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Genetic diversity of a tropical tree species *Guazuma crinita* Mart. (Malvaceae) in the Peruvian Amazon

PhD thesis

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Declaration

I, Lady Laura Tuisima Coral, hereby declare that I have elaborated this thesis independently and quoted only sources listed in References.

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Lady Laura Tuisima Coral

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A Adenine

ADD Air-dry density

AFLP Amplified Fragment Length Polymorphism

AMOVA Analysis of Molecular Variance

ANOVA Analysis of Variance

BD basic density

bp Base pair

BSA Bovine Serum Albumin

C Cytosine

CV Coefficient of variation

CTAB Hexadecyltrimethylammonium bromide

DNA Deoxyribonucleic Acid

dNTPs Deoxynucleotide triphospahte

EDTA Ethylenediaminetetraacetic acid

G Guanine

GD Green Density

ICRAF The World Agroforestry Center

IAA Isoamylalcohol

IIAP Research Institution of the Peruvian Amazon

ISSR Inter Simple Sequence Repeat

m.a.s.l. Meters above sea level

MC Moisture Content

ODD Oven-dry density

PCR Polymerase Chain Reaction

PCoA Principal Coordinates Analysis

PF Polymorphic Fragments

PPB Percentage of Polymorphic Bands

PPF Percentage of Polymorphic Fragments

R Radial

RAPD Random Amplified Polymorphic DNA

SD Standard deviation

SHR Shrinkage

SPG Specific gravity

SSR Simple Sequence Repeats

T Tangential

UBC University of British Columbia

UNIA Universidad Nacional Intercultural de la Amazonía

UNU Universidad Nacional de Ucayali

V Volumetric

v version

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Abstract

Fast growing tree species *Guazuma crinita* (Malvaceae) was selected as a priority species for domestication in the Peruvian Amazon due its important contribution to the livelihood of local farmers. Its domestication process is still in an early age, as for many tropical tree species little is known about its genetic variability and we don't know anything about the impact of domestication on its genetic resources. The main objective of this research was to assess the genetic variability of *G. crinita* within and among populations in the Peruvian Amazon by the use of morphological (wood physical traits) and molecular (ISSR and AFLP) markers.

Wood physical properties among six *G. crinita* provenances were evaluated. Wood samples were drilled from the base, middle and top of the stem of 12 randomly selected eight-years-old trees for determination of wood moisture content, density, specific gravity, radial, tangential and volumetric shrinkage and the coefficient of anisotropy. Pearson correlations between physical properties were also determined. All wood physical properties, except green density, differed significantly ($p \leq 0.05$) among provenances. We also found statistically significant variation ($p \leq 0.05$) due to stem level position. The moderately dense wood and the coefficient of anisotropy (1.6) suggested that *G. crinita* has stable wood; they represent important advantages in terms of costs for transport and transformation process. The results suggested potential to select provenances with desirable wood properties for further breeding and domestication. Due to the variation found even in limited tree samples it is recommended further analysis with more extensive number of samples from different provenances and planting zones.

This research presents the first assessment of genetic variability based on inter-simple sequence repeat (ISSR) markers for 44 *G. crinita* genotypes from a clonal garden multiplication established in the Peruvian Amazon Research Institute (IIAP) in Ucayali region. Ten ISSR primers amplified a total of 65 bands of which 61 were polymorphic (93.8%). The range of DNA amplification varied from 260 to 2,200 bp. Among the provenances, overall genetic differentiation (G_{st}) was 0.03, indicating 97% of genetic diversity within provenances. Gene flow (Nm) was 12.9 alleles per generation. Cluster analysis was not related with geographic origin suggesting a common gene pool, which was supported by calculation of weak positive correlation ($r = 0.27$, $p < 0.05$) was found between genetic and geographic distance.

With the use of AFLP markers an insight on how domestication process does impact *G. crinita* genetic resources is also reported on this research work. I was able to generate fingerprint for 58 leaf samples representing eight provenances and three population types, 19

from a natural regenerated population, 15 cultivated in home garden nursery and 24 from a collection of genotypes considered as semi-domesticated population. Seven selective AFLP primer combinations were used. A total of 171 fragments were amplified with 99.42% of polymorphism at species level. Each type of population generated 124, 84 and 93 fragments with 72.51%, 49.12% and 54.39% of polymorphic fragments, respectively. Nei's genetic diversity and Shannon index information were found to be higher in the population of natural regeneration compared to overall semi-domesticated population (*He* = 0.10 and 0.9; *I* = 0.19 and 0.16, respectively). The analysis of molecular variation (AMOVA) showed higher variation within provenances rather than among (84% and 4%, respectively). UPGMA clusters analysis and PCoA did not showed correspondence between genetic and geographic distance, in addition their correlation was not significant. There was a significant genetic differentiation among types of population (PhiRT=0.12 at $p < 0.001$), suggesting slight genetic bottleneck in semi-domesticated populations, yet with relatively high levels of genetic variation.

In situ conservation for populations with high levels of genetic diversity was recommended. In addition proper management of natural regeneration and *ex situ* genotype collections, might be a good conservation strategy to maintain *G. crinita* genetic resources.

The use of morphological (wood physical traits) and molecular markers were successful to reveal genetic variability of *G. crinita* and they could be used for other tropical tree species. For further researches it is emphasis to extent the number of samples and geographic scale.

Key words

Bolaina blanca, genetic diversity, semi-domesticated population, wood physical properties.

Abstrakt

Guazuma crinita (Malvaceae) je dřevina pocházející z peruánské Amazonie a díky svému důležitému významu pro místní zemědělce byla vybrána jako vhodná pro domestikaci. Tento proces je stále ještě v raném stádiu. Tak jako u mnoha jiných druhů dřevin je málo známá její genetická variabilita a stále není jasné, jaký bude mít dopad proces domestikace na její genetické zdroje. Hlavním cílem tohoto výzkumu bylo posoudit genetickou variabilitu *G. crinita* mezi různými populacemi v peruánské Amazonii pomocí morfologických (kvalita dřeva) a molekulárních (ISSR a AFLP) markerů.

První část této studie se zaměřila na variabilitu fyzikálních vlastností dřeva u této dřeviny v rámci jedinců původem z šesti různých lokalit. Vzorky dřeva byly získány ze spodní, střední a horní části kmene. U vzorků dřeva z dvanácti náhodně vybraných stromů, starých osm let, byla stanovena vlhkost, hustota, specifická hmotnost, radiální, tangenciální a objemové smrštění a koeficient anizotropie. Dále byly stanoveny vzájemné vztahy mezi těmito fyzikálními vlastnostmi. Všechny fyzikální vlastnosti dřevin, s výjimkou čerstvé hustoty, se významně lišily ($p \leq 0.05$) mezi jednotlivými lokalitami. Statisticky významné rozdíly byly nalezeny i ($p \leq 0.05$) mezi vzorky z různých částí stromu. Hustota dřeva a koeficient anizotropie (1.6) ukázal, že *G. crinita* má stabilní dřevo, představující významné výhody, pokud jde o náklady na dopravu a proces zpracování. Výsledky ukázaly potenciál pro výběr vhodných genotypů s požadovanými vlastnostmi dřeva pro další výsadbu a domestikaci.

Druhá část studie popisuje výzkum genetické variability na základě využití ISSR markerů u 44 genotypů z klonálního sadu založeného v peruánské Amazonii ve výzkumném ústavu IIAP v oblasti Ucayali. Deset molekulárních markerů (ISSR) bylo amplifikováno v 65-ti lokusech, z toho 61 lokusů bylo polymorfních (93.8%). Rozsah amplifikace DNA se pohybovala od 260 do 2,200 bp. Celková genetická rozdílnost mezi populacemi (G_{st}) byla pouze 0.03, což ukazuje 97% genetické rozmanitosti uvnitř jednotlivýchpopulací . Genetický tok (*Nm*) byl 12.9 allel na generaci. Nenalezli jsme korelaci mezi místem původu a genetickou variabilitou, což značí společný genetický původ těchto jedinců, což dokládá slabá pozitivní korelace ($r = 0.27$ $p < 0.05$) mezi genetickou a geografickou vzdáleností.

Třetí část poskytuje přehled o tom, jaký má domestikační proces dřeviny *G. crinita* vliv na její genetické zdroje. Podařilo se nám zhodnotit 58 vzorků z osmi lokalit tří typů populací, 19 vzorků z přírodní populace, 15 pěstovaných v domácích zahradách a 24 z domestikovaných populací. Použili jsme metodu AFLP se sedmi vybranými primerovými kombinacemi. Celkem 171 fragmentů bylo amplifikováno s 99.42% polymorfismu na úrovni druhu. U testovaných

typů populací byl získán následující počet fragmentů 124, 84 a 93 s úrovní polymorfismu 72.51%, 49.12% a 54.39%. Genetická rozmanitost a Shannon index byly zjištěny vyšší v přírodní populaci ve srovnání s domestikovanou populací (He = 0.1 a 0.9, I = 0.19 a 0.16). AMOVA ukázala vyšší rozdíl v rámci jednotlivých populací než mezi nimi (84% a 4%). UPGMA analýza a PCoA neukázaly statisticky významnou korelaci mezi genetickou a geografickou vzdáleností. Došlo však k výrazné genetické diferenciaci mezi jednotlivými typy populací (PhiRT 0.12 při $p \leq 0{,}001$), což naznačuje zúžení genetické variability u domestikovaných populací, i když genetická variabilita zůstává stále relativně vysoká.

