DEVELOPMENT OF MICROSATELLITE MARKERS IN INDIAN NEEM (*Azadirachta indica* A.Juss. var.*indica*) AND THAI NEEM (*Azadirachta indica* A.Juss. var. *siamensis*)

INTRODUCTION

Neem is an important tropical tree species with a number of medicinal and biopesticidal properties (Singh, 2002). It is a very valuable forestry species in India and Africa and is also becoming popular in Tropical America, the Middle-East countries and in Australia. In addition, neem has been planted in countries world wide and now has become a global tree (Neem Foundation, 2007).

Neem has multiple use applications throughout its tropical distribution with many useful products derived from nearly every part of the plant (Mitscher, 2005). The species is highly efficient in restoring soil, and could useful as a windbreak and in areas of low rainfall and high wind speed (Forster and Moser, 2000). Fire wood and other products derived from neem help to meet basic needs in rural households simultaneously like medicines, pesticides, mosquito repellants, fertilizers, diabetic food, soaps, lubricants, gums, agricultural implements, tooth paste, tooth brush sticks and even contraceptives. In rural areas, villagers take bath in water heated with neem leaves as a preventive against heat rash. They also boil and drink neem water as a cure for skin diseases. Neem oil is used against stomach ulcers, worm infections and rheumatism (Tewari, 1992).

Neem has been studied in much detail, i.e. biology, physiology, growth development and its utilization. In regards to growth and tree improvement program, series of species trials, provenance trials, clonal tests and progenies test have been conducted. The tree improvement program for neem is well underway with the aim of screening the most suitable germplasm for large scale plantation establishment in Kanchanaburi, Thailand.

Chromosomes of neem were previously studied with 2N = 30. Isozyme studies have been were previously conducted in many research institutes (Changtragoon *et al.*, 1996, Krisanapant, 2007). Molecular markers are now being increasingly used as a very effective tool for the understanding of population genetic structure, gene flow, parentage, population variability and, ultimately, to quantify the effects of habitat fragmentation and to guide conservation strategies (Collevatti *et al.,* 1999). Among the different classes of molecular markers, SSR markers are useful in a variety of applications in plant genetics and breeding because of their multiallelic nature, co-dominant inheritance, relative abundance, good genome coverage (Varshney, 2005) and polymorphic between and within species (Stajner, 2005). Besides, they are reproducible and easily detected by polymerase chain reaction (PCR) (Bouhadida, 2007). Despite the usefulness of microsatellite markers for the investigation of population genetic and conservation, reports on the development, characterization and use of SSR loci in tropical tree species are still scarce (Collevatti, *et al*., 1999). Though neem is a very important tree due to its medicinal value and other uses, therefore, development of microsatellite and study in genetic variation in neem is important for the genetic improvement of neem.

OBJECTIVES

The objectives of the present study are:

1. To develop microsatellite markers for Indian neem (*Azadirachta indica* A.Juss. var. *indica*) and Thai neem (*Azadirachta indica* A.Juss. var. *siamensis*).

2. To study genetic variation in population of Indian neem and Thai neem by using developed microsatellite markers.

3. To provide basic information for further genetic studies in neem.

LITERATURE REVIEWS

Origin and geographic distribution

Neem is probably native to Burma (Schmidt and Joker, 2000) although the South of India and Burma are the main area of origin (Forster and Moser, 2000). But it has been widely and extensively cultivated in tropical Asia and Africa (Mabberley *et al.,* 1995). However, some claimed that neem is native to the entire Indian subcontinent; others attribute its origin to dry forest areas throughout all of South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia (National Research Council, 1992).

In India, neem is widely used. It is grown throughout India in deciduous forests (Warrier *et al.,* 1994) from the southern up of Kerala to the Himalayan hills, in tropical to subtropical regions, in semiarid regions to wet tropical regions, and from sea level to about 700 meter elevation (Tewari, 1992).

Neem was introduced to Africa earlier this century. It is now well established in at least 30 centuries, particularly those in the regions along the Sahara's South fringe, where it has become an important source of both fuel and lumber. Although widely naturalized, it has nowhere become a weed. Indeed, it seems rather well "domesticated"; it appears to thrive in villages and towns.

Over the last century or so, the tree has also been established in Fiji, in many other Pacific islands, in Mauritius, the Caribbean, and many countries of Central and South America. In some cases, it was probably introduced by indentured laborers, who remembered its value from their days of living in India's villages. In other cases it has been introduced by foresters. In the continental United States, small plantings are prospering in southern Florida, and exploratory plots have been established in southern California and Arizona (Lemmens, 1995; Forster and Moser, 2000).

Figure 1 Areas where neem trees are found around the world (★).

Source: Adapted from Forster and Moser (2000).

