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Application of molecular markers in cool season food legumes breeding

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Abstract

As the marker density has increased in linkage maps of the food legumes over the last ten years, many markers closely linked to economic traits, both qualitative and quantitative inherited, have been identified and published. Typical with other crops, soybean and maize for example, application of marker assisted selection (MAS) in food legumes has been aggressively pursued primarily by private institutions, due to costs and the more basic nature of public institutions research missions. However, developments in recent years have contributed to increases in both the utility and application of MAS in public and private institutes breeding and germplasm enhancement programs. These developments include

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directly: (1) the development of PCR-based markers, sequence-tagged sites and co-dominant microsatellite markers available for pea, chickpea, lentil per se and the development of cross-taxa markers from Medicago truncatula and Lotus japonicus, (2) the reduction in costs of the MAS technology, and indirectly (3) increases in genomic tools such as the construction of legume EST and BAC libraries and sequencing of Medicago truncatula and Lotus japonicus, along with the elucidation of plant gene functions, particularly from Arabidopsis thaliana. We will review specific examples of the development status of MAS in pea for biotic stresses, chickpea for biotic stresses, lentil traits, other food legumes breeding and germplasm enhancement projects, and conclude with prospects for the future.

Introduction

As the marker density has increased in linkage maps of the cool season food legumes over the last ten years, numerous markers closely linked to economic traits, both qualitatively and quantitatively inherited, have been identified and published. Typical of other crops, soybean and maize for example, application of marker assisted selection (MAS) in food legumes has been aggressively pursued primarily by private institutions, due to costs and the more basic nature of public institutions' research missions. However, developments in recent years have contributed to increases in both the utility and application of MAS in public and private institutes' breeding and germplasm enhancement programs. These developments include directly (1) the development of PCR-based markers, sequence-tagged sites and co-dominant microsatellite markers available for pea, chickpea, lentil *per se* and the development of cross-taxa markers from *Medicago truncatula* and *Lotus japonicus*, (2) the reduction in costs of the MAS technology, and indirectly (3) increases in genomic tools such as the construction of legume EST and BAC libraries and sequencing of *Medicago truncatula* and *Lotus japonicus*, along with the elucidation of plant gene functions, particularly from *Arabidopsis thaliana*. This review will cover the developmental status of MAS for breeding and germplasm enhancement of pea (*Pisum sativum*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*), and conclude with prospects for the future.

The main advantage of MAS is improved efficiency of applied breeding programs in the release of superior cultivars. The cool season food legume breeding programs' goals include improved yield and quality by pyramiding single gene resistance, and accumulating new positive alleles for quantitatively inherited traits, while maintaining positive linkage blocks in elite germplasm. Useful markers are currently polymerase chain reaction (PCR) based and co-dominant markers are the most informative (Table 1). The ideal marker is the gene (sequence) responsible for the phenotype, called 'perfect marker' by Ellis *et al.* (2002).

Development of highly informative co-dominant markers linked to economic traits, especially microsatellites, is crucial for adoption of MAS in cool season food legume breeding. The first extensive set of microsatellites was developed for chickpea (Udupa *et al.*, 1999). The first extensive microsatellite-based linkage maps for chickpea were published (Winter *et al.*, 1999; Winter *et al.*, 2000; Tekeoglu *et al.*, 2002) followed by traits marked by these powerful co-dominant markers (Table 2). In the absence of a consensus map for chickpea, Winter *et al.* (2000) serves as the de facto consensus map in recent publications.

Table 1. Definitions and class of markers used in or available in the future for marker assisted selection to identify superior lines for marker assisted selection in cool season food legumes

