Chapter 6

Marker-Assisted Selection (MAS): A Fast-Track Tool in Tomato Breeding

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76007

Abstract

Marker-assisted selection (MAS) is a complementary tool for conventional breeding where a molecular marker linked to a trait is indirectly selected. Many studies conducted have been able to identify and develop markers for traits such as disease and pest resistance and other abiotic stresses. Despite the availability of these markers, the technology has been extensively used in tomato breeding for the identification of some economic traits in particular disease resistance. In developed countries, MAS is utilized routinely in breeding programs, but this cannot be said for developing countries such as Africa. It is high time Africa as a continent looks at the importance of the technology and invests in it. In addition to MAS, other strategies such as marker-assisted backcrossing and recurrent selection have also been employed for breeding in tomato. The use of MAS in crop improvement will not only reduce the cost of developing new tomato varieties but will also increase the precision and efficiency of selection in the breeding program as well as lessen the number of years required to come up with a new crop variety.

Keywords: tomato, crop improvement, molecular marker, indirect selection, efficiency, variety

1. Introduction

Tomato, *Solanum lycopersicum* L., is the second most important vegetable after potato. Indisputably, it is the most popular vegetable crop in the world [1]. Though a tropical plant, the crop is cultivated virtually all over the world [2]. In most West African countries especially Ghana, the crop is consumed in almost every household daily [3]. Tomato provides vitamins A and C as well as vital

minerals and other nutrients [4]. That notwithstanding, both the fresh and processed tomatoes are the richest sources of the dietary antioxidant lycopene, which debatably protects cells from oxidants linked to cancer [5]. Tomato is also a source of other compounds with antioxidant activities such as rutin, tocopherol, chlorogenic acid, plastoquinones and xanthophylls [6]. Tomato has been commonly used not merely as food, but also as research material. The tomato plant possess many interesting features such as fleshy fruit, compound leaves and a sympodial shoot, which are lacking in other model plants (e.g., *Arabidopsis* and rice). Moreover, tomato belongs to an enormous family Solanaceae, which is closely interrelated with many commercially important plants such as garden eggs, eggplants, peppers, potato and tobacco [7]. Information or knowledge obtained from studies conducted on tomato can be easily applied to these plants, hence making tomato an important research material. For this reason, tomato functions as a model organism for the family Solanaceae and especially for fleshy-fruited plants. Phenotypic selection coupled with traditional breeding was used to develop most commercial cultivars of tomato. Currently, tomato breeding has entered into a new era following the introduction of molecular markers and marker-assisted selection (MAS) technology. Tomato was one of the first crops for which molecular markers were suggested as indirect selection criteria for breeding purposes [8–10]. Molecular markers have been used extensively for genetic mapping as well as identification and characterization of genes for many agriculturally important traits in tomato. The technology also has been utilized for marker-assisted breeding for several economically important traits. The actual use of MAS in tomato breeding began approximately 30 years ago with the use of the isozyme marker acid phosphatase (*Aps-11* locus) as an indirect selection criterion for breeding for nematode resistance [11]. Paradoxically, this isozyme marker is still being used in many private and public tomato-breeding programs for selecting for nematode resistance. However, more recently, with the development of new molecular markers and maps in tomato, MAS has become a routine practice in many tomato breeding programs, in precisely in the private sector. MAS is often used to assess hybrid purity from overseas production by screening seed lots with a panel of molecular markers [12]. MAS is used effectively for quick germplasm screening for disease resistance or fruit quality. Often, a panel of linked markers is used on individual selections or pools of seed or tissue from early generation populations to "index" breeding populations. This aids breeding efforts by informing the breeder about which disease resistances or fruit quality traits are segregating or fixed in a given population. MAS is employed for marker-assisted backcrossing (MAB) after reliable linkages between markers, and simple traits of interest are discovered. Such traits include, but not limited to, disease resistance, fruit color and carotenoid content (e.g., lycopene and *β*-carotene), fruit-ripening-related traits (various genes including *Rin* and *Nr*), jointless pedicel (*j2*) and extended shelf life using various genes such as *alcobaca, nor and rin* [12]. MAS is not only faster than phenotypic selection but also cheaper and more effective. However, the extent to which MAS has been employed in public and private tomato breeding programs has not been clearly determined. This chapter gives a review of the application of MAS in tomato and assesses the current and potential use of MABC in tomato breeding programs.