Natural distribution of neem in Thailand

The exact occurrence of neem in Thailand is not well known (Lauridsen, *et al.,* 1991). Thai neem (*Azadirachta indica* var. *siamensi*s) has a wide natural distribution in Dry Evergreen Forests in Thailand (Figure 2) and seems to occur naturally only along the western coast in a 30-40 kilometer wide belt. In the east along the border with Cambodia, neem is not found until, Sa Kaoe province. In the central north, Thai neem occurs commonly. The distribution of neem in the northeast close to Laos has not been verified yet. In the North and North-West, neem has spread naturally through the valleys along the Ping, Yom, Nan Rivers and has reached 19^{o} North Latitude. In the west, the densely forest-covered mountains along the border to Myanmar, have prevented neem from naturally spreading from Thailand into Myanmar. In southwestern Thailand, neem extends up to approximately 90 North Latitude (Glover and Adams, 1990; Lauridsen, *et al*., 1991).

Plants in Meliaceae are widespread in tropical and subtropical region (Mabberley *et al*., 1995). The family is important chiefly as a source of valuable timber, which comes from *Swietenia* (mahogany), *Cedrela* (West Indian Cedar), and *Khaya* (African mahogany) and *Azadirachta* (neem tree). They are important medicinally and as a source of insecticides. *Melia* (chinaberry/Persian lilac) and *A. indica* are ornamental trees (Judd *et al*., 1999). The Genus differs characteristically from its related genera by its leaves being simply pinnate, glabrous innovation; base of the petiole mostly with 1 pair of orbicular glands and one pair of elongate glands below them.: inflorescence about as long as the leaves; pistil with 3-locular ovary, 2 ovules in each locule, collaterally arranged; style slender, stigma on a whistish ring, 3-lobed; drupe with thin endocarp, 1-seeded (Tewari, 1992, Mabberley *et al*., 1995).

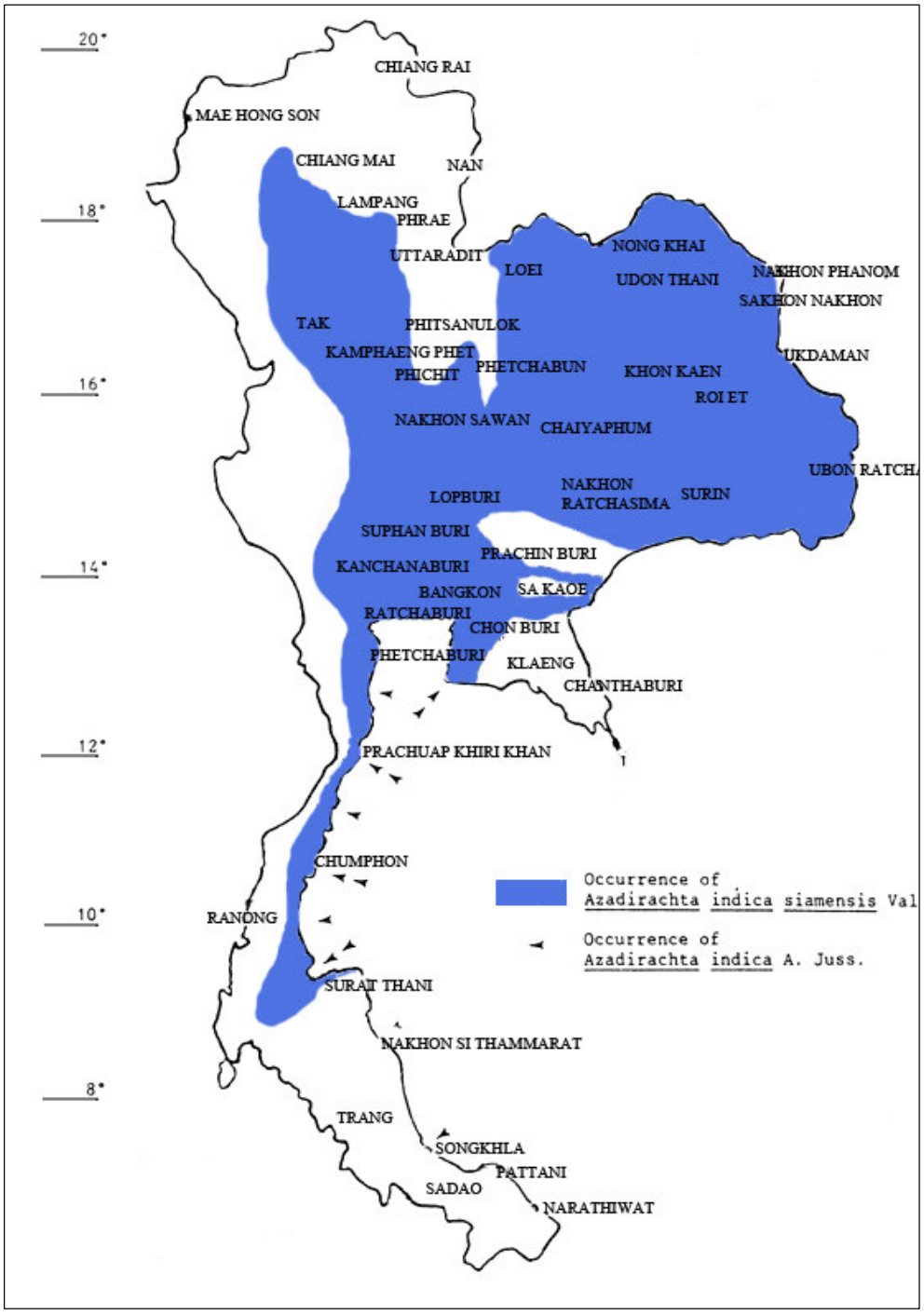

Figure 2 Distribution of neem in Thailand.

