II.6 Molecular Markers in *Vigna* Improvement: Understanding and Using Gene Pools

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1 Introduction

The genus *Vigna* is a large genus with about 90 species distributed worldwide in warm and tropical regions. The genus includes 13 cultigens (Table 1), of these cowpea, mungbean and azuki bean are the most important (Table 2). Perhaps due to *Vigna* cultigens being mainly crops in the developing world, molecular marker and genomic studies have lagged behind those of other major crops. For example, genome designations are known for species in many crop genera, such as *Glycine*, but these have not been established for *Vigna* species. There has recently been considerable progress in using molecular markers to understand *Vigna* genetic resources and in developing genome maps and associating molecular markers to agronomically important traits in the *Vigna* cultigens. However, the actual use of molecular markers in *Vigna* breeding at the end of 2002 is still being planned.

In this chapter we review recent progress in the use of molecular markers in understanding *Vigna* genetic resources, *Vigna* linkage map development, particularly in relation to the location of genes for agronomic traits, and transformation systems.

2 Application of Molecular Markers to Understand the *Vigna* Crop Gene Pools

2.1 Genus *Vigna*

Current understanding of the taxonomy of the genus *Vigna* rests largely on the work of Maréchal and coworkers (1978). Several insights into *Vigna* taxonomy have resulted from molecular analyses of *Vigna*. Restriction fragment length polymorphism (RFLP) analysis supports the taxonomic opinion that *Phaseolus* and *Vigna* belong to a common complex of species particularly when the poorly studied New World *Vigna* species are considered (Fatokun et

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al. 1993). Chloroplast DNA, RFLP and isozyme analyses suggest that both section *Catiang* (the cowpea section) of the subgenus *Vigna* and subgenus *Ceratotropis* are well-defined groups (Jaaska and Jaaska et al. 1988, 1990; Fatokun et al. 1993; Vaillancourt and Weeden 1993)

Table 1. The cultivated and domesticated *Vigna* species

Subgenus *Section*	Cultigen species name (common name)	Presumed progenitor
Vigna		
Catiang	*V. unguiculata* (L.) Walpers subsp. *unguiculata* var. *unguiculata* (cowpea)	ssp. *unguiculata* var. *spontanea* (Schweinf.) Pasquet
Vigna	*V. subterranea* L. (bambara groundnut)[a]	*V. subterranea* L.
	V. luteola (Jacq.) Benth.[a]	*V. luteola* (Jacq.) Benth.
	V. marina (Burm.) Merrill[a]	*V. marina* (Burm.) Merrill
Plectotropis	*V. vexillata* (L.)A. Richard[a]	*V. vexillata* (L.) A. Richard
Ceratotropis		
Angulares	*V. angularis* (Willd.) Ohwi and Ohashi var. *angularis* (azuki bean)	var. *nipponensis* (Ohwi) Ohwi and Ohashi
	V. reflexo-pilosa Hayata var. *glabra* (Roxb.) N. Tomooka and Maxted	var. *reflexo-pilosa*
	V. trinervia (Heyne ex Wight and Arnott) Tateishi and Maxted[a]	*V. trinervia* (Heyne ex Wight and Arnott) Tateishi and Maxted
	V. umbellata (Thunb.) Ohwi and Ohashi (rice bean)[a]	*V. umbellata* (Thunb.) Ohwi and Ohashi
Ceratotropis	*V. mungo* (L.) Hepper var. *mungo* (black gram)	var. *silvestris* Lukaki, Maréchal and Otoul
	V. radiata (L.) Wilczek var. *radiata* (mungbean)	var. *sublobata* (Roxb.) Verdcourt
Aconitifoliae	*V. aconitifolia* (Jacquin)Maréchal (moth bean)	Unknown
	V. trilobata (L.) Verdcourt (jungli bean)[a]	*V. trilobata* (L.) Verdcourt

[a] Species cultivated, but not fully domesticated

Table 2. The production, production area and main areas of production of the main *Vigna* cultigens. (Sources: Poehlman 1991; Lumpkin and McClary 1994; Singh et al. 1997)