2. Breeding history of tomato

Domestication of tomato has activated a wide range of morphological and physiological traits that differentiate domesticated crops from their wild ancestors. At the end of the nineteenth

century, numerous cultivars of tomato were available in different colors and for different purposes. Since these cultivars require no cross-pollination, growers especially tomato farmers get access to seeds effortlessly for the next planting. For the reason that tomato has only 4% chance of outcrossing, tomato produces plants that show resemblance to the parents. As a matter of fact, previous or former tomato cultivars that were carefully chosen and innate in a family got the name heirloom. Heirloom tomato varieties though open-pollinated are unique in shape, size and color [13]. These cultivars could be considered as landraces and products of domestication. The collection, description, propagation and distribution of genetic materials are of the utmost importance in tomato breeding. The Tomato Genetics Resource Center in Davis, California (TCRC) during the latter half of the twentieth century assembled and maintained thousands of wild Solanum species accessions coupled with producing large proportion of monogenic mutants and various genetic stocks of tomato. Currently, the most Solanaceae species in the world were collected and maintained by the Botanical and Experimental Garden (http://www.bgard.science.ru.nl/) in the Netherlands [14] (http://zamir.sgn.cornell.edu/mutants/), which is an isogenic tomato "mutation library" containing a total of 13,000 M(2) families derived from treatment with ethyl methane sulfonate (EMS) and fast-neutron mutagenesis.

Systematic breeding for improvement of the overall horticultural characteristics of tomato actually started in the 1930s. Tomato breeding gained prominence at the beginning of twentieth century in the public institutions predominantly in the USA. Later private companies were formed and engaged in commercial breeding that led to hybrid development. Hybrids give a good combination of characters from both parents. Growers preferred to buy hybrid seeds at higher prices following their enormous benefits over the open-pollinated cultivars. The first hybrid tomato cultivar, which developed through a single cross, was released in 1946 [15].

Currently, most tomato varieties whether fresh market tomato or processed tomato are hybrids. The breeding process involves recognizing and combining certain traits to create a novelty for each market. The final product could be sold in a wide range of shapes such as pyriform, high round, cylindrical, oval and sizes from small cherry tomato to very large beef tomatoes. The breeders' law allows breeders to make new crosses either with their own materials or cultivars of their competitors [16]. To avoid taking many generations to remove deleterious genes, breeders often dodge using wild germplasm to introduce new traits. Crosses are, however, made to produce test hybrids i.e., hybrids developed through F4 to F6 with fixed parental lines. These hybrids then go for testing at on station (breeders site) and finally to the farmers' sites after which the best hybrids are selected for commercial usage. Recently, a number of tomato breeding companies are major players in the world market. It is, therefore, important that seed companies continue to develop new cultivars with added value [17]. It takes approximately 5 years for commercial tomato cultivars to turn over time. As a matter of fact, breeding companies can get return on their investments if prices are high for their seeds. This is typical of the fresh tomato market as the yearly value of worldwide tomato seed market is approximately half a billion euros especially for fresh market.

The goals of public and private tomato breeding programs vary widely depending on location, need and resources. In general, breeding goals in tomato have gone through four phases: breeding for yield in the 1970s, for shelf life in the 1980s, for taste in the 1990s and for nutritional quality currently. To be successful, growers must produce a high yield of high-quality

fruit, while holding production costs as low as possible. Therefore, many of the breeding goals focus on characteristics that reduce production costs or ensure reliable production of high yields with high-quality fruits. The genetics of a quantitative trait is hard to study, since the effect of each gene is small and often influenced by environment or by the interaction with other genes (epistasis). Many important tomato traits as described above are genetically controlled by a combined action of QTLs with favorable alleles often present in the wild species [18–20]. To introgress the wild favorable allele into cultivated tomato, marker-assisted selection plays an important role and the map positions and markers linked to the QTLs provide a basis for breeders to design optimal breeding strategies. To map QTLs in tomato, interspecific populations have been extensively used. However, in an interspecific cross, multiple segregating QTLs at the whole genome level often tend to mask the effects of one another [21, 22].

3. Development of genetic markers

An alternative approach to improving selection efficiency in tomato is to discover genetic markers that are associated through linkage or pleiotropy with genes that control the trait(s) of interest. Genetic markers are biological features that can be transmitted from one generation to another. They can be used as experimental probes or tags to track an individual, a tissue, a cell, a nucleus, a chromosome or a gene. The value of genetic markers as indirect selection criteria has been known to breeders since early 1900s. Genetic markers can be classified into two categories namely classical markers and DNA markers [23, 24]. Classical markers comprise morphological markers, cytological markers and biochemical markers. DNA markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), rapid amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), single nucleotide polymorphism (SNP), etc. have been developed. These DNA markers have developed into many systems based on different polymorphism detecting techniques or methods including northern and southern blotting of nucleic acid hybridization, polymerase chain reaction (PCR), and DNA sequencing [25].

