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Review article

Timelines in conventional crop improvement: pre-breeding and breeding procedures

Hussein Shimelis* **and Mark Laing**

University of KwaZulu-Natal, African Center for Crop Improvement, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa

***Corresponding author: Shimelish@ukzn.ac.za**

Abstract

This article is aimed at highlighting the timelines and breeding procedures of clonally propagated, self-fertilizing and cross-fertilizing crops in conventional crop improvement. Plant breeding is aimed at developing crop cultivars with improved genetic constitution to serve diverse human needs. Cultivar development follows well-defined activities, including plant breeding research or 'pre-breeding', followed by the actual breeding *per se*. These discrete activities are the components that determine the pace at which cultivars are released to growers. The two activities, in turn, depend upon factors, such as breeding goals, genetics and agronomy of the crop, breeder's vision, availability of testing facilities and national cultivar-registration requirements. Given these factors, there are established steps and procedures found in any conventional breeding program. These include parental choice, making crosses among chosen parents, selections from recombined parents followed by extensive field testing at targeted sites, followed by maintenance and multiplication of candidate cultivars for seed production and distribution. The present review outlined the approximate timelines at 7, 9, or 17 minimum breeding generations before the release of an improved cultivar of vegetatively reproducing, self-fertilizing or cross-fertilizing crop, respectively through conventional breeding. The traditional breeding procedures can be complemented with other approaches, such as marker-assisted selection and doubled haploidy breeding to accelerate and shorten the timeline to release of new crop cultivars.

Keywords: breeding procedures; conventional breeding; cultivar development; mating systems; plant breeding; pre-breeding; timeframe.
Abbreviations: CGIAR- the Consultative Group on International Agricultural Research; DH-doubled haploids; GIPB-the Global Partnership Initiative for Plant Breeding Capacity Building; MAS-marker-assisted selection.

Introduction

Plant breeding is aimed at developing genetically improved crop cultivars with economic benefits for small-scale and commercial farmers. Population growth, dwindling agricultural land and global climate change presents increasing risks to crop production. Consequently, plant breeding aims to constantly develop crop cultivars with improved yields and quality and tolerant to droughts, diseases and pests. Use of genetically improved crop cultivars and better management practices are among the best strategies to increase food production and meet a projected doubling of food demand in the next 40 years (Sleper and Poehlman, 2006; Acquaah, 2007; Brown and Caligari, 2008; Miller et al., 2010; Repinski et al., 2011). Both the public and private plant breeding sectors face several constraints, including a shortage of professional plant breeders and breeding technicians, limited budgets, inadequate facilities and lack of access to information (Guimaraes et al., 2006; Morris et al., 2006; Miller et al., 2011; Repinski et al., 2011). Successful plant breeding requires adequate infrastructural investments, in addition to the actual breeding activities. The benefits of plant breeding research and development can only be realized on a long-term basis because of the inherent nature of the crops and the eminent breeding procedures involved. Plant breeding involves two main activities, i.e., pre-breeding/ germplasm enhancement and cultivar development *per se*. These interdependent activities are the controlling factors that determine the pace at which cultivars are released timeously and consistently to farmers. The two phases, in turn, depend upon various factors, e.g., breeding goals, genetics and agronomy of the crop, breeder's long term objectives, availability of testing facilities and national cultivar-registration requirements. Despite these factors, there are certain clear steps and breeding procedures found in any conventional breeding program. These include parental choice, making crosses among chosen parents, and selection from recombined parents followed by extensive field testing of the candidate cultivars. Maintenance and multiplication and distribution of the seed are the ultimate stages of a breeding program. Systematically outlined conventional breeding procedures, timeframes and estimated breeding generations are essential to objectively consider the ultimate timescales required to release new and improved cultivars of all crops. The information may assist plant breeding-students, educators, researchers, project advisors, administrators and funding agencies. The objective of this paper is to outline the timeframes of conventional breeding procedures of clonally propagated, self-fertilizing and cross-fertilizing crops.

