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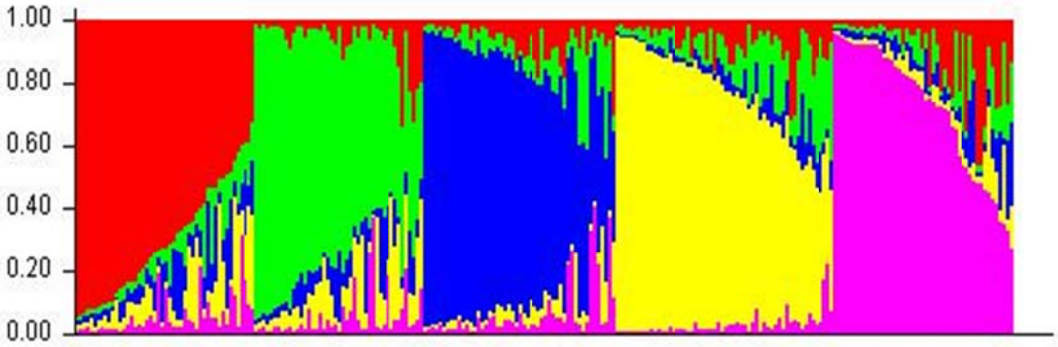
Genetic diversity of teak (Tectona grandis Linn. f.) progenies from Clonal Seed Orchard (CSO) in Myanmar revealed by microsatellite markers



Yazar Minn, Ph.D.
Assistant Director
Forest Genetics and Biotechnology Section
Forest Research Institute

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မြန်မာနိုင်ငံရှိ ကျွန်းမျိုးသန့်ဥယျာဉ်မှ မျိုးဆက်ပင်များ၏ မျိုးရိုးဗီဇစုံလင်မှုကို Microsatellite markers မျိုးရိုးဗီဇအမှတ်အသားများ အသုံးပြု၍ လေ့လာခြင်း

စာတမ်းအကျဉ်း

မျိုးရိုးဗီဇစုံလင်မှုသည် သစ်မျိုးသန့်ပြန့်ပွားခြင်း လုပ်ငန်းများအတွက် အရေးကြီးသည့် အကြောင်းအရာများထဲမှ တစ်ခုဖြစ်ပါသည်။ ၎င်းတို့သည် ဇီဝနှင့်အဇီဝဆိုင်ရာ လွှမ်းမိုးမှုများကို တုန့်ပြန်မှုပေးစေနိုင်သည့် အခြေအခံတစ်ရပ် ဖြစ်ပါသည်။ မျိုးရိုးဗီဇ စုံလင်ကွဲပြားမှုနှင့် စပ်လျဉ်း၍ ဘဘာဝကျွန်း သစ်တောဦးရေစုများကို လေ့လာမှုများစွာ ဆောင်ရွက်ပြီး ဖြစ်ပါသည်။ ၎င်းဦးရေးစုများသည် မျိုးရိုးဗီဇ စုံလင်ကွဲပြားမှုမြင့်မားခြင်း တွေ့ရှိကြပါသည်။ မျိုးသန့်ဥယျာဉ်သည် မျိုးကောင်း မျိုးသန့် သစ်စေ့အမြောက်အမြား ထုတ်လုပ်ပေးသည့် သစ်မျိုးသန့် ပြန့်ပွားရေး လုပ်ငန်းစဉ်တွင် အရေးပါလှပါသည်။ စိုက်ခင်း၏အောင်မြင်မှုနှင့် မျိုးကောင်း မျိုးသန့်သစ်စေ့များ အသုံးပြုတည်ထောင်ခြင်းသည် ဆက်နွယ်လျက်ရှိပါသည်။ မျိုးသန့်ဥယျာဉ်တွင်းရှိ ကလုန်းအရေအတွက် အနည်းအများသည် သစ်စေ့၏အရည်အသွေးပေါ်တွင် ရှိပါသည်။ ပင်တွင်းနှင့် ကလုန်းတွင်းကြား မျိုးဆက်ခြင်းသည် မလိုလားအပ်သည့် ဆိုးကျိုးများဖြစ်သည့် သစ်စေ့ အရည်အသွေး ကျဆင်းခြင်းနှင့် သစ်စေ့ ပေါက်ရောက်မှု အားမကောင်းခြင်းတို့ ဖြစ်ပေါ်လေ့ရှိပါသည်။ ကလုန်းအနည်းငယ်ဖြင့် မျိုးသန့်ဥယျာဉ် တည်ထောင်ပါက မျိုးရိုးဗီဇစုံလင်မှုမှာ နည်းပါးနိုင်ကြောင်း ယေဘုယျ မျှော်လင့်နိုင်ပါသည်။ မြန်မာနိုင်ငံတွင် ကျွန်းမျိုးသန့်ဥယျာဉ်များကို တည်ထောင်ထားသော်လည်း ကလုန်းမျိုးဆက်ပင်တို့၏ မျိုးရိုးဗီဇ စုံလင်မှုကို လေ့လာဖော်ထုတ်ထားခြင်း မရှိသေးပေ။

