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# Marker Assisted Selection for Common Bean Diseases Improvements in Tanzania: Prospects and Future Needs

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George Muhamba Tryphone,  
Luseko Amos Chilagane, Deogracious Protas,  
Paul Mbogo Kusolwa and Susan Nchimbi-Msolla

Additional information is available at the end of the chapter

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## 1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important grain legume for direct human consumption [1]. It is an important source of dietary protein, calories, dietary fibres, and minerals especially iron and zinc in Africa and a primary staple in parts of the Great Lake Regions (GLR) [2, 3]. Beans consuming have medicinal benefits [4]. It is estimated that over 75% of rural households in Tanzania depend on it for daily dietary requirements [5]. Bean production also provides farm households with both income and food for nutrition [1]. Bean is a cash income earner crop where the dry seeds and fresh pods attract a higher market price [6].

Despite the importance of common bean in Tanzania and other developing countries, its production mostly relies on local cultivars [7- 9]. The local cultivars however, are commonly known to produce notoriously low yields as they are highly constrained by several biotic and abiotic factors, including diseases, insect pests, poor seed quality, drought, low soil fertility and poor crop management [1, 10-12]. Yield losses caused by bean diseases are very significant and devastating in the bean industry [11, 13-15]. The economic losses caused by diseases results from reduction of seed quality and yield [16]. Since most of landraces and improved cultivars grown in Tanzania are susceptible to the diseases, there is a need therefore to incorporate resistance against them in adapted cultivars. Currently, none of the commercial genotypes has multiple resistances to common bean diseases. However, using classical breeding, significant strides have been made in crop improvement through pheno-

typic selections for agronomical important traits. Considerable difficulties however, are often encountered during this process, due to genotype-environment interactions [17]. Furthermore, resistance to some diseases is complex as they are quantitatively inherited making it difficult to achieve rapid progress through classical breeding [13]. In addition, breeding is complicated by the pathogens variability and different genes conditioning resistances [1, 13]. The identification of plants carrying two or more resistance alleles of different genes using standard inoculation test is impractical because several races would be needed to screen for specific alleles [16]. Thus classical breeding is limited by the length of screening procedures and reliance on the environmental factors. Hence, deployment of the molecular markers linked to resistance genes could be an alternative, more reliable screening procedure to increase the efficiency of breeding for disease resistance using marker assisted selection (MAS) [13]. Molecular marker available include 23 RAPD and five SCAR markers linked to 15 different resistance genes in addition to QTL conditioning resistance to seven major pathogens of common bean [13, 66]. The use of DNA molecular markers will improve understanding of the genetic factors conditioning these traits and is expected to assist in the selection of superior genotypes [17, 18]. Molecular marker assisted selection can be used to simultaneously screen for resistance to diseases without affecting the growth of the plants [13, 19]. Selection for genetic markers linked with resistance genes and QTL can accelerate development of multiple resistant varieties and increase efficacy [14, 20, 21]. The use of disease resistant cultivars in combination with appropriate cultural practices is essential for the management of bean diseases [14, 22, 23]. This chapter discusses the importance of MAS and how it can be integrated into breeding programs for enhancing selection efficiency in developing disease resistant bean varieties in Tanzania.

## 2. Economic important of common bean

Common bean (*Phaseolus vulgaris* L.) is an important grain legume for the direct human consumption in the World [1]. It is a staple food for more than 100 million people in Africa with per capita consumption of 60 kg/person/year in the Great Lakes Regions (GLR) [24]. Beans represent one of the principal crops in East Africa in terms of total area planted and number of farmers involved in production [25]. Bean production also provides farm households with both a source of income and food for nutrition through sales and consumption of part of the produce. Tanzania ranks 6<sup>th</sup> among top 10 bean producers worldwide [26] and is the largest producer in Africa with 850,000 MT produced per year which is equivalent to a commercial value of US\$ 246,583,000. Production of common beans in Tanzania is higher than any other pulses estimated at 300 000 tonnes annually, representing 82% of the total pulse production [5, 27]. The dry bean is the major product although green beans are also widely consumed. It complements cereals and other carbohydrate rich foods by providing near perfect nutrition to people of all ages. Common bean has the nutritional benefits such as high source of proteins and high mineral contents especially Fe and Zn which combat high prevalence related micronutrient deficiencies [3, 6]. Consuming beans also have medicinal bene-

fits as it is recognized that they contribute to treating human ailments like cancer, diabetes, and heart diseases [2, 4].

### 3. Constraints in bean production

The average yields of common bean has remained low (>500 kg/ha) [11] while the potential of current promising released varieties are at 1500 kg/ha [11, 28]). Across farming systems, biotic and abiotic stresses continue to present the major constraints for increased bean production and high yields with bean diseases representing the major constraints to production by reducing yields and seed quality. In Tanzania and other parts of the world, large yield losses of common bean are due to a great number of diseases affecting the crop. The major diseases affecting bean production in Tanzania include Bean Common Mosaic Necrosis Virus, common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*), halo bacterial blight (*Pseudomonas syringae* pv. *phaseolicola*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotricum lindemuthianum*) and rust (*Uromyces phaseoli*) [11]. On sandy soils the root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) are the main problems [11]. The angular leaf spot (ALS), the common bacterial blight (CBB), the bean common mosaic virus (BCMV) and the bean common mosaic necrosis virus (BCMNV) are diseases which are endemic in Tanzania occurring across all production ecologies [1]. They can cause yield loss up to 100% of the expected yield, depending on the environment and the cultivars used [29]. There is thus a need to breed for high resistance levels and one option is to introgress resistance genes in adapted cultivars grown locally or into one line.