Species	Production (10^3 tonnes)	Area (10^3 ha)	Main areas of production
Cowpea	3000	12,500	64% Western and central Africa
Mungbean	2500–3000	5000	45% India
Azuki bean	600	1000	60% China

2.2 Subgenus *Vigna*

Cowpea was domesticated in Africa, though where is unclear (Pasquet 1999), from the wild annual form (*V. unguiculata* ssp. *unguiculata* var. *spontanea*). Amplified fragment length polymorphism (AFLP), chloroplast DNA and isozyme data reveal that domestication resulted in a major reduction in genetic diversity, suggesting a single domestication event followed by genetic differentiation under domestication (Weeden et al. 1996; Pasquet 1999; Coulibaly et al. 2002). Despite much effort to collect cowpea genetic resources from throughout Africa (Ng and Monti 1990), large gaps in the collection remain (Pasquet 1999). Cultivated cowpea (var. *unguiculata*) can intercross with wild annual (var. *spontanea*) and some wild perennial (various subspecies of *V. unguiculata*) forms (Ng 1995; Pasquet 1996). Gene flow between wild and domesticated cowpea, revealed by AFLP analysis, has resulted in a large crop weed complex and thus broadened the genetic diversity of *V. unguiculata* (Coulibaly et al. 2002).

Chloroplast DNA analysis suggests the cowpea section is phylogenetically closer to subgenus *Plectotropis* than other species of subgenus *Vigna* and this may have importance in relation to introducing useful characters into cowpea (Vaillancourt and Weeden 1993). Studies of diversity within *V. unguiculata* have shown the usefulness of random amplified polymorphic DNA (RAPD) over isozymes for revealing variation (Vaillancourt and Weeden 1993; Mignouna et al. 1998). Microsatellite markers developed by Li et al. (2001) will be powerful tools to understand the genetic diversity of the cowpea gene pool.

RAPD markers have been used to analyse diversity in *V. subterranea* (Amadou et al. 2001), *V. luteola* and *V. marina* (Sonnante et al. 1997). *V. subterranea* accessions were shown to be differentiated based on geographic origin, western and southern Africa. *V. marina* ssp. *oblonga* was found to be more closely related to *V. luteola* than *V. marina* ssp. *marina*, suggesting that taxonomic revision of *V. marina* may be in order.

2.3 Subgenus *Ceratotropis*

The subgenus *Ceratotropis*, which includes eight cultigens (Table 1), is a difficult group of species to distinguish based on morphological characters (Baudoin and Maréchal 1988). However, the application of various molecular marker techniques has greatly improved understanding of the subgenus. Molecular analyses based on AFLP, chloroplast and rDNA variation have supported the division of the subgenus *Ceratotropis* into three sections (Doi et al. 2002; Tomooka et al. 2002a, b). These three sections, section *Angulares* (azuki and rice bean group), section *Ceratotropis* (mungbean and black gram group) and section *Aconitifoliae* (moth bean group), can be considered separate gene pools for breeding purposes. Of the three sections, section *Angulares* is the most complex and speciation appears to be recent as interspecific

genetic divergence is not great, although between some species interspecific hybridization barriers exist (Tomooka et al. 2002b, c). There remains much to be understood about barriers to hybridization and speciation processes in the genus *Vigna*.

Studies of *V. radiata* suggest that Afghanistan-Iran retains more genetic diversity than other regions (Tomooka et al. 1992). However, the presence of wild and weedy races of mungbean, archaeological remains of *Vigna* and landrace diversity suggest that India is the most likely area of domestication (Tomooka et al. 2003). Studies of the diversity of *V. radiata* var. *sublobata*, using RAPD and AFLP methods, suggest considerable geographic variation in this presumed progenitor of mungbean (Savaranakumar et al. 2004), but comprehensive studies of this taxa from throughout its range from Africa to Australia are lacking.

