Chapter 11

Coconut

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1. Introduction

Coconut (*Cocos nucifera* L.) is a member of the monocot family *Arecaceae* (*Palmaceae*) and the subfamily *Cocoideae* that includes 27 genera and 600 species, and is currently the only species of the genus *Cocos*. Coconut possesses a diploid genome with 32 chromosomes (2n=2x=32). Many different varieties and forms of coconut have been described by different workers (Narayana and John, 1949; Gangolly *et al.*, 1957; Menon and Pandalai, 1958 and references there in; Liyanage, 1958) based on morphology, breeding habit and more recently according to their place of origin (Ohler, 1984). There is a great diversity in coconut, for example fruits vary for size, shape and colour and the proportions by weight of fruit components *viz.* husk, shell, endosperm and water (the liquid endosperm). There are also coconut variants having high value, such as: Makapuno, Kopyor and Aromatic coconuts (Maskromo *et al.*, 2015a; Maskromo *et al.*, 2013b, c; Maskromo *et al.*, 2011). Realizing the benefits of coconut as revealed by newest research findings, people are accepting coconut today as a wonder food commodity. With all the health benefits attributed to the coconut, there is a big demand opening in the world market for coconut today and therefore there is a need for increased production and supply of coconut. In some countries (*i.e.* the Philippines, Thailand and Indonesia), breeding for unique and specific characters, such as makapuno and kopyor type solid endosperms and aromatic liquid endosperm, which have higher value have also been initiated (Sudarsono *et al.*, 2015; Novarianto *et al.*, 2014; Sudarsono *et al.*, 2014). Thus collection and evaluation of coconut germplasm and breeding coconut for desirable characters has become a priority in many research programmes in many coconut growing countries.

2. Coconut Germplasm

Germplasm is a collection of genetic resources of the target crops. Availability of diverse coconut germplasm is the pre-requisite for coconut breeding because they are the genetic source used by coconut breeders to develop new cultivars. Coconut germplasm is mainly stored as a living collection of trees in the field (*ex situ*) which is a very resource intensive task. A programme for coconut germplasm collection and conservation has been in place in coconut centers around the world for many years, resulting in entries of 1621 coconut accessions, comprising of at least 1200 tall and 421 dwarf coconuts, deposited in the Coconut Genetic Resources Database (CGRD). Selection of those coconut accession/collections is mainly based on phenotype and ecological distribution. Hence this coconut collection would probably contain redundancies and genetically close accessions. Further, appropriately designed collection strategies have not been in place in coconut for the purpose of identification of exact number of locations and collection of exact number of individual per location to ensure capturing the highest diversity and loosing rare alleles at the lowest probability. For this, within and between population variation and population differentiation statistics have to be developed.

The selection of parents in the coconut breeding in many national and international coconut breeding activities has been arbitrary and based on mostly differences in phenotypes and place of origin. Application of molecular markers could help overcome the above weaknesses and strengthen the germplasm conservation and breeding programmes. Indonesian Palm Research Institute (IPRI), Manado in collaboration with Bogor Agricultural University have validated existence of redundancies and genetically close coconut accessions in the germplasm collections, using molecular markers (Sudarsono *et al.*, 2015, Kumaunang and Maskromo, 2007). Therefore, molecular analysis of germplasm collections is an important task.

3. Molecular Markers

Molecular markers have become an important tool in almost every crop plant to manage germplasm collection and conservation, generate diversity indices, track down origins and thereby accelerate breeding programmes. DNA-based marker techniques are considered more effective as opposed to traditional techniques using morphological and descriptive markers, which are laborious, subjective, time consuming and limited. Attempts to incorporate isozyme techniques in coconut breeding have been reported in the Philippines (Benoit, 1979; Carpio, 1982), India (Geethalakshmi *et al.*, 2004; Parthasarathy *et al.*, 2004), Indonesia (Novarianto. 1988; Novarianto *et al.*, 1988a,b; Novarianto *et al.*, 1993) and in Sri Lanka (Fernando, 1995). However, its use for characterizing coconut populations has been limited. Isozymes are relatively cheap, easy to use, independent of environment, and co-dominant markers; however, its use in coconut breeding is limited because the number of detected polimorphic loci are limited.