### 4. Breeding for disease resistance

The low bean yields in developing countries among others are due to a lack of effective diseases management practices including the lack of disease resistant cultivars and when such cultivars are available, they are not integrated in the disease management packages. The development of cultivars with improved resistance to biotic and abiotic stresses has long been a primary goal for many bean breeding programs [8]. It is considered that the use of resistant cultivars is an efficient, safe and inexpensive technique accessible for bean growers [14]. In fact, this strategy is the most effective and sustainable method for controlling bean diseases [29]. Resistant varieties therefore provide distinct channels for achieving high productivity through productivity maintenance, where benefits are not derived from the avoidance of yield losses associated with disease pressure and the yield gains the resistant varieties can give under disease pressure [30]. The use of resistant varieties leads to a reduction in both production costs especially pesticide cost and lower the quantity of pesticides or their residues released into the environment [14, 16]. Thus, varieties with improved disease resistance can reduce reliance on pesticides in high input systems, avert the risk of yield loss from diseases in low- and high-input systems, and enable more stable bean production across diverse and adverse environments [30]. However, the development of resistant variety is an

obstacle for breeders as most pathogen exhibits a great variability for pathogenicity which mostly overcomes the resistance in the released cultivars. Breeders are thus continuously forced to look for new sources of resistances. The screening procedures to ascertain resistance is another setback as pathogenicity tests need to be reliable by exhibiting comparable and reproducible results [13]. The other constraint is whatever resistances detected with those tests should be efficient in controlling the target diseases in the field. Finally, methods usable by breeders for speeding up the breeding work should be developed. Genomics of *P. vulgaris* appear to be promising in discovering and tagging novel alleles [19, 31]. If closely linked to resistant genes, molecular markers such as Sequence Characterized Amplified Region (SCAR), Simple Sequence Repeats (SSR), Restriction fragment length polymorphism (RFLP), Amplified fragment length polymorphism (AFLP) can enhance the efficiency of breeding programs especially in the so-called marker assisted selection (MAS) and can be used in initial and intermediate stages of the breeding process. The target traits can be achieved indirectly using molecular markers closely linked to underlying genes or that have been developed from the actual gene sequences [32]. MAS can be used to simultaneously screen for resistance to diseases without affecting the growth of the plants. Selection for genetic markers linked with resistance genes and QTL can accelerate development of multiple resistant varieties and increase efficacy [20, 21]. The uses of MAS enable the introgression of resistance genes into a cultivar, decreases population size and ultimately reduce the time required to develop a new variety.

## 5. Molecular markers

Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as signs or flags and they are used as chromosome landmarks to facilitate the introgression of chromosome regions with genes associated with economically important traits [19]. However, such markers themselves do not affect the phenotype of that trait of interest because they are located only near or are linked to genes controlling the target traits [31]. Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as either hybridization-based or polymerase chain reaction (PCR)-based markers [19]. DNA markers are useful particularly if they can reveal difference between individuals of the same species or different species [32, 33].

There are three types of genetic markers: morphological (or classical or visible) markers, which themselves are phenotypic traits or characters, biochemical markers which include allelic variants of enzymes called isozymes and DNA (or molecular) markers, which reveal sites of variation in DNA [19]. Morphological markers are usually visually characterized and include phenotypic characters such as flower colour, seed shape, growth habits or pigmentation [34]. However, these markers are limited in number so only small portion of the genome can be assayed for contribution towards complex characters. Also, the genes controlling morphological markers have pleiotropic effects on the characters under investigation; this eludes the actual location of genes due to distortion of segregation ra-



tio. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The number of useful protein markers is very small. Both morphological and biochemical markers are influenced by environmental factors and/or developmental stages of the plants [20, 16, 19, 34].

However, the properties to be considered desirable for ideal DNA markers for their use as DNA markers in MAS as suggested by authors in reference [32, 33] as: highly polymorphic nature, codominant inheritance (distinguishing homozygous and heterozygous states of diploid organisms), quality and quantity of DNA required, frequent occurrence in genome (reliability), selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices), easy access (availability), easy and fast assay, high reproducibility and easy exchange of data between laboratories. However, it is not easy to find a molecular marker which meets all these criteria. Depending on the type of study undertaken, a marker system can be identified that would fulfill at least a few of these criteria.

### 5.1. Restricted Fragment Length Polymorphism (RFLP)

RFLPs are simply inherited naturally and are Mendelian characters. They have their origin in the DNA rearrangements that occur due to evolutionary processes, point mutations within the restriction enzyme recognition site sequences, insertions or deletions within the fragments, and unequal crossing over. The usefulness of these markers in improvement of common bean have been the assessment of genetic diversity as they are useful in detecting polymorphism among different lines and hence being used to determine how diverse the genome being assessed [35] and they have been found superior over isozymes for their better coverage of the genome and higher level of polymorphism. These markers are useful in breeding for disease resistance when they are linked to disease resistant genes. For example, four RFLPs were found to be linked to *Are* gene for resistance to Anthracnose of common bean [36] and in this matter then the RFLPs can be used to breed for resistance.