RAPD and AFLP markers have also been used to understand the domestication process in azuki bean, *V. angularis* (Yee et al. 1999; Mimura et al. 2000; Xu et al. 2000a, b; Isemura et al. 2002; Zong et al. 2003). The most comprehensive of these studies suggests that there are four different groups of germplasm related to geographic origin, China, Korea (and some Japanese germplasm), Japan and the Himalayan region. Among these, Himalayan germplasm is well differentiated from the other groups (Zong et al. 2003). The results suggest that azuki bean was probably domesticated independently in the Himalayan region and East Asia.

3 Linkage Maps

To date there have been ten different linkage maps based on eight different crosses developed for *Vigna* species. Four have been developed for *V. radiata* (Menancio-Hautea et al. 1992; Lambrides et al. 2000), four have been developed for *V. unguiculata* (Fatokun et al. 1992b; Menendez et al. 1997; Ubi et al. 2000; Ouédraogo et al. 2002a) and two have been developed for *V. angularis* (Kaga et al. 1996, 2000). A comparison among these maps is shown in Table 3. Only one of the maps developed so far has resolved the 11 linkage groups (Ouédraogo et al. 2002a), equivalent to the haploid chromosome number of these three *Vigna* species (Sinha and Roy 1979). To overcome the limited number of marker clones available for *Vigna* species, many DNA clones from related species such as *Glycine max* and *Phaseolus vulgaris* have been used to increase the saturation of the *Vigna* genome maps (Menacio-Hautea et al. 1992; Boutin et al. 1995; Kaga et al. 2000; Lambrides et al. 2000; Chaitieng et al. 2002). Recently, microsatellites have been identified in *Vigna* species based on database searches (Yu et al. 1999) and microsatellite libraries have been specifically developed from cowpea (Li et al. 2001), mungbean (Kumar et al. 2002) and azuki bean (Wang et al. 2004).

Table 3. Genome linkage maps for *Vigna* species

Cross combination	Population (plants/lines) analyzed	Markers used	Linkage groups resolved	Map distance (cM)	Level of distortion (%)	Reference
V. angularis (cv. Erimoshouzu) × *V. nakashimae*	F_2 population (80)	19 RFLP, 108 RAPD and 5 morphological markers	14	1250	19.7	Kaga et al. (1996)
V. angularis (cv. Erimoshouzu) × *V. umbellata* (cv. Kagoshima)	F_2 population (86)	114 RFLP, 74 RAPD, 1 morphological marker	14	1702	29.8	Kaga et al. (2000)
V. radiata var. *radiata* × *V. radiata* var. *sublobata* (from Madagascar)	F_2 population (58)	151 RFLP, 20 cDNA and 1 pest locus	14	1570	12	Menacio-Hautea et al. (1992)
V. radiata var. *radiata* × *V. radiata* var. *sublobtata* (from Australia)	F_2 population (67)	52 RFLP, 56 RAPD, 2 morphological markers	12	758.3	14.5	Lambrides et al. (2000)
V. radiata var. *radiata* × V. *radiata* var. *sublobata* (from Australia)	Recombinant inbreed (67)	113 RAPD, 2 morphological markers	12	691.7	24	Lambrides et al. (2000)
V. radiata (cv. Berken) × *V. radiata* ssp. *sublobata* (ACC41)	Recombinant inbreed (80)	255 RFLP markers	13	737.9	30.8	Humphrey et al. (2002)
V. unguiculata (IT84S-2246–4 improved line) × *V. unguiculata* ssp. *dekindtiana* var. *pubescens* (TVNu-110–3A)	Recombinant inbreed (94)	77 RAPD, 3 morphological markers	12	669.8	21.7	Ubi et al. (2000)
V. unguiculata (IT2246–4) × *V. unguiculata* ssp. *dekindtiana* (TVN1963)	F_2 population (58)	79 genomic, 4 cDNA, 6 RAPD, 2 aphid, 1 seed coat texture markers	10	>800	22	Menacio-Hautea et al. (1993); Fatokun et al. (1997)
V. unguiculata (IT84S-2049) × *V. unguiculata* (524B)	F_2 population (94)	133 RAPD, 19 RFLP, 25 AFLP, 3 morphological, 1 biochemical markers	12	972	18	Menéndez et al. (1997)
V. unguiculata (IT84S-2049) × *V. unguiculata* (524B)	Recombinant inbred (94)	133 RAPD, 36 RFLP, 267 AFLP, 3 morphological, 1 biochemical markers	11	2670	19.7	Ouédraogo et al. (2002a)