ယခု သုတေသနသည် မျိုးသန့်ဥယျာဉ် ကလုန်းမျိုးဆက်ပင်များ၏ မျိုးရိုးဗီဇစုံ လင်မှုကို မျိုးရိုးဗီဇ အမှတ်အသား microsatellite markers ၈ မျိုး အသုံးပြု၍ လေ့လာခြင်းဖြစ်ပါ သည်။ ရေဆင်းပျိုးဥယျာဉ်တွင် ပျိုးထောင်ထားသည့် ပျိုးပင်များမှ နမူနာကောက်ယူပါသည်။ ကျွန်းကလုန်း ၂၉ ခုမှ ရရှိသော သစ်စေ့ များဖြင့် ပျိုးထောင်ထားသည့် ပျိုးပင်များမှ ကလုန်း အဖြစ်သတ်မှတ်ထားသည့် ကလုန်းတစ်ခုလျှင် ပျိုးပင် ၂၅ ပင်နှင့် ကလုန်း အဖြစ် သတ်မှတ် ထားသည့် ကလုန်း ၁၀ မျိုးမှ စာရင်းကောက်ယူခဲ့ပါသည်။ စုစုပေါင်း ပျိုးပင်ပေါင်း ၂၅၀ မှ စာရင်း ကောက်ယူခဲ့ပါသည်။

မျိုးရိုးဗီဇအမှတ်အသားရလဒ်မှ အတည်ပြုချက်အရ အဆိုပါသတ်မှတ် ကလုန်းများ၏ ပျိုးပင်များသည် ၎င်းကလုန်းအသီးသီး၏ မျိုးဆက်များ မဟုတ်ကြောင်း တွေ့ရှိရ

ပါသည်။ ရလဒ်များအရ ကျွန်းမျိုးဆက်ပင်များကြား မျိုးရိုးဗီဇစုံလင်မှုမှာ မြင့်မားကြောင်း တွေ့ရပါသည်။ inbreeding coefficient မှာ အနည်းငယ် အပေါင်းလက္ခဏာဆောင်သည့် အဖြေကို ရရှိသော်လည်း Loci ၈ ခုတွင် ၇ ခုမှာ စာရင်းအင်းနည်းလမ်းအရ သိသာသော inbreeding မရှိသည်ကို တွေ့ရှိရပါသည်။ သို့ဖြစ်၍ မျိုးသန့်ဥယျာဉ်တွင် အပင်တွင်းနှင့် မျိုးတူ ကလုန်းကြားမျိုးစပ်ခြင်း သိသာစွာနည်းပါး၍ အပင်ခြားမျိုးဆက်ခြင်း ပိုမိုများပြားသည်ဟု ဆိုနိုင်ပါသည်။ ထို့ကြောင့် ကျွန်းမျိုးသန့် ဥယျာဉ်အား လုံလောက်သည့် ကလုန်းအရေအတွက်ဖြင့် တည်ထောင်ပါက မျိုးရိုးဗီဇစုံလင်မှုအပေါ် အနှုတ်သဘောဆောင်သော သက်ရောက်မှုမရှိ နိုင်ဘဲ စိုက်ခင်းများအတွက် မျိုးသန့်ဥယျာဉ်မှ စိတ်ချစွာ သစ်စေ့စုဆောင်း၍ ပျိုးထောင် စိုက်ပျိုးနိုင်ပါသည်။

