Commentary Article

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Some progressive steps in coconut research and development in Sri Lanka through utilization of molecular markers

Lalith Perera

Coconut Research Institute, Lunuwila, Sri Lanka

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Abstract

Coconut is the sole species of genus *Cocos* and as such breeding of coconut is limited to the intra-specific level. Furthermore, the long life cycle, massive stature, highly heterozygous nature, lack of vegetative propagation method, limited number of seeds produced per year and bulkiness of seeds, limit the use of many traditional breeding methods employed in other crops in coconut breeding. Obtaining pure line from heterozygous coconut remained unrealistic because of the long vegetative phase. Thus coconut breeding is confined to mass selection of phenotypically superior parent palms, and to inter and intra-varietal hybridization. All these constraints in coconut breeding make the use of molecular breeding techniques highly attractive. Although identifying molecular markers linked to useful traits and characters to strengthen and fasten the selection and breeding of coconut looks the main goal of using markers, they can be efficiently used for many other applications. This article deals with some other useful applications of molecular markers practically used in the coconut breeding programme in Sri Lanka.

Keywords: Breeding, coconut, germplasm, molecular markers

Introduction

Although conventional coconut breeding using standard techniques based on phenotypic selection have been relatively successful, the inherent constraints such as long life span, highly heterogeneous nature and cross pollination behavior make the potential of new biotechnologies in coconut breeding highly attractive. Application of molecular genetics in coconut breeding, particularly the molecular markers began in the early 1990s and their application in coconut have been diverse, ranging from assessing genetic diversity to creating high-resolution genetic maps and identification of pathogens. Initially, the studies were aimed at assessment of coconut genetic diversity and genetic relatedness at the DNA level, using universal marker techniques such as RAPD (Everard, 1996), RFLP (Lebrun *et al*., 1999, 1998), AFLP (Perera *et al*., 1998) and ISTR (Duran *et al*., 1997; Rohde *et al*., 2000). Later, the need for coconut specific markers was felt and accordingly in 1999, two sets of microsatellite markers were isolated by two groups of scientists using cultivar Sri Lanka Tall (Perera, 1999; Perera *et al*., 1999) and Tagnanan Tall (Rivera *et al*., 1999). Microsatellites, as co-dominant markers, have been particularly useful in analyzing highly heterozygous coconut for genetic diversity and genetic relatedness estimates, germplasm characterization and development of co-collections (Perera *et al*., 2000, 2001, 2003), hybridity testing (Perera, 2010) and detecting somaclonal variation in tissue cultured coconut plants (Perera *et al*., 2008) and for construction of genetic linkage maps (Herran *et al*., 2000; Lebrun *et al*., 2001; Baudouin *et al*., 2006) leading to identify marker-traits linkages for yield, fruit characters, pest and disease resistance. A microsatellite kit comprising 14 primers and an associated software for data analysis has also been developed (Baudouin and Lebrun, 2002) standardizing the techniques across

**Corresponding Author: kanthaperera@yahoo.com*

laboratories for comparable results and for efficient detection of diversity and identification of varieties. More recently, DArT markers for coconut have been validated for diversity studies (Perera, 2005).

Selection of parents for breeding and germplasm collection strategies

A high level of genetic diversity in coconut has been observed by Perera *et al.* (2000, 2002, 2003) using microsatellites markers in a collection of 130 coconut individuals representing 51 tall coconut varieties and 49 dwarf coconut varieties sampled across the entire geographic range in the world. The mean genetic diversity values (Nei, 1987) observed by Perera (1999) was 0.647. He has also observed a loss of allelic richness or reduction in the number of alleles detected in dwarf coconut group compared to tall coconut group, with a reduction in amount of genetic diversity in dwarfs. Perera *et al.* (2000) also reported that heterozygous loci were evident not only in cross pollinating talls, but also in dwarfs, but at lower frequency (2.5%) compared to talls (30%). The distribution of genetic diversity between varieties within tall groups was observed to be higher than that of dwarf group. This finding has changed the germplasm collection strategies for dwarf and tall groups in Sri Lanka by focusing on more number of palms per collection for talls and lesser number of palms per collection for dwarfs and king coconuts. A genetic diversity study conducted for coconuts in Sri Lanka (Perera, 1999) led to the finding that the genetic base of Sri Lanka coconut is narrow. This has resulted in the change of the breeding strategies of the Sri Lanka coconut breeding programme and led to the importation of exotic coconut varieties and incorporation of them in the country's breeding programme. Similarly these studies identified redundancies in the germplasm collection. Perera *et al*. (2001) who analyzed 33 coconut populations belonging to Sri Lanka tall variety using SSR markers across the island representing different geographical regions concluded that there was no population differentiation (between population variation was only 5%) within Sri Lankan talls. This has led to the strategic change in the direction of germplasm collection in the ongoing germplasm collection programme by targeting only the phenotypically different and specific populations for certain characters only for further germplasm collection and *ex situ* conservation rather than collecting populations randomly.