Cultivar development

Pre-breeding

The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB)/FAO and Biodiversity International use the term 'pre-breeding' to describe the various activities of plant breeding research that have to precede the stages involved in cultivar development, testing and release (Biodiversity International and GIPB/FAO, 2008). Further, the Global Crop Diversity Trust defined pre-breeding as 'the art of identifying desired traits, and incorporation of these into modern breeding materials.' Pre-breeding aims to reduce genetic uniformity in crops through the use of a wider pool of genetic material to increase yield, resistance to pests and diseases, and other quality traits (http://www.croptrust. org/main/sharingknowled.php?itemid=299). Pre-breeding is routinely applied in commercial breeding programs where desired traits are constantly sought and identified from source genotypes for use in cultivar development. Overall, pre-breeding includes all activities directed at identification of desirable crop traits and/or genes, and their subsequent transfer into a suitable set of parents for further selection. The procedure identifies useful character(s) or genes that can be exploited in cultivar development (Ortiz, 1999). Pre-breeding is involved in the following activities, among others:

Characterization of landrace populations

Landraces are often referred as 'farmers varieties' or 'cultivated native varieties' that are adapted to a specific agro-ecological and farming system without a scientific form of selection. Landraces are invariably heterogeneous and an excellent source of genetic variation for crop breeding programs (Sleper and Poehlman, 2006). Landraces harbor useful genes such as genes for early maturity, yield potential, disease and pest resistance and other desired traits. Landraces are most prevalent in the centers of diversity. They can be characterized using various markers (biochemical, physiological, morphological and molecular markers) for breeding and to determine the level of genetic variation (Podlich et al., 2004; Ortiz et al., 2008). In developing countries landrace varieties are predominantly grown for their farmers-preferred traits (Mulatu and Zelleke, 2002; Ceccarelli and Grando, 2007) and lack of access to well-balanced modern cultivars via an effective production and delivery mechanism of commercial seeds.

Creation of new parent populations to be used as breeding material, with the long-term goal of using the best parents for cultivar development following progeny testing

The success of a crop breeding program relies on choice of the best parents possessing complementary and desired traits. Thus, breeders continuously select potential parent populations from diverse sources including landraces, modern cultivars, obsolete or primitive cultivars, wild or semi-wild species. Parents with high specific or general combining abilities are selected via progeny testing through well-designed recombination. The progenies are evaluated to determine the genetic potential of parents for subsequent breeding and to discern the type of cultivar to be developed, i.e., pure line, hybrid, or open-pollinated. Progeny testing is performed in a set of target and representative environments with half-sibs, full-sibs, testcrosses or recombinant inbreds (Acquaah, 2007; Brown and Caligari, 2008).

Introgression of new traits from other useful sources, usually exotic germplasm or a landrace or related species

The plant breeder transfers one or more desirable traits from unrelated, exotic or semi-exotic, landrace or related germplasm into an intermediate variety with good agronomic potential but lacking a specific trait (Simmonds, 1993). Thus, the new variety will be developed with the introduced novel gene(s) in the existing genetic background. Often the unrelated or exotic germplasm does not have immediate usefulness and as such it has to be selected for adaptation to the target production environment. Exotic germplasm may constitute races, populations, clones, inbred lines, or other forms of genetic structure (Hallauer and Miranda Filho, 1988). When introgressing genes from unrelated, exotic, primitive or wild germplasm, both the desired gene(s) and a considerable amount of undesirable genetic material is introduced into the progeny that has to be removed through a series of backcrosses to the recurrent parent (Brown and Caligari, 2008).

Creation of novel traits, for instance, through mutation breeding followed by backcrosses to good parents

Mutations lead to spontaneous changes of the genetics of individuals that are often heritable. Naturally, mutational events occur at low frequencies, i.e., 10^{-5} to 10^{-8} per locus. Induced mutagenesis through the use of artificial mutagenic agents is an important tool in plant breeding and functional genomics to increase the frequency of mutations and consequently to broaden genetic variation. Induced genetic variations have been used successfully in several crops to create useful mutants (Newhouse *et al*., 1991; van Harten, 1998; Ahloowalia *et al*. 2002; Pozniak and Hucl, 2004; Hohmann *et al*., 2005). The technique can be regarded as an efficient option for germplasm enhancement towards important agronomic traits (van Harten, 1998; Pozniak and Hucl, 2004). The novel mutational events can either be directly developed as essentially derived varieties or novel genes introgressed into candidate parents through a backcross program.