The linkage maps for *V. radiata* have been based on crosses between *V. radiata* var. *radiata* and *V. radiata* var. *sublobata*. In one linkage map the *V. radiata* var. *sublobata* accession came from Madagascar, in the other the accession came from Australia. In the resulting genome maps the order of markers was similar. However, the level of distortion was higher in the cross that involved the Australian accession and regions of distortion did not coincide with those produced using the Madagascar accession. This suggests that *V. radiata* var. *sublobata* has considerable intraspecific genetic diversity and that the Australian form of var. *sublobata* is more distantly related to cultivated *V. radiata* than the Madagascar form (Lambrides et al. 2000).

Of the genome maps reported that between *V. angularis* and *V. umbellata* had the highest level of segregation distortion (29.8 %; Table 3). High levels of segregation distortion may not enable the trait(s) of interest to be found in segregating populations. When using distantly related species in crosses, it is necessary to generate very large segregating populations, since the likelihood of recombination drops and thus, the likelihood of getting the traits needed in the desired background is low. However, large populations may not be easy to obtain as weak or sterile F_2 plants can limit seed production (Kaga et al. 1996). Ways to overcome this problem in *Vigna* section *Angulares* may include using bridging species to facilitate gene transfer (Tomooka et al. 2002c).

The latest and most detailed genetic linkage map of cowpea spans a total of 2670 cM with an average of 6.43 cM between markers (Ouédraogo et al. 2002a). This cowpea linkage map revealed how important using a variety of molecular markers is to obtain a saturated linkage map. Ouédraogo et al. (2002a) added AFLP markers to the cowpea linkage map and this revealed a large segment of 580 cM on linkage group 1 that was undetected when only RFLP and RAPD markers were used to create the linkage map. Thus, further major improvement to the genetic linkage map for cowpea and other *Vigna* species may be expected when microsatellite markers are used.

4 Synteny

4.1 *Vigna* and Other Genera

There have been efforts to understand the comparative genome organization across *Vigna* and related cultigens (Menacio-Hautea et al. 1993; Boutin et al. 1995). Comparisons of genome maps of *V. radiata* with *V. unguiculata* and *Phaseolus vulgaris* have revealed conserved blocks of considerable size some containing loci for important traits. The comparison with *P. vulgaris* showed that average size of conserved blocks is about 36.6 cM with the longest being 103.5 cM (Table 4). Therefore, there is considerable scope for understanding genome organization in cultigens of genus *Vigna* by using probes from and comparison with better developed genome maps in other related species.

Table 4. Comparison of lengths of genome blocks conserved between mungbean and soybean, and common bean and soybean. (Adapted from Boutin et al. 1995)

Species compared	Average length of conserved block (cM)	Standard deviation	Length of the longest conserved block (cM)
Mungbean and soybean	12.2	9.4	37.8
Common bean and soybean	13.9	9.5	34.8

One of the best-developed genome maps among legumes is that of soybean. Comparison of *V. radiata* and *Glycine max* revealed a different type of genome organization than the comparison of *Glycine max* and *Phaseolus vulgaris*. Conserved linkage blocks are smaller and are highly scattered in the *V. radiata* comparison compared to *P. vulgaris* (Table 4). However, specific analysis of a genomic region influencing seed weight in soybean showed colinearity of RFLP markers with mungbean (Maughan et al. 1996).

