APPLICATION OF MOLECULAR TOOLS IN BREEDING CUCUMBER (*Cucumis sativus* L.): A REVIEW

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ABSTRACT
Cucumber (*Cucumis sativus* L.) is a member of the economically important family cucurbitaceae and most likely originated in India (South foot of the Himalayas) and is widely distributed throughout the world. Conventional breeding techniques have been used to improve cucumber yield, quality and resistance against biotic and abiotic stresses but this technique is non-invasive and restrictive in that only sexually compatible plants can be improved. However, recombinant Deoxyribonucleic acid (rDNA) technology, phenotypic and DNA-based markers allow the transfer of genes from one organism to another by overcoming the sexual process. In cucumber breeding, interest is in knowing the association (linkage) of markers to genes controlling the traits to be manipulated. These molecular techniques are providing researchers with the genomic resources and approaches to overcome most of the challenges associated with the use of conventional breeding tools. The development of genetic linkage maps has provided tools for the molecular analysis of important characteristics in cucumber including fruit quality, disease resistance, and yield components.
Keywords: Cucumber (*Cucumis sativus* L.), recombinant DNA, Molecular breeding

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable crop in the cucurbitaceae family that has been cultivated in Northern India and is widely distributed throughout the world (Whitaker and Davis, 1962). With respect to economic importance, it ranks fourth after tomatoes, cabbage and onion in Asia (Eifediyi and Remison, 2011) and second after tomatoes in Western Europe (Thompson and Kelly, 1959). In spite of being native of India sub-continent and endowed with enormous variability and genetic divergence, cucumber remains under-utilized given its economic potential and unexploited from breeding point of view.

However, the production of the fruit in Nigeria is very low due to its limited use. They are produced mainly in the Northern states, south –south and little in the south-east (Ohaji-Egbema) of Nigeria, (Adetula and Denton, 2003). The need to increase the production of cucumber arose due to its numerous importance to man. Cucumber is a good source of vitamins A, C, K, B_6, potassium, pantothenic acids, magnesium, phosphorus, copper and manganese (Thompson and Kelly, 1959). In India, the fruits are used in the preparation of chutney and curries. It is good for diabetic patients as it contains low sugar and helps in the burning of excess fat in the body. Cucumbers are used in skin tonics and other beauty aids; acts as an anti-inflammatory, anti-cancer, relieves arthritis, control insect and pest, its juice are often recommended as sources of silicon to improve the health and complexion of the skin (Duke, 1997).

Inspite of the increasing relevance of cucumber, the production of the crop in Nigeria is mostly by means of the conventional plant breeding tools. Hybridization is often conducted routinely without any problem when individuals from the same species are involved, provided there are no fertility-regulating mechanisms operating. Even when such mechanisms exist, hybridization can be successfully conducted by providing appropriate pollen sources. Sometimes, plant breeders are compelled to introduce desired genes from distant relatives or other species. However, crossing plants from two different species or sometimes even plants from different genuses had been a major challenge of the conventional tools in cucumber breeding.

Conventional plant breeding methods

Conventional plant breeding tools are the first and second generation technologies for plant genetic manipulation. The first generation technology was non-invasive requiring artificial crossing to accomplish gene combinations to create new genotypes while the second generation technology of tissue culture, mutagenesis, and chromosome manipulations allowed more intrusive plant genetic manipulations, targeting cells or the genetic material relatively more directly (Acquaah, 2003). The primary challenge of the conventional plant breeding (first generation technology) method is that the first generation technology requiring artificial crossing of cucumber is restricted in that only sexually compatible cucumbers can be improved this way. Trying to circumvent this challenge, second generation technology was employed such as somatic hybridization and protoplast fusion to overcome such