4. Classical markers

Breeders have used morphological markers to select for superior phenotypes for many decades. During the history of plant breeding, markers mainly used included visible traits such as flower color, leaf shape, seed shape, fruit shape, flesh color, stem length, etc. These morphological markers can easily be identified and therefore usually used in the construction of linkage maps. Some of these markers are also linked with other agronomic traits and thus can be used as indirect selection criteria in breeding. However, morphological markers available are limited, and many of these markers are not associated with important economic traits like yield and quality. In addition, some even have undesirable effects on the development and growth of the plant. In tomato, there are over 1300 morphological, physiological (e.g., male sterility, fruit ripening, and fruit abscission), and disease-resistance genes [26] of which only less than 400 have been mapped [27].

Cytological markers are represented by chromosome karyotype and banding patterns. These markers are not directly used in plant breeding but serve as landmarks on the chromosomes thereby used for identifying linkage groups and subsequently genetic maps are constructed. Biochemical markers or isozymes are alternative forms or structural variants of an enzyme with different molecular weights and electrophoretic mobility but have the same catalytic activity or function. The second generation of isozymes became more popular during 1970s and early 1980s. Although some 41 isozymic genes in tomato have been identified, characterized and mapped [28], these markers are few and less polymorphic [29].

5. DNA markers

In overcoming limitations associated with classical markers, development of DNA markers have proven to be of great significance in enhancing genetics and breeding of crop varieties [30]. A DNA marker is a fragment of DNA showing mutations/variations, which can be used to detect polymorphism between different genotypes in a population. These fragments are usually associated with a specific location within the genome and may be detected using modern molecular tools. In the past, different types of molecular markers have been developed and utilized. This includes both dominant and codominant markers. Dominant markers are markers that are unable to differentiate between homozygotes and heterozygotes, while codominant markers can differentiate between homozygotes and heterozygotes. A lot of molecular markers have been developed for tomato. Notable among them are RFLP markers; however, this marker is time and labor intensive and requires the use of large amount of DNA. As a result, RFLP markers have been replaced with PCR-based markers that are easy to handle (http://solgenomics.net). Other marker techniques that have been developed for tomato include random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) [30]. Large amount of sequence information have been released for tomato species and subsequently, SSR markers developed. These SSR markers are widely used because they are easy to handle and able to detect multiple alleles. Currently, over 20,000 SSR markers have been developed from expressed sequence tag (EST) and BAC-end sequences and used as genetic and genomic tools in tomato species [31]. Single nucleotide polymorphism (SNP), which is now the marker technology of choice, has also been discovered by a resequencing strategy, and several SNP genotyping methodologies have been developed for application in tomato research. As a result, this high-throughput SNP analysis can be performed effectively in a large number of samples by array-based assays as genotyping platforms and applied to the construction of high-density genetic linkage maps and performance of genome-wide association studies [32]. The diversity arrays technology (DArT) platform, which is one of the array-based methods, has also been used to develop polymorphic markers across introgression line (ILs) population of tomatoes [33].

6. Genetic maps in tomato

The first linkage map of tomato was reported in 1968. This linkage map was constructed based on both morphological and physiological markers [34]. The map was later improved

and was assigned to the 12 linkage groups in tomato [35]. This facilitated the development of other maps including the tomato isozyme linkage map that was published in 1980. Then in 1986, another map consisting of RFLP and isozyme loci was also generated. Since then, several interspecific genetic linkage maps have been generated with RFLPs incorporating cleaved amplified polymorphic sequences (CAPS), SSR and SNP markers. Varying number of markers ranging from 93 to 4491 have been used for constructing linkage maps with a coverage of about 50% of the genome. Other intraspecific maps were later constructed using SSR and SNP markers. Identification and construction of these markers and maps, respectively, will be helpful in identifying useful genes or QTLs that can be introgressed into desirable genetic backgrounds for marker-assisted breeding [36]. This may not only hasten the breeding process, but will also allow pyramiding of desirable genes and QTLs from different genetic backgrounds, which will serve as an effective complementary approach to substantial crop improvement.

