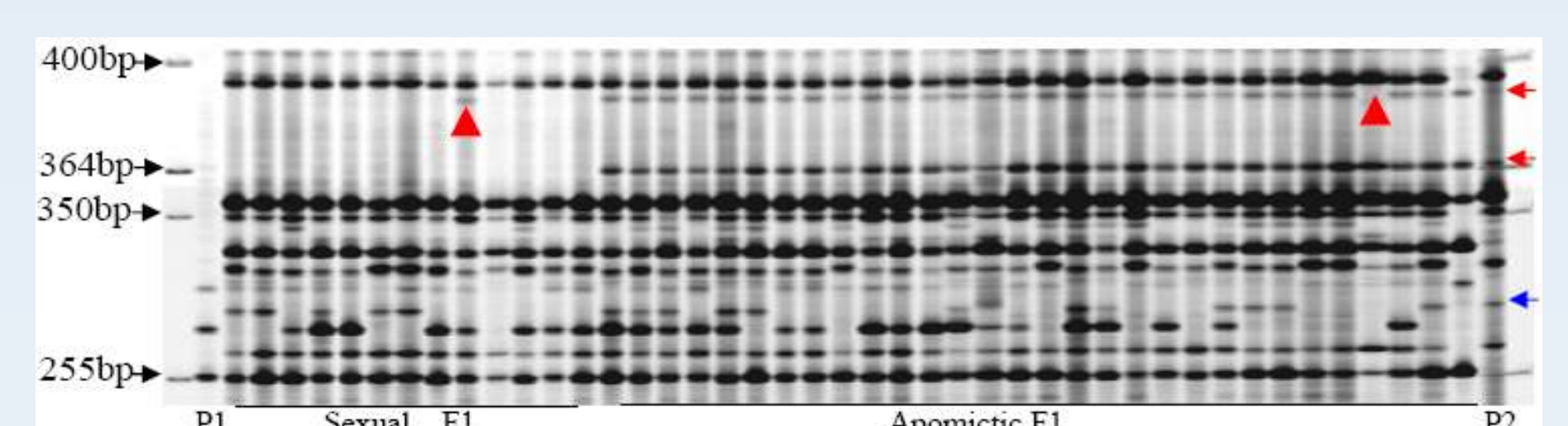


◀ Primers from the conserved region between CTG35-2-33 of P800 and the LTRs of Ofovin

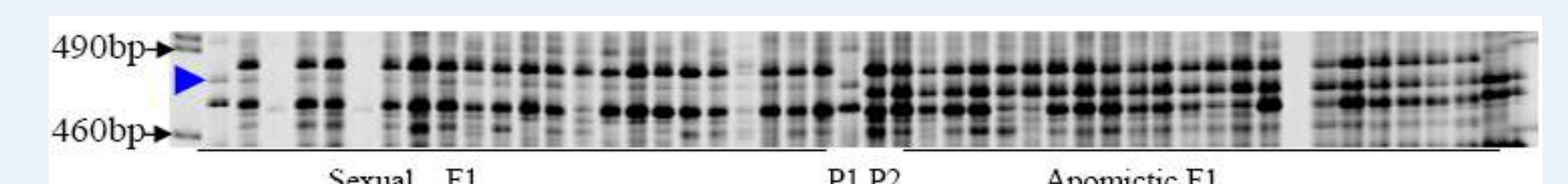
Fifteen products amplified from Ps26 and ASGR-linked BACs (circled) were fully sequenced and aligned



