The *GCP molecular marker toolkit*, an instrument for use in breeding food security crops

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Abstract Crop genetic resources carry variation useful for overcoming the challenges of modern agriculture. Molecular markers can facilitate the selection of agronomically important traits. The pervasiveness of genomics research has led to an overwhelming number of publications and databases, which are, nevertheless, scattered and hence often difficult for plant breeders to access, particularly those in developing countries. This situation separates them from developed countries, which have better endowed programs for developing varieties. To close this growing knowledge gap, we conducted an intensive literature review and consulted with more than 150 crop experts on the use of molecular markers in the breeding program of 19 food security crops. The result was a list of effectively used and highly reproducible sequence tagged site (STS), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and sequence characterized amplified region (SCAR) markers. However, only 12 food crops had molecular

markers suitable for improvement. That is, marker-assisted selection is not yet used for *Musa* spp., coconut, lentils, millets, pigeonpea, sweet potato, and yam. For the other 12 crops, 214 molecular markers were found to be effectively used in association with 74 different traits. Results were compiled as the *GCP Molecular Marker Toolkit*, a free online tool that aims to promote the adoption of molecular approaches in breeding activities.

Keywords Molecular marker · Marker-assisted selection · SSR · SCAR · STS · SNP

Introduction

The twentieth century has witnessed the world's population growing exponentially from 1,650 million to over 6,000 million. At present, almost all growth is taking place in the less developed regions, where food security currently remains an unfulfilled target for more than 800 million people (FAO 2003) and where agriculture is still a key factor in economic growth, poverty alleviation, and income generation (Evenson 2003). This situation has led to an urgent need to increase crop production and productivity.

Plant breeding is a proven way to improve yields in agriculture in a sustainable and time-efficient way. With the development of DNA-based genetic markers in the late 1970s, breeding programs that were

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previously based on phenotypic selection could gradually move towards genotypic selection (Ruane and Sonnino 2007). Marker-assisted selection (MAS) created promising expectations among breeders, mainly because it can substantially increase accuracy and reduce the time needed in the selection process. Some of its other advantages over conventional breeding techniques are described by authors such as Francia et al. (2005), Koebner and Summers (2002), Ragot and Lee (2007), and Xu and Crouch (2008).

Despite the advantages that MAS offers, tangible results are still modest in the public sector and are mostly restricted to major crops such as rice, maize, barley, and wheat. One of the many practical, logistical, and genetic constraints that impede the transfer of promising markers from research to breeding is the validation of these published markers in a range of populations representing breeding materials (Xu and Crouch 2008).

This limited effective uptake of molecular markers in breeding programs contrasts sharply with the avalanche of published studies available on molecular markers (Babu et al. 2004). The overwhelming number of sources, particularly digital, results from the past decade's explosive development of both information and communication technology and genomic tools. The huge amount of information on the Internet is readily illustrated when *Google Scholar* (<http://scholar.google.com.co/>) is used to search for publications on useful markers. For example, on 18 May 2010, accessing the search engine with the combination of the terms “brown planthopper resistance”, “rice”, and “marker” resulted in 314 hits. When “marker” was replaced by “marker-assisted selection”, the number of hits dropped to 186. Even so, finding the targeted practical information still remained an odyssey, considering that it is often of questionable quality or outdated.

Because of their global importance, major crops such as rice and wheat have available a powerful set of molecular and bioinformatic tools and methods that make the search for the required information even more cumbersome.

If an overflow of information is not necessarily a problem, then access to information that is immediately available for use in an accurate and timely manner is indeed a growing challenge. Molecular genetics and genomics is a highly dynamic and

rapidly evolving field of science, where new developments are often published in various, limited-access (i.e., expensive) journals. Such a lack of access to key information frequently limits research progress in developing countries, hindering their advancement towards independent and effective plant breeding.

Existing information resources are currently more related to genomics and molecular biology than to practical plant breeding (Coffman et al. 2004; Collard et al. 2008). An uncommon, but nevertheless well-known, example of a current information source of practical use for MAS is Wheat CAP (<http://mas.wheat.ucdavis.edu/protocols/index.htm>). A similar initiative is that of the Bean Improvement Cooperative (BIC), which keeps a current list of sequence characterized amplified region (SCAR) markers for use in bean breeding on their web page (http://www.css.msu.edu/bic/PDF/SCAR_Markers_2009.pdf). A third example is the website of the Global Partnership Initiative for Plant Breeding Capacity Building (GIPB) (<http://km.fao.org/gipb/>); this multi-institutional initiative aims to enhance plant-breeding capacity by providing all kinds of information related to plant breeding.