Source: Adapted from Lauridsen *et al.* (1991).

The genus *Azadirachta* was named by Antoine Laurent de Jussieu, a French botanist, in 1830 with *Melia azadirachta* Linn. as the only species. He later named it *Azadirachta indica* A.Juss. The origin of the name came from the Persian *azad dhirakat* or *azaddhirakt* meaning "excellent tree, noble tree," referring to the usefulness and the considerable economic importance of the genus (Quattrocchi, 2000).

Tree morphology of Indian neem and Thai neem

Indian neem (*Azadirachta idica* A.Juss. var. *indica*)

Indian neem is a large evergreen that ranges in height between 12-15 meters and exceeds rarely up to 25 meters. It has a clear bole of 3-7.5 m height and 1.8-2.8 m girth, generally branching early and forms a broad rounded crown of bright-green foliage. The bark is reddish-brown or grayish, fissured and flanking in old trees; the inner bark is reddish brown. (Mabberley *et al.,* 1995). Leaves are imparipinnate, and, crowded near the end of branches. The leaflet is 9-13, nearly opposite, 2.5-7.5 x 1.2-4.0 cm. Inflorescence is axillary with many-flowered panicle, shorter than the leaves. Flowers are white, fragrant and smelling of honey, bisexual and male on the same tree (polymanous). Fruit a drupe, 12-18 mm long, yellowish-green smooth, dark yellow or purple when ripe. Outer seed-coat with thick-walled epidermis and three layers of loosely arranged cells; the cells of the inner integument undergo tangential elongation and form the inner seed coat (Tewari, 1992).

Thai neem (*Azadirachta indica* A.Juss. var. *siamensis*)

Thai neem usually grows to a height of 15-20 meters. The bark of the bole of older trees is grayish and longitudinally fissured. The leaves are unpaired and measure up to 45 centimeters in length. The color of the leaflets is medium to dark green. The petioles are short. There exists a type of *A. indica* var. *siamensis* with reddish young leaves and another one with green leaves. The shape of leaflets is asymmetric and the

margins are more or less crenate, sometimes even nearly entire. The basiscopal half is reduced and cuneate, the tip pointed (Schmutterer, 1995).

The most important differences between *A. indica* var. *indica* and *A. indica* var. *siamensis* are summarized in Table 1.

Table 1 Comparison of features of Indian neem and Thai neem (*Azadirchtan* spp.)

Features	**Indian neem**	**Thai neem**
Shape of tree	Crown dense, branches numerous	Crown relatively open, branching moderate
Shape of leaflets	Smaller and thinner	Wider, longer and thicker
Margin and tip of leaflets	Distinctly and regularly serrate, tip pointed. Basal part of anterior part of leaflet strongly curved to mid vein	Irregularly crenate to entire, tip relatively blunt. Basal part of anterior part of leaflet slightly curved to mid vein
Inflorescences and flowers	Panicles loose, open and long; flowers usually axillary, smaller	Panicles dense; glowers stout and bigger, often non-axillary
Flowering period	March, or any other time during the year	November/December; rarely March
Fruit/Seed	Narrower; no dark green layer under brown testa of seed kernel	Wider; dark green layer under brown, parchment-paper-like testa of seed kernel

Source: Sombatsiri *et al*. (1995).

Figure 3 The difference of tree ideotype between Thai neem and Indian neem (A) Thai neem (B) Indian neem.

Figure 4 The differences in bark characteristics between Thai neem and Indian neem (A) Thai neem (B) Indian neem.

Figure 5 The difference of leaflet shapes between Thai neem and Indian neem (A) Thai neem (B) Indian neem.

Natural regeneration

Neem is seldom found growing gregariously. It regenerates naturally from seed, and is also reported to regenerate well by coppice and root-suckers in Andhra Pradesh and Tamil Nadu (Tewari, 1992). Birds play an important role in dispersal of seed. The fruit ripening period coincides with the rainy season and under natural conditions, the fruit ordinarily falls to the ground and germinates within a fortnight. A carpet of new recruits can be seen surrounding mature trees. It is frequently self-sown near gardens and villages and the area near mature neem trees is quickly colonized by seedlings (National Research Council, 1992). Neem reproduces naturally with tolerable freedom and has the capacity to establish itself under the protection of thorny bushes. It also has the ability to survive in dry poor soil, provided it is not subjected to frost. Once established, the seedling is hard to eradicate (Glover and Adams, 1990).