7. Applications of marker-assisted selection (MAS) in tomato breeding

Marker-assisted selection (MAS) is a tool for crop improvement where an associated marker is used for indirect selection of a trait. In this case, you are selecting for a trait based on the genotype of an associated marker rather than the trait itself. It is a technique that has been extensively explored for a wide range of plant traits and can reduce the cost as well as increase the precision and efficiency of selection in breeding. With recent development of molecular tools and genetic maps, MAS has become more attractive and practical than before. Molecular markers are not affected by either genetic or environmental factors, making MAS a useful tool in crop improvement. Markers developed to be used for MAS must be tightly linked to the genes or QTLs. In recent years, it is widely accepted that QTL effects, QTL validation or fine mapping with high resolution is a requirement for MAS [37]. The most important issue in the application of molecular markers in plant breeding is that major effect QTLs or genes should be mapped with high accuracy. In addition, these genes should not have any negative effect on other traits. The use of MAS in tomato breeding started in the 1930s [35] much earlier than in many other crop species. It was employed for the improvement of many morphological, physiological, and disease resistant traits.

Although resistant genes or QTLs have been identified for many fungal diseases in tomato, only few of these are been used for MAS, while with the others, markers associated with resistant genes/loci have been identified, but there are no reports on PCR-based markers developed for resistance breeding. Typical examples are with Alternaria stem canker [38] and gray leaf spot [39] where RFLP markers have been reported, but no PCR-based markers developed; with anthracnose ripe rot, few RADP markers associated with QTLs [40] have been reported but not validated for MAS; with black mold, QTLs [39] have been identified, but there is no report for MAS; with corky root rot, RFLP markers have also been identified and converted to CAPS and additional RAPD markers identified [41], but there is no report of using these markers for MAS; with Fusarium crown root rot, a RAPD marker has been identified, which may be useful for MAS in tomato breeding [42]; with early blight, QTLs have been identified [43], but there is no

PCR-based markers reported; with powdery mildew, several QTLs [44] have been identified, but there are no PCR-based markers closely linked to these QTLs identified; and with Septoria leaf spot, there has been no report of genetic mapping studies for resistance breeding. MAS has, however, been successful for resistance breeding in tomato for Fusarium wilt, late blight, leaf mold and Verticillium wilt. Molecular markers associated with Fusarium wilt resistance *I*, *I-1*, *I-2* and *I-3* [45] conferring resistance to four different races of the pathogen were identified, and PCR-based markers developed for all with the exception of *I-1* and used effectively for MAS; markers associated for late blight resistance *Ph-1*, *Ph-2* and *Ph-3* [46] has also been developed and used for tomato breeding; several PCR-based markers linked to the *Cf* gene for leaf mold [47] and Verticillium wilt [48] has also been reported and widely used for MAS.

QTLs and molecular markers associated with resistance have also been identified in tomato for the various bacterial diseases; however, it is only markers that are tightly linked to RFLPs and PCR-based markers for gene *Pto* in bacterial speck [49] that have been used for resistance breeding via MAS. With the other bacterial diseases including the bacteria canker, bacterial spot and bacterial wilt, QTLs or RFLP markers have been identified and reported but are not commercially used for MAS. With bacterial canker, two QTLs [50] have been developed and could be useful for MAS. RFLP markers associated with *Rx-1* and *Rx-2* and Rx-*3* for bacterial spot have been reported [51], but *Rx-1, Rx-2* and Rx-*3* are independently associated with hypersensitive response in the greenhouse and are not polymorphic in most breeding populations and hence not useful for MAS breeding, while *Rx-3* is associated with both hypersensitive response and field resistance. CAPs markers have been developed for the gene *Rx-3* and used for MAS breeding. Several QTLs have also been identified for breeding for bacteria wilt resistance in tomato; however, two dominant markers associated with the gene *TRST-1* [52] have been suggested to be useful.