Markers that are effectively used in breeding programs were compiled from a literature review and from contacts with crop experts to create the *GCP Molecular Marker Toolkit* (the *Toolkit*). The information given for each marker is of immediate use and includes laboratory protocols, validation processes, and key references. The tool is freely available as a global public good that is searchable via the Internet (<http://www.generationcp.org/sp5/MM-Toolkit>).

As an easily accessible global public good paving the way for the latest advances in molecular plant breeding, the *Toolkit* represents a vital contribution towards modern agricultural science for the benefit of those in developing countries. It also encourages breeders and scientists to actively provide information.

This article aims to illustrate the breadth of information made available through the *Toolkit*.

Materials and methods

The *GCP Molecular Marker Toolkit* was designed to be a readily accessible tool to provide information of molecular markers for immediate application in the respective breeding programs.

Toolkit structure

Users may search and browse for data by selecting one of the 19 listed crop names and the corresponding species of interest in a drop-down menu. For each of the 19 crops, the compiled information is presented as a summary that outlines the current status of MAS, together with a list of further reading. For each effectively used marker included in the crops' breeding programs, the given information is divided into four windows that can be activated separately: (1) general information on the given marker, including marker's name and type, trait to which it is linked, and crop expert's name; (2) the corresponding laboratory protocol (e.g., primers and PCR conditions); (3) added information on the marker's validation (e.g., donor and recurrent parent, and population type and size); and (4) key references of consulted papers and websites. As not all consulted papers have free access status, a link is given to their abstracts.

Crops

We examined 19 food security crops for their current situation with respect to markers for crop improvement programs. Only 12 had markers suitable for improvement through MAS—barley, beans, cassava, chickpea, cowpea, faba bean, groundnut, maize, potato, rice, sorghum, and wheat—with 214 molecular markers being effectively used in association with 74 different traits. For the remaining seven food security crops—coconut, lentils, millets, *Musa* spp., pigeonpea, sweet potato, and yam—MAS is not yet used. For all 19 crops, the *Toolkit* summarizes the current status of MAS, and includes a list of references that were screened for marker information and useful tips for further reading.

Markers

Markers included in the *Toolkit* comply with the following conditions: they are associated with traits relevant for applied crop breeding, and are validated and effectively applied in breeding programs. This first version of the tool includes only sequence tagged site (STS), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and sequence characterized amplified region (SCAR) markers because they are easy to use and highly reliable (i.e.,

reproducible). Regarding type of traits, qualitative as well quantitative trait loci (QTL) are included. Because of the overwhelming number of papers on QTL mapping, markers associated with QTL were restricted to those QTL which explain at least 10% of phenotypic variance and for which the effect had to be observed in at least two different genetic backgrounds.

However, some exceptions were made for including a marker: for chickpea, a marker associated with resistance to the fungus ascochyta blight (AB) (*Ascochyta rabiei*) at the seedling stage and explaining only 2.5% of phenotypic variance was included (Kottapalli et al. 2009; Millan et al. 2006). Apart from being effectively used, this genomic region had also been previously studied by several authors, who confirmed it as being associated with partial AB resistance (often close to 20%) (T. Millan, pers. commun.).

Another exception was SSR markers linked to a QTL for blast (*Pyricularia grisea*) resistance in rice (Noenplab et al. 2006). Although the validation process has not yet been published, the markers were included because they were already effectively used in a breeding program at the Kasetsart University in Thailand to develop a new variety of resistant sticky rice (J. Siangliw, pers. commun.).

For faba bean, although some QTL identified as controlling resistance to the weed broomrape (*Orobanche crenata*) (Román et al. 2002) and the ascochyta blight fungus (*Ascochyta fabae*) (Avila et al. 2004; Díaz-Ruiz et al. 2009; Román et al. 2003) were already validated in different environments and genetic backgrounds, they were not included in the *Toolkit*. Although more tightly linked markers or gene-based markers will be developed in the future, they have not yet been applied in faba bean breeding programs (A.M. Torres, pers. commun.). However, as the related information may be of interest for breeders, they are mentioned in the crop's summary.

Sources of information

Papers and websites

The first step in developing the *Toolkit* was to discover the actual status of MAS in each crop. Reliable Internet sources of institutes, known for their expertise and/or mandate in a specific crop, were consulted. These included CGIAR centers, for example, CIAT's

cassava website at http://www.ciat.cgiar.org/AboutUs/Documents/synthesis_cassava_program.pdf, or well-known National Agricultural Research Systems (NARS) such as the Coconut Research Institute of Sri Lanka with its website at <http://www.cri.lk/research.html>.