Comparative mapping of *V. radiata* and *Lablab purpureus* (hyacinth pea), both belonging to subtribe Phaseolinae, revealed that the order of markers is highly conserved and enabled suggestions of which linkage group belonged on the same chromosome in lablab and mungbean (Humphrey et al. 2002). Surprisingly, the results suggest that mungbean shares a higher level of genome organization with lablab than taxonomically more closely related species in the subgenus *Ceratotropis* (*V. angularis* and *V. umbellata*). However, while mungbean and lablab maintain the same marker order, they have accumulated a large number of deletions/duplications after divergence (Humphrey et al. 2002).

Despite the incompleteness of the genetic map data, comparisons between *Phaseolus vulgaris* and *V. radiata* and *Arabidopsis* have enabled a reconstruction of a proposed ancestral DNA segment in the present genome of soybean (Lee et al. 2001).

4.2 Within the Genus *Vigna*

Early comparison of cowpea and mungbean linkage maps revealed that 90 % (48 out of 53) RFLP probes hybridized with both species. While marker order was often similar, distances between markers varied. Ten regions of the linkage maps of these two cultigens showed syntenic association (Menacio-Hautea et al. 1993). A specific study of the genetics of seed weight resulted in finding quantitative trait loci (QTLs) that accounted for 52.7 and 49.7 % of the variation for this trait in cowpea and mungbean, respectively (Fatokun et al. 1992a). The genomic region with the greatest effect on seed weight spanned the same RFLP markers in the same order (Fatokun et al. 1992a)

4.3 Within *Vigna* Subgenus *Ceratotropis*

Comparison of two interspecific linkage maps (rice bean × azuki bean and azuki bean × *V. nakashimae*) revealed seven conserved linkage blocks (size range 7–115 cM; Kaga et al. 2000). Comparison of the rice bean × azuki bean linkage map with the mungbean linkage map of Menacio-Hautea et al. (1993) revealed 16 conserved segments without regions of inversion and translocation (size range 2–95 cM; Kaga et al. 2000). This study enabled orthologous linkage groups in the different maps to be proposed.

5 Gene Mapping

A list of the loci for major agronomic traits that have been associated with linkage groups in *Vigna* is provided (Table 5). Among these, the progress in cowpea in mapping for resistance to the parasitic plant *Striga* and in mungbean mapping resistance to bruchid beetles and powdery mildew provide the best examples of the state of gene mapping in *Vigna*. These examples are discussed here.

The parasitic plant *Striga gesnerioides* can result in 100 % yield loss in cowpea. AFLP markers were used to finely map *Striga* resistance. Five races of *Striga* are known to affect cowpea. Of these five races, AFLP markers have been found linked to genes for resistance to races 1 and 3 (Ouédraogo et al. 2001, 2002b). The AFLP marker studies revealed that the genes (or alleles) for resistance are clustered on at least one linkage group (linkage group 1 of the genome map of Ouédraogo et al. 2002a; Fig. 1). The identification of molecular markers associated with clustered *Striga* resistance genes are now leading to plans to use molecular marker selection in cowpea breeding (B.B. Singh 2002, pers. comm.).

RFLP analysis was used to map a bruchid resistance gene in wild mungbean. The gene was a single major locus on linkage group 8 (subsequently revised to linkage group 9; Young et al. 1992; Fig. 2a, b). In a mapping population between azuki bean and rice bean the main QTL for bruchid resistance in rice bean was linked to one of the same RFLP probes (pR26) linked to bruchid resistance in mungbean (Kaga 1996). The nearest RFLP marker to the bruchid resistance gene in the mungbean map was 3.6 cM distant (Fig. 2a). Since this resistance gene, from *V. radiata* var. *sublobata* (TC1966), also has an inhibitory activity against bean bug (*Riptortus clavatus* Thunberg) and it was associated with novel cyclopeptide alkaloids, further efforts were made to map this gene (Kaga and Ishimoto 1998). The resulting genetic map enabled the resistant dominant gene to be located to within 0.2 cM of the nearest RFLP markers (Fig. 2c). This map distance may enable the gene to be cloned within a large genomic library for eventual introduction into susceptible mungbean lines or other crops.