Creation of polyploids

The breeder may create new variability through changing the number of chromosomes in a species, either by altering the basic chromosome set or addition or deletion of specific chromosome(s). Individuals with altered chromosome set (euploids) are developed by doubling the number of genome of a species or by crossing unrelated species followed by chromosome doubling of the inter-specific hybrid. Polyploids can be artificially induced by various means such as exposing plant materials to environmental shock (e.g. low or high temperature treatment, x-ray irradiation) or with chemicals (e.g. colchicine) that disrupt normal chromosome division (Sleper and Poehlman, 2006; Acquaah, 2007; Brown and Caligari, 2008).

Acquisition of new information on crop genetics

The breeder constantly looks new genes from diverse sources for enhanced nutritional qualities, early maturity, high yield

Table 1. Mating systems and propagation among selected food security crops.

Crop	Mating system	Commercial Propagation	Cultivar
Cassava (*Manihot esculenta* Crantz)	Cross fertilizing	Vegetative	Clone
Sweet potato (*Ipomoea batatas* (L.) Lam.)	Cross fertilizing	Vegetative	Clone
Potato *(Solamum tuberosum* L.)	Cross fertilizing	Vegetative	Clone
Banana (*Musa acuminata* Colla)	Cross fertilizing	Vegetative	Clone
Maize (*Zea mays* L.)	Cross fertilizing	Seed	Open pollinated, hybrid
Pearl millet (*Pennisetum glaucum* (L.) R. Br.)	Cross fertilizing	Seed	Open pollinated
Rice (*Oryza sativa* L. and *O. glaberima* Steud.)	Self fertilizing	Seed	Pure line , hybrid
Sorghum (*Sorghum bicolor* (L.) Moench)	Self fertilizing	Seed	Pure line, hybrid
Finger millet (*Eleusine coracana* (L.) Gaertn.)	Self fertilizing	Seed	Pure line
Tef (*Eragrostis tef* (Zucc.) Trotter)	Self fertilizing	Seed	Pure line
Wheat (*Triticum aestivum* L.)	Self fertilizing	Seed	Pure line
Dry bean (*Phaseolus vulgaris* L.)	Self fertilizing	Seed	Pure line
Cowpea (*Vigna unguiculata* (L.) Walp.)	Self fertilizing	Seed	Pure line
Pigeon pea (*Cajanus cajan* (L.) Millsp.)	Self fertilizing	Seed	Pure line
Soybean (*Glycine max* L.)	Self fertilizing	Seed	Pure line
Groundnut (*Arachis hypogaea* L.)	Self fertilizing	Seed	Pure line

Crops listed by FAOSTAT for 2006

potential and biotic and abiotic stress tolerance. Understanding the candidate genes and the pattern of inheritance of the genes in controlling these characters is profoundly significant for effective transfer and to improve the efficiency of selection in cultivar development (Ortiz et al. 2008; Meneely, 2009).

Development of new plant breeding techniques

New and modern breeding techniques can assist in improving selection response. These include development of more efficient conventional selection procedures, biotechnology, molecular marker technologies and identification of markers linked to traits of interest, effective gametocides and cytomlasmic sterility systems with a desired genetic background (Acquaah, 2007; Brown and Caligari, 2008; Lusser et al., 2012).

Cultivar development

Cultivar development embraces well-defined breeding procedures directed at the production of improved cultivars with respect to the mating system of the crop. In self-fertilized and vegetatively reproduced crops the various breeding procedures or phases can be differentiated to include selection of parents, crosses, progeny selections, finishing off the selections to create the new cultivar, followed by its maintenance, multiplication and distribution. However, these stages are less lucid with cross fertilizing crops because crosses and selection are closely connected. In cross-fertilizing crops the controlled crosses are performed by the breeder or happens randomly, i.e., in each selection cycle many recombination events occur. Therefore, in cross-fertilizing crops it is not possible to discern a basic procedure but the breeder identifies the best selection method(s) depending on the crop and the breeding objectives. Cultivar development requires well-developed and elite breeding material, generated from an established and relational pre-breeding program. If present, locally screened and adapted germplasm are an ideal starting material in cultivar development. A plant breeder can spend many years on pre-breeding to ensure that locally adapted and the best available parental material is used to meet the demands of a defined set of agro-ecological zone and day-length requirements of a given latitude. Alternatively, the breeder may use elite material (e.g. inbred lines) that other breeders have developed, such as the germplasm available from the CGIAR centers that is not locally adapted. This will allow a plant breeder to release new candidate cultivars, even if they are not adapted to the local agro-ecological and cropping system into which they are to be released. The released cultivar may have some superior traits but the local farmers often reject the new cultivars because they are not bred to include farmers-preferred traits. Farmers' trait preferences are diverse and