Where MAS was indeed effectively applied in breeding programs, the second step was to compile details of targeted traits and associated markers from either peer-reviewed papers or existing crop-specific websites. For crops that had not yet entered the MAS phase, confirmation of this status was sought from crop experts.

Review papers on MAS for a specific crop were a key source of information. In a relatively succinct way, they provided information on available molecular markers or marker–trait associations. Examples of useful review papers included Jena and Mackill (2008) for rice, William et al. (2007) for wheat, Blair et al. (2007) for beans and cassava, Miklas et al. (2006) for beans, Ejeta and Knoll (2007) for sorghum, and Dita et al. (2006) and Varshney et al. (2010) for legumes. The *Toolkit* lists, in the *Further Reading* section of each crop summary, the review papers consulted. Because the information presented in these papers was often not strictly limited to validated or effectively used markers, but also referred to potential markers or the future prospects of MAS, crop experts were contacted to seek confirmation of their effective use.

Many databases on molecular markers are also available, including the well-known Gramene (<http://www.gramene.org>) and GrainGenes (<http://wheat.pw.usda.gov/GG2/index.shtml>). Most of these databases focus on available markers in general and not on those that are effectively used in breeding programs. However, they were very useful for double-checking historical data or providing background information.

Consultations with crop experts

After a literature and database review, a preliminary list of marker data was sent to crop experts to verify the compiled information against their experiences. The number of crop experts consulted varied according to the crop. The contacted persons were selected according to their expertise and to ensure global coverage, according to their geographical distribution. More than

150 crop experts collaborated during the *Toolkit*'s entire development. Crop experts were contacted for a wide range of reasons such as:

- Confirming if markers mentioned in review papers, marker–trait association lists or validated markers are indeed effectively used in a breeding program (sometimes the validation process is published but the marker is no longer applied [M. Baum, pers. commun.])
- Updating information (e.g., if linkage with the associated gene is broken, if a rapid amplification of polymorphic DNA [RAPD] marker was converted into a SCAR marker, if identified markers are already implemented or if markers continue to be used)
- Completing information gaps such as laboratory protocols and verifying if markers have been tested in several genetic backgrounds (i.e., mainly for QTL)
- Finding markers that are related to traits for which phenotyping may still be preferred

Results

State of the art of MAS in crops covered by the *Toolkit*

Based on the literature review and contacts with crop experts, MAS is either not yet applied to coconut, lentils, millets, pigeonpea, sweet potato, or yam, or the markers used do not comply with the conditions for inclusion in the *Toolkit* (those which are associated with traits relevant for applied crop breeding, are validated and effectively applied in breeding programs, and in this first version are STS, SSR, SNP, and SCAR markers only). For all 19 crops, a description is provided of the current state of marker development, or comments are given on their current (for types of markers not included in the *Toolkit*) or future applications.

Marker-assisted selection in *Musa* differs from MAS in the other crops, as the currently used markers are not directed towards selecting genes of interest but towards detecting the presence or absence of pathogens. For example, markers detect endogenous sequences of the infectious banana streak virus (eBSV) present in the *M. balbisiana* genome, particularly

those involved in recognizing particles of this virus (Gayral and Iskra-Caruana 2009; Hohn et al. 2008; Staginnus et al. 2009).

In coconut, markers are currently used for fingerprinting accessions and studying genetic relationships but are not used in breeding programs as yet. However, several QTL linked to interesting traits are given in CIRAD's TropGENE DB database (<http://tropgenedb.cirad.fr/en/coconut.html>).

For lentils, markers have been developed that are mainly related to resistance to diseases such as ascochyta blight (*Ascochyta lentis*) and anthracnose (*Colletotrichum truncatum*). However, the currently effectively used markers are of the RAPD type, which is excluded from the *Toolkit*.

A very successful cultivar developed with MAS technology is the pearl millet variety HHB 67 Improved, derived from the popular, public-sector-bred hybrid HHB 67. Marker-assisted backcrossing had been applied to improve resistance to downy mildew (*Sclerospora graminicola*) (Hash et al. 2006). However, the corresponding markers are not included in the *Toolkit*, as they are of the restriction fragment length polymorphism (RFLP) type. Several markers for QTL traits are still being validated. The original success of MAS in pearl millet was based on the use of ^{32}P -labeled RFLP probes, but currently used markers are confined to STSs and SSRs, together with a small number of single-strand conformational polymorphism (SSCP)-SNP markers (T. Hash, pers. commun.). A cleaved amplified polymorphic sequence (CAPS) marker, linked to early/late flowering alleles at *PHYC* (phytochrome C gene) was reported (Saïdou et al. 2009; Y. Vigouroux, pers. commun.). However, this type is, at present, also excluded from the *Toolkit*.