Table 5. Molecular mapping of agronomically important traits in *Vigna*

Species	Molecular markers used	Trait of interest	Linkage Group (LG)	Reference
V. unguiculata	RFLP	Aphid resistance	LG.1 (Fatokun et al. 1992b)	Myers et al. (1996)
V. unguiculata	AFLP	*Striga* resistance	Resistance to race 1 and 3 on LG.1 and 6 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2001, 2002a, b)
V. unguiculata	AFLP	Cowpea mosaic virus	LG.3 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. unguiculata	AFLP	Cowpea severe mosaic virus	LG.3 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. unguiculata	AFLP	Blackeye cowpea mosaic virus (BlCMV)	LG.8 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. unguiculata	AFLP	Southern bean mosaic virus (SBMV)	LG.6 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. unguiculata	AFLP	Fusarium wilt	LG.3 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. unguiculata	AFLP	Root-knot nematode	LG.1 (Ouédraogo et al. 2002a)	Ouédraogo et al. (2002a)
V. radiata	RFLP	Bruchid resistance	LG.8 (Menancio-Hautea et al. 1992)	Young et al. (1992)
V. radiata	RFLP, RAPD	Bruchid resistance	LG.8 (Menancio-Hautea et al. 1992)	Kaga and Ishimoto (1998)
V. radiata	RFLP, AFLP	Powdery mildew resistance	QTL	Chaitieng et al. (2002)
V. radiata	RFLP	Powdery mildew resistance	QTL	Young et al. (1993)
V. unguiculata	RFLP	Seed weight	QTL	Fatokun et al. (1992a)
V. radiata	RFLP	Seed weight	QTL	Fatokun et al. (1992a)
V. unguiculata	RFLP, RAPD	Multiple quantitative traits	QTL	Menéndez et al. (1997)

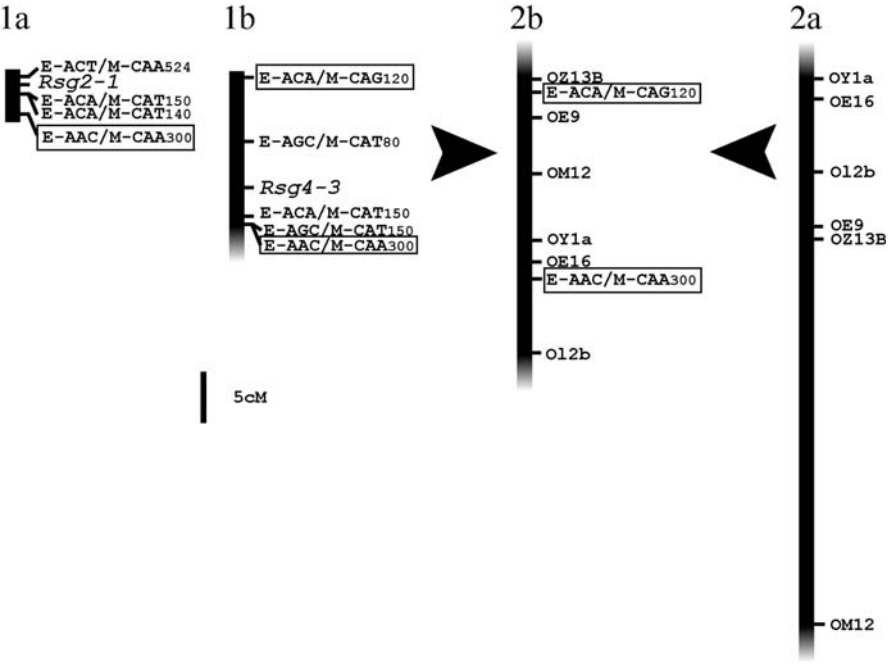

Fig. 1. The use of DNA markers (*boxes*) to finely map the location of genes (alleles) for resistance to *Striga* (based on Ouédraogo et al. 2001, 2002a, b). **1a** AFLP markers found linked to resistance (*Rsg2–1*) for *Striga* race 1 from Burkino Faso in cross Tvx3236 (sus) × IT82D-849 (res). **1b** AFLP markers found to be linked to resistance (*Rsg4–3*) for *Striga* race 3 from Niger in cross IT84S-2246–4(sus)×Tvu14676 (res). **2a** Location of RFLP markers on linkage group 1 of cowpea linkage map. **2b** Location of RFLP and AFLP markers on linkage group 1 of the cowpea linkage map