မျိုးရိုးဗီဇ အမှတ်အသားများဖြင့် မိခင်အပင်များမှ ရရှိသည့် မျိုးဆက်ပင်များသို့မဟုတ် မတူညီသော ဦးရေစုများရှိ အပင်များမှ စုဆောင်းထားသည့် မျိုးဆက်ပင်များကိုသော်လည်းကောင်း ခွဲခြားနိုင်ပါသည်။ ဦးရေစုဖွဲ့စည်းပုံဆန်းစစ်ခြင်းအရ ဦးရေစု (၅)စု တွေ့ရှိရပြီး မျိုးဆက်ပင်များသည် ဦးရေစု (၅)မှ ပေါက်ရောက်ခဲ့သည့် မျိုးသန့်ပင်များနှင့် ဆက်သွယ်နေသည်ဟု ဆိုနိုင်ပါသည်။ ထို့အပြင် နမူနာ (၂၅၀) မှာ ကလုန်း (၁၀) မျိုး ထက်ပိုသော အပင်များမှ ရယူထားသည့် မျိုးဆက်ပင်မှ ဖြစ်သည်ဟု ရလဒ်ကို ခိုင်မာစေပါ သည်။

Genetic diversity of teak (*Tectona grandis* Linn. f.) progenies from Clonal Seed Orchard (CSO) in Myanmar revealed by microsatellite markers

Abstract

Genetic diversity is one of the most important parameters in breeding programs. It forms the basis for their response towards biotic and abiotic influences and thus is regarded as a prerequisite for adaptability. Many studies have been performed with regard to variation in teak in natural populations. All these studies indicated high level of genetic variation within populations. Seed orchards play a vital role in genetic improvement of forest trees. The success of plantations depends on the genetic quality of seeds that orchards produce. Many factors influence the genetic quality of orchard. Inbreeding, particularly selfing, often leads to reduction in seed yields and poor growth of viable seeds. Due to the limited number of clones in the Clonal Seed Orchard (CSO), low level of genetic diversity is generally expected. However, genetic diversity of the established CSOs is still unknown.

This study was conducted to examine genetic diversity of teak progenies derived from CSO in Letpangone Research Station using eight microsatellite markers. Leaf samples were collected from seedlings from the nursery in 2016. Seedlings were reportedly derived from progenies of 29 clones. 25 seedlings from each prior defined clone were sampled. In total, 250 seedlings from 10 clones were sampled in order to analyse mating system of CSO directly.

DNA markers confirm that seedlings were not true progenies of each clone and mixing and mislabelling took place among seedlings of clones in the past. The study showed that genetic diversity of teak progenies from CSO was relatively high. Moreover, there was slight positive inbreeding coefficient F_{IS} of CSO, indicating a slight deviation from Hardy-Weinberg equilibrium. However, inbreeding for 7 out of eight loci were not significant. Thus, it could be suggested that CSO has more outcrossing among different clones rather than selfing and clonal selfing. Moreover, the study indicates that the CSO can not diminish genetic diversity of teak progenies if they were established using sufficient numbers of clones. This present study

suggested that seeds and seedlings from the CSO can also be safely deployed for teak plantations.

DNA markers can also help us to check whether progenies come from a single mother or from different trees from different population origins. Population structure analysis revealed 5 genetic clusters, suggesting that progenies were from 5 different provenances. Moreover, this study can confirm that all sampled seedlings come from more than 10 putative clones.