Použití morfologických (kvalita dřeva) a molekulárních markerů úspěšně odhalilo genetickou variabilitu druhu *G. crinita* a tyto metody mohou být použity i pro jiné druhy tropických dřevin. Pro další výzkum doporučujeme rozšíření počtu vzorků i do dalších geografických oblastí.

Klíčová slova

Bolaina blanca, domestikace, fyzikální vlastnosti dřeva, genetická diverzita

Resumen

Guazuma crinita (Malvaceae) es un árbol de rápido crecimiento que fue seleccionado como especie prioritaria para domesticación en la Amazonía Peruana por su importante contribución al sustento del agricultor local. Su proceso de domesticación aún está en una etapa inicial, como en el caso de muchas especies de árboles tropicales poco se sabe sobre su variabilidad genética y el impacto la domesticación sobre sus recursos genéticos. El principal objetivo de esta investigación fue estimar la variabilidad genética de *G. crinita* dentro y entre poblaciones en la Amazonía Peruana por medio del uso de marcadores morfológicos (características físicas de la madera) y moleculares (ISSR y AFLP).

La variabilidad de las propiedades físicas entre seis procedencias de *G. crinita* fue evaluada. Muestras de madera se obtuvieron de la base, medio y ápice del tronco de 12 árboles de ocho años de edad seleccionados al azar para la determinación de contenido de humedad de la madera, densidad básica, peso específico, contracción tangencial, radial y volumétrica, y el coeficiente de anisotropía. Se determinó también la correlación de Pearson. Todas las propiedades físicas a excepción de la densidad saturada, fueron estadísticamente diferentes ($p \leq 0.05$) entre procedencias. También encontramos variación estadísticamente significativa debido al nivel de posición en el tronco. La moderada densidad de la madera y coeficiente de anisotropía (1.6) sugirieron que *G. crinita* tiene madera estable, además representan importantes ventajas en términos de costo de transporte y proceso de transformación. Los resultados indican potencial para seleccionar procedencias con deseables propiedades de la madera para posterior mejoramiento y domesticación. Debido a la variación encontrada aun en un limitado número de nuestras se recomienda futuros análisis con mayor número de muestras de diferentes procedencias y zonas de plantación.

Esta investigación presentamos la primera evaluación de variabilidad genética basada en marcadores ISSR para 44 genotipos de *G. crinita* de un jardín de multiplicación clonal establecido en el Instituto de Investigación de la Amazonía Peruana (IIAP) en la región de Ucayali. Diez cebadores ISSR amplificaron un total de 65 bandas de las cuales 61 fueron polimórficas (93.8%). El rango de amplificación de ADN varió desde 260 hasta 2 200 pares de base. La diferenciación genética general (G_{st}) fue 0.03, indicando 97% de diversidad genética dentro de las procedencias. El flujo genético (Nm) fue 12.9 alelos por generación. El análisis de agrupamiento no se relacionó con el origen geográfico, indicando un común acervo genético, apoyado por el cálculo de correlación positiva débil ($r = 0.27$, $p < 0.05$) entre las distancias genéticas y geográficas.

Con el uso de marcadores AFLP este trabajo de investigación también provee una percepción de cómo el proceso de domesticación impacta los recursos genéticos de la *G. crinita*. Se generó huellas genéticas para 58 muestras que representan a ocho procedencias y tres tipos de población, 19 de una población de regeneración natural, 15 cultivadas en un vivero de huerto familiar y 24 de una colección de genotipos considerados como población semi-domesticada. Se utilizó siete combinaciones de seleccionados cebadores AFLP. Un total de 171 fragmentos fueron amplificados con 99.42% de polimorfismo a nivel de especie. Cada tipo de población generó 124, 84 y 93 fragmentos con 72.51%, 49.12% y 54.39% de fragmentos polimórficos, respectivamente. La diversidad genética de Nei y el índice de información Shannon fueron altos en la población de regeneración natural comparado con la población semi-domesticada en general (*He* = 0.10 y 0.9; *I* = 0.19 y 0.16, respectivamente). El análisis de variación molecular mostró superior variación dentro de las procedencias que entre ellas (84% and 4%, respectivamente). UPGMA análisis de agrupamiento y el análisis de componentes principales no mostraron correspondencia entre las distancias geográficas y genéticas y su correlación no fue significativa. La diferenciación genética entre las poblaciones fue significativa (PhiRT=0.12 at $p < 0.001$) sugiriendo un ligero cuello de botella genético en la poblaciones semi-domesticadas, aún con niveles de variación genética relativamente altos.

Se recomienda conservación *in situ* de poblaciones con mayores niveles de diversidad genética. Además el manejo apropiado de la regeneración natural y de la colección de genotipos *ex situ* puede ser una buena estrategia para mantener los recursos genéticos de *G. crinita*.

El uso de marcadores morfológicos y moleculares fue exitoso para revelar la variabilidad genética de *G. crinita* y podrían ser usados en otras especies de árboles tropicales. Para futuras investigaciones se debe enfatizar la ampliación del número de muestras así como la escala geográfica.

Palabras claves

Bolaina blanca, diversidad genética, población semi-domesticada, propiedades físicas de la madera

1. Introduction

Tropical forests provide many valuable products including rubber, fruits and nuts, medicinal herbs, floral greenery, lumber, firewood, and charcoal (O' Neils et al., 2001). Natural forest populations usually store great amount of genetic variation Simons et al., 1994). However expanding slash-and-burn agriculture by migration into forest areas is the primary cause of deforestation in many parts of the tropics (Mertz et al., 2008), over-harvesting and other poor forestry practices in many areas of the tropics are reducing tree genetic diversity (O' Neils et al., 2001). Moreover strategic approaches to domestication, conservation and long-term viability of wide tree genetic variation may provide the ability to resist potential inbreeding depression through future generations (Simons et al., 1994; Weber et al., 2001).

The domestication of annual crops began over 10,000 years ago and involved processes of selection, breeding and adaptation of germplasm to fulfil human needs. For trees, the same processes are involved as with annual crops, but the great majorities of tree species, especially in tropics, are still essentially wild or are only in early stage, called incipient domesticates (Jamnadass et al., 2012). The successful cultivation of agroforestry tree species requires the implementation of various researches for development activities that address many issues, opportunities and constraints of the different stakeholders involved (Jamnadass et al., 2012), however the breeding programme must be accompanied with genetic characterization of selected species.

Over the last two decades the characterizations of genetic variation using morphological and molecular techniques have been developed (Jamnadass et al., 2009; Leakey et al., 2012). In general very little research on intra-specific genetic variation in agroforestry tree species in the tropics has been reported (Sotelo-Montes et al., 2000; Hollingsworth et al., 2005; Dawson et al., 2008; Weber and Sotelo-Montes, 2008). Genetic variability is essential to the success of strategies for tree improvement including selection, sustainable management and genetic resource conservation (Zobel and Jett, 1995; Sotelo-Montes et al., 2000; O'Neill et al., 2001).

Morphological measurements made in natural stand, field trials and commercial wood traits have been used traditionally to characterize levels and patterns of diversity; in any case many trees have not been properly evaluated in even in this way (Dawson et al., 2012a). Evaluation of genetic variation in wood traits is important for applied tree improvement programs that use selection and breeding to improve wood production (O'Neill et al., 2001; White et al., 2007). Wood trait variation assessment involves the consideration of a number of

anatomical, physical and mechanical properties. Certain wood properties are reported to be good indicators of timber properties and uses. Limited information on variation of wood properties of timber trees in Peruvian Amazon was reported (Sotelo-Montes et al., 2003; Sotelo-Montes et al., 2006; Sotelo-Montes et al., 2007; Sotelo-Montes et al., 2008; Weber and Sotelo-Montes, 2008). However these traits alone represent only a small portion of the plant genome and are also influenced by environmental factors (Muchugi et al., 2008).

The use of genetic markers based on DNA overcome these disadvantages and are complementarily able to measure the genetic diversity in plant species (Feyissa et al., 2007). Genetic markers can be used to characterize levels and patterns of genetic variation in a species, their evolutionary causes and the effects of genetic drift and gene migration on spatial and temporal patterns of variation (White et al., 2007). Therefore they represent important tools to develop conservation and management strategies (Russell et al., 1999; Aga et al., 2005; Dawson et al., 2012b).