Marker class name	Definition	Type	Ease of use*, scalable†	Published example from food legumes
SNP	Single nucleotide polymorphism	Co-dominant	1, yes	Gao *et al.*, 2004b
INDEL	Insertion/deletion of 2 or more nucleotides	Co-dominant	1, yes	Gilpin *et al.*, 1997
Microsatellite, SSR. STMS	Repetitive elements of 2 to many repeat bases, simple sequence repeats = sequence-tagged microsatellites (Weber & May, 1989)	Co-dominant	1, yes	Winter *et al.*, 2000
STS	Sequence tagged sites, frequently from known clones genes (Olson *et al.*, 1989)	Co-dominant	1, yes	Gilpin *et al.*, 1997
CAPS, dCAPs	Cleaved amplified polymorphic sequences (Konieczny & Ausubel, 1993), derived CAPS (Neff *et al.*, 1998)	Co-dominant	1, yes	Huettel *et al.*, 2002; 2005 Rajesh *et al.*,
SCAR (ASAP)	Sequence characterized amplified region, allele-specific amplified polymorphism (Paran & Michelmore, 1992)	Dominant or co-dominant	1, yes	Yu *et al.*, 1995
AFLP	Amplified fragment length polymorphisms (Vos *et al.*, 1995)	Dominant, few co-dominant	2, yes	Pilet-Nayel *et al.*, 2002
RAPD	Random amplified polymorphic DNA (Williams *et al.*, 1990)	Dominant, few co-dominant	2, yes	Mayer *et al.*, 1997
ISSRs	Inter simple sequence repeats (Salimath *et al.*, 1995)	Dominant	2, yes	Kahraman *et al.*, 2004
RFLP	Restriction fragment length polymorphisms (Burr *et al.*, 1983)	Co-dominant	3, no	Dirlewanger *et al.*, 1994
Isozymes	Assay of different alleles resulting in alternate forms of the same enzyme	Co-dominant	3, no	Weeden *et al.*, 2000

*Ease of use, 1 = easiest, 3 = most difficult; † Scalable for moderate to high through-put, yes or no.

Table 2. Published markers linked to economic traits in cool season food legumes, of which some will be useful for marker assisted selection. The single gene markers for pyramiding positive alleles are listed first for each food legume crop, followed by QTL markers for each food legume crop

Crop/Disease or trait	Inheritance	Gene/Marker type/name	Marker class/distance for qualitative†, LOD or LR‡ & % variance§ for QTLs	Reference
Pea/Fusarium wilt, race 1	Qualitative	*Fw*/SCAR/Y15_999*Fw*	Dominant/4.6 cM	Okubara et al., 2005
		Fw/RAPD/Y15_1050	Dominant/4.6 cM	McClendon *et al.*, 2002
		Fw/AFLP/ACG:CAT_222, ACC:CTG_159	Dominant/1.4 cM, 2.6 cM	
Pea/Fusarium wilt, race 2	Qualitative	*Fnw*/SSR/PSMPSAD171	Co-dominant/7.7 cM	McPhee *et al.*, 2004
Pea/Fusarium wilt, race 5	Qualitative	*Fwf*/SCAR/U693_400*Fwf*	Dominant/5.6 cM	Okubara *et al.*, 2002
Pea/Pea enation mosaic virus	Qualitative	*En*/ASAPs/P256$_{900}$ and B500 $_{400}$	Co-dominant/6 cM, 8 cM, respectively	Yu *et al.*, 1995
Pea/Pea seed-borne mosaic virus, pathotype 1	Qualitative	*sbm-1*/STS/sG05–2537	Co-dominant/4.0 cM	Frew *et al.*, 2002
		sbm-1/STS/sP446	Co-dominant/6.8 cM	
		sbm-1/Perfect Marker/*eIF4E*	Co-dominant/0.0 cM	Gao *et al.*, 2004b
Pea/Pea seed-borne mosaic virus, pathotype 2	Qualitative	*sbm-2*/STS//*eIF(iso)4E*	Co-dominant/0.0 cM	Gao *et al.*, 2004b
Pea/Powdery mildew	Qualitative	*er*/RAPD/OPU-17	Dominant/10.7 cM	Janila and Sharma, 2004
		er/SCAR/ScOPD-10$_{650}$	Dominant/3.4 cM	
		er-1/RAPDs/PO-18$_{1200}$, PE-16$_{1600}$, PL-6$_{1900}$	Dominant/0 cM, 4 cM, 2 cM,	Tiwari *et al.*, 1998
Pea/Basal branching	Qualitative	*rms1*/Perfect marker (CAPS) / PsMAX4	Co-dominant	Foo *et al.*, 2005
Pea/Basal branching	Qualitative	*rms2*/RAPD/AD4-1000	Dominant/~10 cM	Rameau *et al.*, 1998
		rms3/isozyme, RAPD/*Aat-p*, T3-650	Co-dominant and dominant/9.5 cM	
		rms4/isozyme, RAPD/*Aat-m*, C12$_{500}$	Co-dominant and dominant/5.3 cM	
		rms6/RAPDs/K2-750 and R3-2000	Dominant/<10 cM, NR	Rameau *et al.*, 2002
Pea/Aphanomyces root rot	Quantitative	*Aph1*/RAPDs/N14.950 and U326.190	Dominant/14.5 LOD, 26% and 5.1 LOD, 16%	Pilet-Nayel *et al.*, 2002;
		Aph1/AFLPs/E7M4.251 and E2M4.292	Dominant/20.4 LOD, 47% and 2.7 LOD, 10%	Pilet-Nayel *et al.*, 2005