### 5.2. Microsatellite markers (SSR-Simple Sequence Repeats)

They essentially belong to the repetitive DNA family. Fingerprints generated by these probes are also known as oligonucleotide fingerprints. The methodology has been derived from RFLP and specific fragments are visualized by hybridization with a labelled microsatellite probe. Microsatellites or short tandem repeats/simple sequence repeats consist of 1 to 6 bp long monomer sequence that is repeated several times. These loci contain tandem repeats that vary in the number of repeated units between genotypes and are referred to as variable number of tandem repeats. Microsatellites thus form an ideal marker system creating complex banding patterns by simultaneously detecting multiple DNA loci. Some of the prominent features of these markers are that they are dominant fingerprinting markers and codominant sequence tagged microsatellites markers. If many alleles exist in a population, the level of heterozygosity is high and they follow Mendelian inheritance.

These markers have been utilized in a variety of ways in bean improvement since they are linked to disease resistance genes and in diversity analysis [37]. The SSR markers have been used in diversity assessment in common bean because of their utilities like low costs, high efficiency, whole genome coverage, robustness and minimum DNA requirements [20, 37]. In addition these markers are preferred for use because of being highly polymorphic, co-dominant, being PCR based and easily detected [19, 37]. The SSR markers have been utilized in assessing the genetic structure and diversity among common beans [38]. In MAS some SSR markers have been identified to be linked to disease resistance genes as the case for Angular leaf spot genes where the primer *PV-atct001* was found to be linked to resistant allele to ALS [16, 39] and some markers have been used in Marker assisted backcrossing [29].

### 5.3. Random Amplified Polymorphic DNA (RAPD)

This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequences. In this reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermocyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals.

These markers have been used in a variety of ways in genetic analysis. They have been used in gene pyramiding especially where conventional procedures couldn't solve the problem when there is epistasis between resistance genes to be pyramided [40, 41]. Some of the RAPD markers have been used to pyramid three rust resistance alleles *Up2*, *Ur-3* and *B-190* [42] with other epistatic resistance alleles from plant introduction collection [40, 43]. Similarly, pyramiding was also suggested in reference [44] where the two genes linked to *I* and *bc-3* genes for resistance to bean common mosaic virus disease and bean common mosaic necrosis virus disease respectively were incorporated in elite cultivar/line.

These markers have also been used in the assessment of genetic diversity of common bean. In reference [45] reported the potential of using RAPD markers as compared to RFLP, DAMD-PCR, ISSR and AFLP for assessing diversity of common bean and in this finding it shows that these markers were able to produce higher percentage of polymorphism than the others used hence being very useful in detecting polymorphism.

### 5.4. Sequence Characterized Amplified Region (SCAR) markers

In SCAR markers, the RAPD marker termini are sequenced and longer primers are designed (22–24 nucleotide bases long) for specific amplification of a particular locus [16]. The presence or absence of the band indicates variation in sequence. These are better reproducible than RAPDs. SCARs are usually dominant markers, however, some of them can be converted into codominant markers by digesting them with tetra cutting restriction enzymes and polymorphism can be deduced by using simple non denaturing gels to detect whether the products has different restriction sites for the different alleles. Compared to arbitrary pri-

mers, SCARs exhibit several advantages in mapping studies (codominant SCARs are informative for genetic mapping than dominant RAPDs), map-based cloning as they can be used to screen pooled genomic libraries by PCR, physical mapping, locus specificity, etc. SCARs also allow comparative mapping or homology studies among related species, thus making it an extremely adaptable concept in the near future [16, 19]. These markers have been widely used in breeding for disease resistance especially where the disease is controlled by dominant gene since these markers are dominant in nature. Different SCAR markers have been identified linked to resistance genes to many common bean diseases [46].

### **5.5. Sequence Tagged Sites (STS)**

RFLP probes specifically linked to a desired trait can be converted into polymerase chain reaction (PCR)-based Sequence-Tagged Sites (STS) markers based on nucleotide sequence of the probe giving polymorphic band pattern, to obtain specific amplicon. Using this technique, tedious hybridization procedures involved in RFLP analysis can be overcome. This approach is extremely useful for studying the relationship between various species. When these markers are linked to some specific traits, for example the powdery mildew or stem rust resistance genes in barley, they can be easily integrated into plant breeding programmes for MAS of the trait of interest [47].

### **5.6. Amplified Fragment Length Polymorphism (AFLP)**

The technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. The fingerprints are produced, without any prior knowledge of sequence, using a limited set of generic primers. The number of fragments detected in a single reaction can be 'tuned' by selection of specific primer sets. AFLP technique is reliable since stringent reaction conditions are used for primer annealing. This technique thus shows an ingenious combination of RFLP and PCR techniques and is extremely useful in detection of polymorphism between closely related genotypes. Due to their characteristics, these markers are useful in assessing diversity of common bean and in case the marker is linked to a trait of importance in common bean then it can be useful for MAS in selecting or screening genotypes for that particular trait [48, 49]. For example, AFLP studies conducted to determine genetic relatedness of two near-isogenic Teebus lines and Teebus of common bean to CBB resistance [44, 50]. These markers despite being useful, their analysis is too difficult and troublesome, for this they can be converted to other types of markers like SCAR or STS which is also a difficult thing to achieve.

