CHAPTER 9
MOLECULAR MARKERS FOR ANALYSIS OF RESISTANCE TO STRIGA GESNERIOIDES IN COWPEA

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Cowpea is one of the most important food and forage legumes in the semi-arid tropics. Losses due to attack by the root hemiparasitic weeds Striga gesnerioides (witchweed) and Alectra vogelii are among the major constraints to cowpea production in West and Central Africa. At least seven races of S. gesnerioides have been identified within the cowpea-growing regions of West Africa based on host differential response and genetic diversity analysis. Race-specific resistance genes have been identified and mapped to two linkage groups (LG1 and LG6) of the cowpea genetic map. Molecular markers have been identified that are associated with specific resistance genes, and at present two markers have been developed as sequence-confirmed amplified regions and are available for germplasm evaluation and efficacy testing on field populations. Marker-assisted selection has yet to be implemented in cowpea but the groundwork has now been laid for its development.

1. Introduction
Cowpea (Vigna unguiculata L.) is one of the most important food legumes in the semi-arid tropics covering Asia, Africa, Southern Europe, Southern United States and Central and South America. It serves a critical role in the lives of millions of people in Africa and other parts of
the developing world, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops. In addition to its nutritional value, cowpea is a valuable and dependable commodity that produces income for farmers and helps to restore soil fertility for succeeding cereal crops grown in rotation with it.

Yields of cowpea grain are reduced by a variety of biotic and abiotic constraints of which attack by two root parasitic angiosperms, *Striga gesnerioides* and *Alectra vogelii*, are a major concern. *S. gesnerioides* causes extensive damage to cowpea in the Sudano-Sahelian belt of West and Central Africa. Annual yield losses range from slight to moderate in most regions, however, total crop loss is not unusual in some parts of Nigeria, Niger, and Burkina Faso. *A. vogelii* also infects a number of grain legume crops in an agroecological range extending from the northern agricultural regions of South Africa and Swaziland, through Central Africa to Burkina Faso and Mali in the west and Kenya in the east. In addition to cowpea, soybean, bambara groundnut (*Vigna subterranea*), common bean, mung bean (*Phaseolus radiata*) and many legume fodder crops, including *Lablab purpureus*, *Siratro* (*Macroptilium atropurpureum*) and velvet bean (*Mucuna pruriens*) are also parasitized by these two noxious pests.

Several control strategies have been developed for parasitic weeds including improved cultural practices, breeding using wild and cultivated germplasm as sources of resistance, and the use of chemical control. These topics are covered elsewhere in this volume.

The breeding of improved cultivars with pyramided desirable agronomic traits and multiple disease and pest resistances requires effective screening and selection procedures. Cowpea is considered to have been domesticated in Africa and likely to have co-evolved with *Striga* and *Alectra*. Thus, it may have many of the requisite genes for resistance. Resistance against most parasitic weeds, including *S. gesnerioides* and *A. vogelii* is often difficult to assess due to numerous confounding factors in the field, including parasite variability, unpredictable environmental influences, and imprecise selection criteria. Despite these difficulties, significant success has been achieved in the identification of heritable sources of resistance to both *S. gesnerioides*...
2. Variation in Host Preference

There is considerable variation in host specificity among isolates of *S. gesnerioides*. In addition to cowpea, members of the wild legume genera *Alysicarpus*, *Indigofera*, and *Tephrosia*, and non-legumes such as *Ipomea*, *Jacquemontia*, *Merremia*, *Euphorbia*, and *Nicotiana* are among the known hosts of *S. gesnerioides*. Strains of the parasite growing on cowpea, *Indigofera* spp., *Tephrosia* spp. and *Jacquemontia* spp. would only attack and emerge on the host species from which they had been collected. A strain of *S. gesnerioides* parasitic on tobacco in South Africa and Zimbabwe germinates in the presence of other potential hosts but is only capable of completing its lifecycle on tobacco. Similarly, *S. gesnerioides* parasitic on *Indigofera* species will germinate in the presence of cowpea roots, but is not capable of parasitizing this host.