With regard to finger millet, to the best of our knowledge, no markers have yet been developed in association with agronomically important traits.

As with most legumes, marker development in pigeonpea has been slow (S. de Villiers, pers. commun.). Research is in progress to identify markers for resistance to fusarium wilt (*Fusarium udum*), and fertility restorer genes (Dar et al. 2006; Varshney et al. 2007). The development of a larger number of polymorphic SSR markers and diversity arrays (DArT) (A. Killian, cited in Varshney et al. 2007) is expected to facilitate trait tagging in the near future.

Improvement of sweet potato has been limited but, given the significance of the sweetpotato virus disease (SPVD)—a major constraint to this crop—a MAS system for SPVD resistance is currently being developed. As with coconut, the use of markers for yam is restricted to diversity analysis, and microsatellite markers for breeding are now being developed. The results are expected to be published soon and the markers will be entered into the EBML database (H. Chair, pers. commun.).

For the remaining crops in the *Toolkit*, that is, barley, beans, cassava, chickpea, cowpea, faba bean, groundnut, maize, potato, rice, sorghum, and wheat, markers are being effectively applied in breeding programs. The number and type of these effectively used markers are analyzed below, and the type of traits with which they are associated are described.

The effectively used markers compiled in the *Toolkit*

The number of markers that met *Toolkit* conditions was 214. About three-quarters of these are used in breeding programs for rice (63), wheat (58), and barley (39). In all, 87% of markers in the *Toolkit* correspond with those used in breeding programs of major crops (rice, wheat, barley, sorghum, maize, and potato), whereas only 13% are used for minor crops.

As already mentioned, development of markers for legumes has been slow, as reflected in their low numbers. Only 10% of markers included in the *Toolkit* are destined for use in breeding programs for beans, chickpea, cowpea, faba bean, or groundnut.

An even bigger discrepancy—at 6% versus 94%, respectively—is found between the groups of clonally propagated crops (i.e., *Musa* spp., cassava, potato, sweet potato, and yam) and seed-propagated groups (remaining crops).

Of the 214 markers recorded in the *Toolkit*, 96 are SSR markers, 78 STSs, 10 SNPs, and 30 SCARs. Because all 13 bean markers are of the SCAR type, the markers in the group of minor crops comprise 20 SCARs and only 8 SSRs. Markers for the non-cereals comprise 21 SCARs and 9 SSRs, with legumes being associated with 19 SCAR and 3 SSR markers. The number of SSR and STS markers is nearly the same as for barley with, respectively, 21 and 17. For rice, the dominant type of marker is SSR while for wheat the predominant marker is STS, especially those

Table 1 Number of markers available in the *Toolkit* per crop, per marker type and per type of trait

	Total	Type of marker					Type of trait	
		SSR	STS	SNP	SCAR	Simply inherited	QTL	
Cereals								
Rice	63	31	20	10	2	40	23	
Wheat	58	19	34	0	5	50	8	
Barley	39	21	17	0	1	32	7	
Sorghum	13	10	2	0	1	3	10	
Maize	6	6	0	0	0	4	2	
Legumes								
Beans	13	0	0	0	13	9	4	
Chickpea	3	2	0	0	1	0	3	
Cowpea	2	0	0	0	2	2	0	
Faba bean	2	0	0	0	2	2	0	
Groundnut	2	1	0	0	1	1	1	
Root crops								
Potato	7	1	5	0	1	7	0	
Cassava	6	5	0	0	1	6	0	
Total	214	96	78	10	30	157	57	

linked to abiotic and quality traits. Very few effectively used SNPs were reported. The proportion of each of the four marker types per crop is given in Table 1.

Type of traits for which markers are effectively used

Of the 214 markers in the *Toolkit*, 157 markers are used to screen simply inherited traits and 57 for QTL. Most of the above-mentioned crop groups follow this proportion and have many more markers related to simply than to quantitatively inherited traits. Testing the effectiveness of the QTL in independent populations and different genetic backgrounds is a time-consuming and cumbersome task in which inconsistent or non-significant results are often obtained. This is even more noticeable for crops for which many markers are available, such as wheat, rice, and barley. In legumes, the proportion is less skewed with eight markers linked to QTL and 14 to simply inherited traits. In the group of clonally propagated crops, no markers are available yet for QTL, although results are expected soon (see “[Discussion](#)”). Exceptions are sorghum and chickpea, which have more markers

linked to QTL than to simply inherited traits. Table 1 gives an overview per crop of the number of markers associated with simply inherited traits and QTL.