## **6. Molecular Marker Assisted Selection (MAS)**

By using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased. Use of markers in plant breeding is called marker-assisted selection (MAS) and is a complement of the new discipline of molecular breeding [33]. MAS is the novel approach in which individuals for intercrossing are selected using selection index based on genotypic



data controlled by few or several genes (Quantitative linked traits or QTL). The gain from selection using such index is expected to be higher than phenotypic selection used in conventional recurrent methods [21]. Significant progress has been made through phenotypic selections for agronomic traits. However, difficulties are often encountered due to the genotype x environment interactions [17]. For example, significant progress has been achieved in selecting BCMV and BCMNV resistant lines [13]. However, some of the traits are controlled by multiple genetic loci (Quantitative Trait Loci) and display a strong interaction with the environment. Molecular markers linked to such traits are available and have increased the efficiency of breeding for diseases in MAS programmes [13, 19]. The use of DNA molecular markers will improve understanding of the genetic factors conditioning these traits and is expected to assist in the selection of superior genotypes [18]. The use of disease resistant cultivars in combination with appropriate cultural practices is essential for the management of these bean diseases [2].

MAS is an approach designed to avert problems encountered with conventional/classical plant breeding by increased precision of selection, selecting phenotypes through the selection of genes that control the traits of interest [19, 51]. This is because molecular markers are clearly not influenced by environment and are detectable at all stages of plant growth [20, 28]. With the availability of an array of molecular markers and genetic maps, MAS has become possible for traits governed by single gene or QTLs. MAS is a good approach for bean breeders who also work to improve bean for disease resistance. For MAS to be highly successful, a high correlation and/or tight linkage must exist between the genes for resistance to diseases and molecular markers, and the markers must be stable, reproducible and easy to assay [52].

MAS provide an effective and efficient breeding tool for detecting, tracking, retaining, combining, and pyramiding disease resistance genes [31]. DNA based markers can be effectively utilized for the following basic purposes (i) tracing favorable alleles (dominant or recessive) across generations and (ii) identifying the most suitable individual (s) among the segregating progeny, based on allelic composition across a part or the entire genome [20, 32].

## 7. Why MAS in plant breeding

Justifications for the application of MAS in plant breeding fall into four broad areas that are relevant to almost all target crops [53, 54, 55] (i) traits that are difficult to manage through conventional phenotypic selection because they are expensive or time-consuming to measure, or have low penetrance or complex inheritance; (ii) traits whose selection depends on specific environments or developmental stages that influence the expression of the target phenotypes; (iii) maintenance of recessive alleles during backcrossing or for speeding up backcross breeding in general; and (iv) pyramiding multiple monogenic traits (such as pest and disease resistances or qualitative traits) or several QTL for a single target trait with complex inheritance (such as drought tolerance or other adaptive traits). Introgression and pyramiding of multiple genes affecting the same trait is a great challenge to breeding programs.

The target cropping environments of many breeding programs require a combination of diverse biotic stress resistances, agronomic and quality trait profiles, plus abiotic stress tolerances to improve performance, yield stability, and farmers' acceptance. The greatest impact from MAS will only be realized when breeding systems are adapted to make best use of large-scale genotyping for both multiple target traits and the genetic background. The greatest benefits from this type of integrated molecular breeding approach will be to achieve the same breeding progress in a much shorter time than through conventional breeding, and from pyramiding combinations of genes that could not be readily combined through other means.

## 8. MAS' requirements

Success of marker based breeding system depends on several factors as described by [20, 19], a genetic map with an adequate number of uniformly-spaced polymorphic markers to accurately locate desired QTLs or major gene (s); close linkage between the QTL or a major gene of interest and adjacent markers; adequate recombination between the markers and rest of the genome; an ability to analyze a large number of plants in a time and cost effective manner.

## 9. Applications of MAS

The key success of integrating MAS into breeding programmes lies in identifying applications in which markers offer real advantages over conventional/classical breeding methods or complement them in a novel way. MAS offer significant advantages in cases where phenotypic screening is expensive, difficult or impossible or traits are of low heritability and/or the selected trait is expressed late in plant development. Also, for incorporating genes for resistance to diseases or pests that cannot be easily screened due to special requirements for the genes to be expressed; the expression of the target gene is recessive; there is a need to accumulate multiple genes for one or more traits within the same cultivar, or improving perennial/biennial crops with long life cycle using a process called gene pyramiding [13, 20, 32, 33]. The success of MAS depend upon the distance between the markers and the target gene, the number of target genes to be transferred, the genetic basis of the trait, the number of individuals that can be analyzed and the genetic background in which the target gene has to be transferred, the type of molecular marker (s) used and the availability of specific technical facilities [20, 21].

Conventional breeding has been successfully applied in several crops' breeding programmes and a large number of varieties or lines possessing multiple attributes have been produced. However, the difficulties associated with this method are due to the dominance and epistasis effects of genes governing the target disease resistances, for example, the CBB resistance in case common bean. Therefore, MAS has been especially suggested for increas-

ing the selection efficiency and timely delivery of cultivars in the particular case of breeding for resistant cultivars. The benefits from the use of genomics tools include (i) more effectively identify, quantify and characterize genetic variation from all available germplasm resources; (ii) tag, clone and introgress genes and/or QTLs that are useful for enhancing the target trait using either genetic transformation, facilitating pyramiding or recurrent selection, by differentiating and selecting particular genotypes in breeding populations [20, 32].