complex such that the total crop value is more important than absolute crop yield (Witcombe and Virk, 1997). Other farmers-preferred traits include: cooking quality, taste, market acceptability, storability (Tripp et al., 1997) and the quantity of utilizable parts of the crop left after harvesting, processing and storage (Cromwell et al., 1992; Mulatu and Zelleke, 2002). McGuire (2008) indicated that despite 25 years of sorghum breeding in Ethiopia most of the released cultivars had been poorly adopted by the small-scale farmers. Thus, a balance between farmers-preferred traits and solutions to production constraints should be the breeders' goal in order to enhance cultivar uptake by farmers. Crop breeding units with adequate resources should run two parallel programs concurrently. Firstly, a pre-breeding program to continually develop new and improved parent materials with superior traits of commercial importance. This is vital to the ongoing improvement of cultivars. Without the pre-breeding program, the development of new cultivars will stall because there will be no new genetic material. Secondly, the breeding program should have a cultivar-development program that will take the selected superior parents and develops this into cultivars, followed by multi-location testing in the target growing environments. If there is no cultivar-development program, then none of the best parent material of the pre-breeding program will reach the farmers. Based on their natural modes of reproduction, crops are classified as clonally propagated, self-fertilizing or cross-fertilizing. To these, hybrids are included as man-made entities. The predominantly common mating systems and propagation of the main food security crops of Africa are summarized in Table 1. The timeframes of the traditional breeding procedures of the common field crops are schematically presented in Figs 1, 2 and 3. It takes at least 7 (Fig 1) or 9 (Fig 2) or 17 (Fig 3) minimum breeding generations to create an improved cultivar of vegetatively reproducing or self-fertilizing or cross-fertilizing crop, respectively. Typically, the estimated minimum timeframes of breeding different crops would be fairly similar unless different selection approaches are adopted to shorten the timeframe for releasing new crop cultivars, e.g., use of double haploid technology and marker-assisted breeding, which are briefly described below:

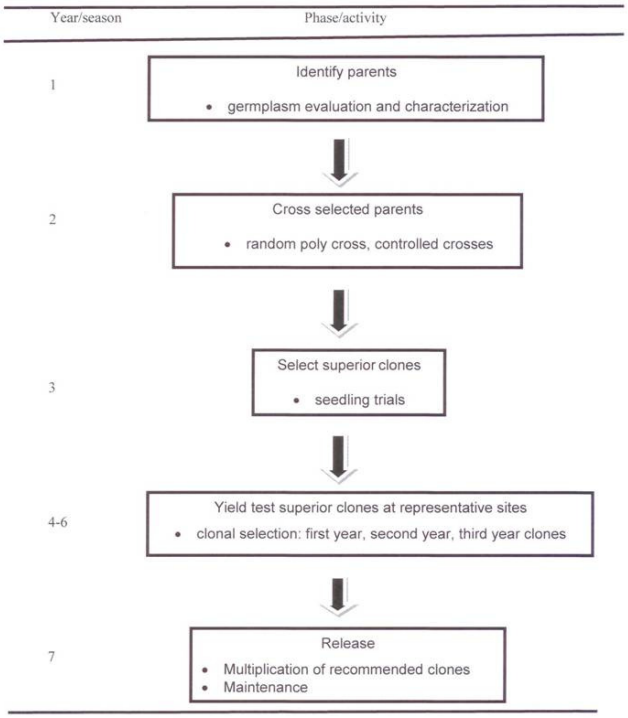

Fig 1. Scheme showing the minimum breeding generations and phases/activities in clonally propagated crops: cassava, sweet potato, potato, banana.