A domestication program for tropical tree species started in the Peruvian Amazon in mid-90s, led by World Agroforestry Centre (ICRAF), and identified *Guazuma crinita* Mart. (Malvaceae family) as a priority timber species for agroforestry systems (Sotelo-Montes and Weber, 1997). This species can be inter-cultivated with food crops because it has a small crown with thin branches, and the older branches naturally self-prune in the lower crown. It provides wood products at an early age of about 8-10 years, can be coppiced for successive harvests and contributes significantly to farmers' income (Labarta and Weber, 1998; Putzel et al., 2013). Due to its fast initial height growth (up to 3 m per year) it has been promoted in reforestation programs and agroforestry systems (Sotelo-Montes et al., 2000; IIAP, 2009) across Amazon region. It is recognized as a versatile pioneer tree species, with excellent adaptability to a wide range of sites, including degraded areas of exploited forest and areas of poorly drained heavy clay soils (Maruyama et al., 1989; Maruyama et al., 1997; Flores, 2000; Soudre, 2012). This species produces a soft and light wood, with good quality and excellent properties for panelling, interior joinery, mouldings and housing construction (Maruyama et al., 1997; Reynel et al., 2003; Putzel et al., 2013).

A provenance test and a provenance/progeny test of *G. crinita* were established in the Aguaytia watershed in Peruvian Amazon to investigate genetic variation on quantitative traits and environmental differences among planting zones in the watershed (Sotelo-Montes et al., 2000; Rochon, 2004; Rochon et al., 2007; Weber and Sotelo-Montes 2008; Weber et al., 2011). Results from the provenance test indicated that the provenance from the local watershed (Aguaytia) would perform better in terms of growth than provenances from other watersheds

in the Peruvian Amazon Basin (Sotelo-Montes et al., 2000). In addition, methods for germplasm's conservation (Maruyama et al., 1997), micropropagation and grafting technologies (Villegas, 2008; Rollo, 2009; Soudre, 2012) have been developed. However, up to now, there are no published reports on genetic diversity of *G. crinita* using any type of molecular markers.

The present research was aimed to assess genetic variability of *G. crinita*, among various natural and semi-domesticated populations in the Peruvian Amazon and also to evaluate its wood physical properties.

2. Literature Review

2.1 Genetic Markers

Population genetics is about assessing genetic variation and the way it is structured in an organism, and then using this information to help explain aspects of the biology of a species and thereby help determine appropriate management options (Dawson et al., 2012b). The factors determining the level and structure of genetic variation within plant species, include, evolutionary history characteristics, population density, mating system and mechanisms of gene flow (Thangjam, 2014). Genetic variation can be estimated by the use of different type of genetic markers (Farooq and Azam, 2002). Morphological traits are the oldest and most widely used genetic markers, and they may still be optimal for certain germplasm management applications (Azofeira-Delgado, 2006).

To design a molecular marker study properly, a basic understanding of the forces involved in structuring genetic diversity within and among populations is required, forces such as mutation, migration, recombination and selection (Muchugi et al., 2008). Dawson et al., (2012b) described these forces; mutation is a permanent structural alteration in DNA and the ultimate source of genetic variation, which results in the development of new alleles (the variant forms of a gene at a particular locus). Migration is the movement of any form of genetic material from one population to another. It can occur through the natural dispersal of seed and pollen, or it may be facilitated by humans. Recombination is the process of generating new mixtures of genetic diversity by exchanging parts of the chromosomes that make up the genome of an organism. Selection is a natural and human-induced process that occurs when the genetic variation in an organism influences its fitness (ability to survive and reproduce in a particular environment).

In tree species, genetic variation have been assessed using phenotypic measurements made in natural stands and field trials, or by asking the people that use trees about the characteristics they observe and value. Most tree improvement programs assess variation in growth, form, adaptability and pest resistance but do not include wood traits (Zobel and Jett, 1995). These traits alone present only a small portion of the plant genome and are also influenced by environmental factors, thereby limiting their utility in describing the potentially complex genetic structures which may exist within and between taxa (White et al., 2007; Dawson et al., 2012b). To overcome these constraints, in the last two decades various independent DNA markers have been developed, they were used and still being utilized in plant breeding programmes with well-defined objectives, (Farooq, 2001; Aga et al., 2005; Cao et al., 2009).

2.1.1 Molecular Markers

The information for different genes and gene systems in higher plants is stored in the DNA sequences of the nuclear chromosomes, chloroplast and mitochondrial (Bretting and Widrlechner, 1995; Azofeira-Delgado, 2006). The DNA content of plant genome differs significantly between species, (Farooq and Azam, 2002). DNA provides many advantages that make it especially attractive in studies of diversity and relationships; (1) Freedom from environmental and pleiotropic effects. DNA characters have a much better chance of providing homologous traits; (2) A potentially unlimited number of independent markers are available; (3) DNA characters can be more easily scored as discrete states of alleles or DNA base pairs; (4) Many molecular markers are selectively neutral (Kumar et al., 2009). Bretting and Widrlechner (1995); Farooq and Azam (2002); Azofeira-Delgado (2006) described some desirable properties for an ideal molecular marker such as (A) highly polymorphic nature: It must be polymorphic as it is polymorphism that is measured for genetic diversity studies, (B) codominant inheritance: determination of homozygous and heterozygous states of diploid organisms, (C) frequent occurrence in genome: A marker should be evenly and frequently distributed throughout the genome, (D) selective neutral behaviours: The DNA sequences of any organism are neutral to environmental conditions or management practices (E) easy access (availability): It should be easy, fast and cheap to detect, (F) easy and fast assay, (G) high reproducibility (H) easy exchange of data between laboratories.

Several polymerase chain reactions (PCR)-based DNA markers have been widely used for the detection of genetic variation and structure in many crops and trees species (Aga et al., 2005; Russell et al., 1999). Among these, inter simple sequence repeats (ISSR) markers combines some advantages of other markers such as reproducibility, low costs and no need to develop species-specific primers for analysis (Feyissa et al., 2007; Thangjam, 2014). Therefore it was chosen to perform a preliminary analysis with the first sample collection.

In the second molecular analysis, Amplified Fragment Length Polymorphism (AFLP) technique (Vos et al., 1995) was used, it is able to provide high levels of resolution to allow the selective amplification of subsets of genomic restriction fragments (Cao et al., 2009). Such advantage was the reason why it was decided to use this technique for the assessment of genetic diversity among three types of populations (natural regeneration, home garden nursery and semi-domesticated populations).

Both technologies are accessible and quickly provide large numbers of polymorphic markers with universal reagents (Russell et al., 1999; Bérgamo de Souza et al., 2013).

2.1.2 ISSR Markers

Inter-simple sequence repeat is a technique, reported by Zietkiewicz et al. (1994) based on microsatellites and they are utilized to amplify inter-SSR DNA sequences. ISSR analysis is based on DNA amplification with a single 15- to 20-bp primer, which is homologous to a microsatellite repeat and has a short (2–4 bp) random degenerated sequence (an anchor) at the 3' or 5' end. With an anchor sequence, ISSR primers are more selective and amplification patterns better reproducible (Gupta et al., 1994; Wu et al., 1994; Zietkiewicz et al., 1994). About 10–60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size. ISSR-PCR process was schematized in Figure 1. Because ISSR is a multilocus technique; disadvantages include the possible non-homology of similar sized fragments. ISSRs segregate mostly as dominant markers following simple Mendelian inheritance (Gupta et al., 1994; Tsumura et al., 1996; Wang et al., 1998). However, they have also been shown to segregate as co-dominant markers in some cases thus enabling distinction between homozygotes and heterozygotes (Wu et al., 1994; Akagi et al., 1996; Wang et al., 1998; Sankar and Moore, 2001). The main advantage of ISSRs is that no prior sequence data for primer construction are needed (Joshi et al., 2000). As the analytical procedures include PCR, only low quantities of template DNA are required (5–50ng per reaction) (Kojima et al., 1998). The technique is simple, quick, and the use of radioactivity is not essential.

A number of authors reported the use of ISSR markers with different purposes such as the assessment of genome polymorphism at the inter-specific level and establishment phylogenetic relationships and taxonomic ranks (Salimath et al., 1995; Wolff et al., 1998). Naik et al. (2009) assessed genetic diversity in *Gmelina arborea* Roxb., (Verbenaceae) the markers yielded 95% polymorphic loci. High level of gene differentiation was observed at species level (G_{st} =0.6) indicating that the populations were subjected to genetic isolation; reduction and fragmentation of forest cover could be one of the main causes that led to an increase of genetic differentiation and reduce gene flow between such a populations. ISSR markers were used also to determine genetic mapping in Citrus, genetic diversity in the endangered tropical tree species *Hagenia abyssinica* J.F. Gmel., in *Centella asiatica* (Linn.) Urban., in *Parkia. timoriana* (DC.) Merr., (Sankar and Moore, 2001; Fayissa et al., 2007; Zhang et al., 2012; Thangjam, 2014). The information obtained through genetic analysis led to selection of adequate germplasm for plant management and conservations of target sited in order to maintain high genetic bases.