Utilization of neem

Neem has now been universally accepted as a “wonder tree” due to its multitude of uses (Dhaliwal *et al.*, 2004). Neem wood is moderately heavy, stable, and resembles mahogany in appearance. In its strength properties, it resembles teak but is more resistant to shock. It is straight grained and seasons easily except for end splitting.

The wood is moderately resistant to fungi and repugnant to most borers but difficult to impregnate with preservatives (Lemmens *et al*., 1995). It is used for making furniture, carts, axles, yokes, boards and panels, cabinets bottoms of drawers, packing-cases, ornamental ceilings, ship and boat building, helms, oars, oil-mills, cigar boxes, carved images, toys, drums and agricultural implements (Forster and Moser, 2000). The neem tree provides good fuel wood and has long been used, in arid zones of India (Tewari, 1992).

Neem bark contains tannins which are used in tanning, dyeing, etc. Compounds extracted from neem bark are used in production of some dental-care products. Neem bark is also tapped for gum.

Neem seed pulp is useful for methane gas production. It is also useful as a carbohydrate-rich base for other industrial fermentations (Neem Foundation, 2007).

Dried flowers of neem are either eaten raw or used as ingredients in curries and soups; or prepare as fried dish in South India. They are useful in some cases for dyspepsia and general weakness (Mitscher, 2005). The flowers provide plenty of nectar.

The leaves of the versatile neem trees also have many uses. Neem leaves are not only useful for pest and disease control, they are also fed to livestock mixed with other fodder in several feed in several parts of the country. Neem leaves are used in some parts of India as fertilizer in rice fields (National Research Council, 1990). In some countries, neem leaves are used as mulch in tobacco and tomato fields. Neem leaves are spread over the plant roots to retain moisture, kill weed etc. neem leaves can also be used to protect stored woolen and silk clothes from insects (Glover and Adams, 1990).

Neem oil contains several compounds which have proven medicinal an agricultural uses of high value. Neem oil is, however, not used generally for these purposes. The most common use of neem oil is for soap production. This indicates vast scope for expanding neem oil production. The collection of neem seeds to be supplied to the crushers can be important means of supplementary employment and income for the poor household, especially for rural women, since the task of seed collection is highly suited to them (Lauridsen *et al.,* 1991). There are several other uses of neem oil as well. Neem oil can be used as an illuminant. In India, neem oil is widely burnt as fuel in lamps for lighting and repelling mosquitoes. Neem oil is used for mosquito net impregnation which seems to be gaining popularity. Neem oil is also useful for lubrication purposes (Neem Foundation, 2007).

Various parts of the Thai neem tree are usable. The inflorescences before flowering and the young leaves are commonly eaten as vegetables (Schmutterer, 1995). Neem seed are used in pest control for vegetable production. Neem is also used for fuel wood. As an excellent multipurpose tree species, more genetical research is encouraged so that more possibilities are discovered for additional potential uses.