Perera *et al*. (2003) have constructed a phenetic tree diagram showing the genetic relationships among 51 tall coconut varieties and 49 dwarf coconut varieties across the world. Instantly, this phenetic tree divided all tall coconuts into two main groups, the first group comprising all the tall varieties from Southeast Asia, the Pacific and the West coast of Panama and all dwarfs in a sub-cluster within the tall cluster. The second group consisted of talls from South Asia, East Africa and West Africa. Interestingly, none of the dwarf coconuts is grouped with the second main tall group. These results improved the knowledge of coconut breeders in Sri Lanka as well as breeders internationally in identification and utilization of parents for the coconut hybridization programme for maximizing the heterosis. This indicated that selection of parents from two genetically diverse groups, one from Southeast Asia and Pacific and one from South Asia and Africa will give increased heterosis rather than selecting and crossing parents within a genetically similar group. Further, it was realized that crossing dwarfs with tall materials from South Asia and Africa will give higher heterosis than crossing dwarfs with Southeast Asian and Pacific originated tall coconuts. These findings changed the breeding directions and guided the import of exotic coconut genetic materials to Sri Lanka from Southeast Asia and Pacific regions. The exotic crosses developed by pollen import are now being evaluated in the field and promising results have been shown while same finding lead to terminate one of the field trials which have been developed by crossing between populations within the country. Furthermore, genetic relationship studies in coconut described above suggests that maximum segregation of traits in coconut can only be obtained by crossing between varieties of Southeast Asia and the Pacific group and Indo-Atlantic group. Hence, this finding lead the coconut breeders involved in developing segregation population for coconut aiming at linkage maps, towards selecting parents from two divergent groups. The failure in the mapping population developed by coconut scientists from Europe prior to molecular marker information is a

good example for this, where mapping population composed of parents belonging to just one genetic group, the Southeast Asia and Pacific group (Malayan Yellow Dwarf x Laguna Tall) was used and as a result, about 84 per cent of molecular markers generated in the mapping population were non-polymorphic indicating that the two parents share identical alleles at a large number of loci.

Confirmation of legitimacy and purification of genetic materials

Perera *et al*. (2010) reported the successful use of microsatellite molecular markers to uniquely identify coconut varieties used as parents in the Sri Lankan coconut breeding programme and the resulting hybrids from them. This is very important in confirming the identity of varieties and their hybrids in very likely events of seed-nut lots mixing, mislabeling of seed-nut lots in coconut nurseries and to check the legitimacy of hybrid seedlings. Two molecular markers have exhibited the potential for distinguishing coconut varieties; Sri Lanka Tall, Sri Lanka Green Dwarf and Sri Lanka Yellow Dwarf used as parents in the breeding programme of Sri Lanka uniquely and thus for confirming the hybridity of two commercially growing coconut hybrids; the Sri Lanka Green Dwarf x Sri Lanka Tall and Sri Lanka Yellow Dwarf x Sri Lanka Tall. Similarly, Bandaranayake and Kearsey (2005) have developed markers specific to the San Ramon variety for identification of uncontaminated materials. These makers have been used in identification of pure San Ramon from a mixed and likely to be contaminated populations of San Ramon for the purpose of multiplication in planting in the seed gardens. These markers have also indicated that yellow petiole coloured hybrid seedlings, used as a phenotypic marker in culling illegitimates among hybrid seedling, is not 100 per cent accurate as some of the yellow dwarf seedlings are of the hybrid type. Further, this indicates the possibility of utilizing those hybrid seedlings also in the planting programme once the screening methodology become cheap. A very good example of utilizing molecular markers in variety identification is the confirmation of the dwarf identity and uniqueness of the recently identified Sri Lanka Brown Dwarf variety during the germplasm exploration programme from the rest of the dwarf varieties (Perera *et al*., 1993). Two high yielding hybrids resulted by utilizing this variety in the breeding programme were released for commercial planting in 2012. A genetic material purification program has also been launched to identify the true dwarf yellow palms in a mixed population as had been recognized by morphological variation of dwarf yellow palms used in the hybrid seed production programme at in the isolated seed garden, Ambakelle (Perera, 2012) aiming to reduce the genetic variation in the hybrids.