Evidence for the existence of distinct races of *S. gesnerioides* that attack cowpea is also based upon the observation that some cowpea cultivars are differentially resistant to various geographic isolates of the parasite. It was proposed that there are five distinct races of *S. gesnerioides* in west and central Africa based on their ability to differentially parasitize different cowpea lines. A broader collection of *S. gesnerioides* isolates from this region was analyzed using genotypic profiling with molecular markers and host differential resistance response studies, and at least seven distinct races of the parasite were recognized. The races were designated as follows: SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 (Benin), SG4z (localized to the Zakpota region of Benin), SG5 (Cameroon), and SG6 (Sénégal). SG1 and SG5 are the most closely related, while SG4 and SG3 are the most diverged. SG6, one of the new races of the parasite identified in Sénégal, was genetically most similar to SG4. The hypervirulent isolate of *S. gesnerioides* from Zakpota (SG4z) is genotypically distinct from other populations of SG4 located in this region and elsewhere in Benin.
Geographic variation in host preference has also been observed in *A. vogelii*. *A. vogelii* populations from West Africa and Cameroon attack cowpea and groundnut. Isolates from eastern Botswana and northern portions of South Africa parasitize cowpea, groundnut, and mung bean, while those from the eastern portions of South Africa, Kenya, Malawi and Zimbabwe parasitize cowpea, groundnut, mung bean, and bambara groundnut. *A. vogelii* also has distinct races that differentially parasitize cowpea.

For example, the cowpea landrace B301 is resistant to *A. vogelii* in Kenya, but susceptible to isolates from Malawi, Botswana, and some areas of South Africa and the cowpea breeding line IT81D-994 is resistant to *A. vogelii* in Nigeria, but susceptible to isolates from Malawi (C.J. Botanga, N. Skizim, and M.P. Timko, unpublished).

### 3. Mechanism of Resistance

At least two mechanisms of resistance to *S. gesnerioides* have been described. Neither type is resistance due to reduced parasite germination or failed haustorial formation as the parasite succeeds in attaching to the potential host and initiates penetration of the host tissue. Penetration of the resistant cowpea cultivar 58-57 from Sénégal by *Striga* was associated with rapid necrosis of the host cells around the point of infection, leading to the death of the parasite in 3 to 4 days. This mechanism of resistance is analogous to the hypersensitive response shown in plant-pathogen interactions. The response in the host was specific with rapid death of cowpea tissue localized to the sites of parasite invasion.

The second type of resistance mechanism was observed in cultivars B301 and IT81D-994, where resistance to *S. gesnerioides* parasitism was not as dramatic. In these interactions, the majority of *Striga* seedlings penetrated the cortex and reached the host stele. Although tubercles began to develop on the host root surface, these did not enlarge, remaining less than 0.5 mm in diameter (on B301), or failing to expand their cotyledons (on IT81D-994). In these same studies, the host resistance response was also dependent on which race of *S. gesnerioides* was used. Tubercle arrest is also seen during interactions of *Striga* strains adapted for growth on one host species, when attempting to
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parasitize a non-host. In these cases, careful examination has revealed that the parasite neither forms vascular bundles (no xylem-xylem connections are evident with the host) nor develops proper internal organization.

4. Genetics of Resistance to Parasitic Plants in Cowpea

The genetic basis of resistance to *S. gesnerioides* and *A. vogelii* parasitism has been examined by a few laboratories. Monogenic dominant inheritance has been demonstrated in the progeny of the crosses between Suvita 2, 58-57 or B301 and susceptible lines. The region where the study was conducted and the strain of *S. gesnerioides* involved is not usually specified when the results of inheritance are mentioned, which limits the interpretation of these results.