Currently, many DNA based marker techniques have been employed in various genetic studies to reveal variations at the DNA level. Out of these, the most popular and widely used DNA marker techniques are Restriction Fragment

Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP). Simple Sequence Repeats (SSRs) or microsatellites have also been developed to screen individuals. Microsatellites are tandem repeats of short sequence motifs occurring randomly in the eukaryotic genomes, in which the basic repeat unit is around 1- 6 base pairs. These simple repeats in the nucleotide sequences are mostly located in the centromere and are mostly represent non-coding regions of the genomic DNA. Recently, with the availability of sequencing data from transcriptome and genome sequencing projects, Single Nucleotide Polymorphism (SNP) have been developed and utilized to support coconut breeding programs (Mauro-Herrera *et al.*, 2006). All of molecular markers rely on the existence of polymorphism. Polymorphism is simply variation at the DNA base sequences or at the restriction sites (site where the restriction enzyme cleaves the DNA) due to natural occurrence of base substitution or insertion-deletion (InDel) mutations. Several combinations of PCR-based molecular techniques reveal the existence of high degree resolution of polymorphism (variation) and these form of variations could be used as screening methods to ascertain the identity of the individual plant and its relationship to the population.

4. Molecular Markers Availability and their Use in Coconut

Currently, there are many powerful molecular marker techniques and the choice of the markers depend upon several factors such as the information content of the marker, ease of performance, reproducibility, expense and availability of the experts and the facilities. A variety of molecular markers have been used in coconut but the most popular type of markers currently or exhaustively used in coconut include Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR) or microsatellites markers. With many DNA sequencing projects becoming reality for coconut, it would be possible to evaluate nucleotide diversity among gene sequences in coconut and identify the existence of single nucleotide polymorphisms (SNPs). Single nucleotide amplified polymorphism (SNAP) marker may be generated based on the presence of SNPs in the sequences of target gene (Maskromo *et al.*, 2013).

The first DNA marker system reported to be used in coconut was the RAPD (Everard, 1996; Ashburner *et al.*, 1997; Duran *et al.*, 1997; Wadt *et al.*, 1999;). The principle of RAPD analysis is amplification of random DNA segments with single short arbitrary primers resulting in the amplification of several discrete DNA products which can be separated on agarose gels by electrophoresis (Williams *et al.*, 1991; Welsh and McClelland, 1991). This method is fast and simple and requires no prior sequence knowledge or the use of radioactivity and requires only small amounts of DNA for the analysis (Babu *et al.*, 2014). However, the disadvantages of this method are that it is a dominant marker and most probably less reproducible between assays and laboratories (Rafalski *et al.*, 1996; Powell *et al.*, 1996a). However, RAPD markers have contributed to the wealth of information in coconut. Manimekalai and Nagarajan (2006) used RAPD markers to derive inter-relationship among coconut germplasm accessions and reported low genetic similarity among South Pacific and South East Asian coconut accessions. Rajesh *et al.* (2013) have

identified a RAPD marker capable of differentiating tall and dwarf coconut palms. Sequence characterized amplified region (SCAR) primers were designed from the unique RAPD amplicons and validated. RAPD markers have also been used to analyze intra-population diversity of Bali Tall, Mapanget Tall, Sawarna Tall and Tenga Tall in Indonesia (Novarianto *et al.*, 2001; Pandin, 2009a, b).

RFLP markers have also been employed in coconut (Lebrun *et al.*, 1999a; Lebrun *et al.*, 1998), though in a limited manner. RFLP (Beckmann and Soller, 1983) was one of the first techniques to be used widely to detect variation at the DNA level. Such markers are expected to be numerous, developmentally stable and co-dominant. The major drawbacks of RFLPs are that the process is very labour intensive, expensive and requires large amount of good quality DNA (Powell et al., 1996b). The use of AFLPs in coconut is also limited but a good coverage of genetic diversity and genetic relationships of coconut of Sri Lanka (Perera *et al.*,1996) and entire geographic range of coconut cultivating area in the world (Perera *et al.*, 1996; Teulat *et al.*, 2000) have been published.