Genetic diversity of teak (*Tectona grandis* Linn. f.) progenies from Clonal Seed Orchard (CSO) in Myanmar revealed by microsatellite markers

Introduction

Genetic diversity is one of the most important parameters in breeding programs. It is defined as the total amount of genetic variation carried by individuals within or among genetic unit, it forms the basis for their response towards biotic and abiotic influences and thus is regarded as a prerequisite for adaptability (Hedrick 2001; Reed and Frankham 2003).

Many studies have been performed with regard to variation in teak in natural populations. Natural teak varies in phenology (Bedell, 1989; Kaosa-ard, 1999), in floral biology and seed biology (Tangmitcharoen and Owens, 1997; Gunaga and Vasudeva, 2002; Vasudeva et al., 2004) and in fruit size, weight and viability among natural provenances with different ecological conditions (Palupi and Owens, 1998; Indira, 2003). Wood quality also varies among teak provenances in India (Kjaer et al., 1999; Varghese et al., 2000; Bhat and Priya, 2004). Teak provenances in Myanmar differs in growth, morphological characteristics and correlation with geo-climatic factors (Lwin et al., 2010), with local provenances outperforming than others (Lwin et al., 2010).

Genetic variation of teak has been investigated using RAPD (Nicodemus et al., 2003; Parthiban et al., 2003), AFLPs (Shrestha et al., 2005; Minn, 2007) and microsatellites or Simple Sequence Repeat (SSRs) (Fofana et al., 2009). All these studies indicated high level of genetic variation within populations. Minn et al 2014 reported that there was no significant difference between adults and regeneration in the natural populations while there was strong genetic differentiation between two major regions such as the North and South regions of Myanmar. Moreover, there were trong genetic clusters among two regions and within regions.

Teak has been widely planted not only not only in its native countries but also in other tropical regions, amounting to 4.3 million ha (Kollert and Cherubini 2012). Teak plantation is being established using seeds or clonal materials. Genetic quality of seeds is considered to be one of the important aspects of successful plantation. Improved seeds are generally available through establishment of Seed Production Areas and CSO. Generally, seeds from unknown origins are likely to diminish genetic gain while carefully selected seeds with known origins or from CSO generally tend to produce genetic gains from a minimum range of 2 to 4 percent to a maximum range of 10 to 40 percent.

Seeds, though not genetically identical to mother trees, play an important role in the establishment of teak plantation program in developing countries like Myanmar. Improved seeds from CSO serve as an important source reforestation programs. Considering importance of improved seeds, Forest Department established the first teak Clonal Seed Orchard in Letpankhon Research Station, Oktwin in 1981. However, genetic diversity of the CSO was still unknown.

Many factors influence the genetic quality of orchard. Inbreeding is one of the major concerns for small effective population like CSO when design of CSO is not appropriate. Inbreeding, particularly selfing, often leads to reduction in seed yields and poor growth of viable seeds. The number of clones used in CSO may also affect genetic diversity of their progenies. CSO is generally established using seeds from plus trees in different provenances.

With the application of DNA, population structure among different populations has been successfully identified (Minn et al, 2014). DNA markers can also help us to check whether progenies come from a single mother or from different trees from different population origins.
The present study will deal with assessment of genetic diversity of progenies and their genetic structures in progenies population.

Objectives

The general objective of the study is to provide information for sustainable utilization of teak resources deployed in tree improvement program.

Specific objectives:

The specific objectives of the study are as follows;

- To estimate genetic diversity of progenies derived from CSO and
- To determine population structure of seedlings population.

Materials and Methods

Study species

Teak is a tropical hardwood species being well known for its strength and durability. It naturally occurs in India, Laos, Myanmar and Thailand and is reported to be naturalized in Indonesia. The areas of natural teak forests is estimated to be 29 million ha; Myanmar stands as a heavy weight country possessing the largest share amounting to 13.5 million (Kollert and Cherubini 2012). Due to its economic importance, it is artificially planted not only in its native countries but also in other tropical regions, amounting to 4.3 million ha (Kollert and Cherubini 2012).
Teak is a strong light demander1. It naturally grows in a wide range of climatic conditions (from 800 mm to 3500 mm rainfall). Teak normally occurs <1000 m above sea level1. Well drained alluvial soil is the most preferential site for the best growth of teak2. Teak thrives best on soils with pH (6.0 to 7.0). Calcium and phosphorous play a vital role in the growth of teak 3. Teak flowers are bisexual and hermaphroditic, having 1,200- 3,700 flowers. The flowers open for only one day. Teak is a mainly insect-pollinated species. Teak is partially self-incompatable species. However, it is also reported that teak has mixed mating system (Selfing and outcrossing).