▶ Six LTR primers for S-SAP were designed based on their comparison

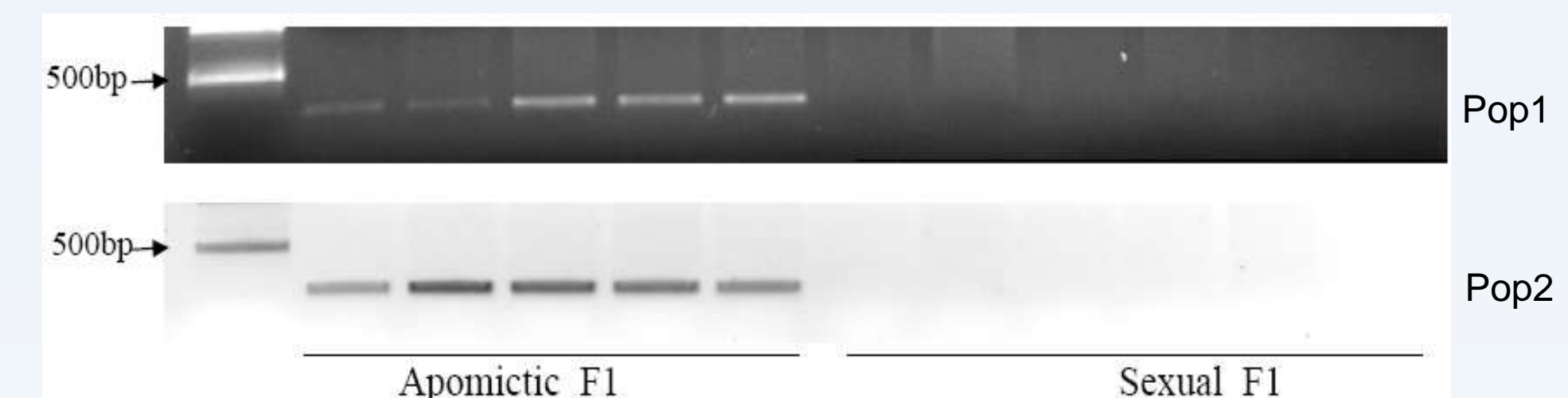


▶ LTR/*EcoRI*/*MseI*-S-SAP image. Red arrow: apomixis-linked marker. Blue arrows: band segregation. Red arrow heads: recombined bands



▶ LTR/*PstI*/*MseI*-S-SAP image. Blue arrow heads: Potential sexual reproduction-linked marker

◀ Genetic linkage analysis



▶ A SCAR marker (P56-25-400) derived from an S-SAP fragment confirms segregation with the ASGR against two F1 populations

▶ Expression of gypsy-LTR-retrotransposon (P56-25-400) in ovary and leaf



## Conclusions and References

- ▶ 247 single dose markers were generated using the LTR primers. One hundred forty-seven markers (60%) co-segregate with apomixis. Twenty seven closely linked apomixis-linked markers were also discovered.
- ▶ A genetic map was constructed using Joinmap with the 247 markers plus one previous AFLP marker (Pq365) and SCAR marker (Ugt197).
- ▶ One potential marker showed linkage with sexual reproduction
- ▶ One SCAR marker (P56-55-400) was developed from a co-segregated apomixis-linked fragment which show high similarity to a putative gypsy-type retrotransposon from rice; this SCAR marker was confirmed with two different F1 populations, and its expression was observed in ovary and leaf of *P. squamulatum*;
- ▶ Based on the genetic map and physical location of AFLP markers, sixteen additional markers are being recovered.
- ▶ The retrotransposon-based markers will be used to increase the BAC coverage of the ASGR in *P. squamulatum*
- ▶ Expression of gypsy-retrotransposon in sexual plants and its transposition in apomictic plants might be checked in the future

- 1 Hanna WW (1995) *Adv Agron* 54:333-350.
- 2 Ozias-Akins P, Akiyama Y, Hanna WW (2003) *Funct Integr Gen* 3:94-104
- 3 Ozias-Akins P, Roche D, Hanna WW (1998) *Proc Natl Acad Sci USA* 95:5127-5132
- 4 Roche D, Conner JA, Budiman MA *et al.* (2002) *Theor Appl Genet* 104:804-812
- 5 Akiyama Y, Conner JA, Goel S *et al.* (2004) *Plant Physiol* 134:1733-1741
- 6 Porceddu A., Albertini E., Barcaccia G. *et al.* (2002) *Mol Genet Genomics* 267: 107-114

We thank Anne Bell, Evelyn Morgan, Laura Ramos, Ye Chu and Yajuan Zeng for the technical assistance.