		Aph2/AFLP/E3M3.167	Dominant/10.1 LOD, 32%	
		Aph3/RAPD/U370.900	Dominant/4.0 LOD, 11%	
		Aph3/AFLP/E1M3.154	Dominant/4 6 LOD, 12%	
Pea/Aphanomyces root rot		*Aph*/RFLP/P393	Co-dominant/NR	Weeden *et al* , 2000
		Aph / Isozyme/PgmF$_{-390}$	Co-dominant/NR	
Pea/Ascochyta blight	Quantitative	*Asc1.1*/RFLP/c206	Co-dominant/LOD = 5.26, 35.9%	Timmerman-Vaughan *et al.*, 2002, 2004
		Asc2.1/RAPD/M02-835	Dominant/LOD = 7.13, 19.6%	
		Asc2.1/SCAR/sM2P5-234	Co-dominant/LOD = 5.87, 16.2%	
		Asc3.1/SCAR/M27	Co-dominant/LOD = 5.19, 16.9%	
		Asc3 2/RAPD/J12-1400	Dominant/LOD = 3.97, 16.1%	
		Asc4.2/RAPDs/C12-680, W17-150	Dominant/LOD = 2.84, 12%	
		Asc 4 3/RFLP/P346	Co-dominant/LOD = 2 96, 7.3%	
		Asc5.1/SCAR/sY16-1121	Co-dominant/LOD = 3.12, 11.9%	
		Asc7 1/AFLP/ M2P2-193	Dominant/LOD = 2.95, 13.9%	
		Asc7.2/SCAR/sB17-509	Dominant/LOD = 2 59, 12.3%	
		Asc7 3/RAPD/S15-1330	Dominant/LOD = 2 97, 7 8%	
Pea/Ascochyta blight	Quantitative	*mpIII-1*/RAPD/V03-1200	Dominant/LOD = 18, 42%	Prioul *et al.* 2004
		mpIII-3/SRR/PSMPSAA175	Co-dominant/LOD = 3.2, 6%	
		mpVa-1/SRR/PSMPSAA163.2	Co-dominant/LOD = 7.2, 10%	
		mpVII-1/SRR/PSMPSAA399	Co-dominant/LOD = 3.2, 5%	
		mpVI-1/RAPD/G04-950	Dominant/LOD = 9.3, 15%	
Pea/Ascochyta blight	Quantitative	*II ccta2*/AFLP/NR	Dominant/LOD = 2 9, 5%	Tar'an *et al.* 2003b
		IVcccc1/AFLP/NR	Dominant/LOD = 3 3, 19.1%	
		VIacct1/AFLP/NR	Dominant/LOD = 3.1, 16.8%	
Pea/Seed weight/seed number	Quantitative/ 2 traits	*num1.1, wt1.1*/RFLP/P445	Co-dominant/LOD = 3 94 to 15.69, R^2 = 9-27%	Timmerman-Vaughan *et al.*, 2005
Pea/Seed weight/seed number/harvest index	Quantitative/ 3 traits	*num1.2, wt1.2, hi1.1*/RAPD/P11-520	Dominant/LOD = 3.64-8.86, R^2 = 7-10%	Timmerman-Vaughan *et al.*, 2005
Pea/Yield *per se*/seed number/harvest index	Quantitative/ 3 traits	*yld3 1, hi3 1, num3 1*/AFLP/M2P2-370	Dominant/LOD = 3.71-3.04, R^2 = 7-20%	Timmerman-Vaughan *et al* , 2005
Pea/Yield *per se*/seed weight	Quantitative/ 2 traits	*yld4.2, wt4.1*/SCAR or RFLP/P628	Co-dominant/LOD = 3.31 to 8.84, R^2 = 7-15%	Timmerman-Vaughan *et al.*, 2005

