

COMMUNITY RESOURCES FOR HIGH THROUGHPUT GENOME MAPPING AND DIVERSITY ANALYSES IN 1EBN POTATO SPECIES

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Introduction

For potato breeding programs, wild species are a valuable source of agriculturally significant genes. We specialize in the *Stellata* superseries encompassing 20 wild potato species sexually incompatible with cultivated potato (Tab. 1). To improve access to noteworthy traits from these wild potato species, we have initiated the development of a DArT (Diversity Array Technology, Fig. 1) microarray for comparative structural genomic studies and mapping agriculturally significant genes.



Series*	Species	EBN	Reported Disease Resistance
I. Moeabelloma	<i>S. clurum</i>	7	Early blight, PVM, Verticillium
	<i>S. moeabelloma</i>	7	Blackleg, Bacterial wilt, Early blight, Late blight, PLRV, PVM, Ring rot, Verticillium, Wart
II. Bulbocastana	<i>S. bulbocastanum</i>	1	Late blight, Ring rot, Verticillium, Wart
	<i>S. cardiophyllum</i>	1	Blackleg, PLRV, PVM, Ring rot, Verticillium
	<i>S. jamaei</i>	1	Black leg, Early blight, Late blight, PLRV, Ring rot
III. Pinnatisecta	<i>S. pinnatisectum</i>	1	Blackleg, Early blight, Ring rot, Verticillium
	<i>S. elongophyllum</i>	1	Late blight, Ring rot
	<i>S. larai</i>	1	Late blight, PLRV, Ring rot, Verticillium
	<i>S. viticosum</i>	1	Ring rot, Verticillium wilt
IV. Polyadenia	<i>S. polyadenium</i>	1	Blackleg, Late blight, PLRV, PVA, PVM, Ring rot, Verticillium
V. Comerosolana	<i>S. comerosolana</i>	1	Bacterial wilt, Blackleg, Early blight, Ring rot, Verticillium
VI. Circaeifolia	<i>S. circaeifolium</i>	1	Bacterial wilt, PVM, PVS, Rhizoctonia, Wart
VII. Lignicaula	<i>S. lignicaula</i>	1	Fusarium wilt, Rhizoctonia, Verticillium, Wart
VIII. Omosiana	<i>S. omosiana</i>	?	
IX. Yungasiana	<i>S. anaco</i>	2	PVA, PVF, PVX, PLRV, Verticillium
	<i>S. chacoense</i>	2	PLRV, Verticillium
	<i>S. huancabambense</i>	2	Verticillium
	<i>S. longirostre</i>	2	
	<i>S. yungasense</i>	2	

Tab. 1. *Classification scheme for series *Stellata* of Hawkes (1990) with modifications after Rodriguez and Spooner (1997), Lara-Cabrera and Spooner (2004), and Spooner et al. (2004).
 *Resistance data from personal experience, published accounts [Austin et al. 1993; Helgeson et al. 1998; Micheletto et al. 2000; Naess et al. 2000; Song et al. 2003; Vossen et al. 2003; Vossen et al. 2005], and USDA Potato Genebank website. Highlighted species are those used for DArT array construction.

Materials & Methods

The utility of DArT was demonstrated via phylogenetic analysis of 1EBN potato species using an array of about 7500 markers (Fig. 2). An expanded version of this array now includes 16500 markers, 9500 of which are derived from *Stellata* species. The array has been especially enriched for markers derived from *S. bulbocastanum* and *S. commersonii*. To support mapping efforts, evaluation of late blight resistance has been conducted for different accession of *S. commersonii*, *S. chacoense*, *S. polyadenium* and *S. pinnatisectum* (Tab. 4).