The *Toolkit* possesses markers for 74 different traits, most of which (44) are biotic. The numbers of abiotic and quality traits targeted in MAS are almost the same, with 16 and 14, respectively. A similar pattern is found for most of the crops (Table 2), faba bean being an exception, with the only trait targeted by MAS being the absence of tannins, a quality trait. Wheat breeding programs target many quality traits and therefore the number for wheat is higher than for other major crops. As rice is a major staple crop, its breeding programs have recently been oriented towards yield improvement in less favorable agricultural areas. Hence, rice breeding programs that use markers are oriented towards abiotic traits such as phosphorus deficiency, drought, and submergence tolerance.

Unpublished information

Consultations with experts resulted in practical information for breeding that cannot be traced back to published papers or public databases. The *Toolkit* therefore includes unpublished markers and

Table 2 Number of biotic, abiotic, and quality traits per crop for which molecular markers can be used

	Total	Type of trait		
		Biotic	Abiotic	Quality
Cereals				
Wheat	21	10	4	7
Barley	13	8	4	1
Rice	11	3	5	3
Sorghum	6	3	3	0
Maize	4	2	0	2
Legumes				
Beans	8	8	0	0
Cowpea	2	2	0	0
Groundnut	2	2	0	0
Chickpea	1	1	0	0
Faba bean	1	0	0	1
Root crops				
Potato	3	3	0	0
Cassava	2	2	0	0
Total	74	44	16	14

laboratory protocols, practical laboratory tips, and cost–benefit analyses for some markers.

Discussion

Is there a need for a toolkit? Impact and future of the *Toolkit*

One may question whether, in this era of booming electronic information sharing, another online information source for breeders and biotechnologists such as the *Toolkit* is needed, or whether its release could possibly have a significant impact on progress in breeding programs. Xu and Crouch (2008) describe the several difficult steps in translating promising markers into high-scale breeding applications. Limited access to useful markers, however, is not listed as constituting an obstacle to implementing MAS. Nevertheless, experience with the GCP's Genotyping Support Service (GSS) (<http://www.generationcp.org/sp5/?da=0994853>) suggests that plant breeders in the developing world need a succinct, easily accessible data source on effectively used markers.

The novelty of the *Toolkit* lies in its selecting and comprehensively compiling those markers that are used in MAS, as opposed to those markers that have been identified but whose usefulness has not yet been established. It also refers to several crops, contrasting with similar initiatives such as the Wheat CAP and the BIC SCAR list for bean breeding (see “[Introduction](#)”) that refer only to one or two crops.

The *Toolkit*'s succinctness is clearly illustrated by the way it reduces the hundreds of hits found when searching *Google Scholar* for information on crop-specific markers. Apart from its comprehensiveness, the *Toolkit* also differs from other databases by extensive personal input from crop experts. Although publications were envisioned as the main source of information in developing the *Toolkit*, the input of crop experts proved to be of equal if not greater importance.

A possibly successful impact of this tailor-made breeding information resource is, and will be, related to the impact of the GSS and Integrated Breeding Platform (<http://mbp.generationcp.org/>), as the *Toolkit* is integrated with both support services. Key to significant impact is ensuring that the *Toolkit* reaches

its target audience through release to appropriate breeding platforms and workshops.

As well as general information platforms such as the GIPB website, the *Toolkit* could ideally be disseminated through crop-specific platforms such as the blog of the Red LatinPapa (<http://jorgealonso.posterous.com/release-of-gcp-toolbox-on-molecular-markers>). However, such platforms are unfortunately not available for all target crops. An alternative is to introduce the *Toolkit* to developing country breeders at workshops whereby immediate feedback can be received from the target group.

Apart from questioning the *Toolkit*'s impact, a critic may also challenge its durability. Major advances have occurred in the development of DNA markers, starting with RFLP and amplified fragment length polymorphism (AFLP) markers, which were followed by those used today—SSR, STS, SCAR, and SNP markers. Non-SNP marker systems are widely believed likely to become outdated in the future. DArTs are also promising as a generic and cost-effective genotyping technology that overcomes some of the limitations of other molecular marker technologies such as RFLP, AFLP, and SSR. DArT technology has been successfully developed for *Musa*, barley, cassava, coconut, chickpea, groundnut, rice, wheat, pearl millet, pigeonpea, potato, sweet potato, yam, and sorghum. Work is in progress for other crops (see <http://www.diversityarrays.com/genotypingserv.html>).