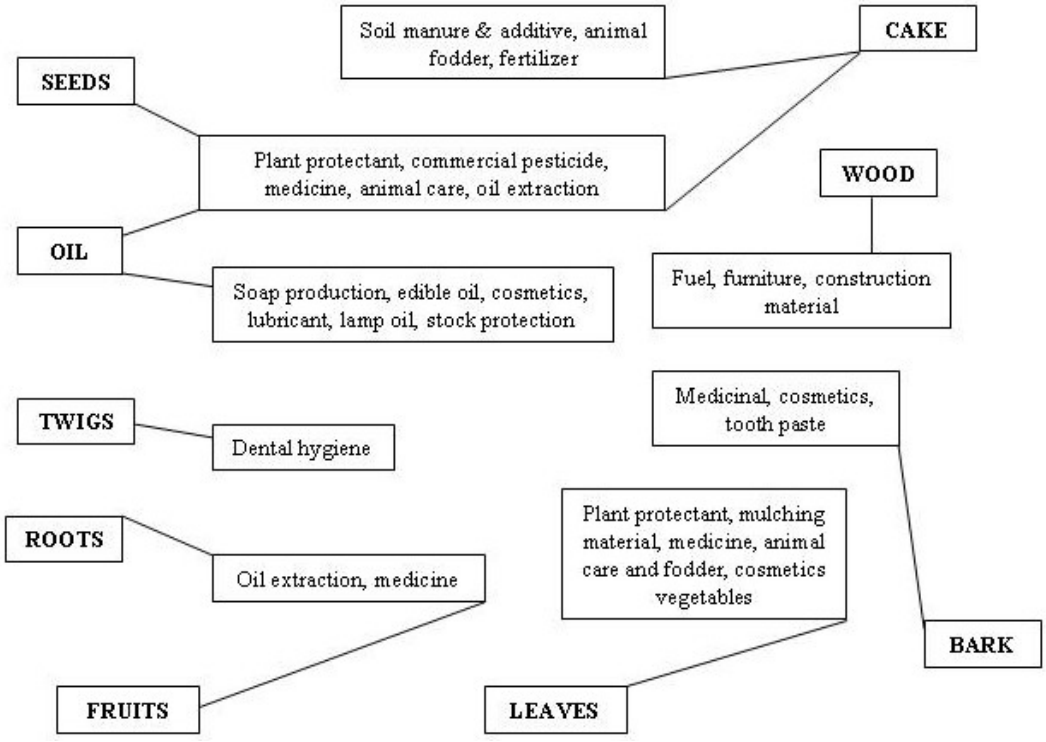

Figure 6 Parts of neem tree and their potential usage.

Source: Dhaliwal *et al.* (2004)**.**

Molecular markers

Genetic markers are polymorphic genetic property that can be used to distinguish the parental origin of alleles (Andersen and Lubberstedt, 2003) and are complementary tools for morphological approaches in reliable and precise genotype identification (Omrani-Sabbaghi, *et al*., 2007). It is often encountered as a particular enzyme or DNA fragment having a defined position on an electrophoresis gel. Any given 'band on a gel' generally does not gain marker status, however, unless two or more forms (alleles) of it exist at a single chromosomal location (locus). At their most basic level, alleles are simply the DNA sequence variants found at a locus (Echt, 1999).

In recent years, different marker systems such as Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, Single Nucleotide Polymorphisms (SNPs) have been developed and applied to a range of crop species (Korzun, 2003) and tree such as pinus (Mariette *et al*., 2001), citrus (Dong *et al*., 2006), Pear (Wunsch and Hormaza, 2007) and avocado (Ashworth et al., 2004). As these tools come into more widespread used, they will provide new insights into the processes of population-level differentiation (Judd, 2002). The relative advantages and disadvantages of these techniques are shown in Table 2.

Table 2 Comparison of the most common used marker systems

Features	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (?g)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	high	high	moderate	moderate	high
PCR-based	no	yes	yes	yes	yes
Number of polymorphic loci analyzed	1.0-3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of use	not easy	easy	easy	easy	easy
Amenable to Automation	low	moderate	moderate	high	high
Reproducibility	high	unreliable	high	high	high
Development cost	low	low	moderate	high	high
Cost per analysis	high	low	moderate	low	low

Source: Korzun (2003).

Comparison between RAPD, AFLP and SSR markers in different species has revealed that co-dominant SSRs detect the highest level of polymorphism per locus as it surveys the hyper-variable microsatellite regions of the genome (Singh, 2002). Beside polymorphism, they are reproducible and easily detected by PCR (Bouhadida *et al.,* 2007).

Microsatellite markers

Among different classes of molecular markers, microsatellites are useful for a variety of applications in plant genetic and breeding because of their ubiquitous distribution in the genome (Fujimori *et al*., 2003). Microsatellite DNA, or simple sequence repeats (SSRs), were firstly characterized as highly informative genetic markers in humans (Weber and May, 1989), and have since been found in practically all organisms. Microsatellite sequences are composed of a particular 1 to 6 DNA nucleotide motif, arranged head-to-tail without interruption by any other base or motif. Such repeated sequences are unstable and prone to errors in replication (Judd, 2002). The mutations are generally manifested as gain or loss of individual repeat

units, referred to as step-wise mutations, thus giving rise to a series of alleles that have discrete size differences. Within most species genomes, within their complement of chromosomal DNA, SSRs are abundant, widely distributed, and frequently surrounded by non-repetitive, unique DNA sequences (Gillet, 1999). It is this latter property that allows use of the polymerase chain reaction technique to amplify a specific single SSR locus from a small sample of DNA. As articulated by Echt (1999) by using PCR methodology many informative SSR loci can be genotyped very quickly from small amounts of tissue, as shown in Figure 7.