Genetic markers

Studies on genetic variation of forest tree species has advanced with the development of DNA markers including restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs; or microsatellites), and single nucleotide polymorphisms (SNPs). Microsatellites are nowadays widely used for many studies on population genetics, gene flow, mating systems and parentage analysis. They are co-dominant and highly polymorphic markers. Microsatellites primers are mostly species specific. Microsatellite

primers for teak have been developed by Verhaegen et al 2005 and have been successfully applied in teak genetics.

Plant materials

Genomic DNA was extracted from dried leaves. Sampled leaves were immediately stored in silica gel to prevent fungal contamination. Silica gel was periodically checked and replaced to keep material dry. Leaf samples were collected from seedlings from the Yezin Nursery in 2016 (Fig 1). Seedlings were reportedly derived from progenies of 29 clones from the Letpangone CSO after seed collection in 2014. These provenances of 29 clones were Bodaung, Yonepin, Kabaung, Kai, Saiya, Yemathin. The CSO comprised of 76 clones located in different blocks. Due to secondary data collection through nursery seedlings, with assumption of mixing and mislabelling the clones, 25 seedlings from each putative clone was sampled. In total, 250 seedlings from 10 putative clones were sampled in order to analyse mating system of CSO directly. All lab works were conducted in Lab of Forest Biology, Kyoto University, Japan in 2016.

DNA isolation

Genomic DNA was isolated from dried leaves by using the protocol of CTAB protocol. DNA was stored at – 20°C.

Genotyping of SSRs

Eight specific SSR primers were applied in three different multiplex PCR (Verhaegen et al., 2005) (Table 1). The PCR amplification of SSRs was carried out in Takara/ABI GeneAmp 2700 Thermocycler in a volume of5 µl containing 0.9 µl of DNA (> 20 ng), 0.05 µl of each forward and reverse primers (20 µm), 2.5 µl of 10x PCR Qiagen MasterMix and 1.5 µl of milliQ.

The amplification was performed under the following conditions: initial denaturation at 95°C for 15 min, 27 cycles (Multiplex A and B)/ 29 cycles (Multiplex C) of denaturation at 94°C for 30 s, primer annealing at 51°C for 1 min 30 s, primer extension at 72°C for 1 min 30 s, and final extension at 72°C for 10 min. The forward primers were fluorescently labelled with 6-FAM, VIC or NED (see Table). The SSR markers were scanned on ABI 3130xl Genetic Analyzer (Applied Biosystems) with internal size standard (GS Liz 500) from Applied Biosystems. The sizes of fragments were identified by GeneMapper.

Teak progenies (1 yr old) in the nursery of Forest Research Institute

Figure 1 Teak progenies in the nursery of Forest Research Institute

Table 1 8 SSR primers used in this study (Verhaegen et al. 2005)