Although there has been reports on the identification of the resistant gene *Cmr* for the cucumber mosaic virus [53], *pot-1* gene for Potyviruses [54] and two QTLs associated with the tomato mottle virus, there are no reports of use of these markers in tomato breeding. With the tomato mosaic virus, PCR-based markers for *Tm-1*, *Tm-2*, and *Tm-2*^{2}-resistant gene have been reported to be used for MAS [55]. Several genes have also been reported to be resistant to the tomato spotted wilt virus; however, PCR-based markers for only resistant gene *Sw-5* have been reported to be developed and utilized by most tomato breeding programs [56]. With the tomato yellow leaf curl virus, PCR-based markers have been identified for and developed for *Ty-1*, *Ty-2*, *Ty-3* and *Ty-4*-resistant loci [57]; hence, these markers are not very consistent and hence the challenge in using them for MAS. In the early 1980s, linkage association between the gene *Mi* [58] controlling nematode (*Meloidogyne incognita*) resistance and *Aps-1*^{1} locus was reported [59]. RFLP markers associated with the *Aps-1*^{1} locus and PCR-based markers associated with the *Mi* gene [60] have been routinely used for the selection of root knot nematode resistance in tomato. The *Mi* gene has also been reported to be resistant to two biotypes of the whitefly *Bemisia tabaci*. Several studies have tried to identify genes or QTLs for insect resistance in tomato; however, there are fewer reports on the identification of these genes/QTLs [61]. This may be attributed to difficulties in phenotypic screening for insect resistance, linkage drag and ease of using pesticides for insect control. However, with the increasing crusade on integrated pest management and restrictions on the use of pesticides, new discoveries in marker development, it is expected

that more efforts will be devoted to the identification, development and use of markers for insect resistance improvement in tomato. In tomato, molecular markers have been used to map genes or QTLs for abiotic environmental stresses (such as salinity, drought and heat) and many flower and fruit-related characteristics including exerted stigma, petal and sepal characters, fruit size, shape, color, soluble solids content, pH, lycopene, acidity, flavor, ripening, and many others. However, there is very little indication of the use of MAS for manipulating QTLs for these complex traits, although attempts are being made to improve some quantitative traits. Although MAS is as an effective tool for crop improvement, most breeding programs especially in Africa are not using it routinely. It is imperative that MAS is employed in our breeding programs to enable us ripe the benefits.

8. Marker-assisted backcrossing approach in tomato breeding

8.1. Marker-assisted backcrossing

The backcrossing method has been used extensively in plant breeding to incorporate one or a few genes from one plant possessing a unique trait (donor parent) into a desired adapted or elite variety (recurrent parent) that lacks few qualities such as disease resistance. In most cases, the parent used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics [62]. The application of molecular markers in backcrossing has increased the efficiency of selection. Marker-assisted backcrossing involves the use of molecular markers to track either the target locus or the background of the recurrent parent. The outcome of such a process is a cultivar that contains only the major gene that is obtained from the donor parent, while the genome of the recurrent parent remains intact.

8.2. Marker-assisted backcrossing approaches

Whereas [63] proposed two types of selections (foreground and background selection) under marker-assisted backcrossing, [64] identified three levels of marker-assisted backcrossing (foreground selection, recombinant selection and background selection).

8.3. Foreground selection

In the foreground selection, target locus of the donor parent is tracked using the selected molecular marker of interest [65]. The objective is to maintain the target locus in a heterozygous state (one donor allele and one recurrent parent allele) until the final backcross is completed. This is done to ensure that the locus that is targeted for improvement remains in a state of heterozygosity in the progeny for both donor and recurrent parent. The progeny are then selfed to ensure segregation and recombination in the next generation. Individuals that are found to be homozygous for the allele of interest are identified and selected [66].

The foreground selection is an efficient method to introgress favorable alleles into farmer-preferred varieties and elite cultivars of crops including maize. This approach ensures that only the gene of interest is transferred, while the genetic background of the elite cultivar remains intact. The resulting variety is the same as the original recurrent parent except the

new gene. This prevents the need to promote the new variety [67]. This method is useful for traits that have laborious or time-consuming phenotypic screening procedures. It is also very effective for selecting reproductive traits at the seedling stage, so that only best plants are identified and tagged for backcrossing. Application of marker-assisted backcrossing enables the successful transfer of recessive alleles, which is difficult to do when using conventional approaches. Visscher et al. [68] reported that resistance in barley was improved following a successful tracking of a marker linked (0.7 cM) to the *Yd2* gene for resistance to barley yellow dwarf virus in the progeny population. They observed that BC_2 F_2-derived progenies containing the linked marker showed fewer leaf symptoms and gave much higher grain yield though they were together with progenies that lacked the marker (**Figure 1**). The method has also been successfully used to improve salinity tolerance in rice. This selection involved the use of markers tightly linked to salt tolerance in rice to screen BC_1F_1 progenies for the presence of salt tolerance QTL. They were able to successfully identify individuals that carried homozygous loci from the heterozygous ones though they were phenotypically the same. These heterozygous individuals were then selected for further evaluation in the program.