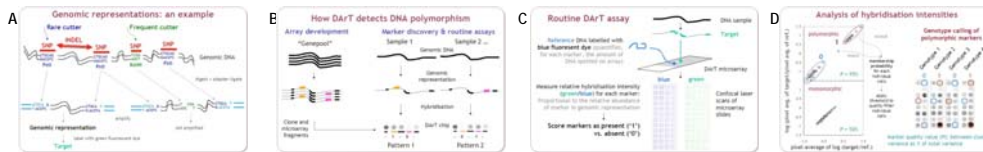
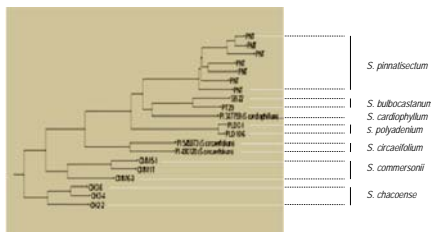


Fig. 1. DArT microarray development. (A) Digestion and selective amplification; (B) polymorphism detection; (C) DNA hybridization and data matrix creation; (D) Data validation.

Results

Validation of a first generation DArT array was carried out via phylogenetic analysis of seven *Stellata* species (fig. 2). Cluster analysis of DArT data identified seven species-specific clusters, as expected.

Fig. 2. Phylogenetic analyses of different accessions of seven wild potato species using DArT markers.



Comparison with previously reported phylogenies based on other molecular marker systems (Spooner et al., 2004) revealed general concordance. Preliminary results obtained by a first hybridization of F₁ *S. commersonii*, *S. bulbocastanum* F₁ hybrids are reported in Tab. 2 and 3, respectively.

<i>S. bulbocastanum</i>					
Parents	Progeny	Markers		Segregation ratio	
		Total	Mappable		
Aa x aa		705	515	1:1	
Aa x Aa		249	180	3:1	
AA x aa		356	0	no segr.	
Total		1310	695		

<i>S. commersonii</i>					
Parents	Progeny	Markers		Segregation ratio	
		Total	Mappable		
Aa x aa		641	578	1:1	
Aa x Aa		160	160	3:1	
AA x aa		343	0	no segr.	
Total		1144	738		

Tab. 2-3. Preliminary results obtained from hybridizations carried out with 92 and 61 F₁ hybrids of *S. bulbocastanum* and *S. commersonii*, respectively. (P=0,05).

Late blight resistance screening (Table 4) demonstrated that resistance to *P. infestans* US8 can be found in several genotypes.

Tab. 4. Results of foliar resistance to *Phytophthora infestans* US8.

Species	Number of accession tested	Resistant #	Susceptible #
CMM	63	13	50
CHC	133	11	122
PLD	2	2	0
PNT	2	2	0
Tot.	198	28	172

CMM: *S. commersonii*; CHC: *S. chacoense*; PLD: *S. polyadenium*; PNT: *S. pinnatisectum*. Disease index ranging from 1 to 9. Resistant ≤ 4; Susceptible ≥ 5.

Future research

Stellata species will be systematically screened for new resistance traits. Ongoing molecular investigations focus on:

- *S. commersonii*, *S. bulbocastanum* map analysis;
- wild potato-tomato genome structure comparison;
- mapping and cloning of interesting resistance genes.

Bibliography:

Austin S. (1993) *Am Potato J* 70:485-495; Hawkes J.G. (1990) Smithsonian Institution Press, Washington, D. C.; Helgeson J.P. (1998) *Theor Appl Genet* 96:738-742; Lara-Cabrera S.I. (2004) *Plant Syst Evol* 248:129-142; Micheletto S. (2000) *Theor Appl Genet* 101:902-906; Naess S.K. (2000) *Theor Appl Genet* 101:697-704; Rodriguez A. (1997) *Syst Bot* 22:31-43; Song J. (2003) *Proc Natl Acad Sci U S A* 100:9128-9133; Spooner D.M. (2004) *American Society of Plant Taxonomists, U.S.*; Vossen EAGvd. (2005) *Plant J* 44:208-222; Vossen Evd. (2003) *Plant J* 37:867-882.