Single dominant genes confer resistance to SG1, SG2 and SG3 in the cultivar B301 (Table 1). Resistance to SG3 in the cultivars B301 and IT82D-849 may be conferred by different alleles at the same locus or tightly linked genes, as two types of resistance response are manifested. Contradictory to other reports, resistance to *S. gesnerioides* race SG3 in Niger was conferred by a single recessive gene in IT82D-849. Prior studies indicating the presence of a recessive gene for resistance to *S. hermonthica* and *S. asiatica* in sorghum were cited in support of their interpretation. These results could indicate that more than one race of *S. gesnerioides* is present in Niger or that the response to SG3 in Niger is influenced by level of parasite infestation, or environmental factors.

The inheritance of resistance to SG1 in Burkina Faso, was studied using two resistant cowpea varieties, HTR (from Niger) and Wango-1 (from Burkina Faso). Resistance in HTR was controlled by one or two dominant genes that are nonallelic and independent of the resistance gene active against SG1 in IT82D-849 and B301 but possibly linked to the SG1 resistance gene in IT81D-994. Resistance to SG1 in Wango-1 is conferred by a single dominant gene probably allelic to the resistance gene in Gorom, and possibly linked to the resistance gene in IT81D-994. Unfortunately, no supporting data are provided for these findings. However, more compelling data are available for SG1 resistance in the cowpea cultivar IT81D-994 conferred by a single dominant gene.
Table 1. Inheritance of resistance to \textit{S. gesnerioides} in some cowpea lines

<table>
<thead>
<tr>
<th>Cultivar/line</th>
<th>Inheritance</th>
<th>Race of \textit{S. gesnerioides}</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B301</td>
<td>Single dominant</td>
<td>SG1 (Burkina Faso), SG2 (Mali)</td>
<td>26,34,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG3 (Niger), SG3 (Nigeria)</td>
<td>32,34,35,43</td>
</tr>
<tr>
<td>IT82D-849</td>
<td>Single dominant</td>
<td>SG1 (Burkina Faso), SG3 (Nigeria)</td>
<td>34,35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG2 (Mali)</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Single recessive</td>
<td>SG3 (Niger)</td>
<td>34</td>
</tr>
<tr>
<td>Suvita 2</td>
<td>Single dominant</td>
<td>SG1 (Burkina Faso), SG2 (Mali)</td>
<td>34,35</td>
</tr>
<tr>
<td>IT81D-994</td>
<td>Single dominant</td>
<td>SG1 (Burkina Faso), SG2 (Mali)</td>
<td>37,53</td>
</tr>
<tr>
<td>HTR</td>
<td>1 or 2 dominant</td>
<td>SG1 (Burkina Faso)</td>
<td>36</td>
</tr>
<tr>
<td>Wango-1</td>
<td>Single dominant</td>
<td>SG1 (Burkina Faso)</td>
<td>36</td>
</tr>
</tbody>
</table>

Approximately 650 local cowpea varieties and exotic accessions were screened for resistance to \textit{A. vogelii}. Landraces B301 and B359 from Botswana were among the most resistant genotypes.\textsuperscript{38,39} The superiority of B359 as a source of resistance for southern Africa was demonstrated when it was shown to remain completely resistant to isolates of the parasite from Malawi, while B301, IT90K-59 and IT90K-76 (two lines derived from B301 as parent), all supported the emergence of parasites of a population from Malawi.\textsuperscript{41} B359 was resistant in pot trials to isolates of \textit{A. vogelii} from different locations in east, southern and west Africa, including Botswana, Cameroon, Mali, Malawi, Nigeria and South Africa.\textsuperscript{22,39-41}