8.4. Background selection

The approach involves the use of flanking markers that are tightly linked to the genomic regions for recombinant selection and unlinked markers to select for the genomic background of the recurrent parent [69, 70]. Background markers are markers that are unlinked to the target gene. Therefore, these markers can be used to select against the donor genome. Individuals that are homozygous for as many alleles of the recurrent parent are selected for full recovery of the recurrent parent genome [71, 72].

The breeder selects the genome of the recurrent parent using marker alleles for all the genomic regions of the recurrent parent except the target locus. The target locus is then selected based on the phenotype. Sometimes, elite genes are colocated in the same genomic regions and may affect the final product if transferred together. Elimination of such regions is very difficult in conventional approaches. The application of marker-assisted backcrossing approaches using background selection enables the introgression of just the target locus. The background method of selection is important in eliminating such deleterious genomic regions of the donor parents that may negatively affect the final product. This is extremely useful because the recurrent parent recovery can be greatly accelerated. Conventional backcrossing takes a minimum of six backcross generations to recover the genome of the recurrent parent, with some fragments of the donor genome still remaining intact. However, the genome of the recurrent parent can be achieved at the BC_2, BC_3 or BC_4, thus shortening the process by two of the four backcross generations when markers are involved [69, 70, 72–74] (**Figure 1**).

8.5. Recombinant selection

This method of MABC approach is used to reduce the number of deleterious genes (linkage drag) that are transferred from the donor parent. It involves the simultaneous tracking of the genetic background of the recurrent parent and the allele of the donor parent in a heterozygous state [75]. Many undesirable genes that negatively affect crop performance may be linked to the target gene of the donor parent, and the rate of decrease of this undesirable