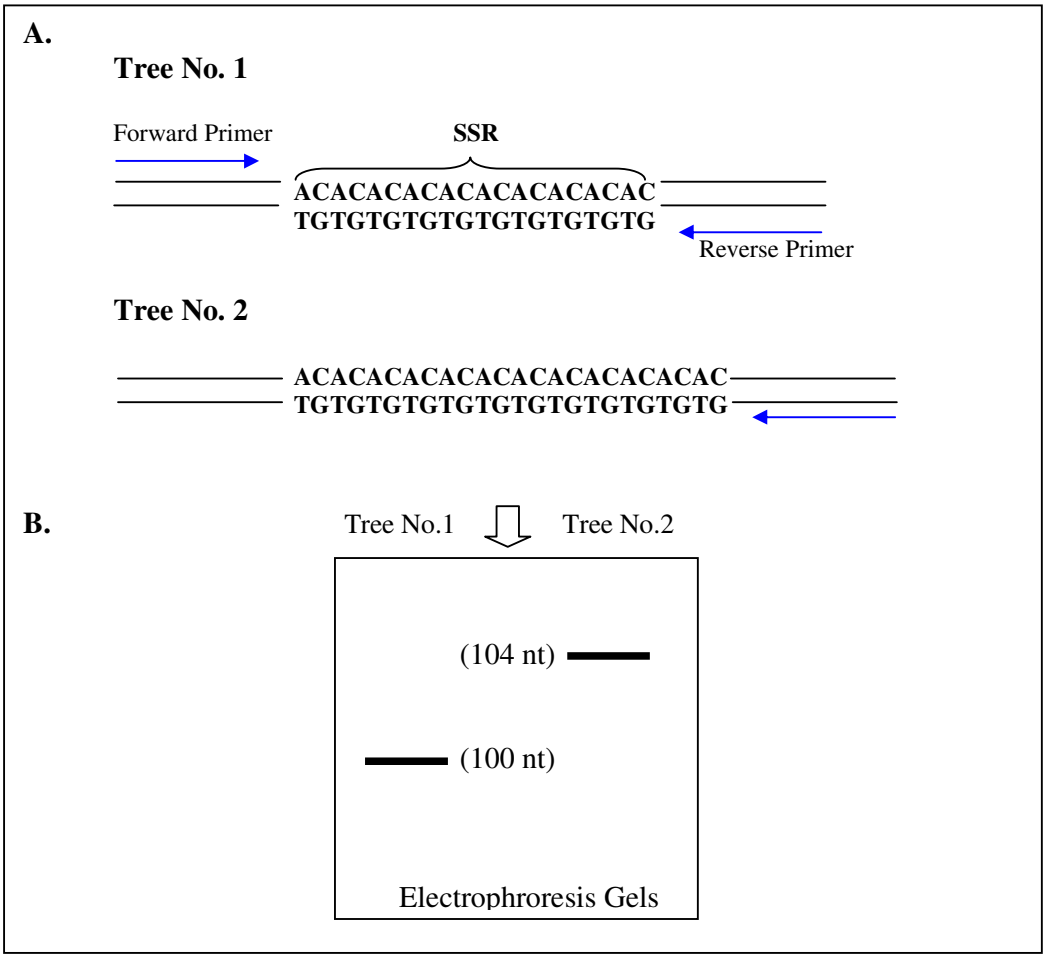

Figure 7 Schematic representation of the genetic basis of phenotypic differences of two Simple Sequence Repeat alleles (SSRs) by their length, one has100 nucleotides (nt) and the other 104 nt, each representing two different trees.

Source: Echt (1999).

Mechanistic basis for microsatellite instability

The characteristic that makes loci that contain microsatellite repeats particularly useful for evolutionary and genetic studies is their inherent instability. The mutation rates at most microsatellite loci are usually orders of magnitude higher than mutation rates at other loci within the same genome. Although many types of mutations occur at microsatellite loci, the elevated mutation rate is primarily caused by an elevated rate of one particular class of mutations: changes in the length of the repeat tract. Thus the term 'microsatellite instability' is frequently used to refer specifically to these tract-length changes since most of these tract-length changes result from changes in the integral number of copies of the repeat, they are also frequently referred to as copy number changes.

To have a complete understanding of the mechanism of microsatellite instability one must also explain why stability varies both within and between species. Clues to the cause of this variation have come from the identification of factors the correlate with the level of microsatellite instability. Such factors include size of the repeat unit, number of copies of the repeat, presence of variant repeats, and amount of transcription in the region of DNA containing the repeat (Eisen, 1999).

Microsatellite mutation models

The mechanism of microsatellite instability has focused on two competing. One model proposes that microsatellite instability caused by an elevated rate of unequal crossing-over (UCO) within microsatellite repeats. Unequal crossing-over is the result of recombination between homologous chromosomes that are imperfectly aligned. The UCO microsatellite instability model suggests that UCO occurs at an elevated rate in microsatellites because the presence of repeats increases the likelihood of misalignment between homologues. A similar proposal has been made to explain the high rates of copy number changes observed in tandemly repeated genes (Eisen, 1999).

An alternative model proposes that microsatellite instability is caused by an elevated rate of slip-strand mispairing (SSM) errors during DNA replication. The SSM process begins with the DNA polymerase 'slipping' during replication, causing the template and newly replicated strands to become temporarily unaligned. For replication to continue, the strand must be aligned. Mutations will be generated if this realignment is imperfect. The SSM microsaellite instability model proposes that SSM occurs at an elevated rate in microsatellites because the presence of repeats increases the likelihood of misalignment after slippage (since repeats can easily be looped out of the DNA double-helix). However, SSM alone does not provide a full picture of this mutation process. Not all SSM errors become mutation –some are 'repair' by error-correction mechanisms. The two error-correction pathways that have been shown to be important in repairing SSM errors are exonucleolytic proofreading and post-replication mismatch repair. Thus a complete description of the mutation process must include both the generation of replication errors by SSM and the correction of some of these errors by mismatch repair and proofreading (Schlotterer and Tautz, 1992) as shown in Figure 8.