In coconut, use of SSR markers provides wider coverage as its applications have been very diverse ranging from assessing genetic diversity in natural populations to creating high-resolution genetic maps (Perera *et al.*, 1999, 2000, 2001, 2003; Teulat *et al.*, 2000, Rivera *et al.*, 1999; Meerow *et al.*, 2003; Akuba, 2002). SSR markers have also been used to study pollen dispersal and determined the level of self and cross pollination among Dwarf and Tall Coconuts (Larekeng *et al.*, 2015a,b). Although SSRs are one of the most effective candidate marker system for population analysis (Karp, 1999), their development used to be expensive as isolation of DNA fragment carrying SSR sequences and DNA sequencing are needed for each and every plant. Some examples of cross amplification of SSRs between closely related species are however possible and an example of such study has also been published (White and Powell, 1997a). Despite the high cost involved in isolation and development of SSRs, substantial number of coconut SSR primers have been developed and published (Perera *et al.*, 1999, 2000; Rivera *et al.*, 1999; Teulat *et al.*, 2000). A set of 39 coconut specific microsatellite primer pairs have been developed by Perera *et al.* (1999) from a small insert genomic library enriched for CA repeats using genomic DNA from Sri Lankan Tall coconut variety, following the method described by White and Powell (1997b). Moreover, another set of 38 polymorphic microsatellite primer pairs have been isolated by Rivera *et al.* (1999) from a small insert genomic library enriched for several types of microsatellite repeats following the method described by Edwards *et al.* (1996), using genomic DNA from the Philippines coconut variety Tagnanan Tall (primer sequences are available in Teulat *et al.*, 2000).

Despite the differences in molecular marker system, laboratory where the study was conducted, number and type of accessions or varieties used among the investigations, the results obtained (Rohde *et al.*, 1995; Lebrun *et al.*, 1998; Perera *et al.*, 2003; Teulat *et al.*, 2000) generally revealed distribution of genetic diversity of coconut and genetic relatedness in coconut accessions. A high level of genetic diversity of tall coconut was observed in all studies compared to dwarf with dwarfs showing great loss of allelic richness. The distribution of genetic diversity between accessions in talls was also found to be higher than that of dwarf group. These finding

have led to proposal of changes to the germplasm collection strategies for dwarf and tall groups (Perera, 1999; Ashburner *et al.*, 1997). The phenetic tree (Perera *et al.*, 2003; Teulat *et al.*, 2000) constructed in these studies have revealed the genetic relationships of coconut in the world: all tall coconuts were divided 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 grouped with the second main tall group. These results were very much in agreement with the conclusions of Harries (1978) on the evolution and dissemination of coconuts based on morphological, phenotypical and ethno botanical evidences. According to Harries (1978) naturally evolved coconuts; characterized as 'Niu Kafa' type, predominate in South Asia, West and East Africa, the Caribbean and the Atlantic coast of Central America while coconuts selected under cultivation; characterized as 'Niu Vai' type, predominate in Southeast Asia, some Pacific islands and the West coast of Central America. It is generally accepted that the coconut palm came into existence on the Atlantic coast of Africa, South America and around the Caribbean region for only about 500 years (Child, 1974; Purseglove, 1972) and that there is a great similarity between these coconuts and those coconuts in East Africa, India and Sri Lanka (Harries, 1978). The grouping of Panama Tall (Panama Manarge and Panama Aguadulce varieties; both from the Pacific coast of Panama) with Southeast Asian and Pacific Talls is in agreement with Whitehead's (1976) observation of an eastward movement of coconuts from Southeast Asia to the Pacific region and subsequently from there to the Pacific region to the Pacific coast of America. These results are largely in agreement with the results from ISTR (Inverse Sequence-Tagged Repeats) analysis (Rohde *et al.*, 1995), which grouped Panama Tall with Polynesian varieties/populations of coconuts.

The grouping of all dwarf forms from different geographical regions in a single cluster within the main South Asia and Pacific group and the 'Niu Vai' type of coconuts and loss of allelic richness observed in dwarfs suggest that all dwarfs have a common origin and evolved from the Southeast Asia/Pacific group of talls. 'Niu Vai' type of coconuts in the Southeast Asia/Pacific region, were domesticated there and only later introduced to the other regions. The results of Teulat *et al.* (2000) strongly support a common origin of dwarf varieties. Manimekalai and Nagarajan (2007) used SSR markers to derive genetic relationship among coconut accession belonging to different geographic regions. Dwarf and intermediate accessions showed highest similarity among them. The tall accessions belonging to South East Asia, South Asia and South Pacific were clustered based on their geographical regions, but dwarf and intermediate accessions were clustered separately. Clustering of accessions belonging to Atlantic and America revealed the spread of coconut from Far East to South Pacific.