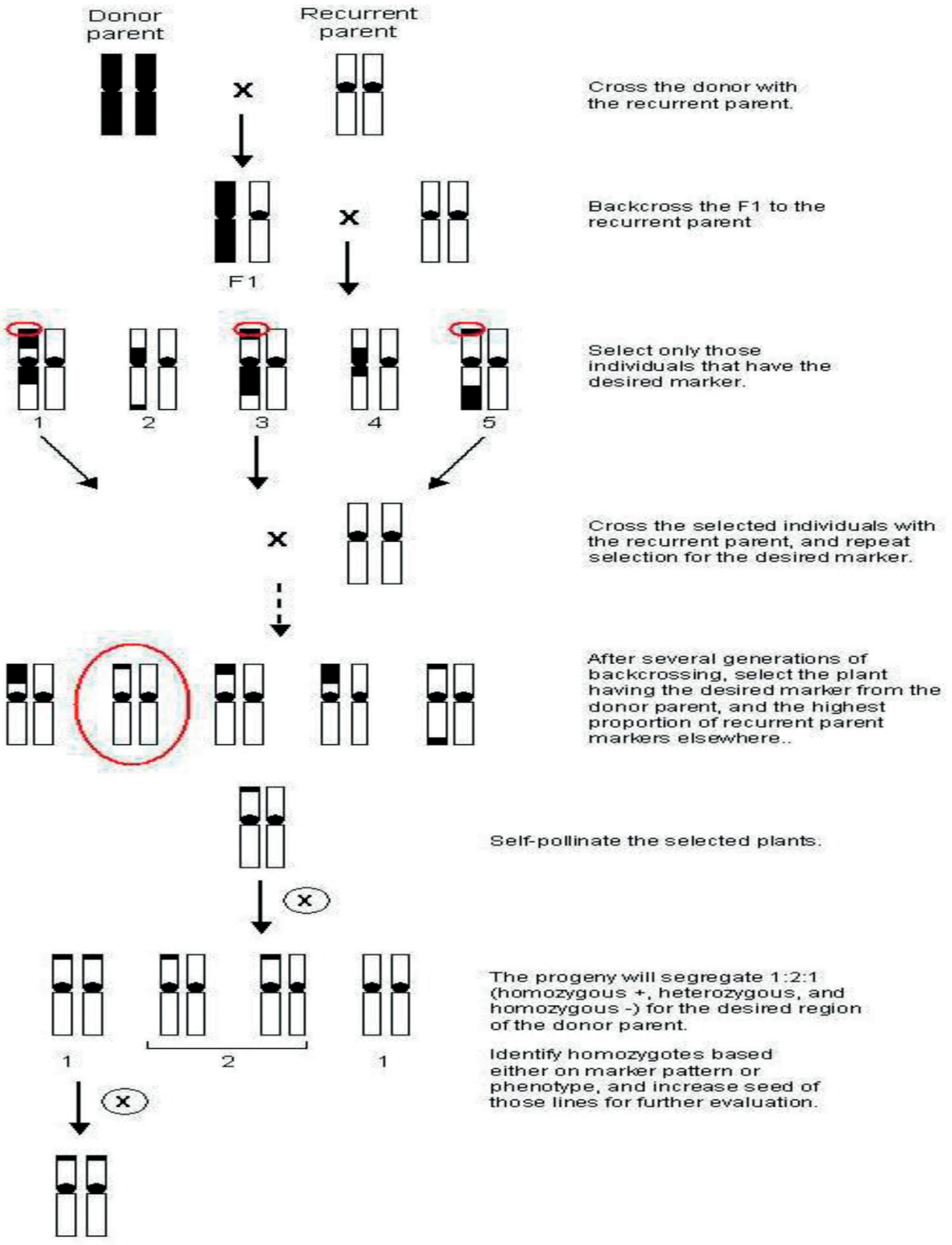

Figure 1. Flowchart of foreground and background selection scheme. Source: http://passel.unl.edu/Image/siteImages/MASFigure7Lg.jpg.

segment is slower than the unlinked regions [76]. After identification of individuals using foreground markers, single and double recombinant individuals carrying the donor alleles as well as the recurrent parents are selected [77, 78]. The use of flanking markers are able to greatly reduce the undesirable segment of the donor parent compared to the conventional

approaches that may carry large segments of the donor parent even after several generations [79, 80]. Compared with conventional backcrossing approaches, marker-assisted backcrossing enables faster recovery of the recurrent parent genome especially when foreground and background selection are combined. In practice, both foreground and background selections are conducted simultaneously in the same backcross program.

9. Progress and prospects of MAS in tomato breeding

In most developing countries such as Ghana, the development of new cultivars, for example tomato, maize, groundnut, cowpeas, has been achieved through conventional plant breeding method rather than transgenic breeding. It generally comprises of sequences of imbrications of three corresponding stages:

- Assembling germplasm like landrace, wild types, improved and/or exotic types of tomatoes as sources of genetic diversity for the major breeding activities to create different recombinants.
- Identification of superior recombinants through selection and testing. This comprise of the selection environment (e.g., promising against biotic and abiotic stresses), selection time (e.g., early against late generation), and the number of years and locations of testing.
- Releasing, distribution, and utilization of new cultivars [81–83].

This breeding method can take over five generations leading to increase in the number of years to develop an elite variety of a particular plant. Backcrossing is the breeding method, which involves transfer of alleles at one or more loci from a donor to an adapted variety or a desirable line [83, 84]. Recurrent backcrossing is the traditional backcrossing program based on the assumption proposed by [85] that the quantity of the recurrent parent genome is recovered at a rate of $[1–(1/2)^t + 1]$ where t is the number of generations of backcrossing. Thus, the expected recovery of the recurrent parent genome after six generations of backcrossing would be 99.2%, a situation called near-isogenic. An imperative objective of recurrent backcrossing is to reduce the effect of the donor genome, as the aim is to move just a few of its genes responsible for the target trait into the recurrent parent's genetic background. It is generally used to improve qualitatively inherited traits such as pests and diseases resistance, since the existence of target trait genes must be confirmed by individual phenotype in the successive cross-generations. Thus, individual phenotypic performance is a key indicator of the genotype, provided genes have a major effect on phenotypic performance and the phenotypic uncertainty is insignificant [86]. However, due to linkage between a target gene and nearby genes (which could code for economically undesirable traits) from the donor parent [87] and/or chance (stochastic or nonrandom positions of chiasmata), any specific backcross progeny will digress from this expectation. This digression has been experienced in couple of plants, for instance, where one tomato cultivar developed after 11 backcrosses still had the complete chromosome arm carrying the gene from the donor parent and introgressed fragments as large as 4 centimorgan (cM) found in tomato cultivars developed after 20 backcrosses, [88]. This was also found in a study conducted by [89] where the fragments around the introgressed genes in barleys diverse from about 1–14 cM in seven (7) generation backcrossed lines. Consequently, two main limitations of recurrent backcrossing approach have been identified:

- The number of generations, thus time, necessary to achieve the introgression objective
- The simultaneous transfer of other genes flanking the gene of interest of the donor parent i.e., linkage drag [90].