## 10. Molecular markers assisted selection for bean diseases

Breeders used to rely on visual screening of genotypes to select for traits of economic importance. However, successful application of this method depends on its reproducibility and heritability of the trait. Therefore, the use of molecular markers in the bean breeding programmes has improved the accuracy of crosses to carry out and allowed breeders to produce germplasm with combined traits that were hazardous and difficult before the advent of DNA technology [13].

### 10.1. Angular leaf spot

Resistance genes against *Phaeoisariopsis griseola* the causal agent of ALS are controlled by major genes, that are either dominant or recessive, acting singly or duplicated and which may interact in an additive manner with or without epistasis [56]. Inheritance of resistance is controlled by a single recessive gene [57], but in an earlier study, resistance to ALS was reported to be controlled by a single dominant gene. This shows that inheritance of ALS resistance is complex, involving both dominant and recessive genes that may be or may not be independent. Major and minor genes mediate angular leaf spot (ALS) resistance in beans (*P. vulgaris*) and a number of sources for these resistance genes have been identified [56]. Diverse sources of resistance to angular leaf spot in bean genotypes have been reported [58]. Examples of resistant cultivars include A 75, A 140, A 152, A 175, A 229, BAT 76, BAT 431, BAT 1432, BAT 1458 and G5686, MAR 1, MAR 2 [59]. In reference [60] found the ALS resistance in AND 277 to race 63:23 to be conferred by a single dominant gene (*Pgh-1*). Cornell 49-242 has *Pgh-2* which confers resistance to *P. griseola* pathotype 31:17 [61] while [41] found that resistance to ALS in Mexico 54 is due to a single dominant gene that confers resistance to pathotype 63:63 and G06727 has resistance to *P. griseola* pathotype 63:59. In reference [120] reported that 'Ouro Negro' had resistance to 8 pathotypes, including *P. griseola* race 63:63 from Brazil. G5686 and Mexico 54 display fairly good levels of resistance to nearly all races [59]. These cultivars are not only good sources of resistance to *P. griseola* but could also serve as reliable indicators of new races of the pathogen in the future [62]. Mexico 54 has shown to be resistant to all *P. griseola* isolates characterized in Africa [63]. Resistance in G5686 is conditioned by two dominant epistatic genes and Amendoim by two recessive genes [64]. Resistance to specific isolates of *P. griseola* has been reported to be simply inherited and molecular markers have been identified for some of these resistance genes [14, 41, 65, 66]. Sources of resistance reported from Africa include GLP 24, GLP X-92, GLP - 806 and GLP 77 [59]. Resistance to various diseases is monogenically determined, but cases of duplicate,

complementary and other interactions have been reported [67]. The breed for ALS resistance, molecular markers linked to angular leaf spot resistance genes have been identified in beans. SCAR markers for selecting for genes for resistance to ALS include SH13 for *phg-1* gene in linkage group 6 [68] and SNO2 for *phg-2* gene in linkage group 8 [69, 61]. Others include, SAA19 [68], SBA16 [68] and SMO2 [68] which is ouro negro dominant gene.

## 10.2. Common bacterial blight

The control of common bacterial blight (CBB) disease caused by *Xanthomonas axonopodis pv phaseoli* (*Xap*) is challenging due to its complexity and seed borne nature [67]. The number of genes involved in resistance to *Xap* range from one to several genes with varying degrees of action and interactions [70, 71]. Breeding for CBB resistance is complicated pathogen genetic diversity and coevolution [72, 73] different genes conditioning resistance in leaves, pods and seeds [16, 73, 74, 76] and linkage of resistance with undesirable traits [16, 76]. Resistance of CBB is quantitatively and qualitatively controlled depending on the source of germplasm with pod and leaf resistance being controlled by different genes [9, 67, 77]. Quantitative inheritance was observed after making original interspecific crosses between resistant *P. acutifolius* 'tepary 4' and susceptible *P. Vulgaris* [67]. Sources of resistance to *Xap* in common bean have been reported [66, 78]. Other sources of resistance have been identified in tepary bean (*P. acutifolius*) [79, 80], and runner bean, (*P. coccineus*) [81]. Resistance to common bacterial blight has been reported in *Phaseolus acutifolius* [77], *P. coccineus* and lines of *P. vulgaris* [82]. CIAT lines VAX 3, VAX 4, VAX 6, and XAN 159 have also been reported to have good level of resistance to common bacterial blight [67]. Increased resistance can be developed by selecting for horizontal resistance [83].

Albeit, genetic studies have shown that resistance to CBB is quantitatively inherited, it involves a few major genes [13]. The identification of QTL influencing resistance to CBB combined with phenotypic data implying the involvement of few genes, suggests that MAS may be useful in combining resistance sources to CBB in common bean. To date, SCAR markers used in selecting resistance to CBB are dominant and are scored as presence or absence of a single band on an agarose gel. SCAR markers available in screening are SU91, BC420, SAP 6, BAC 6, R7313 and R4865. SU91 is linked to a QTL for CBB resistance in bean in the linkage group B8 [16, 84]. BC420 is linked to a QTL for CBB resistance on bean linkage group B6. SAP 6 is for a major QTL in the linkage group B10 [84], BAC 6 for a major QTL in linkage group B10 [85] R7313 for a major QTL in linkage group B8 [86] and R4865 for another major QTL [86]. Thus, molecular markers allow distinct QTLs to be screened and consequently provide an opportunity to pyramid multiple QTL for CBB resistance into a single genotype.