crossing barriers have been largely unsuccessful. Successful hybridization between *C. hystrix* Chakr (2n = 2x = 24) is a notable exception (Chen *et al*., 1997). In the genus *Cucumis*, the "African horned cucumber" (*C. metuleferus* E. Meyer ex Naudin), west Indian geherkin (*C. anguria* L.) melon and cucumber are the only species commonly cultivated for their fruit (Morton 1987; Baird and Thieret 1988). Although there are 32 species in *Cucumis*, cucumber is genetically isolated within the genus since it is not readily cross compatible with any other species (Kirbride, 1993). Chromosome number (x = 7) is a major crossing impediment since cucumber deviates from other *Cucumis* species which posses x = 12 (or its multiples) haploid chromosomes x = 12, (Lower and Edwards,1986). Although cucumber is cross-compatible with a feral, sympatric, botanical variety of the same species (*C-sativus var. hardwickii* (R.) *Allef.* (x = 7, hereafter referred to as (*C.s. var. hardwickii*), cross compatibilities between cucumber and x = 12 *Cucumis* species are extremely rare. The reproductive isolation of cucumber within *Cucumis* has made it difficult to broaden the narrow genetic base of cucumber. Cucumber genetic diversity has been assessed by several types of genetic markers thereby bringing about novel ways of solving conventional breading problems.

Molecular breeding technique

Molecular breeding is used to describe the use of a variety of tools for manipulating the DNA of plants which may or may not involve rDNA to improve them for specific purposes. Organism developed by the rDNA procedure is called a genetically modified organism (GMO). Steps common to rDNA:

The DNA of interest that is to be transferred (the transgene) is extracted from the source organism, the specific DNA sequence of interest is cut out using special enzymes, insertion of the transgene into a special DNA molecule (a cloning vector) to produce a new rDNA molecule, the rDNA is transferred into and maintained in a host cell (bacterium) by the process of transformation, the vector replicates, producing identical copies (clones) of the insert DNA, the host cells with the cloned transgene are identified and isolated from untransformed cells. The cloned transgene can be manipulated such that the protein product it encodes is expressed by a host cell.

The recombinant DNA (rDNA) technology allows the transfer of genes from one organism to any other, for instance, a desirable gene from a watermelon(gene that controls taste or colour) can be transferred into a cucumber genome thereby overcoming the sexual process of the conventional plant breeding method.

Molecular markers

Genetic markers are landmarks on chromosomes that serve as reference points to the location of other genes of interest when a genetic map is constructed (Acquaah, 2003). In cucumber breeding, interest is on knowing the association (linkage) of markers to genes controlling the desirable traits. The logic of markers is that an easy-to-observe trait (markers) is tightly linked to a more difficult to observe and desirable trait, hence, selection for trait of interest by indirectly selecting for the marker (that is detected or observed), when a marker is observed or detected, it signals that the trait of interest is present (by association). Molecular markers have several uses in plant breeding programs, including genetic biodiversity assessment, analysis of germplasm collection, cultivar identity, genetic similarity estimation, fingerprinting, genetic map construction, gene tagging, genotyping of individuals, identification of closely linked DNA markers, speeding, up breeding programs by identifying the gene of interest to be transferred and marker-assisted selection. (Collard *et al*., 2005). However, the ideal markers for use in plant breeding programs are condominant (able to distinguish heterozygotes), easy to develop and use, robust (repeatable and tolerable to slight changes in detection), abundant amenable to high throughout systems and low cost (Staub *et al.*, 1996; Gupta *et al.*, 1999; Collard *et al*., 2005). Although morphological (visualized as a phenotype, such as flower colour) and biochemical markers (allelic variants of functional enzymes also referred to as isozymes) were historically valuable, their paucity and variability due to environmental conditions and developmental stages limit their effectiveness in plant genetics and breeding. The large majority of currently utilized markers are DNA-based because they are relatively abundant, not influenced by the environment, and do not effect phenotype (Staub *et al.*, 1996; Gupta *et al*., 1999; Collard *et al.*, 2005).

Molecular Marker-assisted the cucumber breeding includes; Isozymes, Restriction fragment length polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP), Simple Sequence Repeats (SSR), Single Nucleotide Polymosphisms, (SNP), Sequence Amplified Characterized Region (SCAR). The application of the genetic markers of MAS follows three major recurring cycles regardless of marker type. Markers are identified as potentially useful, and subsequently developed into efficient and effective genotyping systems. These markers are then placed on a genetic map and associated with QTL through progeny analysis for their subsequent use in MAS.