A number of cowpea lines were screened for resistance to \textit{S. gesnerioides} and \textit{A. vogelii} and the landrace B301 was resistant to both parasites.\textsuperscript{32,35,42,43} In contrast, line IT82D-849 is resistant to \textit{S. gesnerioides} but susceptible to \textit{A. vogelii}, Suvita-2 (Gorom local) is resistant only to \textit{S. gesnerioides} in Burkina Faso but susceptible elsewhere and susceptible to \textit{A. vogelii}, and IT81D-994 is moderately resistant to \textit{S. gesnerioides} as well as \textit{A. vogelii}. While resistance to \textit{S. gesnerioides} in B301 is controlled by a single dominant gene designated \textit{Rsg}1, resistance to \textit{A. vogelii} in this cultivar is controlled by duplicate genes, \textit{Rav}1 and \textit{Rav}2.\textsuperscript{32,35} The data also indicate that the genes
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conferring resistance to *A. vogelii* in B301 are non-allelic and independent of each other and not linked to the *Striga* resistance gene *Rsg1*. In subsequent studies the duplicate dominant genes for *Alectra* resistance in B301 were determined to be nonallelic to a single gene for resistance found in IT81D-994. *Rav1* and *Rav2* are used to designate the genes for resistance to *A. vogelii* in B301 and *Rav3* for the resistance gene in IT81D-994.

5. Genetic Mapping of *Striga* Resistance Genes

The most complete genetic map currently available was drawn by Ouédraogo, et al. It is based on segregation in 94 recombinant inbreds derived from a cross between IT84S-2049, an advanced breeding line of African origin (Nigeria), and 524B, a Blackeye type which encompasses the genetic variability available in cowpea cultivars in California. The cowpea genetic map consists of 11 linkage groups (LGs) spanning a total of 2670 cM, with an average distance of ca. 6 cM between markers. It includes 242 AFLP and 18 disease or pest-resistance-related markers, plus 133 RAPD, 39 RFLP, and 25 AFLP markers from the map of Menéndez, et al., for a total of 441 markers, of which 432 were assigned to a specific LG. The various agronomic and disease resistance trait loci that have now been placed on the cowpea genetic map are listed in Table 2.

Three AFLP markers are linked to *Rsg2-1*, a gene that confers resistance to SG1 present in Burkina Faso, and six AFLP markers linked to gene *Rsg4-3*, a gene that provides resistance to SG3 from Nigeria (Fig. 1). Two of the AFLPs were associated with both *Rsg2-1* and *Rsg4-3*. Two AFLP markers are closely linked to *Rsg1-1*, a gene that also confers resistance to SG3 in Nigeria. Five markers were subsequently found linked to the *Rsg994-1* gene on LG6 that also confers resistance to SG1.

The *Striga* resistance genes mapped thus far cluster in two locations in the cowpea genome (Fig 1.). Markers linked to the *S. gesnerioioides* race SG1 and SG3 resistance genes (*Rsg2-1*, *Rsg1-1* and *Rsg4-3*) present in the resistant cowpea lines B301, IT82D-849 and Tvu 14676,
respectively map to LG1, whereas markers linked to the *S. gesnerioides* race SG1 resistance genes *Rsg*3-1 and *Rsg*994-1 present in Suvita-2 and IT91D-994, respectively, were located to LG6.37,44,46

### 6. Molecular Markers and Marker-Assisted Selection

Marker-assisted selection (MAS) is the identification of DNA sequences located near genes that can be tracked to help in the selection of traits that are difficult to observe. In practice, MAS is a tool to more efficiently assemble alleles of interest into an improved cultivar and thereby increase the overall efficiency and effectiveness of crop...
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Fig. 1 Location of molecular markers linked to *S. gesnerioides* race-specific resistance genes in cowpea. Portions are shown of linkage groups 1 (LG1) and 6 (LG6) of the cowpea genetic map developed by Ouédraogo et al. indicating the location of AFLP, RAPD, and other markers linked to resistance to *S. gesnerioides* race 1 (SG1) (Rsg2-1 and Rsg1-1) and race 3 (SG3) (Rsg4-3) (left side) and *S. gesnerioides* race 1 (SG1) (Rsg3-1 and Rsg994-1) (right side). The relative map distances are given in centimorgans (cM). AFLP markers indicated by an asterisk are being used to develop sequence confirmed amplified regions (SCARs).