SSR markers have also been utilized for ensuring the legitimacy of coconut hybrids, which overcomes unreliable morphological traits, like petiole colour, which were utilized earlier as a marker for hybrid identification (Perera, 2010; Rajesh *et al.*, 2012). This technique paves the way for ensuring authenticity and quality of hybrid

seedlings of coconut before distribution to the farming community. Furthermore Sudarsono *et al.* (2015) have recently reported an innovative approach of identifying better parental combination for producing better coconut hybrids based on SSR markers. Use of SSR markers to evaluate pollen dispersal in Dwarf and Tall coconut has also been demonstrated (Larekeng *et al.*, 2015a,b). Parentage analysis has been used to evaluate pollen dispersal among Dwarf and Tall Kopyor coconut in two different kopyor coconut production centers in Indonesia. Investigations have been undertaken to elucidate the dispersal of pollen, the rate of self and out-crossing and the distance of pollen travel in both Pati Dwarf and Kalianda Tall Kopyor coconut population using six SSRs and four SNAPs.

A recent trend in identification and development of cost-effective SSRs has been mining publicly available ESTs (expressed sequence tags). Even though the extent of polymorphism revealed by EST-SSRs is low compared to genomic SSRs, they possess many advantages which include easy access, a high level of transferability to related species and probably represent functional genetic variation. Within the species studies conducted at the global level allowed identifying two large genetic groups, known as "Pacific" and "Indo-Atlantic" which can be seen as sub-species. The role of humans in the dissemination of coconut was highlighted by Gunn *et al.* (2011).

Recently Xiao *et al.* (2013) have scanned 57,304 coconut unigenes for di-, tri-, tetra-and hexa-nucleotide repeat sequences and an additional 30 microsatellites have been developed. These markers have been utilized to evaluate coconut genetic diversity in 30 individuals representing accessions from China (12 samples) and Southeast Asia (18 samples). Based on the results, it was inferred that Chinese coconuts did not evolve independently of the Southeast Asian populations. Combining population structural analyses and historic information, they have proposed a possible explanation for coconut dispersal patterns from Southeast Asia to China: sea currents could have carried coconuts into the Hainan province, while human dispersal from Southeast Asia may have brought coconuts to the Yunnan province.

With the recent advances in genomic research, there has been a shift towards utilization of gene-targeted, functional markers in lieu of random DNA markers. One such novel marker system is the start codon targeted polymorphism (SCoT), which was utilized for assessment of genetic relationship and diversity among 23 coconut accessions (10 talls and 13 dwarfs), representing different geographical regions (Rajesh *et al.*, 2015).

Although the general trends in placing coconut accessions in sub-clusters within the phenetic trees are generally consistent and are in accordance with their origin and geographical locations, consistency in placing individual accessions relative to each other within them is questionable. Different phenetic trees based not only on different molecular marker systems, but also on the same molecular marker systems has shown that placing the different individual accessions relative to the other accessions is different. Different marker systems are being applied by different research groups; most often both RAPD and SSR as the major tools. Only very few studies have been carried out in the world to compare the results

obtained from different marker systems to see the consistency of the results and these represents recent studies on barley (Russell *et al.*, 1997) and cultivated potato (Milbourne *et al.*, 1997). Inconsistent results with different markers systems demonstrated on barley (Russell *et al.*, 1997), soybean (Powell *et al.*, 1996a), and *Brassica* (Thormann *et al.*, 1994), have led to an alarming situation that each PCR-based marker system needs careful evaluation before being applied with a new crop. To date, large number of coconut collections have been made and planted *ex situ* and further collecting of accessions are in progress in view of conserving more diversity. However, lack of adequate knowledge on the amount and distribution of genetic variation in coconut have hindered the implementation of appropriate conservation strategies (*i.e.* number of populations, number of individuals per population *etc.*). Molecular marker based characterization provide efficient method to quantify the genetic variation and to estimate genetic relationships between populations. Selection of molecular marker/s for assessing plant gene pool and germplasm analysis however has to be made with great care as studies on barley; soybean and *Brassica* have demonstrated inconsistent results with different marker assays. Therefore, comparison of outcome of different molecular marker systems is necessary to determine optimum number of markers and number of individuals per accessions with a particular marker system, for effectively determining the correct and consistent genetic relationships (genetic distances and similarities) among the accessions. This procedue will, when applied with different markers system, determine the cross- comparison of the results between systems and guide scientists in different regions or countries with the specifications (which marker to be used with which number of individuals per accession). The results of this kind of a study will help to develop a suitable molecular marker approach for coconut and for molecular characterization. In view of these objectives, highly diverse coconut varieties (*viz.* talls, dwarf greens, San Ramon and King Coconut), each variety represented by 20 individuals or more, should be studied with 20 or more numbers of markers with RAPD primers being the choice for less resource countries and SSR primers the choice of resource and skillful countries. Similarity matrices should be constructed from data for each marker systems with differing number of primer pairs and differing number of individuals per population. Dendrograms would be generated from these matrices to see the genetic relationships to check any changes in the relationships with respect to changing the number of markers and the number of individuals. The amount of genetic diversity should also be studied with the changing number of primers and the number of individuals. This whole procedure should enable estimation of the appropriate number of markers and the number of individuals per population for a proper DNA assay for each marker system. Same similarity matrices should also be used to study the improvement in correlation between the results obtained from different system with changing number of markers and numbers of individuals thus an appropriate package can be designed with each marker system. Based on the allele frequencies of the sample, exact number of individuals that should be collected from a population to capture the maximum genetic diversity would be estimated for future conservation. The effectiveness of each system should be determined in terms of number of loci revealed by each marker system and the amount of polymorphism detected. At the