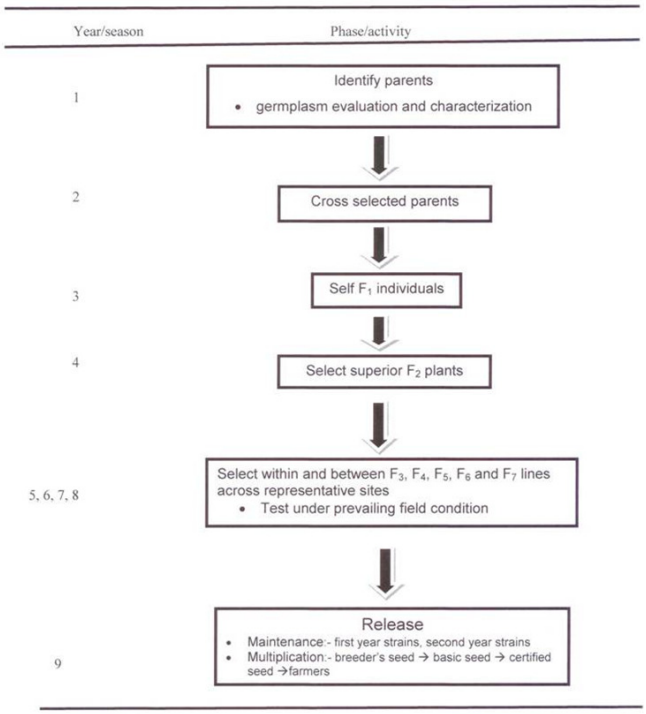

Fig 2. Scheme showing the minimum breeding generations and phases/activities in self-fertilizing crops: rice, sorghum, finger millet, tef, wheat, dry bean, cowpea, pigeon pea, soybean, groundnut.

Applications of doubled haploids in plant breeding

In vitro production of haploid plants followed by doubling of somatic chromosomes is the quickest means to produce pure breeding doubled haploids (DHs) (Choo *et al*., 1985; O'Donoughue and Bennet, 1994). Haploids are produced through various means, such as anther culture (Henry and De Buyser, 1990), or genome elimination following distant hybridisation (Barclay, 1975; Laurie and Bennett, 1988; Matzk and Mahn, 1994; Singh *et al.*, 2001). During wide crosses the entire genome of one of the parents are lost from a hybrid embryo and endosperm in the early cell divisions. In wheat breeding, genome elimination, in particular the use of a wheat by maize cross is usually more reliable than anther culture (Kisana *et al*., 1993; MuJeeb-Kazi *et al*., 1995). The DH lines are fully fertile and manifest only additive genetic variance which provides a basis for efficient selection with a minimum sequence of a breeding cycle. The doubled haploid method has several advantages in crop breeding programs. Firstly, production of doubled haploids leads to homozygosity in a single generation after recombination of selected parents. This is unlike the conventional selection