Pea/Yield *per se*/seed weight/seed number	Quantitative/ 3 traits	*yld7.1, wt7.1, num7.1*/RFLP/MAPKinase	Co-dominant/LOD = 4.98 to 14.4, R^2 = 7-21%	Timmerman-Vaughan *et al.*, 2005
Pea/Yield *per se*	Quantitative	*yld7.2*/RFLP/I7	Co-dominant/LOD = 3.95 to 6.80, R^2 = 6-15%	Timmerman-Vaughan *et al.*, 2005
Pea/Lodging resistance	Quantitative/ 2 QTL	*Ld*/AFLP (SCAR)/cacc4 (A001) *Ld*/AFLP/acct1	Dominant/LOD = 14.5, R^2 = 47% Dominant/LOD = 3.5, R^2 = 26%	Tar'an *et al.*, 2003b
Pea/Plant height	Quantitative	*Ph*/AFLP/cttg7 *ht1*/RAPD/AD12-800 *ht2*/RAPD/U08-1650 *ht3*/SSR/AB33	Dominant/LOD = 21.5, R^2 = 56.9% Dominant/LOD = 4.4, R^2 = 4% Dominant/LOD = 39.9, R^2 = 63% Dominant/LOD = 13.0, R^2 = 3%	Tar'an *et al.*, 2003b Prioul *et al.*, 2004
Pea/Basal branching	Qualitative	*rms2*/RAPD/AD4-1000 *rms3*/isozyme, RAPD/*Aat-p*, T3-650 *rms4*/isozyme, RAPD/*Aat-m*, $C12_{500}$ *rms6*/RAPDs/K2-750 and R3-2000	Dominant Co-dominant and dominant Co-dominant and dominant Dominant	Rameau *et al.*, 2002 Rameau *et al.*, 1998
Pea/Green seed color	Quantitative	*QTL Y*/STS/P108 *QTL U*/RAPDs / I05 530, K02_1700	Co-dominant/LOD13.5, R^2 = 61% Dominant/LOD = 4.70 /R^2 = 56%	McCallum *et al.*, 1997
Lentil/Ascochyta blight resistance	Qualitative, 2 genes	*ral1*/RAPD/UBC 227_{1290} *AbR1*/SCAR/$B18_{680}$ *ral2*/SCAR/OPD-10_{870} *AbR1*/RAPD/RW19 (OPW19_{700}), RB18 ($B18_{680}$)	Dominant/NR Dominant/16 cM Dominant/6 cM. 14 cM, respectively	Tar'an *et al.*, 2003a Chowdhury *et al.*, 2001 Ford *et al.*, 1999
Lentil/Anthracnose	Qualitative	95B36 isolate/RAPD/OPO6_{1250} *LCt-2*/RAPD/PEO6_{1250} ,UBC-704_{700}	Dominant/specific to isolate Dominant/6.4 cM, 10.6 cM	Tar'an *et al.*, 2003a Tullu *et al.*, 2003
Lentil/Fusarium wilt	Qualitative	*Fw*/STMS/SSR59-2B *Fw*/AFLP/p17m30710	Co-dominant/3.5 cM Dominant/8.0 cM	Hamwieh *et al.*, 2005
Lentil/winter hardiness	Quantitative	LG1 QTL/ISSR/ubc840-3 LG4 QTL/ISSR/ubc808-12	Dominant/2.3 LOD, 9.5% Dominant/7.3 LOD, 28.8%	Kahraman *et al.*, 2004
Lentil/frost tolerance	Qualitative	*Frt* / RAPD/ OPS16_{750}	Dominant/9.1 cM	Eujayl *et al.*, 1999
Chickpea/Fusarium wilt race 0	Qualitative	*foc0$_1$* /STMS/TR59 *foc0$_1$* /RAPD/OPJ20_{600}	Co-dominant/2 cM Dominant/3 cM	Cobos *et al.*, 2005