same time, this approach should also study their outcome with phenotypic diversity of coconut. Once an appropriate marker package is developed, approximately 100 coconut populations from the coconut biodiversity conservation programme should be evaluated and characterized in order to develop core collections, eliminate duplicates and identify priority germplasm for breeding.

In view of an appropriate package of a DNA assay for coconut, a microsatellite kit has been developed to identify coconut cultivars (Baudouin and Lebrun, 2002). Its construction involved as stated by Baudouin and Lebrun (2002); several steps such as gathering more than 600 DNA samples belonging to 113 reference populations in order to represent accurately the coconut genetic diversity worldwide, producing a set of 80 polymorphic microsatellite markers, screening them on a reduced set of coconut genotypes, testing the remaining 14 markers on the 600 coconut genotypes, devising protocols, adapted to the use in producing countries and devising suitable statistical methods, in order to identify the reference population, which is the most likely source of a given DNA sample. However this microsatellite kit is unable to distinguish between some coconut cultivars (personal observation).

Further to the considerations above, how the markers are distributed within the coconut genome and whether the markers are genomic or functional (ESTs) are important considerations as depending on those factors the results seems to vary. Thus a framework map of coconut and an improved coconut marker system is necessary. Studies on genome mapping in coconut are still limited, preliminary and recent. The first genome map for coconut (Rohde *et al.*, 1999) was developed based on ISTR markers. This was further extended with a different mapping population using AFLP, ISTR, RAPD and ISSR markers. Three hundred and eighty two makers have been placed in the map resulting in 16 linkage groups and identifying six QTLs for early germination (Herran *et al.*, 2000). Further, QTL for other traits such as, leaf production, girth and height has also been identified for the same mapping population (Ritter *et al.*, 2000). In addition to this, another mapping population in Ivory Coast resulted in 280 [markers] mapped on 16 linkage groups and QTLs related to nut number, bunch number and fruit component related traits (Lebrun *et al.*, 2001, Baudouin *et al.*, 2006) identified. Bandaranayake and Kearsey (2005) stated in their publication that the size of the mapping population is critical in any genome map. Bandaranayake *et al.* (2005) concluded through a simulation study that between 200 to 400 individuals is the effective size of a mapping population for coconut for a steady map resolution. The coconut linkage maps described in Herran *et al.* (2000) and Lebrun *et al.* (2001) were based on less than 65 individuals. Based on the experience and the information generated, a mapping population comprising more than 250 individuals has been developed in Sri Lanka but genotyping results indicated that only 16 out of 300 microsatellite markers are polymorphic between the parents thus making the F_1 not sufficiently polymorphic to be used for construction of a genetic map. Use of new mapping populations is being focused in Jamaica (unpublished) and in CIRAD, France (Luc Baudouin, unpublished data). A mapping population with 120 individuals has also been generated using F_2 intercross derived from Nias Yellow Dwarf x Kopyor Tall Homozygous coconut to find SSR and SNAP marker loci linked to kopyor mutant

character in Indonesia. The mapping populations have been genotyped using 51 SSR and 17 SNAP functional marker loci and they were analyzed using linkage analysis software. Preliminary results of the analysis indicated that two SSR loci (CnCIR226 and CnCIR_J2) and two SNAP loci (SUS#3 and WRKY2#1) are probably linked to kopyor character in coconut (Maskromo, 2015). Once a proper framework map of coconut using all the available markers is developed, the choice of markers for the marker package should be developed based on their distribution in the genome. This should include functional markers such as EST-SSRs and SNAP markers too. Results generated utilizing either genomic markers or only with functional markers may differ depending on the genetic variation in the target areas of the genome.