Powdery mildew resistance is a multi-genic trait. The first attempt to map resistance in a breeding line of mungbean identified three QTLs on three different linkage groups accounting for 58% of the total variation (Young et al. 1993). Using a different powdery mildew-resistant line to develop a mapping population, 96 RFLP probes failed to identify any QTL associated with resistance (Chaitieng et al. 2002). Subsequently, 100 AFLP primer pair combinations were tested and 4 out of more than 5000 AFLP bands were found to be associated with resistance. The main QTL associated with resistance was found on a new linkage group and accounted for 68% of the total variation.

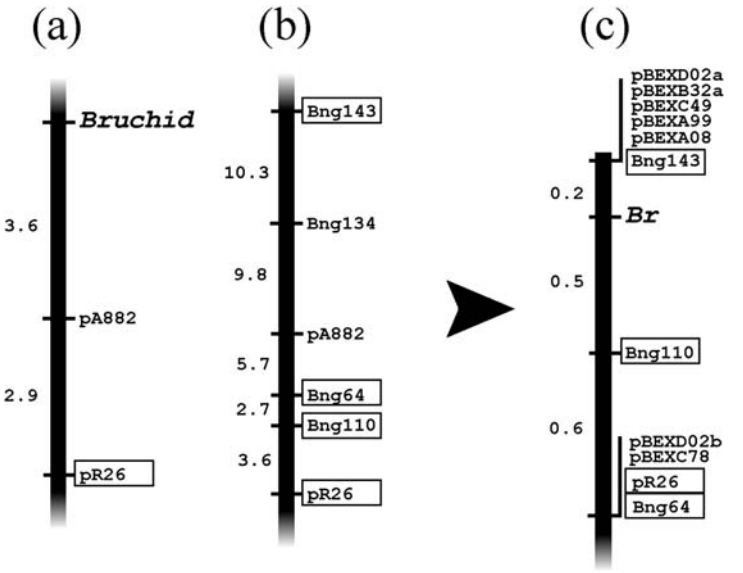

Fig. 2. Use of RFLP markers (*boxes*) to finely map the location of bruchid resistance on the mungbean linkage map. Distances in cM. **a** Initial location of bruchid resistance in the cross *V. radiata* (VC3890) × *V. radiata* var. *sublobata* (TC1966) (Young et al. 1992). **b** Revised linkage map of part of linkage group 9 with information from Bng probes added (Mungbean: U–Minnesota-9 at http://beangenes.cws.ndsu.nodak.edu/). **c** Fine mapping of the location of bruchid resistance in the cross *V. radiata* (Osaka-ryokuto) × *V. radiata* var. *sublobata* (TC1966) based on combination of previously used RFLP probes and newly developed probes from RAPD markers (Kaga and Ishimoto 1998). Distance differences between these figures reflect different parents in the crosses and mapping population size

6 Transformation Systems

Only in azuki bean (*V. angularis*) has genetic transformation been reported to result in improved breeding lines. Ishimoto et al. (1996) developed a bruchid-resistant azuki bean line that had an α-amylase inhibitor gene driven by a seed-specific promoter from common bean (*Phaseolus vulgaris*). This gene was introduced using *Agrobacterium*-mediated gene transfer. The transgenic azuki bean could completely block larvae development of three bruchid species that are the major storage pests of mungbean and cowpea. The method was refined by Yamada et al. (2001) and was found to be reproducible with high transformation efficiency. Using the same method, 15 independent transgenic lines with sGFPs65T (modified green fluorescent protein gene) and 15 independent lines with mt-sHSP (heat shock protein gene) from tomato have been produced (Kaga et al. 2003). Thus, the transformation system of azuki bean can be used for routine transformation.