Primers	PCR Multiplex	5′ dye label	Allelic size	Primer sequences
CIRAD1TeakA06	A	VIC	172-207	F: 5′-CAAAACAAAACCAATAGCCAGAC-3′ R: 5′-TTTCATCATCATCATCAACATCC-3′
CIRAD2TeakB07	A	NED	197-325	F: 5′-GGGTGCTGATGATTTTGAGTT-3′ R: 5′-CTAAGGAGTGAGTGGAGTTTT-3′
CIRAD2TeakC03	A	6-FAM	245-285	F: 5′-AGGTGGGATGTGGTTAGAAGC-3′ R: 5′-AAATGGTCATCAGTGTCAGAA-3′
CIRAD4TeakFO2	A	NED	197-224	F: 5′-CCGGTAAAAAGGTGTGTCA-3′ R: 5′-GAGTGGAAGTGCTAATGGA-3′
CIRAD3TeakDa09	B	6-FAM	173-199	F: 5′-CTCGCTTCTTTCCACATT-3′ R: 5′-ATCATCGCGCATCGTCAA-3′
CIRAD1TeakG02	B	NED	117-158	F: 5′-TTAACGCCAAATCCCAAAG-3′ R: 5′-CACAAAGAGAACCGACGAG-3′
CIRAD3TeakB02	C	NED	197-235	F: 5′-ATGAAGACAAGCCTGGTAGCC-3′ R: 5′-GGAAGACTGGGGAATAACACG-3′
CIRAD3TeakF01	C	6-FAM	174-213	F: 5′-GCTCTCCACCAACCTAAACAA-3′ R: 5′-AAAACGTCTCACCTTCTCACT-3′

Data Analysis

In order to analysis mating system of teak, SSR loci of seedlings from each block were checked manually and also run by CERVUS version 3.0.3 (Kalinowski et al. 2007). However, it was unable to match seedlings to putative clones as open pollinated families. Thus, all data set was treated as one population.

Expected heterozygosity (H_E) (Nei, 1978), observed heterozygosity (H_O) (Nei, 1978) and number of alleles (A) were calculated to estimate genetic diversity within sampled population using Genetic Data Analysis (GDA) ver. 1.0 (Lewis et al., 2001). Inbreeding coefficients (F_{IS}) for the whole population were estimated using FSTAT ver. 2.9.3.2 software (Goudet, 2001) after 1000 permutations. The polymorphic information content (PIC) for each locus in the complete set of samples was calculated using the software CERVUS version 3.0.3 (Kalinowski et al. 2007).

Bayesian clustering approaches implemented in STRUCTURE ver. 2.3.1 (Pritchard et al., 2000) were used to infer the population structure using admixture model. The number of populations (K) was estimated with 5 replicates each for K = 1 to K = 10 using 100000

iterations of Markov Chain and 100000 iterations of burn-in periods. The best estimated *K* was determined with the highest *Ln* (*P* (D)) with lowest deviation. Delta *K* was also estimated using Evanno et al. (2005) method by the web-based STRUCTURE HARVESTER program (Earl and vonHoldt, 2011).

Results

Genetic diversity at progenies of CSO

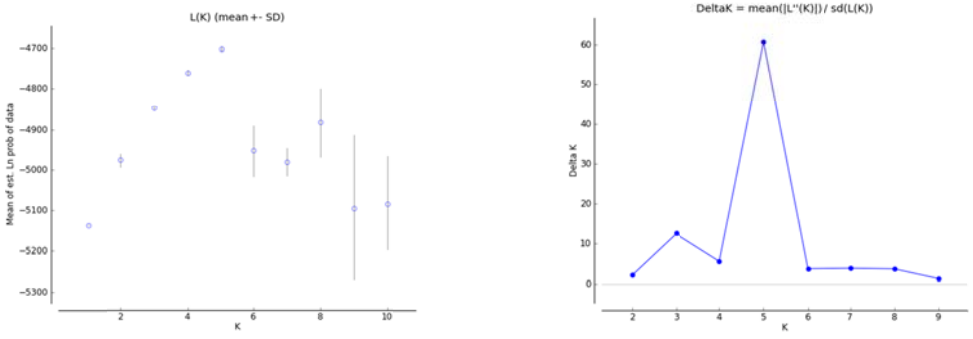
The number of alleles (A) ranges from 5 at G02 to 17 at B02. A total of 10 alleles were detected in all progenies. The expected heterzygostity (He) ranges from 0.176 at G02 to 0.859 at C03. The average value of the inbreeding coefficient was 0.011, indicating a slight deviation from Hardy-Weinberg equilibrium. The total expected heterozygosity was 0.571. The strength of a molecular marker for analyzing genetic diversity is evaluated on the basis of its polymorphic information content (PIC). The PIC of a marker is estimated from the number and frequency of alleles which provides an indicator of genetic diversity. The mean PIC for all eight markers were >0.500 (Botstein et al., 1980) supporting the suitability for analysis of genetic diversity in teak.