## 10.3. Bean common mosaic virus and bean common mosaic necrosis virus

Genetic resistance to both potyviruses is conditioned by a series of independent multi-allelic loci in common bean is affected by four different loci: bc-1, bc-2, bc-3 and bc-u [87]. Resistance controlled by alleles at these loci is inherited as recessive characters [88]. In addition to the recessive bc genes, the dominant I gene in *P. vulgaris* confers resistance to BCMV and other potyviruses through a hypersensitive response [88, 89] and has also been the focus of



positional gene cloning activities [90]. The *I* gene located on B2 [91], is independent of recessive resistance conditioned by three different *bc* genes. The *bc-3* gene is located on B6 [84, 92, 93], whereas the *bc-12* allele was mapped to B3 [84]. The non-specific *bc-u* allele, needed for expression of *bc-22* resistance, also resides on B3 based on the loose linkage with the *bc-1* locus [94].

The independence of the BCMV resistance genes provides opportunities to use gene pyramiding as a strategy in breeding for durable resistance. Bean breeders recognize that the combination of the dominant *I* gene with recessive *bc* resistance genes offers durability over single gene resistance to BCMV and BCMNV, since the two types of genes have distinctly different mechanisms of resistance [95]. The dominant *I* gene is defeated by all necrotic strains, whereas the three most effective recessive genes (*bc-1*, *bc-2* and *bc-3*) act constitutively by restricting virus movement within the plant, probably through the virus movement proteins. The action of the dominant *I* gene is masked by the recessive *bc-3* gene, so as efforts to incorporate the *bc-3* gene into new germplasm proceed, the risk of losing the *I* gene in improved germplasm increases, since direct selection for the *I* gene is not possible. Linked markers offer the only realistic opportunity to maintain and continue to utilize the *I* gene as a pyramided resistance gene in future bean cultivars.

A marker tightly linked to the *I* gene [96] has been demonstrated in many laboratories to be effective across a wide range of germplasm from both gene pools. Breeders have used markers linked to the *I* gene to develop enhanced germplasm with the *I* + *bc-3* gene combination. In addition, [92] developed SCAR markers from the OC11350/420 (ROC11) and OC20460 RAPD markers linked to the *bc-3* gene to improve their utilization. The use of these markers in MAS, however, has been limited due to a lack of polymorphism and reproducibility across diverse genetic backgrounds and gene pools of common bean [91].

Direct screening with strains of BCMV and BCMNV is still required to confirm the presence of the *bc-3* gene. To efficiently introgression the *bc-3* gene for resistance to BCMV and BCMNV into susceptible bean cultivars, there is a need to identify more robust DNA markers tightly linked to the *bc-3* gene that will demonstrate reproducibility across laboratories and be functional in different genetic backgrounds. Similarly, the hypostatic *I* gene is retained in the presence of the *bc-3* gene by MAS for the SW13 SCAR [69, 96]. This combination of a dominant and a recessive gene, likely possessing different resistance mechanisms, should provide more durable resistance to bean common mosaic virus.

At CIAT, bean cultivars have been bred which combine *I* gene and recessive resistance genes. These have been evaluated in areas of East Africa where BCMNV is known to occur [96]. Several commercial varieties combining the *I* gene and recessive resistance genes are now available [97, 99].

#### 10.4. Anthracnose

Two new sources of anthracnose resistance within the Andean gene pool were identified in germplasm from Brazil [10, 100; 101]. The two independent genes were identified as Co-12 in Jalo Vermelho and Co-13 in Jalo Listras Pretas and represent unique resistance patterns.



These are significant findings as the multiallelic Co-1 locus with five alleles was the only resistance sources previously known in Andean germplasm. This is particularly important given the recent breakdown of the Co-12 gene by race 105 in Manitoba. The rapid evolution of this new race underscores the need to monitor the pathogenic variability in different production areas. The availability of new resistance genes of Andean origin offers breeders more choices for pyramiding genes with the more common Middle American resistance sources.

### 10.5. Root rots

Root rot of dry bean is a yield-limiting disease problem for growers in the North-Central region of the U.S. [102]. In North Dakota and Minnesota, *Fusarium solani* was considered to be the most common causal agent of root rot followed by *Rhizoctonia solani* [103]. However, recent findings have highlighted the ability of other *Fusarium* species to cause root rot in dry beans [104, 105]. Little is known about the prevalence and virulence of the four subspecies of *Rhizoctonia solani* that are found on common bean. Crops grown in rotation with beans, such as sugar beets, are also hosts for *R. solani*. [106] found low genetic diversity among 166 isolates of the *Fusarium* wilt pathogen from the U.S. Central High Plains using RAPD markers. Resistance to *Fusarium* wilt in race Durango dry beans CO 33142 and Fisher were controlled by a single dominant gene, whereas polygenic control ( $h^2$  ranged from 0.25 to 0.60) was found for resistance in race Mesoamerica cultivars Rio Tibagi and Jamapa [107, 108]. In addition, limited research has been conducted on *Aphanomyces euteiches* f.sp. *phaseoli*, but this fungus occurs frequently in the sandy soils in the Upper Midwest.