Considering long-term physiological adaptation of the coconut palms (Peries, 1993), a collection and conservation of coconut representing different eco-geographical regions in Sri Lanka was initiated in 1991 (Perera *et al.*, 1996). A molecular marker study in Sri Lanka (Perera *et al.*, 2001) on 33 such tall coconut accessions using SSR markers revealed that there was no genetic differentiation among these accessions as the estimated variation between population accounted only less than 1 per cent. This has led to a change in the strategic plan of coconut germplasm collection in Sri Lanka; further random collection of coconut accessions discontinued and only biased collection is being carried out based on phenotypic characters. Among 33 accessions of coconut only few accessions were characterised to survive a very long and severe drought. The underlining mechanism of survival of those putative drought tolerant palms could be due to genetic factors contributed by drought tolerant genes/QTLs. Thus, a set of genomic and limited number of functional markers or combination of them DNA molecular marker based assay is seriously required. Can the genomic markers which are located in noncoding regions of the genome are qualified for capturing the functional genetic variation (variation in the cording regions) in plants and thus used to make inferences in a germplasm collection? Can the redundancies identified based on molecular markers be actually redundancies? Can co-collections developed based on molecular marker data guarantee that all the useful genes were made available to the breeders? Can even the limited number of functional markers are qualified for capturing the genetic variations occurred in the cording regions in plants, for example pest and disease resistance which are usually govern by major genes? Coconut molecular biologist needs to address these issues seriously and need to use molecular marker data rationally and cautiously depending on the objective of studies. There are instances that molecular markers cannot differentiate coconut accessions which are phenotypically highly distinguishable for many characters or distinguish by single characters. For example Raja brown dwarf, Tebing Tinggi Dwarf and Malayan Red Dwarf are indistinguishable by 12 genomic microsatellites (Perera, 2001). Similarly the Ambakelle special coconut accession, a drought tolerant selection Sri Lanka tall and its original population; Ambakelle tall accessions are indistinguishable by molecular markers (unpublished data). Availability of sequences of the same gene from different accessions would make it possible to evaluate nucleotide diversity among sequences of the target genes and identification of single nucleotide polymorphisms (SNPs), towards this end. SNAP markers could be generated based on the identified SNP in the target gene sequences. If the SNPs exist in the coding

region and they are non-synonymous SNPs, it should be possible to evaluate the association between changes in amino acid residues, possible changes in protein function and the possible changes in phenotypes. As functional marker, SNAP is expected to be better than EST-SSR since SNPs are more frequently found in the genes and most probably are more polymorphic. Use of SNAP markers based on identified SNPs of eight different coconut genes (ABI, SACPD, SUS, and five WRKY genes) with 528 accessions of coconut germplasm collections (Dwarf and Tall coconuts) belonging to IPRI, Manado have been reported (Sudarsono *et al.*, 2015). Results of the analysis confirm the informativeness of the evaluated SNAP markers for analyzing coconut germplasm collections. Subsequently, the same SNAP markers have also been used to analyze diversity of Indonesian Kopyor coconuts collected from different locations in Indonesia. Results of the evaluation further confirmed the informativeness of the develop SNAP markers and their usefullness for cluster analysis of coconut germplasm collections.

Of all the described markers above, breeders who utilize them must be cautious when using information generated only through molecular markers. Combining molecular and phenotype data of the individual accessions and using the combine data to manage the existing coconut germplasm would be more resource efficients. Such combined data can be used to design more efficient hybridization and conservation strategies to develop new and more productive coconut cultivars. Thus evaluation and characterization of all germplasm accessions for their morphological characters and yield and yield related characters, for their responses to biotic and abiotic stresses and for their adaptation to different agro-climatic conditions are of also vital importance.

5. Data Analysis and Interpretation

Analysis and interpretation of molecular marker data is of another vital consideration. Analysis and interpretation of molecular marker data need careful examination of the data, their cross compatibility between assays and among laboratories and its repeatability. Need also arises for the involvement of a conventional breeder for sensible data interpretation. For example the most informative polymorphic marker system and the choice of marker today is the SSRs. (Tautz and Renz, 1984). Their high information content, which is directly related to the effective number of alleles at each locus (multi-allelic), hence, that SSRs are an ideal tool for many genetic applications (Bruford and Wayne, 1993; Queller *et al.*, 1993; Dallas *et al.*, 1995). Moreover, these markers are entirely defined by the sequence of the primer and thus can be easily exchanged between laboratories as primer sequences which facilitate collaboration between research groups. Comparison of SSR diversity levels has revealed higher levels of polymorphism compared to other molecular assay procedures and indicates that SSRs are ideal for germplasm assessment and varietal identification due to their high level of allelic diversity (Powell *et al.*, 1996b; Russell *et al.*, 1997). The observed hyper-variability of SSRs is thought to be due to the unique mechanism by which SSR variation is generated. These slippage events during DNA replication are currently the preferred explanation of the origin of SSR polymorphism and are thought to occur more frequently than the point mutation, insertion and deletion events responsible for