Table 2 Genetic diversity at SSR loci

Locus	n	A	H_E	H_O	F_{IS}
A06	243	10	0.788	0.819	-0.039_{ns}
B02	242	17	0.752	0.748	0.005_{ns}
B07	246	9	0.324	0.280	0.133_{ns}
C03	237	15	0.859	0.882	-0.027_{ns}
Da09	245	8	0.750	0.678	0.096*
F01	244	9	0.548	0.520	0.050_{ns}
F02	244	6	0.386	0.418	-0.082_{ns}
G02	250	5	0.165	0.176	-0.066_{ns}
All	244	9.875	0.571	0.565	0.011

n= number of samples, A= number of alleles, H_E = Expected heterozygosity, H_O = Observed heterozygosity, F_{IS} = inbreeding coefficient, ns= not significant at 0.05, *= significant at 0.005,

Population structure



(A) (B)

Figure 2 Population structure of T. grandis populations at SSRs. Detection of the true number of clusters K. (A) distribution of mean of estimated Ln probability of data based on 8 SSR loci for the admixture model (B) magnitude of ΔK as a function of K (Evanno et al., 2005).

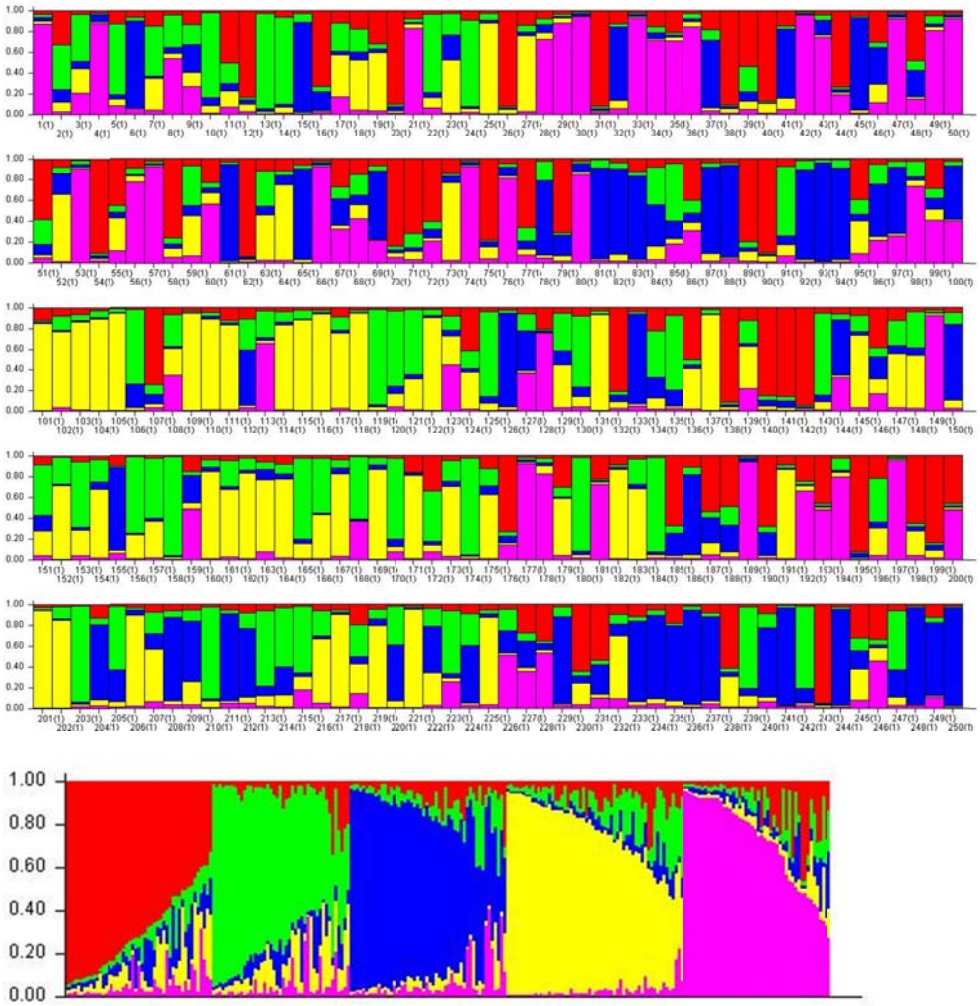


Figure 3 Clusters of *T. grandis* populations based on the admixture model at SSRs.

Population structure at Bayesian analysis

The Bayesian analysis of population structure was conducted using the correlated allele frequency model, detecting five groups (K = 5) (Fig. 3). The number of populations (K) was determined by the highest absolute probability value Ln (P (D)) (Fig. 2A). Additionally, the optimal number of population (K = 5) was also chosen by Evanno et al. method based on the highest ΔK value (Fig. 2B). Assignment of individual genotypes followed the prior defined clones as most individual genotypes were not assigned to the prior cluster of each region.

Discussion

Genetic diversity within teak natural populations is generally high (Minn et al 2014). The genetic diversity of progenies derived from CSO is also comparable to that of natural populations (Minn et al. 2014). This high level of genetic diversity could be due to considerable number of clones from different provenances in the CSO. It also suggests that

the design of CSO did not affect genetic diversity, but genetic gain from this CSO should be investigated further.

One of the most important factors affecting fitness and growth of most species is inbreeding. It refers to mating between genetically related individuals including self-fertilization and is usually quantified by the coefficient of inbreeding F. It affects all loci indiscriminately and causes genome-wide excesses of homozygosity (Hedrick 2005). Inbreeding mostly results in a fitness decline due to the increase in homozygous genotypes with deleterious alleles. Inbreeding was not observed in the study; thus, it can also be suggested that there was no preferential individual selfing or clonal selfing in the CSO. Moreover, due to the absence of inbreeding, seeds can be safely deployed for reforestation programs.