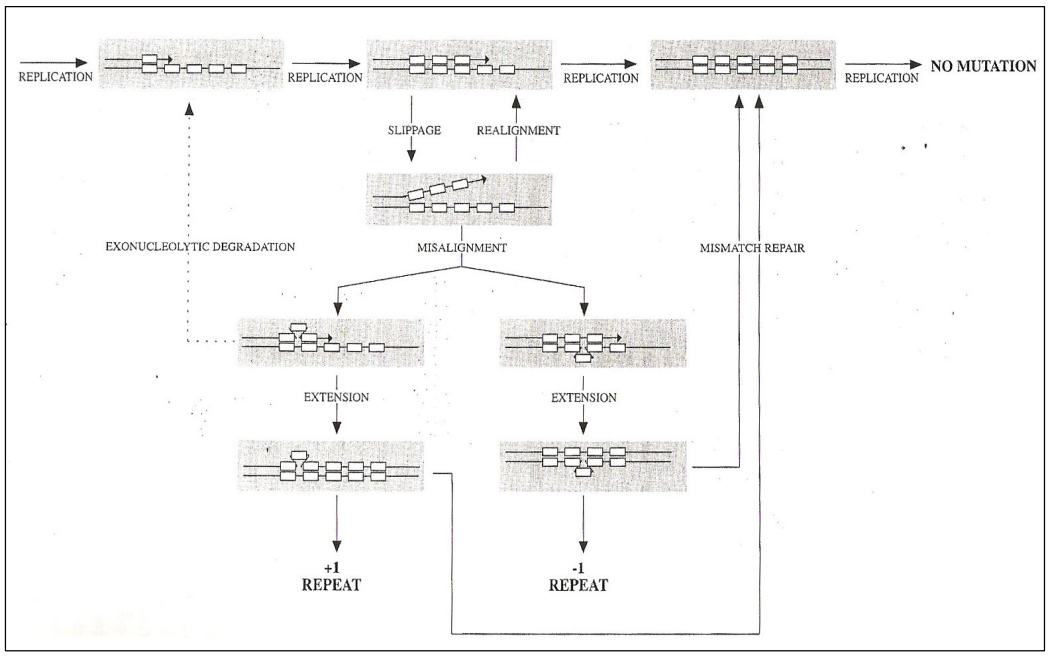

Figure 8 Model of the mutation process at microsatellite loci. Cartoons of double-stranded DNA containing a microsatellite repeat are shown at different stages of the replication and mutation process. In the cartoons, DNA strands are represented by thin lines, microsatellite repeats by small boxes, and ongoing replication by small arrows. Flow arrows point down for steps that lead to mutations, up for steps that prevent mutations from occurring, and to the right for steps in the DNA replication process. The exonuclease step is shown with a dashed line, since it has only a limited role in regulating microsatellite mutations.

Source: Eisen (1999).

Development of microsatellite markers in plants and their applications

Microsatellite markers are developed and widely used for different tree species. In neem, several studies have been carried out using molecular marker to characterize different genotypes and to establish the genetic relationships among the species. Initially, isozymes were used for analysis genetic diversity of 3 *Azadirachta* species (*A. indica*, *A.indica* var.*siamensis* and *A. excelsa*). The average genetic distance between populations of *A. indica* var. *siamensis* was low (0.090). On the other hand, the three taxa were separated by a very high genetic distance and a cluster analysis showed strong genetic divergence among the investigated taxa (Changtragoon *et al.,* 1996).

In another plants, Collevatti *et al.* (1999) reported the development and characterization of ten microsatellite loci for the endangered tree species *Caryocar brasiliense*. Mendelian inheritance and segregation were confirmed for all ten loci in open-pollinated half-sib families as well as the absolute transferability of these ten loci to five other species of the same genus. The number of alleles per locus ranged from 10 to 22 with a mean value of 16 and expecting heterozygosity varying from 0.84 to 0.94, clearly demonstrating that SSR multilocus genotypes are likely to be unique and capable of readily discriminating individuals of *C. brasiliense*.

Omrani-Sabbaghi, *et al*. (2007) studied the molecular characterization of Iranian olive collections using microsatellite markers and to assess the genetic relationships of imported and local varieties of six olive collections in Iran. Forty-seven accessions of 18 cultivars from six olive collection of Iran, were analyzed along with 30 imported cultivars using 16 microsatellite primer pairs. A two-dimensional scatter plot analyzing principle components reveals a clear separation of most of the Iranian olives from Syrian and other introduced cultivars.

Ritschel*, et al.* (2004) developed new microsatellite markers for melon based on a genomic DNA library enriched for microsatellite sequences. One-hundred and forty-four new markers were developed from the *Tsp*-AG/TC genomic library and 67

microsatellite markers were tested for their usefulness. Mapping analysis was initiated with 55 newly developed markers and most primers showed segregation according to Mendelian expectations. Linkage analysis indicated that the markers developed are dispersed throughout the genome and should be very useful for further genetic analysis of melon.