For the past three decades, an optimal number of molecular markers have been identified to be linked to traits of agronomic importance. These markers have been used as gene benchmarks to facilitate the introgression of genes of economic importance into elite varieties [91, 92]. Molecular markers are being used intensively to increase the efficiency of backcross breeding programs. This is what is termed as marker-assisted backcrossing (MAB) (also known as marker-assisted introgression, marker-assisted selection or molecular breeding). In the context of recurrent backcrossing, MAB amplified the pertinence of recurrent backcrossing at least in the following facets. Firstly, for traits that are simply inherited, but challenging or costly to identify phenotypically, and/or that do not have a reliable phenotypic expression under certain specific selection conditions, the efficiency of phenotypic selection is low. The use of markers for foreground selection makes the transfer of target genes feasible and economic. Secondly, quantitative traits, which are generally not targeted by a recurrent backcrossing approach, can be improved using recurrent backcrossing, if major quantitative trait loci (QTL) affecting the trait have been identified. Thirdly, markers provide an effective option to control linkage drag and to speed up the recovery of recurrent genome and make the use of genes contained in unadapted resources easier [93, 94]. Lastly, the number of backcross generations and the time required to eliminate unwanted fragments of donor parent genome to reach high level of similarity to the recurrent parent are lessened.

MAB is an accurate and an efficient process of introgression of major gene controlling a desired trait while retaining the vital features of the recurrent parent [95, 96]. MAB is the process of selecting an individual plant as the parent in a subsequent generation of a genetic improvement program using the results of DNA tests. Molecular markers used to perform DNA test are not influenced by the environment; hence, problems associated with conventional plant breeding (i.e., selection based on phenotype) are eliminated. Here, selection is concentrated on genes that control the desired traits directly and are detectable at all stages of plant growth. With the availability of an array of molecular markers [97] and genetic maps, MAB has become possible both for traits governed by single gene and quantitative trait loci (QTLs) [98]. The philosophy in marker development and implementation can be divided into three broad categories: genetic mapping [99], analyses of links between molecular markers and the trait of interest, and MAB [85, 94, 100].

Gene mapping is the method used to locate the locus of a gene and the distances between genes [101].

The closer a target gene is to another gene, the more likely they are inherited together [94, 100]. Therefore, the preferred condition for MAB is when a direct markers or gene assisted selection is used. This is a situation where molecular markers cosegregate or are closely linked with the desired trait [102]. The effective development of a marker that can be linked to a gene of interest leads to success of MAB. Hence, the assumption that the ideal distance between a molecular marker and a desirable gene initially isolated from wild germplasm be as close as 2 cM, while that of a marker and a target gene from elite into elite lines be close as 12 cM. This

reduces the required size of the backcross population and the time taken to obtain the desirable results [103, 104]. MAB has been effectively used to introgress disease-resistance gene and improve fruit quality in tomatoes [27].

The challenge associated with the utilization of MAB in the developing countries like Africa is the initial cost of developing the markers and the requisite laboratory equipment. For it to be welcomed and used effectively in these regions, the economic returns on their usage must far exceed the cost of using the conventional backcrossing. The initial cost could be funded with aids from donors [105].

10. Conclusion

Tomato breeding evolved from conventional breeding where breeders directly selected for the traits of interest, to the use of morphological and physiological traits, differentiated domesticated crops from their wild ancestors. The limitations of these morphological markers gave rise to more efficient approaches with the emergence of genetic marker technologies since the turn of the nineteenth century. The discovery of DNA markers that are closely associated with the desired phenotypes has been used to track tissue, cell, chromosome or a gene in individuals and increased selection efficiency. A DNA marker is a fragment of DNA that contains large amounts of sequence information and closely linked to traits of importance. The close association of DNA markers with morphological and physiological traits has facilitated the development of several linkage maps and enhanced the selection efficiency in marker-assisted tomato breeding programs. Marker-assisted selection has been explored to increase precision and efficiency of selection for many economic traits in tomato breeding. One classical marker-assisted approach in tomato breeding is marker-assisted backcrossing, which targets either the genetic background of the recurrent parent (background selection) or tracking the gene of interest (foreground selection) through the use of flanking markers. Marker-assisted backcrossing enables faster recovery of the recurrent parent genome compared with conventional backcrossing approaches. Due to the long duration of recurrent backcrossing approaches, adoption of marker-assisted backcrossing approaches will enhance selection efficiency and shorten the breeding process. The potential genetic and economic benefits of marker-assisted backcrossing need to be compared with conventional breeding programs to determine their viability.

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