Application of microsatellite markers to study any possible somaclonal variation in limited number of clonal plants of coconut regenerated from various explants through somatic embryogenesis and successfully field planted in Sri Lanka have been reported by Fernando *et al*. (2004). The microsatellite markers have confirmed the absence of variants among plants within clones (Perera *et al*., 2008).

Genome map construction and linkage mapping

Studies on coconut genome mapping and marker assisted selection (MAS) were started comparatively recently. The first genome map for coconut was developed for an East African Tall x Laguna Tall F_1 population based on ISTR markers (Rohde *et al*., 2000). This work was extended with a mapping population developed in the Philippines from a cross between Malayan Yellow Dwarf x Laguna Tall using AFLP, ISTR, RAPD and ISSR markers. Three hundred and eighty two markers have been placed in the map resulting in 16 linkage groups and identifying six QTLs for early germination, early flowering and high yield in coconut (Herran *et al*., 2000). This was the first report of the opportunity for MAS in coconut. Further, QTL for other traits such as, leaf production, girth and height has also been identified for same mapping population. In addition to this, another mapping population in Ivory Coast derived between Cameroon Red Dwarf and Rennel Island Tall has been used to map 280 markers on 16 linkage groups resulting in identification of several QTLs related to nut number, number of bunches and traits related to fruit components (Lebrun *et al*., 2001; Baudouin *et al*., 2006). Generation of new mapping populations including phytoplasma-resistant

breeding material is being focused in Jamaica (unpublished).

The size of the mapping population and selection of parents for the maximum segregation of traits are critical when producing a genome map of any crop (Bandaranayake and Kearsey, 2005). The size of the mapping population is particularly a crucial issue in coconut because obtaining a reasonable number of seed nuts from a particular crossing combination is a difficult task as a result of very low seed production in coconut *i.e.*, about 100 seed nuts per palm per year. Bandaranayake and Kearsey (2005) concluded that between 200 to 400 individuals constitutes the effective size of the mapping population for coconut for a standard and consistent map resolution through a simulation study. Furthermore, genetic relationship studies in coconut suggests that maximum segregation of traits in coconut can only be obtained by crossing between varieties of Southeast Asia and the Pacific group and Indo-Atlantic group. Based on the experience and the information already generated, a large mapping population has now been developed in Sri Lanka comprising 350 individuals from a cross between 30 genetically identical Sri Lanka Red Dwarf (Southeast Asia and Pacific group) and a single Sri Lanka Tall (Indo-Atlantic group) to obtain maximum segregation of traits and sufficient number of meioses to analyze. The dwarf red population was previously analyzed with SSRs to identify the identical palms. However analysis of the molecular marker data indicated that a much better linkage map can be derived from a F_2 generation segregating mapping population derived from this F_1 population. Another mapping population, particularly for segregating for tolerance to *Aceria* mite, has been constructed in Sri Lanka between tolerant Sri Lanka Yellow Dwarf and a highly susceptible Sri Lanka Tall palm (Perera, 2005) and this has been planted in the Pallama Seed Garden.

Efforts are being undertaken to investigate the possible synteny of the oil palm and the coconut genomes as both palm species are diploid and have the same chromosome number, 2n = 32 (unpublished). Synteny would possibly speed up research by increasing the marker density on the respective linkage maps by the exchange of DNA markers. Development of a number of EST-SSRs from oil palm has been done and their cross-applicability is under investigation.

Screening parents for resistance breeding

PCR - based molecular markers are very useful and reliable tools for identification of pathogens over the identification of pathogens based on visual symptoms. A good example of the use of molecular diagnosis of pathogen is the identification of WCLWD phytoplasma in coconut palms in southern province (Perera *et al*., 2012). Its application in reliable screening of resistant parents for the resistant breeding programmes in the disease control/management programmes, where individual trees are tested for the presence of pathogen which can be detected even at the latent stage before using in the breeding. This application is being used in screening parents for the current WCLWD resistance programme in the southern province.

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