Stajner *et al.* (2005) reported on the isolation and characterization of the hop plant microsatellite using genomic libraries enriched in GA, GT, AT, TAA, ACA, AGA, CAG and ACTC repeats. Sixty pairs of primers of newly isolated microsatellites were tested for amplification on a set of four hop genotypes. Twenty-five of the developed microsatellites were further used for the assessment of polymorphism by the analysis of 67 different hop accessions focusing on the inheritance of 15 markers in the F1 population. This study highlights the isolation efficiency of the enrichment procedure, the abundance of microsatellites in hop, the high information content of the developed markers and their potential usefulness in hop genome mapping and individual identification.

Baleiras-Couto and Eiras-Dias, (2006) used nuclear SSR markers in order to identify single-varietal and blends of two varieties of musts prepared in laboratory and single-varietal wines produced in microvinifications. Preliminary results on multivarietal musts indicated a possible relationship between the proportion of each variety in the mixture and the signal intensity of the alleles, suggesting that it could be possible to quantify the presence of each variety in mixture.

Sanchez-Perez *et al.* (2005) used seventeen peach SSR markers to characterized eight apricot cultivars, breeding lines, and new releases from the breeding program in Spain, examination of the genetic relatedness among these genotypes. Results allowed the molecular identification of all the apricot genotypes assayed. Apricot genotypes clustered into seven principal groups in accordance with their origin and pedigree.

Portis *et al*. (2005) used five SSR loci to investigate variation in seven populations of wild cardoon. As a result of the geographical isolation, the Sardinian and Sicillian populations were clearly differentiated, forming two distinct gene-pools. Most of the genetic variation was partitioned within, rather than between, populations.

Martinez *et al.* (2006) used six SSR loci, previously developed for grapevine, as a tool for characterizing the genetic variation and cultivar relatedness in 25 autochthonous *Vitis vinifera* varieties from Peru and Argentina. The result showed polymorphism information content ranged from 0.70 to 0.88 indicating that the SSRs were highly informative. Varieties were clustered following a general pattern of shared morphological and enological traits, rather than geographical origin.

Mason *et al*. (2006) developed a collection of producible and highly informative microsatellite markers for use in quinoa. A total 1276 clones were sequenced from three microsatellite-enriched (CA, ATT, ATG) libraries. The most commonly repeated motifs, other than CT, ATT and ATG, were GA and CAA. Flanking primers were designed for 397 microsatellite loci. Two hundred and eight (52%) of the microsatellite markers were polymorphic amongst the quinoa accessions. The number of observed alleles raged from two to thirteen, with an average of four alleles detected per locus. Sixty-seven markers (32%) were highly polymorphic. These microsatellite markers are an ideal resource for use in managing quinoa germplasm, trait mapping and marker-assisted breeding strategies.

Shokeen *et al*. (2007) developed microsatellite-based STMS (Sequence Tagged Microsatellite Site) markers from genomic libraries and used these for assessing genetic diversity in a collection of *Catharanthus roseus* germplasm. Sixty-five microsatellite motifs were identified, from which 38 functional STMS primer pairs were designated. Out of these, 24 STMS markers were used to evaluate the genetic polymorphism in 37 genotypes. Sequence analysis of the length of variant alleles at the three STMS loci revealed that the variation in the copy number of repeat motifs was the major source of length polymorphism within *C. roseus*. However,

isolated point mutations in the microsatellite flanking regions of homologous loci from other species also contributed to size homoplasy and allelic size variation.

Shiran *et al.* (2007) determined the genetic characterization, diversity and relatedness among Iranian almond cultivars and their interrelationships using PCR-based molecular markers RAPD and SSR. Eighteen (out of 26) SSR primers were selected for their reproducibility and high polymorphism. The number of presumed alleles revealed by the SSR analysis ranged from three to ten alleles per locus. Both techniques discriminated the genotypes very effectively. For both markers, a high similarity in dendogram topologies was obtained, although some differences were observed. All dendograms, including those obtained by the combined use of marker data, depicted the phonetic relationships amongst the cultivars and species, depending upon their geographic regional and/or pedigree information. Almond cultivars clustered with accession of *Prunus communis* showed their close relationship. *P. orientalis* and *P. scoparia* were clustered out of the rest of *P. dulcis*.

Singh *et al*., (2002) evaluated intra-population genetic variation in *Azadirachta indica* A.Juss by using AFLP and SAMPL (Selectively Amplified Microsatellite Polymorphic Loci). The phenogram based on unweighted pair group method of averages analysis depicted the neem growing in district Kanpur were genetically distinct from the Thai accession as two were linked at a low genetic similarity value of 0.41. Based on AFLP and SAMPL analysis, it is concluded that neem maintains high levels of genetic variation at intra-population level.