Chickpea/Fusarium wilt race 1	Qualitative	*foc1*/RAPDs/CS-27_{700}, UBC170_{550} *foc1*/ASAPs/CS-27_{700}F, CS-27_{700}R UBC170_{550} F, UBC170_{550}R	Dominant/7 cM, 9cM Co-dominant/7 cM to *foc-1*, 7.2 cM to *foc-4*, 4 cM, 4 cM to *foc-5*	Mayer *et al.*, 1997 Tekeoglu *et al.*, 2000
Chickpea/Fusarium wilt race 3	Qualitative	*foc-3*/STMS/TA96, TA27 *foc-3*/STMS/TA194	Co-dominant/0.6 cM Co-dominant/14.3 cM	Sharma *et al.*, 2004
Chickpea/Fusarium wilt race 4	Qualitative, 2 genes	*foc4*/STMS/TA96 *foc4* RAPD/CS27_{700}, UBC170_{550} *foc4*/DAF/R-2609 *foc4*/RAPD/OPU17-1	Co-dominant, Dom./3.4, 3.5 cM Dominant/both 9 cM Dominant/4.1 cM Co-dominant/2 cM	Winter *et al.*, 2000 Tullu *et al.*, 1998 Benko-Iseppon *et al* ,2003
Chickpea/Fusarium wilt race 5	Qualitative	*foc5*/STMS/TA27, TA26	Co-dominant/3.5 cM	Winter *et al.*, 2000
Chickpea/Ascochyta blight	Quantitative	QTL1 = LG IV = *ar2b* STMS/TA146, TA130 STMS/GA2 DAF/OPS06-1, UBC181A RAPD / UBC181a	Co-dominant/11.1 LR Co-dominant/50.2 LR Co-dominant/9.5% Dominant/above 5 LOD, < 50% Dominant/17.23 LOD, 31.5%	Udupa & Baum, 2003 Flandez-Galvez *et al.*, 03 Collard *et al.*, 2003 Rakshit *et al.* 2003 Santra *et al.*, 2000
Chickpea/Ascochyta blight	Quantitative	LGII = *Ar19* (or *Ar21d*)/GA20	Co-dominant/3.08, NR	Cho *et al.*, 2004 Udupa & Baum, 2003
Chickpea/β-carotene concentration	Quantitative	QTL1/ STMS/TS19 QTL2/STMS/TA64, STMS28 QTL3/STMS/GA11, TA122 QTL4/STMS/TR26	Co-dominant / 3.9, 3.0, 2.1, 2.1 LOD, respectively; % NR	Abbo *et al.*, 2005
Chickpea/lutein conc.	Quantitative	QTL1/STMS/TA25	Co-dominant / 2.4 LOD; % NR	Abbo *et al.*, 2005
Chickpea/seed weight	Quantitative	QTL1/STMS/GA24, STMS11, GA2 QTL2/STMS/GA11 QTL3 / STMS / TA120, TR40	Co-dominant / 3.8, 3.2, 2.4 LOD, respectively; % NR	Abbo *et al.*, 2005

†Reported in centiMorgan map units (cM).

‡Highest LOD score (base-10 log likelihood ratio test statistic) reported using interval mapping statistics in cited publication under Reference column cited by the publication under Reference column. LR = likelihood ratio test (LR) is –2log (L0/L1). % indicates use of regression analysis to identify QTL.

§ The highest percentage of the genetic variation explained by the QTL cited by the publication under Reference column.

NR not report

For pea, a consensus map comprised of RFLPs, STSs, ASAPs, RAPDs, isozymes, and morphological markers has been available (Weeden *et al.*, 1998). Until recently, trait marker discover has focused on dominant markers, especially RAPDs and AFLPs (Table 2; Laucou *et al.*, 1998), but not exclusively. Notable are RGA and STS markers published by Gilpin *et al.* (1997), Weeden *et al.* (2000) and Timmerman-Vaughan *et al.* (2000). Burstin *et al.* (2001) published 31 polymorphic pea microsatellites discovered from pea sequences in the Genbank and EMBL databases. In identifying the first microsatellite marker for Fusarium wilt, McPhee *et al.* (2004) mapped 186 pea microsatellites in a RIL population of 187 inbred lines. The linkage data were combined to form the first microsatellite-based consensus map of pea (Loridon *et al.*, 2005).

The first medium density linkage maps of lentil contained primarily dominant markers, comprised of RAPD, AFLPs, RFLP, ISSRs, RGAs, and morphological markers (Eujayl *et al.*, 1998a; Rubeena *et al.*, 2003). Co-dominant microsatellites are under development and 41 have been published for lentil (Hamwieh *et al.*, 2005). Lentil microsatellites were developed from a genomic clone library of lentil (Hamwieh *et al.*, 2005). The development and mapping of microsatellite markers in the existing map of lentil could be substantially increased, thereby providing the possibility for the future localization of various loci of agronomic interest.

Several markers closely linked to economic traits, both qualitatively and quantitatively inherited, have been identified and published for the cool season food legumes (Table 2). This review will cover specific examples of MAS in pea, chickpea and lentil. The authors apologize in advance for this exclusive review due to space constraints and wish this to be a window into the rapidly expanding literature on applications of markers to cool season food legume breeding and germplasm enhancement.

MAS in Pea

PCR-based sequence characterized amplified region (SCAR) markers have been developed for *er-1*, conferring resistance to powdery mildew; *En*, conferring resistance to pea enation mosaic virus; *sbm-1*, conferring resistance to pea seed-borne mosaic virus and *Fw* conferring resistance to Fusarium wilt race 1. SCAR markers have not been developed to date for *Fnw* and *Fwf*, conferring resistance to Fusarium wilt races 2 and 5, respectively.