generating polymorphism detectable by RFLP, AFLP and RAPD analysis (Tautz and Rentz, 1984; Tautz *et al.*, 1986). SSRs are assumed to follow a stepwise mutation model in comparison to the infinite allele mutation model (Valdes *et al.*, 1993; Di Rienzo *et al.*, 1994). The basic idea of the stepwise model is that mutations create new alleles that differ from their previous state by an increase or decrease of one step in the number of repeats. As empirical evidence suggests that mutational changes are often of one repeat unit (Weber and Wong, 1993), the stepwise mutation model has been 'revisited' (Shriver *et al.*, 1993; Valdes *et al.*, 1993; Di Rienzo *et al.*, 1994). Since the SSR data did not always appear to conform to a stepwise mutation model and coconut SSR data seemed to follow the same trend (*i.e.* certain loci did not exhibit the characteristic symmetrical, unimodal allele distribution) genetic distances should be calculated based on the proportion of shared alleles. In addition, it has previously been shown that this distance metric is most suitable for assessing genetic relationships between recently diverged taxa below the species level (Bowcock *et al.*, 1994; Provan *et al.*, 1999).

Although the AFLPs, in assessing the level and distribution of genetic diversity in coconut is a valuable technique, the dominant nature of the AFLP markers limits the amount of information that can be obtained compared to that of co-dominant marker systems such as RFLPs and SSRs. For example co-dominant markers detect heterozygotes from the homozygotes and allelic diversity within a locus. They also allow the direct study of the changes in allele frequencies under natural and artificial selection. However, the level of resolution achievable with RFLPs is limited and the procedure is expensive and laborious and therefore greatest possibility exists for SSR analysis. Moreover as SSRs are sequence tagged sites (STS's), they can also be used for construction of physical maps by STS mapping. Therefore SSR markers on coconut are not only be of value for diversity studies and DNA fingerprinting but also would particularly be beneficial for future studies which will involve genetic linkage mapping and monitoring of gene transmission through generations with collaborations between laboratories.

6. Coconut Genomics: Deciphering the Information from the Genome Sequence

The progress of next generation sequencing (NGS) technology has opened up avenues to understand plant and other organisms at the genome level. NGS techology has been used to generate genome sequences of a number of orphan crops and develop better understanding for the crops at molecular level. The potential progress in alleviating future problems made by availability of such genome sequences remains to be seen. However, breeders for some orphan crops have started to capitalize the potential of genome sequence availability to support their breeding programs. The determination of the whole genome sequence is essential to develop and exploit high-throughput breeding methods and mine the essential genes.

Coconut has also benefitted by the progress in NGS technology. The NCBI GenBank DNA database (http://www.ncbi.nlm.nih.gov/) is one of the repository keeping up the wealth of DNA sequences of almost all studied organisms, including coconut. Searching the NCBI GenBank nucleotide database (http://www.ncbi.

nlm.nih.gov/nucleotide, accessed: 10 Feb 2016, at 10:25) using key word of "*Cocos nucifera*" results in 212,603 entries and out of those 211,744 entries are nucleotide sequences of either chloroplastic, mitochondrial, or nuclear genomes originated from coconuts. Most of the entries come out from either the EST or the NGS sequencing data of coconut.