Marker development in cucumber has occurred in several marker systems (Isozymes, Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), amplified Fragment Length Polymorphisms (AFLP), Simple

Sequence Repeat (SSR), and Single Nucleotide Polymorphisms (SNP) where changes between marker systems were driven by the steady progression of technological advances. In each case, the goal was the development of moderately saturated maps (i.e 150-200 markers to provide 90-95% coverage period. Between 1984 and 1992 work in the U.S. progressed on the development of Isozymes and RLFP markers leading to the construction of unsaturated maps (Knerr and Staub, 1992; Meglic and Staub, 1996; Kennard *et al.*, 1994).

The use of these codominant markers was assessed, and their development was terminated because of their utilization costs and the paucity of polymorphic markers when RAPD technologies were introduced (1992-2000). Although dominant in nature, RAPDs and subsequent AFLPs markers were attractive because of their comparatively low technological costs and methodological simplicity (RAPD) and their potential to produce multiple polymorphic markers from single assay. (RAPDs and AFLPs), in the case of RAPD technology, putative polymorphism declaration (i.e., number of bands) was relatively high (10-15 bands per primes) but reproducibility and fit to 3:1 genetic ratios for many putative marker loci was also low (recovery rate= ~ 50 of 1,000 markers evaluated; Staub and Bacher, 1996). This level of recovery is typical of many other marker systems in cucumber.

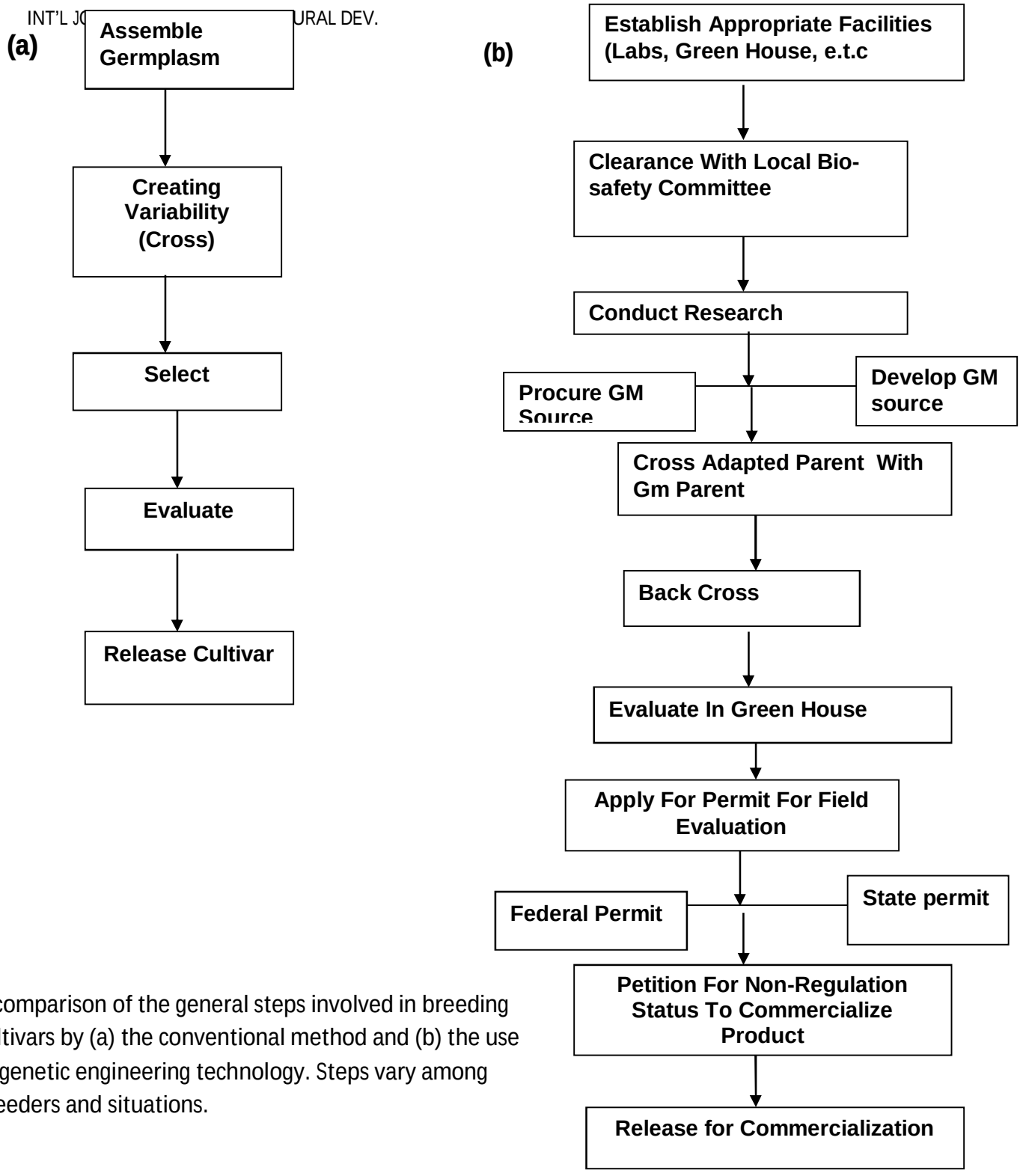
Dominant markers (RAPD and AFLP) were useful initially in the development of moderately saturated maps (Serquen and Bacher, 1976) but are not preferred in breeding programs.