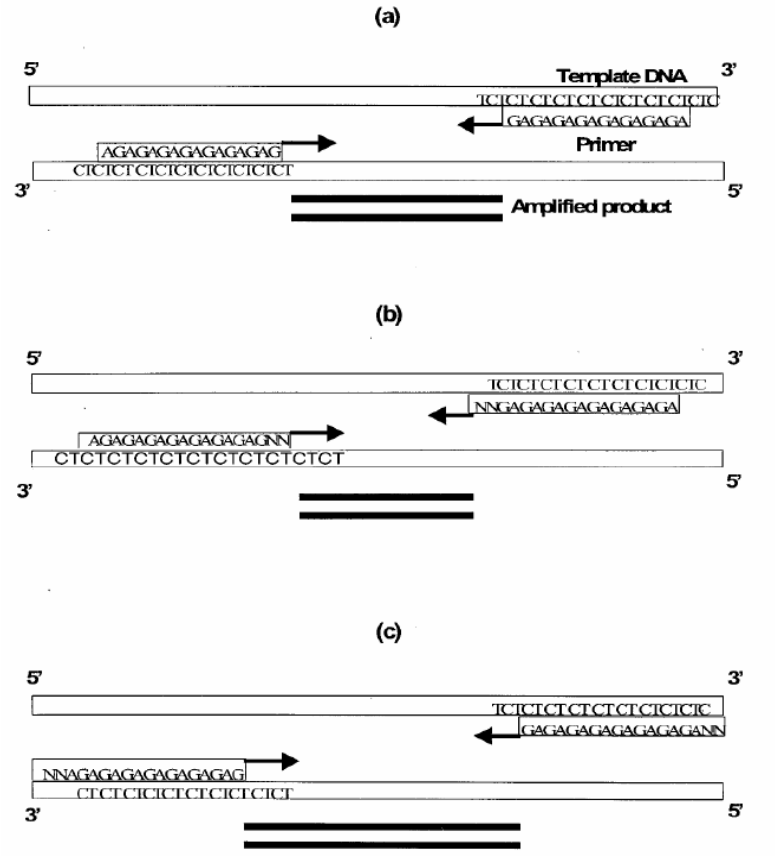

Figure 1 A schematic representation of ISSR-PCR

With a single primer (AG)8, unanchored (a), 3'-anchored (b) and 5'-anchored (c) targeting a (TC)n repeat used to amplify inter simple sequence repeat region flanked by two inversely oriented (TC)n sequences. (a) Unanchored (AG)n primer can anneal anywhere in the (TC)n repeat region on the template DNA leading to slippage and ultimately smear formation; (b) (AG)n primer anchored with 2 nucleotides (NN) at the 3' end anneals at specific regions on the template DNA and produces clear bands; (c) (AG)n primer anchored with 2 nucleotides (NN) at the 5' end anneals at specific regions and amplifies part of the repeat region also leading to larger bands (Reddy et al.,. 2002).

2.1.3 AFLP Markers

AFLPs are fragments of DNA that have been amplified using directed primers from restriction digestion of genomic DNA (Mueller and Wolfenbarger, 1999; Kumar et al., 2009). AFLP technique combines the power of RFLP (hybridization technique) with the flexibility of PCR-based technology by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998). The key feature of AFLP is its capacity for "genome representation": the simultaneous screening of representative DNA regions distributed randomly throughout the genome.

AFLP markers can be generated for DNA of any organism without initial investment in primer/probe development and sequence analysis. Both good quality and partially degraded DNA can be used for digestion but the DNA should be free of restriction enzyme and PCR inhibitors (Vos et al., 1995). The technique involves four steps: (1) restriction of DNA and ligation of oligonucletide adapters (2) pre-selective amplification (3) selective amplification (4) gel analysis of amplified fragments (Gaiotto et al., 1997; Kumar et al., 2009). The advantages of AFLP include; (1) it is highly reliable and reproducible (Mueller and Wolfenbarger, 1999; Powell et al., 1996; Jones et al., 1997), (2) it does not require any DNA sequence information from the organism under study, (3) It is information-rich due to its ability to analyse a large number of polymorphic loci simultaneously (effective multiplex ratio) with a single primer combination on a single gel (Russell et al., 1997), (4) Co-migrating AFLP amplification products are mostly homologous and locus specific (Waugh et al., 1997; Qi et al., 1998), with exceptions in polyploidy species. Figure 2 shows the steps of AFLP technique.

AFLP markers have been used in the assessment of genetic variability in a number of tree species (*e.g.*, Cao et al., 2009; Sirelkhatem and Gaali, 2009; Kariba, 2013). Gaiotto et al. 1997 estimated outcrossing rates in *Eucalyptus urophylla* S.T. Blaket., population (Brazil), they indicated predominant outcrossing and suggest maintenance of adequate genetic variability within families. Russell et al. 1999 analysed nine populations of *Calycophyllum spruceanum* Benth., in the Peruvian Amazon, the result specified 91% of variation within population and 9% among populations. Miller and Schaal (2006) assessed genetic diversity in clonally propagated domesticated stands, and sexually reproducing wild populations, of a fruit tree, *Spondias purpurea* L. by AFLP markers. They found out significant genetic diversity within agricultural habitats. Cultivated plants of *S. purpurea* were found to be lower distinctly than wild population. The genetic structure was most similar among stands of backyard stands and wild population. Living fence and orchard stands had a third more variability within populations, probably due to the relatively high levels of vegetative reproduction.

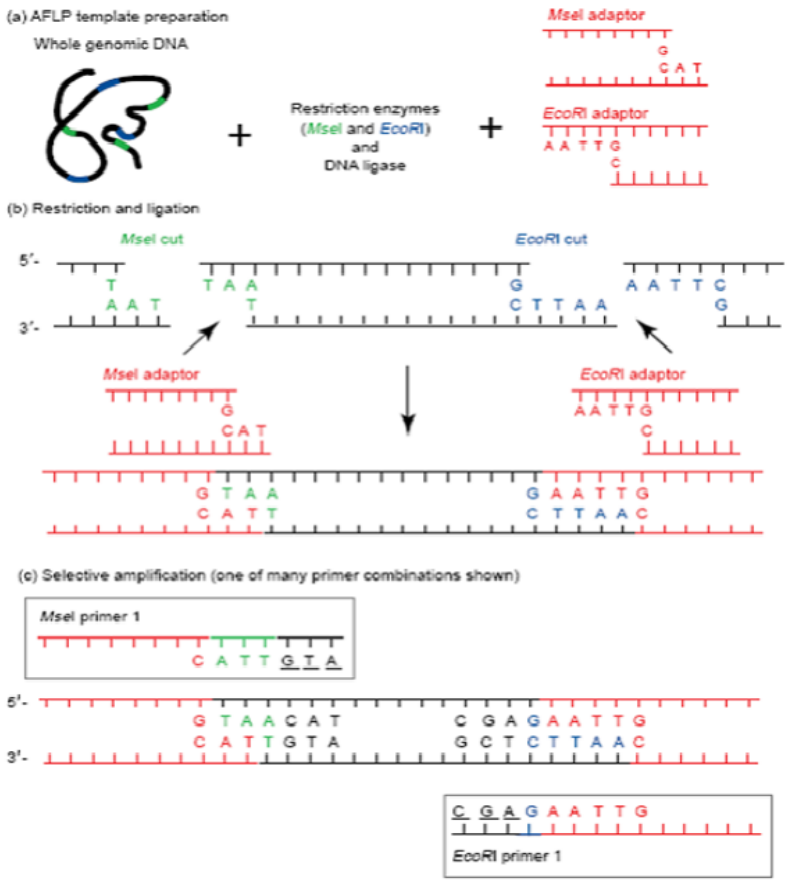

Figure 2 Steps in AFP analysis

Small amounts of DNA are digested with two restriction enzymes (a), and adaptors are joined (ligated) to these ends (b). The end sequences of each adapted fragment consist of the adaptor sequence (in red) and the remaining part of the restriction sequence (in blue and green). To achieve amplification of a subset of these fragments, primers are extended into the unknown part of the fragments [underlined base pairs (bp)], usually one to three arbitrarily chosen bases beyond the restriction site (c, in black). The first PCR (pre-amplification) is performed with a single-bp extension, followed by a more selective primer with up to a 3-bp extension (Mueller and Wolfenbarger, 1999)

2.2 Molecular markers used in tropical tree species

Molecular techniques have been used to analyse several tropical tree species (Leakey et al., 2012). For example; Gillies et al. (1997) assessed genetic variation within and between 10 populations of *Cedrela odorata* L., (Meliaceae) in Costa Rica using RAPDs markers, the analysis revealed high genetic variability within populations and lack of genetic differentiation within populations between regions. That may be an indication of an effective gene flow between the populations. Moreover the results also proposed some areas that need to be adequately conserved to maintain the full breadth of genetic variation. In addition plantations of *C. odorata* being established in areas where it is rare should be careful to use seed from the same zone to reduce risk of genetic pollution.