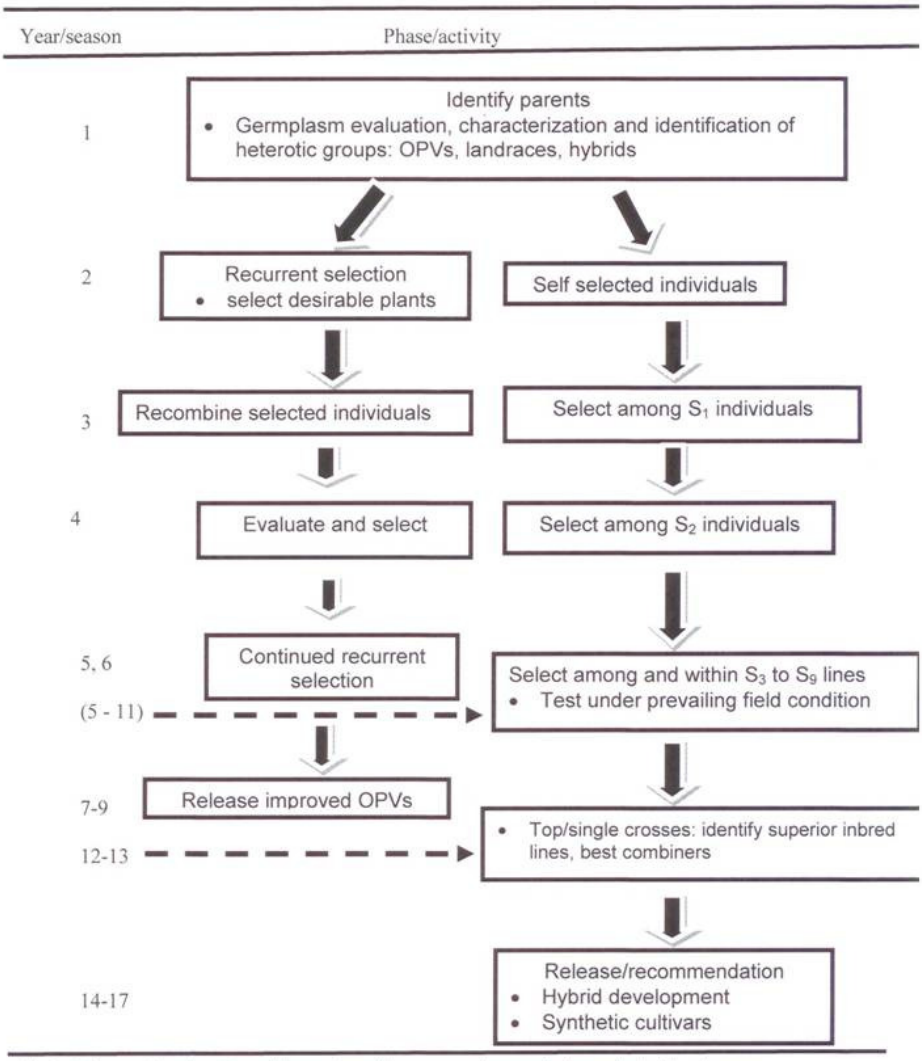

Fig 3. Scheme showing the minimum breeding generations and phases/activities in cross-fertilizing crops: maize, pearl millet.

method that requires six to seven selfing generations to achieve a practical level of homozygosity (Fig 2). Secondly, selection is more efficient for oligogenic or polygenic traits in DHs because the genes are fixed in a homozygous background, limiting dominance genetic variation and segregation (Choo *et al*. 1985). Thirdly, the DH method prevents losses of valuable genetic variations better than the conventional selection method. Traditionally, early generation segregating populations are selected in a single environment where certain genotypes perform poorly are discarded. These genotypes may have carried useful genes that would be expressed in other target environments.

Owing to the gains in speeded up cultivar development and the creation of desirable genetic backgrounds, doubled haploids are widely utilized in breeding as well as in genetic studies of various crops and traits (O'Donoughue and Bennet, 1994; Steffenson *et al*., 1995; Maluszynski, 2004; Arzani, 2008). Overall, the DH technology allows for the creation of stable haploids after recombination of parents with broad genetic variation. Thus, DH derivatives can be selected for improved traits such as yield, earliness, plant height, nutritional quality and pest and disease resistance, in a fully homozygous state. Selected genotypes can be used as homogenous varieties or as breeding parents in the ensuing crosses and selection cycles.

DNA based molecular markers and their applications in plant breeding

Molecular markers reveal genetic differences in the primary structure of DNA between individuals. Compared to protein markers, DNA based polymorphisms are more stable, and can reveal subtle changes in the genomic DNA (Powell et al., 1996; Horacek et al., 2009). Different DNA based marker techniques have been successfully used such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and

single nucleotide polymorphisms (SNP) (Powell et al., 1996; Lusser et al., 2012). Molecular markers are 'landmarks' on chromosomes that serve as reference points to the location of other genes when a genetic map becomes available. If genetic maps are constructed, then the plant breeder establishes association between markers and desirable phenotypic traits. The trait of interest is then selected by indirectly selecting for the marker which is readily assayed or observed (Podlich et al., 2004; Goodman, 2004). In plant breeding, markers are used to locate the chromosomal positions of candidate genes, to determine genomic organisation among different gene pools and to conduct marker-assisted breeding. Identification of DNA markers associated with traits of interest may be facilitated by comparative mapping, i.e., by cross-referencing to the maps of model crop species, owing to gene synetny. These markers may facilitate inter-generic gene transfers and help to minimize linkage drag (Podlich et al., 2004).