Because of this constant evolution of marker systems and their adoption and use in molecular breeding, the *Toolkit* is subject to continuous updating. As it is already integrated into the GCP's Integrated Breeding Platform, it will be adjusted and extended according to plant breeders' needs, particularly those in developing countries.

Far fewer markers effectively used in breeding programs than published markers associated with agronomic traits

Marker technology is used to assess and enhance diversity in germplasm collections, to identify genes that control key traits, and to follow the introgression of valuable traits from new sources, as is the case for MAS. The number of markers destined to be used in MAS is only a very small percentage of available markers. Also, not all markers classified as being

applicable in MAS are effectively used in breeding programs. The *Toolkit* contains only 214 markers, which indicates that routine implementation in ongoing plant breeding programs is still limited (Babu et al. 2004). Phenotyping is sometimes preferred to genotyping, even though useful markers are available. This may occur when the phenotypic expression of a trait is clear-cut, little affected by the environment, and easy to score early in the cropping cycle. The rationale is that when the cost–benefit ratio favors phenotyping, then genotyping tends to become redundant.

Despite this low number of effectively used markers, the importance of their effect cannot be ignored, as some markers tackle some of the biggest biotic constraints worldwide such as bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and blast (*Pyricularia grisea*) in rice; several rusts in wheat (stem rust is caused by *Puccinia graminis* f. sp. *tritici*, leaf rust by *P. tritricina*, and stripe rust by *P. striiformis* f. sp. *tritici*); rust (*Uromyces appendiculatus*) and common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) in beans; and cassava mosaic virus in cassava (Dwivedi et al. 2007). Bacterial blight and blast resistance in rice account for the largest number of markers per disease in the *Toolkit* at, respectively, 12 and 18.

Differences in numbers of markers for major versus minor crops

Unsurprisingly, major crops represent most of the markers in the *Toolkit*—87%. Results from the *Toolkit* clearly show that marker research in minor crops is far behind that in major crops. The fact that minor crops are literally treated as orphans is reflected not only in the *Toolkit* but is also illustrated by figures reported by Dwivedi et al. (2007). SSR development is lagging, not only for legume crops such as lentils and faba bean (Varshney et al. 2009b) but also for *Musa* and many other clonal crops (Dwivedi et al. 2007), which have very few or no markers in the *Toolkit*. However, because of the importance attributed to minor crops, specifically to legumes, in enhancing food security breeding, efforts are clearly catching up (Nelson et al. 2004; Varshney et al. 2009b, 2010). Most of these legume species are expected to soon have larger numbers of SSR markers and high-throughput SNP assays as a result

of advances in sequencing and genotyping technologies, several international collaborations, and declining costs of sequencing technologies (Varshney et al. 2009b).

Compared with 5 years ago, significant progress can already be seen in the development of genomic resources for model species of *Medicago*, *Lotus*, major legumes (soybean, common bean, peanut), and minor legume crops (cowpea, chickpea, pigeonpea) (Sato et al. 2007; Varshney et al. 2009b). The knowledge obtained through advances in the genomics of major crops may potentially benefit minor crops. Such a clearly allied model crop system is not available for clonally propagated crops such as yam, potato, and cassava. However, the success of transferring advanced science from model species to their more neglected relatives is only guaranteed when it is accompanied by strong conventional breeding efforts (Nelson et al. 2004), which are not always present.

Quantitative trait loci are still in the minority

Markers linked to simply inherited traits clearly outnumber those associated with QTL. This became clear during the literature research. Although an increasing number of identified and mapped QTL is reported, very few are actively applied in breeding programs.

During the *Toolkit*'s development, markers linked to QTL were observed to be evolving rapidly with an increasing number of tightly linked markers being sought. Because of climate change and the increasing cultivation of more crops in suboptimal agricultural areas, the use of QTL associated with drought and other abiotic traits is expected to increase. Rice is one of the very few crops where QTL introgression has been successfully employed in breeding lines, for example for submergence tolerance (*Sub1*) (Xu et al. 2000), salt tolerance (*Saltol1*) (Bonilla et al. 2002; Ren et al. 2005), and tolerance of phosphorus deficiency (*Pup1*) (Heuer et al. 2009; Wissuwa et al. 1998, 2002). As multiple abiotic stresses are commonly experienced in farmers' fields, the next target in rice breeding is pyramiding multiple tolerance QTL in the same recurrent parent (Ismail et al. 2007).