Russell et al. (1999) analysed genetic variation of nine populations of a riverine tree *C. spruceanum* (Rubiaceae) along river tributaries of the Peruvian Amazon employing AFLP markers. Their results indicated most variation among individuals within populations and low degree of structuring among populations. Therefore the importance of germplasm evaluation and conservations of this species should be focused on sites considering of their wide genetic base.

Moderate but significant degree of differentiation (F_{st} = 0.097) of *Swietenia macrophylla* King., (Meliaceae) across Brazilian Amazon was reported by Lemes et al. (2003) using microsatellites markers. Furthermore some populations exhibited a significant inbreeding coefficient. These findings underscored the need for *in situ* conservation of multiple populations of *S. macrophylla* across its distribution in the Brazilian Amazon. In addition, the occurrence of microgeographical genetic differentiation at a local scale indicated the importance of maintaining populations in their diverse habitats.

The effect of long-term fragmentation on the genetic diversity of populations of the neotropical tree species, *Terminalia amazonia* (J. F. Gmel) Excell., (Combretaceae) was studied using RAPDs analysis. The research suggested long-term fragmentation of populations of *T. amazonia*, on average, had a negative effect on the genetic diversity of this species However, their data suggested that sites with sufficient cover may acquire higher levels of genetic diversity, possibly as a result of increased gene flow. Ultimately, it is likely that the effect of fragmentation will be species specific and will depend on each species' reproductive biology and in particular the response of their pollen and seed dispersal vectors to the modified landscape (Pither et al., 2003).

Kelly et al. (2004) studied the impact of human activities on temporal and spatial genetic variation of *Vitellaria paradoxa* G. Don., (shea tree) in Africa where little variation was found

at microsatellite loci between sampled stands with no evidence for genetic bottleneck events in agroforests, possibly due to the buffering effect of extensive gene flow between unmanaged and managed populations.

Hollingsworth et al. (2005) published the first report on comparative geographically matched sampling for natural and planted tree stands of *Inga edulis* Mart., from the Peruvian Amazon. Levels of allelic variation were lower in planted than natural stands, it may supports the perspective that human intervention in the Amazonian rain forest is impacting on levels of genetic variation of populations in this species. However an evaluation of absolute levels of SSR diversity indicated that planted populations have not experienced extreme bottlenecks.

Genetic variation of forest coffee trees (*Coffea arabica* L.) from four regions of Ethiopia was investigated using ISSR markers by Aga et al. (2005); results indicated that most of the variability was between populations than within populations, therefore it reflected high level of genetic differentiation perhaps because of substantial gene flow between adjacent populations in each region assisted by man in the process of transplantation or by wild animals such as monkeys. The information provided advice to select sites for *in situ* conservation. Masambuko and Bryngelsson (2006), also evaluated coffee accessions in Tanzania to estimate levels of genetic similarity, mean dissimilarity values between provenances (0.56-0.85) were higher than within provenances (0.37- 0.68), results confirmed the limited genetic diversity present in cultivated *C. arabica* in Tanzania. It also evidenced a different group of coffee accessions in Tanzania that could be of interest to the coffee improvement program in Tanzania.

Molecular characterization of 25 *Hevea brasiliensis* Müll. Arg., (Euphorbiaceae) clones from Asia, South and Central America was realized by Hernández et al. (2006). Clones originated in South America were most different of others introduced from Asia. The analysis led to understand relationship among the clones which could be useful in breeding programs.

The identification and use of sex-specific molecular markers suggest that the sex of young plants of *Uapaca kirkiana* Müll. Arg., can be differentiated, and that the relevant genes are autosomal (Mwase et al., 2010). This result has important implications for tree domestication of dioecious species in the future. As the understanding of genetic variation based on these genomic studies increases, there are likely to be rapid advances in tree domestication.

2.3 *Guazuma crinita* Mart. (Malvaceae)

2.3.1 Origin and distribution

G. crinita is a fast-growing pioneer species that colonizes the floodplain and disturbed secondary forests in the lowland jungle (below 1,000 m elevation) in the Amazonian basin of Peru, Ecuador, and Brazil (Freytag, 1951; Encarnación, 1983). It is very wide in the neotropics from Central America to the Amazon region to southern Brazil and Bolivia, mostly up to 1500 m.a.s.l (Reynel et al., 2003).

Figure 3 *Guazuma crinita* distribution

Sites where specimens of *G. crinita* are collected, indicating the distribution of the species in the Western Amazon region. Data acquired from Missouri Botanical Garden and New York Botanical Garden online herbaria. (Map: M. Agus Salim/CIFOR)

It is recognized as a versatile tree species, with excellent adaptability to a wide range of sites, including degraded areas of exploited forest and areas of poorly drained heavy clay soils and (Maruyama et al., 1989; Maruyama et al., 1997; Flores, 2000; Soudre, 2012). This species occurs naturally in great abundance in the floodplain forests of the Ucayali River watershed in the Peruvian Amazon (Figure 3).

2.3.2 Taxonomy and botanical description

Guazuma crinita Mart. belongs to Malvaceae family, formerly to Sterculiaceae family (Latta, 2008). DNA evidence has shown that many of the genera in those Sterculiaceae form evolutionary lineages, leading the 2009 Angiosperm Phylogeny Group (APG III) to include them in a larger single family rather than maintain artificial lineages (ones that do not share a common ancestor) (APG, 2009). *G. crinita* known with common names as "Bolaina" or "Bolaina blanca" botanical synonyms: *Guazuma poeppig rosea*. Is a fast-growing tree with an initial growth rate of up to 3m in height per year (Maruyama et al., 1989; Maruyama et al., 1997). It can reach up to 25-80 cm in diameter and 15-30 m in total height, with cylindrical stem, the base of the stem straight, ramification from the last third (Reynel et al., 2003).

Smooth outer bark to finely cracked, light brown to grey. Inner bark fibrous tissue forming a finely reticulated, pale yellow to brown rapidly oxidized, it appears to be pulled into strips. Terminal twigs circular, dark when dry, about 3-4 mm in diameter, usually with ferruginous pubescence towards the apical parts, the bark peels off in strips fibrous to be pulled (Reynel et al., 2003).

Leaves simple, entire, chartaceous, margin strait, glabrous and shiny on the upper surface and little tomentose on the under surface, slightly discolored, 5–10 cm long by 3–6.5 cm wide, on petiole 5–15 mm long (Flores, 2004).

Inflorescences are axillary panicles about 8-12 x 3-6 cm flowers. Flowers are small, 8-12 mm long, hermaphrodite, with calyx and corolla present, the pedicels 4-8 mm long, the calyx 2-3 mm long, the corolla 6-12 mm long, pink with five petals, each spoon-shaped with two long appendages at the end, the androecium consists of five columns in the stem end bearing many anthers, gynoecium with ovary superior, ovoid, small (Rodriguez and Sibille, 1996).

Fruits globose capsule about 4-8 mm in diameter with surface densely covered with long hair, about 3-4 cm in length (Reynel et al., 2003). The seed's form is ovoid about 1 mm size.

The main morphological characteristics of *G. crinita* are presented in Figure 4.

Figure 4 Main morphological characteristics of *G. crinita.*
A. Flowers[1] , **B.** Flowering branch[1], **C.** Fruiting branch[1] , **D.** Stem and external bark[2], **E.** *G. crinita* seedling[2]. Photos: [1]A. Gentry, Useful tropical plants database 2014, [2] Medardo Miranda 2015

2.3.3 Uses

It produces a soft and light wood, with good quality, durability and excellent properties for panelling, interior joinery, mouldings and matches (Maruyama et al., 1997; Reynel et al., 2003). In recent years it is increasingly being used in the plywood industry and housing construction (Putzel et al., 2013). The density of this species is medium (417 kg/m^3), with stable coefficient of anisotropy (Weber and Sotelo-Montes, 2008, Sebille-Martina, 2006). It is used in carpentry, making small tools and palettes lollipops, toothpicks, and craft sticks matches. The fibrous inner bark is used locally as tie material (Reynel et al., 2003). *G. crinita* is an important timber-tree species for reforestation and agroforestry plantations in the Peruvian Amazon Basin, they serve as indicator of soil fertility (Rochon et al., 2007). The fibrous inner bark is used locally as tie material (Reynel et al., 2003). The main uses of *G. crinita* are presented in Figure 5.

Figure 5 Main uses and products of *G. crinita* timber tree species

A. Narrow boards[1], **B**. Joined narrow in natural drying process[1], **C.** Manufacture home[2], **D.** Management of natural regeneration[2], **E.** Pallets of *G. crinita*[2]. Photos: [1]Jonathan Cornelius 2007, [2] Medardo Miranda 2015.