### 10.6. Rust

Two new races of rust have been recently reported in Michigan and North Dakota. The new races have reoccurred in Michigan since 2007 and in North Dakota since 2008. Preliminary results are showing that both races are similar, but not identical [109]. Resistance to both races is conditioned by the Ur-5, Ur-11, and CNC genes. A new source of resistance was mapped to LG 4 near the Ur-5 and Ur-Dorado108 loci in black bean populations derived from Tacana [110]. Several new cultivars with different combinations of rust resistance genes have been released [111]. Salient among these are six unique great northern bean germplasm lines named BelDakMi-RMR-8, to -13. These are the first great northern beans that combine four genes for rust resistance and two genes for resistance to the two bean common mosaic potyviruses. These beans combine two Andean (Ur-4 and Ur-6) and two Middle American (Ur-3 and Ur-11) rust resistance genes [111]. Other rust resistant cultivars include great northern bean cultivars ABC-Weihsing (Ur-3 and Ur-6) [112], and Coyne (Ur-3 and Ur-6) [113], and Pinto CO46348 (Ur-4 and Ur-11) [114]. In the case of soybean rust, the common bean lines Compuesto Negro Chimaltenengo (CNC) and PI 181996 were among the most resistant to all six isolates. Inheritance of SBR resistance in CNC was studied by crossing Mx309/CNC. Based on severity, the segregation for SBR resistance in the  $F_2$  population fit a 9 resistant to 7 susceptible ratio.

## 11. Other case studies of MAS

MAS has been proposed as the most practical and realistic approach to provide efficient long term control of bean anthracnose, ashly stem, bean common mosaic virus, common mosaic necrosis virus, bean golden mosaic virus [69], bean rust [115] and common bacterial blight [16, 64]. It has been or is being used to assist the simultaneous transfer of resistance genes for rust, anthracnose and angular leaf spot into Brazilian commercial cultivars [29]. Several lines resistant to rust [115, 116]; bean golden mosaic virus [69] and anthracnose [117] are being obtained using MAS.

## 12. Cost effectiveness of MAS to conventional screening method

As conventional breeding systems attempt to combine more and more target traits, there are tends to lose overall of breeding gains and an increase in the number of breeding cycles required to generate a finished product. In contrast, MAS offers the potential to assemble target traits in single genotypes more precisely, with less unintentional losses and in fewer selection cycles [20]. By means of MAS, breeding programmes have reported twice the rate of genetic gain over phenotypic selection for multiple traits such as yield, biotic and abiotic stress resistance and quality attributes [29, 32].

It has been described that the time, precision, number of traits and efficiency for traits with low heritability has increased with MAS. The cost-effectiveness of MAS depends on four parameters which are: the relative cost of phenotypic versus marker screening; the time saved by MAS; the time and temporal distribution of benefits associated with accelerated release of improved germplasm; the availability in the breeding program of operating budgets [20]. For example, in [16] estimated the cost for using SCAR and RAPD markers to analyse 100 bean samples (lines) would be \$4.24 and 4.59 per data point, respectively after the markers were developed. This included the costs of labour to plant seeds, watering the plants daily for eight days, extract genomic DNA and conduct PCR and electrophoresis as well as the costs for chemicals and greenhouse space, but not the initial costs of developing the markers. Conversely, conventional greenhouse screening was estimated to cost approximately \$6.99 per data point. This included the costs of labour to prepare inoculums, inoculate the plants, take care of plants for 32 days (fertilizer application, daily plant watering, insect control, growth room cleaning) and rate disease symptoms as well as the cost of greenhouse rental.

## 13. Historical background of common bean improvement in Tanzania

Bean production in Tanzania is affected by many problems that range from diseases to poor soil fertility as well as drought as the production is heavily rain-fed [11]. Some of the major bean production areas have acid soils with pH <5.5 which limit crop productivity [1].

Effort has been put on developing varieties that are resistant to biotic and abiotic stresses. This came in when breeding programs that set up across the country. Since the initiation of the breeding programme in Tanzania in 1959 [11], the white haricot beans was produced for the canning industry though it is susceptible to bean rust disease and has a poor seed quality. The objectives were to i) determine the reasons for poor bean yields among smallholders in the Southern Highlands and ii) to select high-yielding cultivars. It was established that diseases were the major yield-limiting factor and disease resistance became the main thrust of the programme. Therefore, its first step was to identify resistance sources among the available lines. The first line adapted in East Africa as being resistant to rust with good quality was Mexico 142 [11].

Since 1984, CIAT has introduced a number of varieties with different attributes into its breeding programmes for the mid- and high altitude areas of central, eastern and southern Africa. Twenty three bean varieties have been released in Tanzania since 1970 and several of these have been CIAT lines or were selections made in Tanzania from CIAT crosses [11, 118, 119].

Classical breeding methods were also used by CIAT in East Africa to develop a population from multi-parent crosses among genetically diverse lines from Andean and Mesoamerican gene pools. Several new lines were selected with combined resistance to ALS, root rot, low soil N, low soil P and low soil pH. These lines are being evaluated in seven countries in the region including Tanzania [121]. The plant breeders in the national and regional breeding programmes have been able to release a number of varieties in Tanzania as shown in Table 1 [119]. However, none of those varieties have been developed through marker assisted selection technique.