Applications of molecular markers in plant breeding

When molecular markers are available, conveniently co-segregating with candidate genes, marker-assisted selection (MAS) or marker-aided selection may improve the efficiency of selections of simple traits in conventional plant breeding programs (Knapp, 1998; Podlich et al., 2004). Broadly, molecular markers are applied in plant breeding in the following areas:

1. To screen for useful single gene traits e.g. disease resistance. This may facilitate the introgression of new genes from a non-adapted parent and in pyramiding desired alleles into enhanced lines of candidate cultivars.
2. To accelerate backcross breeding programs through identification of the gene of interest and to eliminate the undesirable genome of the donor parent. Unlike conventional backcrossing, this method reduces linkage drag and requires few numbers of repeated backcrosses to recover the genotype of the recurrent parent.
3. To characterize diverse germplasm and establish heterotic patterns. Markers are useful to determine the magnitude of genetic diversity for crop improvement and to assign exotic (or non-adapted) germplasm into an appropriate breeding pool. In inbred lines markers assist in establishing heterotic patterns in order to guide the selection of parents for use in a hybrid breeding program. Marker information may be used in combination with phenotypic and pedigree analyses to ascertain genetic differences between lines of different heterotic groups to enable the breeder to predict the performance of hybrids to be developed from different intergroup crosses (Xiao et al., 1996)
4. To identify and protect commercial cultivars through fingerprinting.

Conclusions

This paper summarizes the timeframes involved in the two interdependent breeding activities in crop-improvement programs, namely, pre-breeding/germplasm enhancement, and the actual breeding aimed at cultivar development. It would take at least 7, 9 or 17 generations to release an improved cultivar of a crop that is vegetatively reproducing (e.g. as tubers or suckers), self-fertilizing (pure line, open-pollinated or hybrids) or cross-fertilizing (open-pollinated or hybrids), respectively. As such, both private and public breeding programs require a pool of highly trained and motivated plant breeders to run both activities, which is contingent upon continued and sustained funding support over these time scales. Additionally, the following points should be taken into consideration during planning plant breeding programs:

- The indicated timeframes should be seen as a generic framework for pre-breeding and cultivar development of some African food-security crops, leading to the release of superior cultivars for small-scale farmers;
- The new cultivars need to be locally adapted for the specific agro-ecological systems and latitudes (day-lengths) of the region in which they are to be grown. Very few modern cultivars of relatively few crops in Africa are so adaptable that they can be grown across wide agro-ecological zones. Breeders need to develop novel cultivars of each crop to be specifically adapted to each and every agro-ecological systems and latitude, particularly for day length;
- In self-pollinating and vegetatively propagated crops, it is easy to discern and follow the described breeding procedures. However, the procedures are more varied and difficult to predict with cross-pollinating crops. Thus, a breeder may apply varied selection procedures, such as recurrent selection, half-sib family selection, full-sib family selection or mass selection for the development of improved open-pollinated varieties or to create synthetic or hybrid cultivars. If male gametocides or male-sterility systems are available for a self-pollinating crop, then these procedures can also be applied to produce hybrid cultivars.
- Marker-assisted selection (MAS) should be integrated with traditional breeding methods to enhance the efficiency of cultivar development. The application of MAS is currently limited to Mendelian traits, whereas it is less efficient for complex quantitative traits such as yield and drought tolerance.
- In the present paper specifics on the detailed breeding methods and mating and experimental designs used for different crops are omitted to maintain a focus on the overall breeding timelines.

In conclusion, the speed at which novel crop cultivars can be released by breeders depends upon many variables, in particular:

- The reproductive biology and growth habit of crop plants;
- The resources available to the breeder;
- The scale of pre-breeding that is needed, and the timescales of the pre-breeding operations that have to be followed, before the actual breeding or cultivar development can take place;
- The timescales of the cultivar-development to be followed (e.g., an open-pollinated versus a hybrid cultivar);
- Institutional requirements unique to each crop in each country (e.g., how many years [seasons] and locations [sites] of field testing that are required before registration of a cultivar).

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