Dwivedi et al. (2007) reports that, between 1991 and 2005, about 500 articles on useful QTL were published in high-impact journals and referred predominantly to cereal crops such as barley, maize,

rice, and wheat—a very similar list of crops to that found in the *Toolkit*. The number of articles reporting validation of QTL was much lower—80. The same was experienced during the *Toolkit*'s development, in which the number of QTL was even lower than 80, probably because a commercially interesting crop such as soybean is not included.

The complexity of QTL made it difficult to determine those markers that are effectively used in breeding programs and therefore which to include in the *Toolkit*, especially for the biggest constraints of major crops such as wheat, barley, rice, and maize, which are simultaneously tackled by research teams around the globe. For such cases, seeing the wood for the trees is often hard, as is keeping track of progress when research results are scattered throughout several information sources.

Review papers on QTL research illustrate the complexity of such traits, even as they give a comprehensive overview of the globally obtained results so far for a particular disease or disease group in a crop. Koide et al. (2009) assembled a list with reported DNA markers for blast resistance genes. Wisser et al. (2005) synthesized and published data on quantitative and qualitative disease resistance in rice to evaluate the distributions of and associations among resistance loci. Wisser et al. (2006) made a similar synthesis for maize, consulting 50 publications that, together, reported the locations of 437 QTL for disease, 17 resistance genes, and 25 resistance gene analogs. Buerstmayr et al. (2009) focused on QTL mapping and MAS for fusarium head blight resistance in wheat. Singh et al. (2007) gave an overview of progress towards linkage mapping for salt tolerance components in rice, and Courtois et al. (2009) developed a database solely on QTL related to rice root traits.

Even for a minor crop such as chickpea, a list of QTL for resistance to ascochyta blight has been assembled (Aryamanesh et al. 2010). As with rice, maize is a major crop that is grown worldwide, and has numerous reports on QTL available online, for example at www.maizgdb.org/qtl.php and www.gramene.org/qtl. However, unlike rice, maize cannot show examples of successful QTL introgression in its breeding lines.

Very few QTL could comply with the conditions for inclusion in the *Toolkit*. Through QTL mapping, their location in the genome and the markers closest

to them are established. However, this information is not always useful for application in MAS, which, as explained above, requires the identification of tightly linked markers that are useful in different genetic backgrounds. Validation of markers linked to QTL and of QTL themselves are time-consuming processes that complicate effective application in breeding schemes, despite the considerable amount of research being conducted on QTL identification and mapping.

The difference with a decade ago is that, today, QTL research is no longer confined to major crops but is also conducted for minor crops, which are growing in importance due to climate change and the challenge of feeding a growing world population. For coconut, agronomically interesting QTL are shown in CIRAD's TropGENE DB database at <http://tropgene.db.cirad.fr/en/coconut.html> (Ruiz et al. 2004) (see "Results").

Markers have been developed for quantitative traits in cassava but have not yet been applied. To identify more reliable and closely linked markers to apply in breeding (E. Okogbenin, pers. commun.), further mapping is being carried out for markers associated with early root bulking (Okogbenin and Fregene 2002), cassava bacterial blight resistance (*Xanthomonas axonopodis* pv. *manihotis*) (Jorge et al. 2001), high beta-carotene content, waxy cassava starch, delayed postharvest physiological deterioration, high dry matter content, and drought tolerance (Blair et al. 2007).

Because the importance of minor legumes in the diet of resource-poor people of the semi-arid tropics was recognized only 10 years ago, QTL research for most of the minor legumes has only just started. In chickpea, most research is done on the quantitative trait ascochyta blight (*Ascochyta rabiei*) resistance. The many reports on this disease have been summarized by Anbessa et al. (2009), Aryamanesh et al. (2010), and Millan et al. (2006). The last set of authors, however, point out that MAS for this trait has been limited—as supported by the *Toolkit*, which contains only three markers associated with ascochyta blight resistance. Other quantitative traits in chickpea that were investigated included time to flowering (Lichtenzweig et al. 2006) and carotenoid concentration (Abbo et al. 2005), although with no effective application. The latter set of authors confirmed that, except for this published paper, no

further work was done with SSR markers for carotenoids in chickpea.

In lentils as well, most QTL research is concentrated on resistance to ascochyta blight (*Ascochyta lentis*) (Rubeena et al. 2006) but, at the time of developing the *Toolkit*, all the associated markers and candidates still needed to be validated in a breeding program (R. Ford, pers. commun.). QTL mapping of winter hardiness genes in lentils is reported in an earlier publication (Kahraman et al. 2004). To the best of our knowledge, the markers developed in the winter hardiness study are not currently being used in the breeding program, although plans are being made to use the markers for further research into the genetics of the trait and to further refine the QTL regions that confer winter hardiness (G. Muehlbauer, pers. commun.). A more recent publication shows the results of QTL analysis for earliness and plant height in the same crop (Tullu et al. 2008).