Bayesian analysis revealed that there were 5 distinct clusters for all progenies derived from CSO, indicating that progenies were from at least 5 different provenances. It is noted that assignment of individual genotypes followed the prior putatively clones as most individual genotypes were not assigned to the prior cluster of each region. It indicates that seedlings were not correctly assigned to putative mother clones and mixing among clones might have been taken place in seed collection and/or nursery stage. Thus, this study can confirm that all sampled seedlings come from more than 10 putative clones. The population structure analysis offers us opportunity to identify the number of possible populations even in the progenies stages, notwithstanding it was unable to assign clones into specific populations in this study.

Conclusion and recommendations

This study solely provides genetic diversity and structure of progenies from CSO in Myanmar revealed by SSRs. Due to high level of genetic diversity maintained in CSO, seeds from the CSO can be safely deployed for reforestation programs. In original plan, it was to infer the mating system of CSO but it was to estimate genetic diversity and structure of CSO population as the marker analysis confirmed that there was mislabelling and mixing in seed collection and nursery stages.

Population structure analysis of CSO detected five distinct genetic clusters. Thus, origins of seeds could still be tracked back even in the progenies stages. Genetic identities could still be maintained in the regeneration stages. Strong genetic differentiation among progenies could also contribute to high genetic diversity and thus suggesting the level of clones in these CSO are good enough to maintain the level of genetic diversity. Moreover, due to high level of genetic diversity, the seeds of CSO could safely be deployed for reforestation program. The study provides important information for sustainable utilization of teak resources deployed in tree improvement program.

The following points are recommended for further actions:

- Mating system of CSO should be examined using seeds from CSO directly.
- Genetic identity of clones should be checked if they are really clones or from host plant.
- Genetic quality of seeds and seedlings from different clones should also be checked further.

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