SN	Name of varieties	Year of release	Institutions involved	Yield (t/ha)	Reaction to diseases
1	Canadian wonder	1977	ARI Selian	1.1-2.4	Moderately resistant to halo blight and bean common mosaic virus
2	Kabanima	1980	ARI Uyole	1.5-1.8	Resistant to anthracnose and rust
3	Uyole 84	1984	ARI Uyole	1.5-2.0 (non staked) 2.5-4.0 (staked)	Resistant to anthracnose and halo blight
4	Uyole 90	1990	ARI Uyole	1.5-2.0	It is tolerant to halo blight and angular leaf spot
5	Uyole 94	1994	ARI Uyole	1.0-1.8	Resistant to <i>ascochyta</i> and rust, tolerant to Bean Common Mosaic Virus and Angular Leaf Spot
6	Uyole 96	1996	ARI Uyole	1.0-1.8	Tolerant to rust, <i>ascochyta</i> and Bean Common Mosaic Virus

SN	Name of varieties	Year of release	Institutions involved	Yield (t/ha)	Reaction to diseases
7	Uyole 98	1998	ARI Uyole	1.2-2.0	Resistant to anthracnose, angular leaf spot and rust. Tolerant to halo blight and <i>ascochyta</i>
8	Ilomba	1990	ARI Uyole	1.5-2.5	Resistant to anthracnose, halo blight and rust, Tolerant to <i>ascochyta</i>
9	Lyamungu 85	1985	ARI Selian	1.2-1.5	Resistant to anthracnose, angular leaf spot, Bean Common Mosaic Virus and intermediate to common bacteria blight.
10	Lyamungu 90	1990	ARI Selian	1.2-1.6	Resistant to leaf rust and anthracnose
11	Selian 94	1994	ARI Selian	2.5-3.5	Moderately susceptible to anthracnose and angular leaf spot
12	Jesca	1997	ARI Selian	2.0-3.4	Resistant to anthracnose, Bean Common Mosaic Virus and halo blight, moderately resistant to bean rust, angular leaf spot, common bacterial blight
13	Selian 97	1997	ARI Selian	2.0-2.8	Resistant to anthracnose, Bean Common Mosaic Virus and halo blight, moderately resistant to bean rust, angular leaf spot, common bacterial blight
14	Rojo	1997	SUA	2.2	Resistant to Bean Common Mosaic Virus, moderately resistant to common bacterial blight and nematodes.
15	Wanja	2002	ARI Uyole	1.5	Drought tolerant.
16	Bilfa	2004	ARI Uyole	1.5-2.5	Tolerant to Halo blight, Drought resistant Resistant to Anthracnose and bean rust
17	Uyole 04	2004	ARI Uyole	2.0 – 2.5	Resistant to Bean rust, Anthracnose and Tolerant to Halo blight and drought
18	Pesa	2006	SUA	0.9-1.5	Moderate resistant and Angular Leaf Spot. Resistant to Bean Common Mosaic Virus
19	Mshindi	2006	SUA	0.9-1.5	Moderate resistant to Angular Leaf Spot and Resistant to Bean Common Mosaic Virus
20	Selian 05	2005	ARI Selian	1.0-1.6	Resistant to Bean rust, Anthracnose, Mosaic Virus, and Halo blight
21	Selian 06	2007	ARI Selian	2.5-3.0	Resistant to Bean rust, Anthracnose, Mosaic Virus, and Halo blight

SN	Name of varieties	Year of release	Institutions involved	Yield (t/ha)	Reaction to diseases
22	Cheupe	2007	ARI Selian	2.5-3.0	Resistant to Bean rust, Anthracnose, Mosaic Virus, and Halo blight
23	Njano Uyole	2008	ARI Uyole	2.5 – 3.0	Resistant to Anthracnose

Source: MAFSC, 2008 [119]

**Table 1.** Common bean varieties released in Tanzania since 1970s and their characteristics

## 14. Conclusion

Plant breeders have traditionally and routinely used various recurrent selection methods to cumulate favourable alleles for yield and other polygenic traits. This selection will provide the population or breeding lines with diverse genetic recombination. The selection methods using classical breeding should be compared with that of MAS. To make it successful to the breeder, gains made from MAS must be more cost effective as compared to gains through classical breeding. It is anticipated that the applications and technology improvements will result in a reduction in the cost of markers, which will subsequently lead to a greater adoption of using molecular markers in plant breeding. The obstacles in using MAS are equipment, infrastructure, skilled man power and supplies or consumables. The available projects in Tanzania which involves the use MAS are time based and focuses on few bean pathogen. The available projects are facing several problems such as timely purchase and acquisition of consumables for molecular biology laboratories is frustrating even when funds are available. The main reasons include the reduced number of commercial flights between the supplier countries and Tanzania, the lack of proper cold chains in the supply chain and inappropriate policies hampering imports. The benefits of using MAS need to be critically compared to those achieved or expected from any existing classical breeding programmes. This is because; although classical breeding programme have their limitations, they have also shown over time that they can be highly successful. The use of molecular tools should not be a substitute for classical breeding methods but these two approaches should complement one another so as to achieve the benefits of both in crop breeding programmes. Development of comprehensive crop improvement programmes that will deploy the available sources of resistance to diseases and make proper use of MAS in selection is very important and this can in a proper way leap the benefits associated with these new tools and technologies as MAS in breeding for disease resistance. That can be true if government, donors and private sectors can join efforts to invest on facilities which can be shared for cost effective and efficiency delivery of services using MAS in breeding for disease resistance.



## Author details

George Muhamba Tryphone, Luseko Amos Chilagane, Deogracious Protas,  
Paul Mbogo Kusolwa and Susan Nchimbi-Msolla

Department of Crop Science and Production, Faculty of Agriculture, Sokoine University of  
Agriculture, Chuo Kikuu, Morogoro, Tanzania

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