2.3.4 Reproduction and ecology

Trees can begin flowering after 2–3 years in open-grown conditions (Weber and Sotelo-Montes, 2008), and the lightweight, feathered capsules are dispersed by both wind and water. The species can potentially produce a dense stand of natural regeneration in open patches (Rochon et al., 2007). In the Peruvian Amazon the flowering time begins by June until August. Its fructification period by August to September and its dissemination time from September to December (Flores, 1999). At the beginning of the rainy season; the seeds are minute and abundant, and germinate readily on alluvial soils common in the region (Diaz González, 2007). In floodplain fallows, *G. crinita* grows quickly, with a rotation age of 6–12 years in the Peruvian Amazon (Weber et al., 2011). The trees grow in dense stands of up to 1200 trees/ha in a young fallow (<3 years) and up to 500 mature trees (≥ 25 cm dbh) per hectare after management by smallholders for sawtimber (Padoch et al., 2008). Local farmers manage this natural ecological niche of *G. crinita* while engaging in other agricultural activities. Clearings made to prepare land for crops are an ideal habitat for bolaina blanca. Because *G. crinita* is tolerant of flooding, it is ideal for management in seasonally flooded areas near rivers and streams (Putzel et al.,

2013). These reproductive characteristics probably result in extensive gene flow, which would produce high levels of genetic variation within populations and relatively low genetic differentiation among populations (Hamrick et al., 1992; Ouborg et al., 1999).

2.3.5 Genetic diversity

There are still very scarce information about genetic diversity of *G. crinita*, the mating system of the tree has not been studied yet, but since most tropical trees are predominantly out-crossing it is assumed the same for this species (Bullock, 1985; Muchugi et al., 2008).

A provenance test and a provenance/progeny test of *G. crinita* were established in the Aguaytia watershed in Peruvian Amazon to investigate genetic variation in growth and wood density, correlations between growth and density, and environmental differences among planting zones in the watershed (Sotelo-Montes et al., 2000; Rochon, 2004; Rochon et al. 2007; Weber and Sotelo-Montes 2008; Weber et al., 2011). Results from the provenance test indicated that the provenance from the local watershed (Aguaytia) would perform better in terms of growth than provenances from other watersheds in the Peruvian Amazon Basin (Sotelo-Montes et al., 2000). Rochon et al. (2007) observed significant genetic variation in tree growth at 12 months age. Wood density also varies genetically among provenances (Weber and Sotelo-Montes 2008). Results from the provenance and provenance/progeny test suggest that fast-growing provenances can be selected at an early age without significantly reducing wood density (Rochon, 2004; Weber and Sotelo-Montes 2008).

In addition, methods for storage of alginate-encapsulated germplasm for conservation (Maruyama et al., 1997), vegetative propagation, micropropagation and grafting technologies (Villegas, 2008; Rollo, 2009; Paredes et al., 2010; Soudre, 2012) have been developed. However, there is not published research on molecular genetic diversity in *G. crinita*. To my best knowledge there are no information published about genetic variability of this species on molecular level.

3. Objectives and Hypotheses

In designing strategies for genetic resource management for tree species, it is important to consider the genetic structure revealed by both neutral (molecular markers) and adaptive traits (wood physical properties) (Eriksson, 1995).

The main objective of this study was to assess the genetic variability of *G. crinita* within and among natural and populations in the process of domestication in the Peruvian Amazon by the use of either morphological (wood physical traits) or molecular markers.

Specific objectives:

- To evaluated and compare wood physical properties among six *G. crinita* provenances.
- To assess the level of genetic variation and structure within and among *G. crinita* provenances established in a genotype collection revealed by ISSR markers.
- To analyse the impact of domestication in the genetic diversity of *G. crinita* through AFLP analysis.
- To propose strategies for management and conservation of this species.

Based on the literature review it was hypothesized that; (i) there is significant variation in wood physical properties among *G. crinita* provenances, however (ii) low levels of population differentiation on molecular genetics level, combined with high within-population variability that is expected in species like *G. crinita*, which have potential for long-distance seed dispersal and gene flow, (iii) there is a positive correlation between genetic and geographic distances and (iv) as a result of selection, there is a significant genetic bottleneck in semi-domesticated population.

4. Materials and methods

4.1 Plant material

All plant material used for the different evaluations and analysis were collected from the Ucayali and Huanuco regions in the Peruvian Amazon. Individual trees represented regions where *G. crinita* occurs naturally or is extensively grown by local people. The study area was located in the western part of the Amazon region (Peruvian Amazon) (Figure 6).

Figure 6 Map of the study area

(i) Tree sampling for wood physical evaluation.

A seed orchard established in the National Institution for Agricultural Research (INIA) in Pucallpa CFB km 4, Ucayali region of Peru, represented six tree provenances where wood samples were obtained for evaluating wood physical measures. The seed orchard lies between 08º 22' 00''S and 74º 31' 00''W at 154 m.a.s.l. Generally, mean annual temperature is 25.2ºC, mean annual rainfall 2,344 mm and relative humidity around 84%. An important source of variation was expected because the seed orchard gathers trees of second generation progeny test originated from several provenances across various watershed described by Rochon et al. (2007). Eight-year-old trees in good health condition, two from each of the six provenances/ population (altogether 12 trees) were randomly chosen (Table 1, Figure 7).

Table 1 Six tree provenances included in the evaluation. Number of tree and wood samples evaluated.

Provenances	UTM Coordinates		Tree N°	Height* (m)	DBH* (cm)	n**
	East	North				
Puerto Inca (PI)	503328	8958727	1	10.4	13.1	12
			2	13.8	14.3	12
Nueva Requena (NR)	518637	9095053	3	13.3	14.3	11
			4	12.4	13.0	11
Curimana River (CR)	491115	9074726	5	12.1	14.2	11
			6	13.7	16.6	12
San Alejandro (SA)	486156	9021267	7	10.6	13.3	12
			8	12.4	14.0	11
Aguaytia River (AR)	461057	9025361	9	13.2	13.7	11
			10	13.6	13.1	10
Tournavista Road (TR)	497745	9035717	11	9.8	10.7	10
			12	14.5	19.2	12

*Height and Diameter at breast height at eight years old stand.

**n = number of wood samples, four samples in average were taken from the base, middle and top stem for each tree. Total N = 135

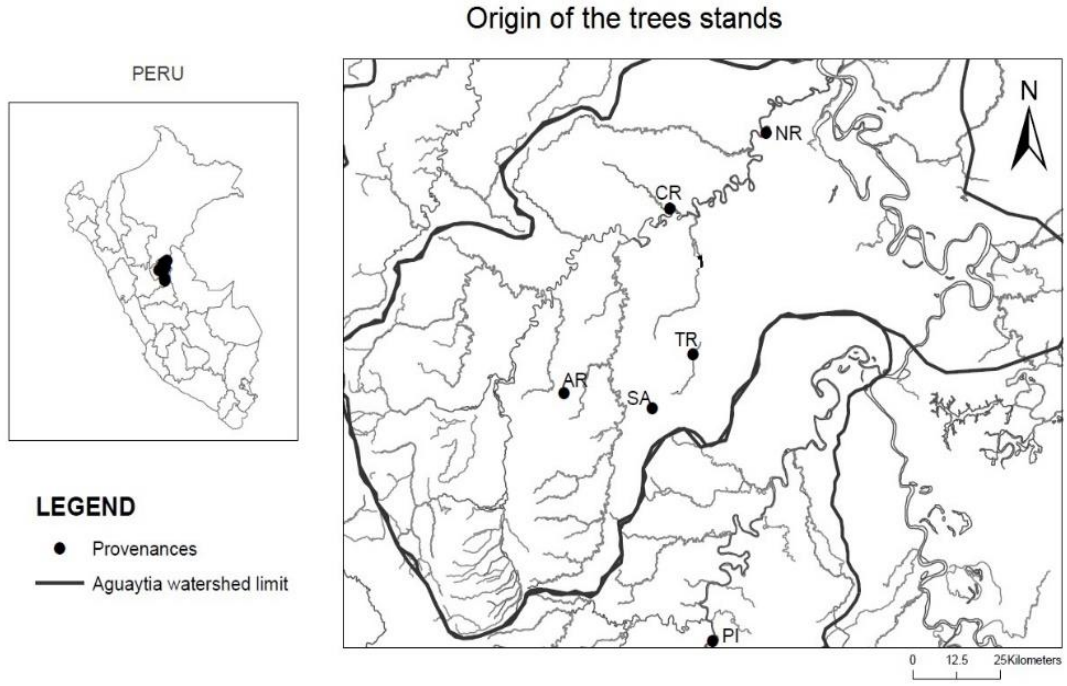

Figure 7 Geographic origin of the tree stands used for wood physical properties evaluation NR=Nueva Requena, CR= Curimana River, TR= Tournavista road, SA= San Alejandro, Aguaytia river and PI= Puerto Inca.