Further exploration in the NCBI GenBank SRA database (http://www.ncbi.nlm.nih.gov/sra), where publicly accessible outputs of NGS technology are stored, using key word of "*Cocos nucifera*" (accessed: 10 Feb 2016, at 10:25) results in eight entries of research activities describing the transcriptome studies (RNA Seq) of coconuts. Those coconut RNA Seq data deposited in the NCBI GenBank SRA database include: trascriptome profiling of (1) leaf (SRX534428), (2) embryo (SRX534380), (3) embryogenic callus (SRX472157), (4) leaf of root (wilt) disease susceptible cultivar (SRX437650), (5) leaf of root (wilt) disease resistant cultivar (SRX436961); and (6-8) coconut palm (SRX400192, SRX198908, and SRX518095), The sizes of deposited raw data ranged from 4.9 G bases sequences in the form of 3.3 Gb downloadable data up to 24.1G bases sequences in 16.5 Gb dowloadable data. Those are huge publicly available coconut transcriptome data ready for use and further exploration by scientist working with coconut. Since October 2012, at least four institutions have contributed to the wealth of those coconut transcriptome data, including: Academia Sinica (https://www.sinica.edu.tw/); Central Plantation Crops Research Institute (CPRI, http://cpcri.gov.in/); Coconut Research Institute (CRI, http://www.cri.gov.lk/), and University Putra Malaya (UPM, http://www.upm.edu.my/). In November 2015, Bogor Agricultural University (IPB) has initiated the transcriptome data generation by conducting RNA-Seq for normal coconut and Kopyor mutant coconut from zygotic embryo tissues. These data should be available by the end of 2016.

Transcriptomics generated EST sequences from different tissues, different development, or from cultivar with resistance – susceptible to certain disease, may actually be important to idetify the genes involved in the essential processes in coconut. Understanding pathways of fatty acid and other important biosynthetic precesses in coconuts may also results in better understanding of and the genes involved in the processes. Transcriptome and RNA Seq data generation followed by analysis of differentially expressed (DE) genes from such transcriptome data should be able to answer such questions. Published information from some of those coconut RNA seq studies have illustrated the possibilities of gaining such knowledge (Fan *et al.*, 2013; Huang *et al.*, 2013). Using such extensive transcriptome sequencing data, Fan *et al.* (2013). identified 57,304 unique genes from coconut and Huang *et al.*,*2013* identified the complete chloroplast genome of coconut.

The initial idea to sequence coconut genome has been around since October 2011 in the form of Coconut Genetic Resources Network (COGENT) research strategies (http://www.cogentnetwork.org/48-coconut-projects/research-ideas/110-sequencing-coconut-genome). As follow up of this, the steering committee of COGENT which met in Kochi, India (July 2012) has decided to set up an international thematic group on coconut genomics under the leadership of India to sequence coconut genome and develop tools for coconut improvement. Unfortunately, the

initiated idea of sequencing coconut genome has not yet been realized. The second record of the attempt to sequence coconut genome was announced by Alsaihati *et al.* (2014) of Joint Center for Genomics Research, a cooperation between KACST Riyadh, Saudi Arabia and CAS, China. The attempt was announced in a presentation at Plant and Animal Genome XXII conference in Singapore in 2014 (https://pag.confex.com/pag/xxii/webprogram/Paper10752.html). The report stated that the estimated coconut genome size is 2.6 Gb and it contain 50-70 per cent repeat sequences. The assembly of the raw sequence data generated an estimated 0.97 – 2.47 Gb (37-94.5 per cent) of coconut genome sequence coverage (Alsaihati *et al.*, 2014). It also reported a total gap (per cent of draft size) of 251 – 749 Mb (10-29 per cent of draft size). Since that announcement, no further follow up information is reported by the group.

At the end of 2015, Indonesian Palm Genome Project was initiated as a collaborative project among BBBiogen, IPB, and IPRI under KKP3S Project. Part of the KKP3S Project targets are generating genome sequences of coconut and sugar palm. In the project, the genomes of a Tall (Tenga Tall) and a Dwarf (Pati Kopyor Green Dwarf) coconuts have been sequenced. The coconut genome sequencing result in a 148,109,277,288 total read base (bp) for the Dwarf coconut and 99,306,500,882 bp for the Tall one. The total GC and AT content of the total read data for Dwarf coconut are 38 per cent and 62 per cent while for Tall coconut are 37 per cent and 63 per cent, respectively (Lestari *et al.*, 2016). Assembly and downstream analysis of the raw data are on going and hopefully by 2017, preliminary reference of draft genome of coconut would be available for general publics. The generation of a reference coconut genome should be a landmark for coconut molecular breeding.

All molecular marker, transcriptomic, and genomic studies have generated a large amount of data. Most of them have been deposited in a public databases, TROPgeneDB (Tropgene: http://tropgenedb.cirad.fr/tropgene/JSP/index.jsp) and NCBI GenBank Database (http://www.ncbi.nlm.nih.gov/nucleotide and http://www.ncbi.nlm.nih.gov/sra). Making as much information as possible available to the community of coconut breeders should be continued. It is also emphasized here that necessary infra-structure facility and trained staff should be made available to all coconut growing countries in carrying out their own modern biotechnological research in coconut.

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