Another well-established transformation system is in moth bean (*V. aconitifolia*) using protoplasts. Although it has been shown that transformation success is cultivar-dependent, the efficiency is the highest among *Vigna* species. Plant transformation systems have been reported for six *Vigna* cultigens (Table 6).

Since the success of *Agrobacterium*-mediated gene transfer, on which most methods rely, is largely influenced by a combination of culture condition, tissue type or genotype of cultivar, the direct gene transfer is an attractive alternative approach to overcoming these complicating factors. Using particle bombardment, Bhargava and Smigocki (1994) obtained transformants of mungbean, black gram and moth bean, but molecular evidence of gene integration was not shown. In planta electroporation-mediated gene transfer has been demonstrated in cowpea and several other grain legumes (Chowrira et al. 1996) and may be a more practical and rapid method to produce plants in *Vigna* species. Plans have been made to transform cowpea using gene constructs encoding *Bt* toxin, α-amylase inhibitor and cysteine proteinase inhibitor for resistance to *Maruca* pod borers and cowpea bruchids. A separate project has been proposed to transfer gene constructs for resistance to cowpea aphid-borne mosaic virus (de Vries and Toenniessen 2001).

Further information on transformation and regeneration systems in *Vigna* cultigens can be found in Nagl et al. (1997).

Table 6. Status of plant transformation in the main *Vigna* crops

Species	Transformed plants produced	Method for gene transfer
V. unguiculata	*hpt* (hygromycin phosphotransferase) gene[a]	*Agrobacterium*
	uidA (β-glucuronidase, GUS) gene[b]	In planta electroporation
	uidA and *nptII* gene[c]	*Agrobacterium*
V. radiata	*uidA* and *nptII* genes[d]	*Agrobacterium*
	uidA and *nptII* (neomycin phosphotransferase) genes[e]	Particle bombardment
	uidA, *nptII* and *hpt* genes[f]	*Agrobacterium*
V. angularis	α AI (α-amylase inhibitor) and *nptII* genes[g]	*Agrobacterium*
	sGFP(S65T) (modified green fluorescent protein), *uidA* and *nptII* genes[h]	*Agrobacterium*
V. aconitifolia	*nptII* gene[i]	Electroporation
	nptII gene[j]	*Agrobacterium*
	uidA and *nptII* genes[e]	Particle bombardment
V. mungo	*uidA* and *nptII* genes[e]	Particle bombardment

[a] Muthukumar et al. (1996); [b] Ignacimuthu (2000); [c] Chowrira et al. (1996); [d] Pal et al. (1991); [e]Bhargava and Smigocki (1994); [f]Jaiwal et al. (2001); [g]Ishimoto et al. (1996); [h]Yamada et al. (2001); [i]Köhler et al. (1987); [j]Eapen et al. (1987)

7 Conclusions

The genome size of cowpea and mungbean are small, ranging from 470–613 Mb, about half that of soybean (Murray et al. 1979; Arumuganathan and Earle 1991). Therefore, *Vigna* species are good candidates for more in-depth genome analysis in the future. The results of such research would likely have an impact beyond *Vigna* as the discussion above on comparative genome mapping suggests.

Published information on the actual use of molecular markers in *Vigna* improvement is lacking. It is only in the last year that a *Vigna* linkage map has resolved the 11 linkage groups that correspond to the haploid chromosome number of diploid *Vigna*. The latest cowpea linkage map has associated many agronomically important traits to molecular markers, thus, we may also be "cautiously optimistic" (Young 1999) that molecular markers will play a role in the future improvement of the *Vigna* cultigens.

This is a field where information rapidly becomes out of date. Recent information related to this topic may be found at:

- http://beangenes.cws.ndsu.nodak.edu
- http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=3913

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