Genetic resistance to powdery mildew, caused by *Erysiphe pisi* Syd., is controlled by two recessive genes, *er-1* and *er-2*. Several RAPD markers linked to *er-1* have been identified and PCR-based SCAR markers developed (Timmerman *et al.*, 1994; Tiwari *et al.*, 1998). Timmerman *et al.* (1994) converted the $PD10_{650}$ positioned 2 cM from *er-1* to a SCAR marker and Tiwari *et al.* (1998) identified three tightly linked RAPD markers, OPO-18_1200, OPE-16_1600 and OPL-6_1900, and developed SCAR ma
in coupling and no recombinants were identified among 57 homozygous F_2 individuals. Both OPE-16 and OPL-6 were linked in repulsion and positioned 4 and 2 cM from *er-1*, respectively. All four markers have application in MAS. Pea enation mosaic virus can be epidemic in proportion; however, genetic resistance conferred by the single dominant gene,

En, is present in the germplasm. Yu *et al.* (1995) identified a RAPD marker, $P256_{900}$, located 6 cM from *En* and developed a SCAR marker as a selection tool.

Seed-borne mosaic virus can be a devastating disease and genetic resistance to pathotype P-1 is conferred by *sbm-1* mapped to linkage group VI by association with the RFLP GS185, located 8cM from the gene (Timmerman *et al.*, 1993). Gilpin *et al.* (1997) reported an additional RFLP marker, P446, located more proximal to *sbm-1* which was converted to the SCAR, sP446 (Frew *et al.*, 2002). Frew *et al.* (2002) converted the G05_2537 RAPD marker to a SCAR that co-located with *sbm-1* at 4.8 and 3.5 cM in two populations, respectively. These markers when tested against a set of genotypes of known reaction were highly correlated with the expected phenotype making them excellent candidates for MAS. Two additional markers, eIF(iso)4E and eIF4E, were placed on the pea genetic map in close proximity to known groups of viral resistance genes on LG II and VI, respectively (Gao *et al.*, 2004a). Further analysis of the eIF4E gene demonstrated its critical role in viral replication whereby a mutation in this gene conferred resistance (Gao *et al.*, 2004b). The direct role of this gene in resistance makes the markers linked to eIF4E 'perfect' markers for *sbm-1* and resistance to pea seed-borne mosaic virus.

Fusarium wilt is an important disease and pea is susceptible to four races, 1, 2, 5 and 6, of *Fusarium oxysporum* f. sp. *pisi*. Genetic resistance is present in germplasm and is conferred by independent dominant genes. McClendon *et al.* (2002) identified two AFLP and one RAPD marker, Y15_1050, located 1.4, 2.6 and 4.6 cM, respectively, from *Fw*, conferring resistance to wilt race 1. Okubara *et al.* (2005) report development of a SCAR marker based on the Y15 RAPD marker. *Fnw* has been more difficult to position on the pea genetic map due to difficulty in phenotypic evaluations; however, McPhee *et al.* (2004) have tentatively placed *Fnw* on LG IV and further effort is required to identify more closely linked markers for MAS. Okubara *et al.* (2002) identified a RAPD marker, U693a, located 5.6 cM from *Fwf* and suggest that conversion of this marker to a SCAR would be useful for MAS.

MAS for QTL in Pea

Aphanomyces Root Rot Resistance

Aphanomyces root rot, caused by the soil-borne fungus *Aphanomyces euteiches*, is a major disease of pea. Since genetics of resistance is known to be quantitative, QTL mapping studies for *Aphanomyces* resistance have been recently developed. From a RIL population derived from the cross Puget (susceptible) x 90-2079, Pilet-Nayel *et al.* (2002) identified 7 QTL associated with partial field resistance over two years (1996, 1998) and two locations in the USA. Three QTL, *Aph1*, *Aph2* and *Aph3*, were considered consistent for they were detected either in at least two environmental conditions, or for at least two of the three different resistance traits assessed. *Aph1*, located on linkage group IV, was highly consistently detected and explained from 10 to 47% of the phenotypic variation, depending on the environment (47% of explained variation at LeSueur, MN; 1998). *Aph2* and *Aph3*, located on linkage groups V and I, accounted for 8-32% and 11-14% of the phenotypic variation,

respectively. These three QTL were also detected from controlled conditions scores, either towards both the US SP7 and the French Ae106 isolates (*Aph1* and *Aph3*) or only towards the French Ae106 isolate (*Aph2*) (Pilet-Nayel *et al.*, 2005). Seven minor-effect additional QTL were specifically detected with one of the two isolates studied and were not identified for partial field resistance in the US (Pilet-Nayel *et al.*, 2005).