The mapped RAPD loci were, nevertheless, strategically important during early map construction (Serquen and Bacher, 1976), and were therefore subjected to conversion to more preferable Sequence Amplified Characterized Region (SCAR) markers by silver staining- mediated sequencing (Horejsi *and* Staub, 1999). Although 62 (83%) of the 75 RAPDs were successfully cloned, only 48(64%) RAPD markers were successfully converted to SCARs markers and 11 (15%) of these reproduced the polymorphism observed with the original RAPD markers. The emergence of automated sequencing technologies made possible the development of codominant SSR and SNP technologies ,and the reassessment of RAPD to SCAR as well as SCAR to SNP marker conversion (Robbinson, 2006).

Two sources of sequence data (SCAR marker fragments and BAC library (Nam *and Lee*, 2006) clones) were employed to convert RAPD to SCAR and SNP markers for increased efficiency (multiplexing) and effectiveness (stable and codominant markers). A total of 39 new markers (SCAR and SNP) have recently been developed, seven of which have proven effective when multiplexed in MAS. The multiplexing potential of the remaining markers and those recently created from EST libraries (unpublished) has yet to be determined.

Markers based on single nucleotide polymorphisms (SNP) are gaining popularity and are the current marker of choice for cucumber and several species of crop plants (Gupta *et al*., 2001). This popularity is based on the idea that as more genomic resources are being made available SNPs are best able to fit the ideal marker for use in plant breeding. SNPs are usually codominant and robust markers. The number of SNPs in any given genome is much higher than any other marker type (estimated at 1in100 to 1 in 1000 base pairs) Gupta *et al*., 2001.

The first genetic linkage maps in cucumber were reported almost 20 years ago and were based solely on phenotypic markers (Fanourakis and Simon, 1987). The first molecular markers mapped were isozymes (Knerr and Staub, 1992), which were subsequently combined with phenotypic markers (Meglic and Staub, 1996), however, as DNA-based molecular markers were developed (RFLP and RAPD), they were combined with existing marker types in linkage maps (Kennard *et al*., 1994). The development of genetic linkage maps has provided tools for the molecular analysis of important characteristics in cucumber including fruit quality (Wenzel and Kennard, 1995), disease resistance, and yield components. General steps involved in breeding cultivars by the conventional method and the use of genetic engineering technology.



A comparison of the general steps involved in breeding cultivars by (a) the conventional method and (b) the use of genetic engineering technology. Steps vary among breeders and situations.

Source: Principles of Plant Genetics and Breeding(George, A.2007)

CONCLUSION

The adoption of a maker-assisted selection (MAS) or marker-aided selection in a breeding program hinges on the availability of useful molecular markers. Fortunately, this resource is becoming increasingly available to many species, curtsey of the advances in biotechnology. This breeding approach is applicable to improving both simple and complex traits, as a means of evaluation of a trait that is difficult or expensive to evaluate by conventional methods by identifying a marker that co-segregates with a major gene of the target trait. MAS is more beneficial to breeding quantitative traits with low heritability, while genetically, the rDNA of cucumber can easily be manipulated using molecular tools thereby transferring desirable traits from one organism to another for specific purposes.

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