In some cases, MAS can allow smaller populations to be used, reduce the number of generations needed to reach a goal, or increase the accuracy of evaluations. MAS offers the only practical method to combine multiple resistance genes into one cultivar to provide more durable resistance.
genes and agronomic traits including genes for *S. gesnerioides* resistance.\(^{37,44,46}\)

Two molecular markers linked to *S. gesnerioides* resistance have been developed as sequence confirmed amplified regions (SCARs) suitable for use in MAS. One marker, designated 61R (E-ACT/M-CAA), was initially isolated as a marker associated with resistance to SG1 on LG1.\(^{37,44}\) The second SCAR is SEACTMCAC83/85 linked to SG3 resistance on LG1.\(^{50}\) Both 61R and a modified version of it termed MahSE2\(^{51}\) are effective in identifying resistance to races SG1 and SG3, as well as SG5. At present, these two markers are available for germplasm evaluation and efficacy testing on field populations. Work is also currently underway to identify markers linked to resistance to SG2 from Mali and SG4z from Zakpota, Benin.

### 7. Conclusions and Perspective

Cowpea largely remains an underexploited crop where relatively large genetic gains can be made with only modest investments in both applied plant breeding and molecular genetics. One of the major goals of cowpea improvement programs is to combine resistances to numerous pests and diseases and other desirable traits (such as those governing maturity, photoperiod sensitivity, plant type, and seed quality) in agroecologically adapted cultivars. Landraces and local cultivars with many of the desired disease and pest resistance traits (e.g., resistance to cowpea weevil, cowpea aphid, bacterial blight, CABMV, root knot nematodes) and resistance to one or more of the defined races of *A. vogelii* and *S. gesnerioides*, have been identified and are presently being integrated in various cowpea breeding programs around the world.\(^{1,2}\) A decade, more or less, is needed to breed a superior improved line using traditional selection and hybridization strategies depending on the source of the trait being introgressed.

The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers linked to important disease and pest resistance traits and the establishment of breeder friendly protocols that will allow marker-assisted selection (MAS) and marker-assisted breeding (MAB) to be readily employed. The hope is that MAS and
MAB will complement and extend conventional breeding efforts in cowpea and speed up the delivery of improved cultivars to the farmer. To date, however, progress in marker development and delivery of useful markers has been slow. With well-defined race-specific markers it should be possible to breed cultivars with resistance to all currently defined races of *S. gesnerioides* and *A. vogelii*.

It is also hoped that the application of knowledge being gained from basic genomic research on other crop plants and “model species” will also contribute to more rapid cowpea improvement. As information on genome structure and composition becomes available from a wide variety of legumes, comparative genomics can be employed for gene / trait identification in cowpea where existing bioassays may not be readily available or are too difficult to conduct. Understanding syntenic relationships is one of the many research areas that will have cross cutting impact on breeding in all legumes.

The integration of genetic engineering and transgenic crops into traditional breeding programs is another issue that needs to be considered. At present, the ability to transform cowpea and generate transgenic lines containing desired resistance and agronomic traits is limited. Without improved selection technologies, it is likely to take as long to introgress a molecularly engineered trait into an improved cultivar as it takes for a natural gene variant, if one is there to be found. Finding recessive mutants is also highly unlikely. So far, low stimulant and low attachment mutants have not been found, as they have with sorghum (Chapter 7). The challenge facing us in the near future is to demonstrate that biotechnologically-based alternative methods can generate knowledge and cost-effective tools that enable germplasm enhancement and product development opportunities that are either complementary or superior to those currently in use. The limitation is how rapidly refinements and changes to plant breeding methodology can be made available to the breeder. We are clearly still at the first of many steps in this long process.
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