From another RIL population derived from the cross MN313 (tolerant) x USO1026 (susceptible), Weeden *et al.* (2000) identified a major gene, located on linkage group IV, controlling tolerance to *Aphanomyces* root rot in the field at one location in the United States (LeSueur, MN). As the two pea lines 90-2079 and MN313 derive from a common partially resistant progenitor, it may be possible that *Aph1* in 90-2079 and the gene associated with *Aphanomyces* root rot tolerance in MN313, correspond to the same locus. Dominant and co-dominant flanking markers at *Aphanomyces* resistance QTL are therefore available for breeding (Table 2). Work is in progress for converting dominant markers into SCARs and identifying more useful markers for MAS, such as SSRs, in various sources of resistance (Coyne, personal communication).

Genetic Loci for Basal Branching (Ramosis)

Basal branching may have an effect on plant standability and possibly on yield determination, therefore may be of interest to the pea breeder. Genes controlling basal branching are also of considerably greater interest for understanding the physiology of apical dominance and the roles of auxins and cytokinins in shoot architecture. Six ramosis genes have been identified (*rms1* through *rms6*). Markers associated with *rms1*, *rms2*, *rms3*, *rms4* and *rms6* are described in Table 2. The gene for *rms1* has been identified (Foo *et al.*, 2005), therefore a perfect marker is available for this gene.

QTL for Ascochyta Blight Resistance

In pea, Ascochyta blight is the term for the economically significant complex of fungal diseases caused by *Ascochyta pisi*, *Mycosphaerella pinodes*, and *Phoma medicaginis* var *pinodella*, or a subset of these pathogens. QTL mapping studies based on molecular linkage maps have identified QTL for resistance to *Ascochyta pisi* Race C (Dirlewanger *et al.* 1994), to *M. pinodes* and *P. medicaginis* (Timmerman-Vaughan *et al.*, 2002, 2004; Ta'ran *et al.*, 2003b, Prioul *et al.*, 2004).

Resistance QTL for *A. pisi* race C were identified on linkage groups I, IV and VI (Dirlewanger *et al.*, 1994), explaining up to 74% of the variation in resistance. The linkage group IV QTL contributes about 45% of the variation in resistance. Consequently, application of MAS in suitable germplasm using markers associated with the linkage group IV QTL region may improve the disease resistance profile of cultivars destined for geographic regions where *A. pisi* race C is an important pathogen. Development and validation of markers suitable for MAS will require additional research, however, to increase map saturation with user-friendly markers.

Implementation of MAS for resistance to Ascochyta blight epidemics that involve

M. pinodes and/or *P. medicaginis* will be challenging because a large number of genetic loci are involved and because interactions appear to occur between plant development (flowering time/plant maturity and height, in particular) and disease development. QTL have been identified for resistance to Ascochyta blight disease caused by *M. pinodes* and/or *P. medicaginis* by Prioul *et al.* (2004), Tar'an *et al.* (2003b) and Timmerman-Vaughan *et al.* (2002, 2004). The Timmerman-Vaughan *et al.* (2002, 2004) and Prioul *et al.* (2004) studies revealed the genetic complexity of resistance, identifying as many as 14 and 12 putative QTL, respectively. QTL have been detected on all seven linkage groups. While most QTL only explain a small fraction of the variation in the disease phenotype, the Timmerman-Vaughan *et al.* (2002, 2004) and Prioul *et al.* (2004) studies have identified genomic regions that explain 20% or more of the disease response variation, some of which colocalizing with QTL associated with plant development. In all QTL mapping studies, resistance alleles have been contributed by both the resistant and susceptible parents, introducing an additional challenge for implementing MAS. Markers associated with Ascochyta blight resistance QTL are listed in Table 2.

In spite of the genetic complexity of resistance and the limitations of the linkage maps published to date, it is possible to speculate that these studies, using diverse sources of resistance, may have identified the same QTL in some cases. If this were so, then these QTL might be the best candidates for implementation of MAS in the first instance because they have been detected in multiple environments as well as diverse germplasm. For example, QTL *mpVII-2* and *mpVII-1* on linkage group VII detected by Prioul *et al.* (2004) are associated with the same genomic regions as QTL *Asc7.1* and *Asc7.2* detected by Timmerman-Vaughan *et al.* (2002, 2004). On linkage group IV, a QTL detected by Tar'an *et al.* (2003b) and QTL *Asc4.1* detected by Timmerman-Vaughan *et al.* (2004) are associated with the genomic region containing the anchor locus P628.