For groundnut, no QTL introgression in breeding lines has yet been reported but major advances are anticipated from QTL analysis for resistance to rust (*Puccinia arachidis*) and late leaf spot (*Phaeoisariopsis personata*) (Khedikar et al. 2010; Mondal and Badigannavar 2010). The development of the first SSR-based genetic linkage map for cultivated groundnut (Varshney et al. 2009a) led to the mapping of QTL that control drought tolerance-related traits (transpiration, transpiration efficiency, specific leaf area, and SPAD chlorophyll meter reading [SCMR]). Because diseases significantly reduce groundnut yield, Leal-Bertioli et al. (2009) worked on identifying candidate genome regions that control disease resistance. They mapped 34 sequence-confirmed candidate disease-resistance genes, and five QTL for resistance to late leaf spot—a major disease.

Nor have QTL been applied in breeding programs for cowpea, although research on drought resistance is well advanced. Muchero et al. (2009) mapped QTL associated with seedling drought tolerance and maturity in cowpea and later conducted candidate-gene mapping (Muchero et al. 2010a) to identify trait determinants underlying QTL of interest. As the associated markers were AFLP, they were not included in the *Toolkit*. Recent research has also involved the identification of QTL for resistance to *Thrips tabaci* and *Frankliniella schultzei* (Muchero et al. 2010b).

Even in earlier published papers, reported QTL research was limited to identification and mapping QTL

such as those with effects on resistance to flower bud thrips (*Megalurothrips sjostedti*) (Omo-Ikerodah et al. 2008) and QTL analysis for seed weight, an important trait related to yield, in the *Vigna* domesticated cowpea and mung bean (Isemura et al. 2007). Pigeonpea may be the only minor legume crop for which QTL are not reported, either in the *Toolkit* or in the literature.

Publicly available markers versus use of markers in private companies

The *Toolkit* reflects only those markers that are publicly available. At the time of publication, no efforts were made to trace those markers that are patented or used by private companies. Estimating the percentage of all trait-related markers that could possibly be used in breeding programs and are publicly available is therefore hard. Although some successful applications of MAS are present in public breeding programs, for quite a while high costs delayed the incorporation of these new technologies into most public breeding programs (Dubcovsky 2004). In contrast, many private breeding companies are known to invest heavily in implementation (Dwivedi et al. 2007; Koebner 2003). However, such companies are interested only in certain crops. For example, a self-pollinating crop reduces the profitability of a private company because growers can save seed from year to year. The public sector is therefore much more involved in developing these crops than cross-pollinators such as maize.

Private companies avoid investing in minor crops, which have limited economic interest, with returns unlikely to cover initial investments. Monsanto announced in 2007 that it would provide academic researchers and public institutions free access to its state-of-the-art soybean cyst nematode marker technology (www.crop-protection-monthly.co.uk/Archives/CPMFeb2007.doc), an initiative that hopefully will be copied for other crops. Such synergetic efforts would certainly result in more efficient and effective result-oriented research, essential for feeding the projected 9,000 million people in 2050.

Conclusions

Modern technologies for breeding and novel breeding approaches are evolving extremely rapidly. To serve

the breeding community more efficiently and keep it up to date with the latest methodologies, the *Toolkit* must continue to expand. The validated and effectively used SSR, STS, SNP, and SCAR markers that are currently included in the *Toolkit* should be complemented with new markers and marker technologies and hopefully an increasing number of validated QTL. As it is often difficult to provide complete and updated information, users are invited to inform of any outdated or missing information found in the *Toolkit* by clicking on a feedback button located at the bottom of each *Toolkit* page. To date, user feedback has been very limited, but an increase is expected once the *Toolkit* is more known in the breeding community.

To reach target audiences, the *Toolkit's* release was announced on several breeding information platforms and presented at conferences and workshops. For those breeders who have no access at all to the Internet, specific information will be sent upon request.

The technical aspects of markers have always been in constant evolution and will continue to be so in the foreseeable future. However, the fundamental need to deliver accurate information on markers that are proven and well documented will not only continue to exist but will also grow as more scientists, working with more crops, become interested and develop the capacity to use them.

As the *Toolkit* continues to provide user-friendly and more complete information, it will enable breeders to access a wider range of choices for developing improved varieties over shorter periods of time and at lower operational costs. Such increased capacity will eventually increase crop productivity for small farmers, thereby giving the world a better chance to feed itself.

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