(ii) Sampling for ISSR analysis

In 2013, young leaf tissue samples of a total of 44 *G. crinita* genotypes (Table 2) were individually collected and stored in micro-test tubes with silica gel for further DNA isolation. This genotype collection was consider as semi-domesticated population, as it came from a progeny test started in 1998 by the collection of seeds from 200 mother trees from several provenances surrounding two representative watersheds in the Peruvian Amazon, and then established in three zones within the Aguaytia watershed in the Ucayali region. The details of the progeny test are described by Rochon et al., (2007). After 10 years and through a process phenotypic evaluation, selection and thinning, the 44 genotypes from 11 provenances (Figure 8), were selected and established in a clonal multiplication garden through vegetative propagation (Soudre, 2012) at the Peruvian Amazon Research Institute (IIAP), located 12.4 km from Pucallpa in the Ucayali Region. The samples were in the sixth step of domestication described by Vodouhe and Dansi (2012) where to improve the quality of the product, farmers may adopt specific criteria to select plants that better satisfied people needs.

Table 2 Original 11 provenances from the Aguaytia (A) and Pachitea (P) river watersheds in the Peruvian Amazon

Provenance	Altitude (m)	No. of samples (code)	UTM Coordinates (Zone 18)	
			East	North
Nueva Requena (ANR)	150	5 (ANR07, 08, 11, 12, 13)	518637	9095053
Neshuya-stream (ANS)	180	4 (ANS8, 09, 10, 12)	499925	9060461
Tahuayo-stream (ATS)	175	3 (ATS01, 02,09)	501915	9062422
Curimana-river (ACR)	170	4 (ACR14, 16, 17, 20)	491115	9074726
Aguaytia-river (AAR)	280	2 (AAR01, 13)	461057	9025361
Von Humboldt (AVH)	220	2 (AVH01, 11)	490269	9023779
San Alejandro (ASA)	230	5 (ASA1, 10, 11, 14, 15)	486156	9021267
Curimana (ACU)	185	4 (ACU03, 04, 06, 07)	504815	9049743
Tournavista-road (ATR)	195	2 (ATR01, 04)	497745	9035717
Puerto Inca (PPI)	290	3 (PPI01, 03, 13)	503328	8958727
Macuya (PMA)	260	10 (PMA01, 11, 16, 23, 24, 33, 36, 42, 44, 46)	498323	8978543

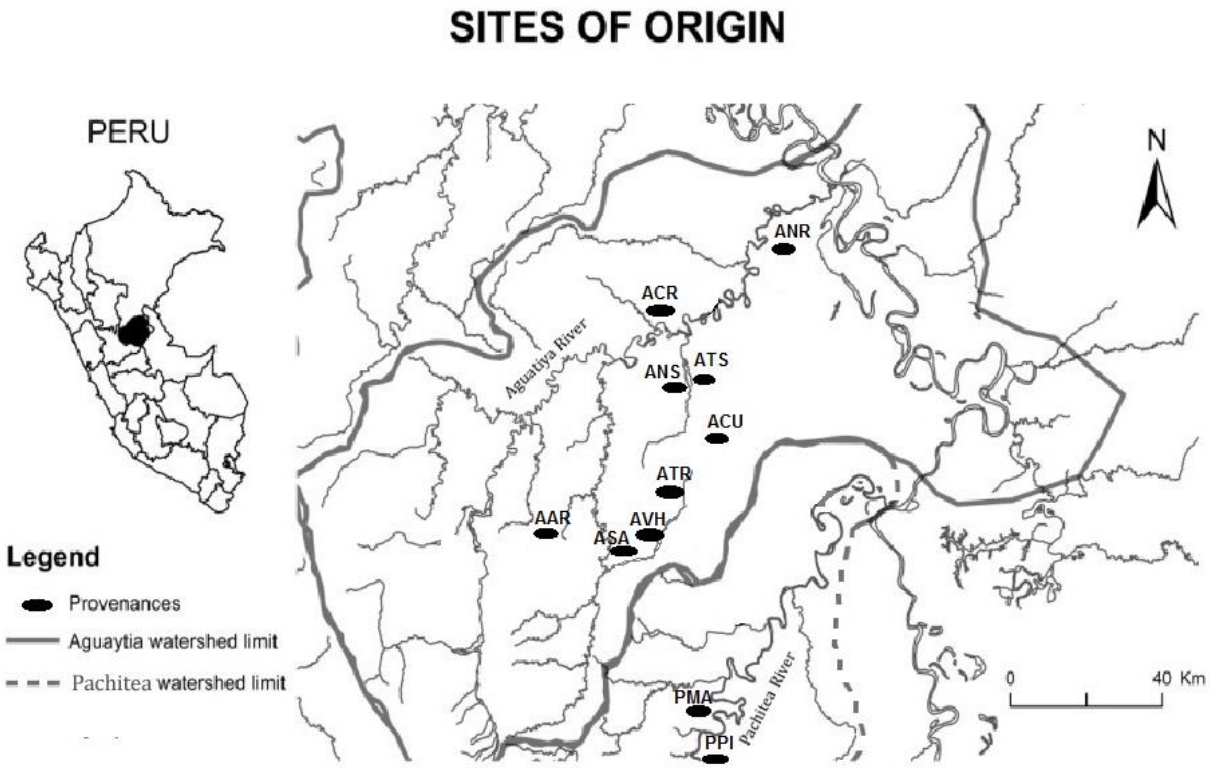

Figure 8 Geographic origin areas of 11 *G. crinita* provenances analysed by ISSR markers.

ANR: Nueva Requena, ANS: Neshuya-stream, ATS: Tahuayo-stream, ACR: Curimana-river, AAR: Aguaytia-river, AVH: Von Humboldt, ASA: San Alejandro, ACU: Curimana, ATR: Tournavista-road, PPI: Puerto Inca, PMA: Macuya.

(iii) Sampling for AFLP analysis

In the last step in September 2014, a total of 58 individuals from three different types of populations (natural, cultivated and semi-domesticated) were sampled. First nineteen individuals from Nuevo Piura village were randomly sampled from a population of natural regeneration (hereafter called natural population) located in Campo Verde district, Ucayali region, lying between 9075717 N and 505356 E at 150 m.a.s.l. Second, in Tingo Maria town, Huanuco region, 15 individuals cultivated (hereafter called cultivated population) in a home garden nursery were sampled, the origin of these samples is in Rio Negro village between 8973025 N and 397584 at 564 m.a.s.l. Third, 24 samples were selected from six provenances of the populations previously described and used for AFLP sampling (hereafter called semi-domesticated population). In total, 58 leaf tissue samples dried in silica gel were used for AFLP analysis (Table 3).

The natural population, was considered in the second step of domestication according to Vodouhe and Dansi (2012) as the trees were wild naturally regenerated and maintained in the fields during land preparation (clearance, burning, and weeding). Meanwhile a population from a home nursery may represent a fourth step in the process of domestication since the reproductive biology of the species is known, and multiplication and cultivation of the species in the home gardens was managed by farmers (Vodouhe and Dansi 2012). At this stage, farmers tend to conduct diverse experiments (date of planting, sowing or planting density, etc.) in order to master mass production of the species in the future. As mentioned before the semi-domesticated population was in the six step of domestication described by Vodouhe and Dansi (2012).

Table 3 Origin of the 58 *G. crinita* individuals used for AFLP analysis

Provenance	Region	No. of samples (code)	Population type (Code)
Nuevo Piura (NP)	Ucayali	19 (NP 01, 02, 03, ... 19)	Natural regeneration (I)
Tingo Maria (TM)	Huanuco	15 (TM 01, 02, 03, ... 15)	Cultivated[1] (II)
Nueva Requena (NR)	Ucayali	5 (ANR07, 08, 11, 12, 13)	Semi-domesticated[2] (III)
Tahuayo Stream (TS)	Ucayali	3 (ATS01, 02,09)	Semi-domesticated (III)
San Alejandro (SA)	Ucayali	5 (ASA1, 10, 11, 14, 15)	Semi-domesticated (III)
Curimana (CU)	Ucayali	4 (ACU03, 04, 06, 07)	Semi-domesticated (III)
Puerto Inca (PI)	Huanuco	3 (PPI01, 03, 13)	Semi-domesticated (III)
Macuya (MA)	Huanuco	10 (PMA01, 11, 16, 23, 24, 33, 36, 42, 44, 46)	Semi-domesticated (III)

[1]Samples cultivated in a home garden nursery
[2]Genotypes established in a clonal garden multiplication

4.2 Wood physical properties evaluation

The 12 trees were felled at 20 cm above ground level and cut with chainsaw to three logs - the base, middle and top stem levels. The cut surfaces of the logs were sealed with glaze paint to maintain the moisture content. For determination of physical properties, wood samples were prepared from each log in the carpentry shop at the National University of Ucayali (UNU), Pucallpa, Peru. The different diameter size did not allow obtain the same amount of samples from each log. In average, four samples were prepared from the base, middle and top stem level of each tree (Table 1: n = 135). The final dimensions of each sample were 20 mm radial (R) by 20 mm tangential (T) by 100 mm longitudinal (L), they were labelled to identify the tree and stem level.