QTL for Yield Components, Seed Traits and Plant Architecture

Mapping studies have also characterised QTL for traits involved in yield including components of yield, plant architecture and seed traits. Yield and yield component QTL have been mapped (Timmerman-Vaughan *et al.*, 2005), including yield *per se* (5 QTL, explaining a total 46-66% of variation), seed weight (9 QTL, explaining a total 43-62% of variation), seed number (9 QTL, explaining a total 15-25% of variation) and harvest index (4 QTL, explaining a total 40% of variation). Notably, QTL for different yield-related traits often coincided in the same genomic region or were even associated with the same molecular markers. Markers associated with yield and yield component QTL that are strongly supported by colocalisation are summarised in Table 2.

Green seed colour quality QTL have also been mapped (McCallum *et al.*, 1997). An important QTL primarily affecting color density (the Y component of the YUV color scale) was identified on linkage group V. Additional QTL affecting U and V were detected on linkage groups II, III and VII. Map refinement since publication of the McCallum *et al.* (1997) paper has clarified linkage group identities. Markers that may be of use for MAS are described in Table 2.

QTL mapping has identified two QTL for lodging resistance, on linkage groups III and VI (Tar'an *et al.*, 2003b; Table 2), and a SCAR marker (A001) has been developed for an AFLP marker associated with the linkage group III QTL. In addition, the application for MAS of two associated markers, A001 and A004 (which is not linked to A001), has been explored (Tar'an *et al.*, 2004). The results of the MAS study suggest that selection of appropriate germplasm using the marker phenotypes A001 (band present)/A004 (band absent) may result in reduced lodging. The lodging resistant QTL on linkage group III coincides with a QTL for plant height, while the linkage group VI QTL coincides with a QTL for resistance to *M. pinodes*.

Genomic regions controlling plant height have also been identified using QTL mapping strategies (Tar'an *et al.*, 2003b, Prioul *et al.*, 2004). Important QTL affecting plant height mapped to linkage group III by both Prioul *et al.* (2004) and Tar'an *et al.* (2003b). The QTL Prioul *et al.* (2004) identified maps in the same genomic region as *Le* and may indicate variation at that genetic locus. Prioul *et al.* (2004) also identified QTL for plant height on linkage groups II and VII. Markers associated with the plant height QTL are described in Table 2.

MAS in Lentil

In lentil, major diseases such as Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lentis*), Ascochyta blight (caused by *Ascochyta lentis*) and anthracnose (caused by *Colletotrichum truncatum*) were shown to be simply inherited and controlled by one or few major resistance genes (Eujayl *et al.*, 1998a; Ford *et al.*, 1999; Chowdhury *et al.*, 2001; Tullu *et al.*, 2003). These genes were tagged with RAPD markers and in case of the anthracnose were also used (Tullu *et al.*, 2003). Eujayl *et al.* (1998b) used a RIL mapping population derived from a cross ILL 5588 (resistant) x L 692-16-1(s) (susceptible) Fusarium wilt to show that RAPD marker (OP-K15$_{900}$) was linked to Fusarium wilt (*Fw*) resistance gene at a distance of 10.8 cM. Further Hamwieh et al. (2005), localized this *Fw* gene on linkage group 6, where the gene was shown to be flanked by a microsatellite marker (SSR59-2B) and a AFLP marker (p17m30710) at distances of 8.0 cM and 3.5 cM, respectively. Ford et al. (1999) tagged a major gene resistance for Ascochyta blight (*ral1* in ILL5588) with two RAPD markers, located approximately 6 and 14 cM from *ral1*. These RAPD markers were converted into SCAR markers and are being used in the Australian breeding programs. Another Ascochyta blight resistance gene *ral2* in cultivar Indianhead was tagged by Chowdhury *et al.* (2001) using RAPD markers. The two identified RAPD markers UBC227$_{1290}$ and OPD10$_{870}$ flanked *ral2* locus at a distance of 12 and 16 cM, respectively. Tullu *et al.* (2003) identified two RAPD markers OPE06$_{1250}$ and UBC704$_{700}$ which are linked to Anthracnose resistance locus (*LCt-2* in accession PI 320937) at a distance of 6.4 cM (in repulsion) and 10.5 cM (in coupling) respectively. Tar'an *et al.* (2003a) used the marker linked to Anthracnose resistance (OPE06$_{1250}$), and markers linked to Ascochyta blight resistance *ral1* (UBC 227$_{1290}$) and *AbR1*(RB18$_{680}$) genes in marker assisted selection to pyramid genes for Anthracnose and Ascochyta blight resistance.