Laboratory measurements on physical properties were conducted in the laboratory of wood properties at the Amazonian Intercultural University in Ucayali (UNIA), Pucallpa, Peru. The saturated masses of the 135 test samples were determined to the nearest 0.001 g using a digital balance, and the three principal dimensions were measured to the nearest 0.001 mm with a digital vernier caliper. The wood samples were continuously evaluated under room condition (25±2°C) and relative moisture 65±5% until moisture content reached approximately 12% and then oven dried at 103±2°C. The wood physical properties were evaluated according to the Peruvian technical standards (PTS 251. 010, 0.11 and 012) (INDECOPI, 2005abc); moisture content (MC) {[(saturated volume - oven-dry volume)/oven-dry volume]x 100}, basic density (BD) (oven-dry weight/saturated volume), green density (GD) (saturated weight/saturated volume), air-dry density (ADD) (air-dry weight/air-dry volume), oven-dry density (ODD) (oven-dry weight/oven-dry volume), and specific gravity (SPG) (oven-dry weight/air-dry volume). Dimensional differences of the samples were used to estimated radial, tangential and volumetric shrinkage {[(saturated - oven-dry dimension)/saturated dimension] x 100} and the coefficient of anisotropy (T/R).

Analysis of variance (ANOVA) was conducted to determine if there were significant differences in physical properties due to the origin (provenances) and tree stem levels position (base, middle and top). The ANOVA model included three main effects (individual tree, provenance, stem level, all treated as fixed effects), two interactions (stem level with provenance and stem level with individual tree), and residual error. Trees within provenances were compared with T-test. Differences among provenances and stem level were tested using Tukey's test ($p \leq 0.05$). Pearson correlation ($p \leq 0.05$) coefficients among physical properties were also determined. All statistical analyses were done using SPSS 19 for Windows.

4.3 Molecular analyses

4.3.1 DNA isolation

The DNA was extracted using CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle 1987). With slight modification adding a trace of polyvinylpyrrolidone (PVP) and 5 µL of RNase (Life Technologies, Czech Republic) to the samples from second collection. Approximately 1.5 g of each leaf tissue sample were grounded in liquid N, then incubated in 500 µL of CTAB (pH 9) + 1% of mercaptoethanol for 45 min at 65 °C with constant shaking. Samples were centrifuged for 10 min at 1200 rpm and the supernatant transferred to fresh 1.5 mL microtube. After 500 µl of chloroform/IAA (24:1) was added and centrifuged for 5 min at the maximum speed. The supernatant was transferred to new microtube and precipitated by 500 µl of cold isopropanol for 30 min at -20 °C. Pallets were washed in cold 96% and 70% of ethanol, dried down and finally suspended in 50 µl of TE buffer. DNA quality was determined by 0.8 % agarose gel electrophoresis and using a Nanodrop Spectrophotometer (Thermo Scientific, USA). The final concentration of all DNA samples was adjusted to 50 ng μl^{-1} for PCR (polymerase chain reaction), and stored at -20 °C.

4.3.2 ISSR amplification

Among the 30 universal UBC-ISSR primers (University of British Columbia, Canada) tested for their ability to produce distinct, reproducible and well-resolved fragments in the 44 individual genotypes, 10 primers were selected for the amplification of all the samples (Table 4). The PCR amplification was performed using T100^{TM} Thermal Cycler (Bio-Rad Laboratories, USA). Each 20 µl PCR reaction mixture was composed of 10 µl of 2x PPP Master Mix [150 mM Tris–HCl, pH 8.8 (25 °C), 40 mM $(NH_4)\ 2SO_4$, 0.02 % Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP, 100 U ml^{-1} Taq-Purple DNA polymerase, monoclonal antibody anti-Taq (38 nM), stabilizers and additives] (Top-Bio, Czech Republic), 10 µM of respective ISSR primer, 2 µl of DNA (50 ng/µl), 0.2 µl of BSA (20 mg/ml) (Thermoscientific, Lithuania) and 7.3 µl PCR H_2O (Top-Bio, Czech Republic).

Table 4 Primers used in the ISSR analysis and summary of amplifications.

No.	Primers	Sequence (5´- 3´)[a]	Annealing temperature (°C)	Total bands	Scorable bands	Polymorphic bands	PPB[b] (%)	Size of bands (pb)
1	UBC807	$(AG)_8$ T	48	236	10	10	15.4	340-1,700
2	UBC809	$(AG)_8$ G	49	217	7	6	9.2	420-1,300
3	UBC810	$(GA)_8$ T	49	211	5	5	7.7	520-1,300
4	UBC812	$(GA)_8$ A	49	164	6	6	9.2	425-1,600
5	UBC814	$(CT)_8$ A	48	115	9	9	13.9	490-2,200
6	UBC828	$(TG)_8$ A	52	126	8	8	12.3	480-2,000
7	UBC834	$(AG)_8$ YT	48	45	1	0	0.0	850
8	UBC841	$(GA)_8$ YC	49	282	11	11	16.9	260-1,250
9	UBC848	$(CA)_8$ RG	47	166	6	6	9.2	340-1,300
10	UBC866	$(CTC)_6$	47	90	2	0	0.0	1020-1,490
Total				1,652	65	61	93.8	

[a] Single letter abbreviations for mixed-base positions: Y = (C, T), R = (A, G).

[b] PPB = percentage of polymorphic bands.

The cycling conditions were as follows: initial denaturation for 4 min at 94 °C, 45 cycles of denaturation for 30 s at 94 °C, primer annealing at 47-52 ºC for 45 s at specific annealing temperature for each primer, extension for 2 min at 72 °C and a final 10 min extension at 72 °C. Amplified products were analysed by electrophoresis on 2% agarose gel in 1x TBE buffer, 60 V, 120 mA for 180 min and detected by ethidium bromide staining (Carl Roth GmbH, Germany) and the bands were observed under UV light (Cleaver Scientific, UK). The size of the amplified products was estimated using Thermo Scientific GeneRuler 100 bp Plus DNA Ladder (Thermoscientific, Lithuania). PCR amplification of the samples with each primer was carried out in duplicate so as to ensure the consistency and reproducibility of the results.

ISSR fragments were scored as a presence (1) or absence (0) of bands from the gel profile. Only strong bands were used to construct a binary matrix. The data were used to calculate percentage of polymorphic bands, Shannon's information index (I, LogBase Ɐ e), Nei's genetic distance matrix, gene diversity (*He*), genetic differentiation (G_{st}) and gene flow (*Nm*) (Nei, 1972) among provenances using POPGENE v1.32 (Yeh et al., 1997). A dendrogram was constructed by unweighted neighbour joining based on Jaccard's dissimilarity index. This analysis was performed using the DARwin5 software (Perrier and Jacquemoud-Collet, 2006). Mantel test in GenAlEx v6 (Peakall and Smouse, 2012) was performed using Nei's unbiased

genetic distances matrix and the matrix of geographic distances to determine whether the geographical and genetic distances between provenances were correlated.

4.3.3 AFLP amplification

Techniques for the AFLP analysis of *G. crinita* were adapted from those described by Vos et al. (1995). Commercial AFLP kits (Stratec Molecular, Germany) were used for the restriction, ligation and pre-amplification steps.

AFLP Core Plant Reagent Kit I (Stratec Molecular, Germany) was used for restriction and ligation. The restriction reaction volume was 5 µl consisted: 1 ul of 5x Reaction Buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM k-acetate]; 0.4 µl of enzyme mixture *Eco*RI/*Mse*I [1.25 U/µl each in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% glycerol (v/v), 0.1% Triton®X-100]; 1.1 µl of sterile water and 2.5 µl of DNA (50 ng/µl). After mixing the reaction was left incubating in a thermocycler at 37°C for 2 hours. Ligation of the adapters contained: 4.8 µl of Adapter/Ligation Solution [*Eco*RI/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl (ph 7.5), 10 mM Mg-acetate, 50 mM K-acetate]; 0.2 µl T4 DNA Ligase [1 U/µl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% (v/v) glycerol]. This volume was added into a microtube with the restriction products from previous reactions. The reaction was left at 37°C for 2 hours.

For pre- amplification AFLP Pre-Amp Mix I (Stratec Molecular, Germany) was used. The cycle profile for pre-amplification PCR was as follows; started with an initial step at 72°C for 2 min, followed by 20 cycles of 94°C for 10 s, at 56°C for 30 s and at 72°C for 2 min and final elongation at 60°C for 30 min; containing 4.0 µl of pre-amplification mix, 0.5 µl of 10x Buffer for RedTaq Polymerase [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 11 nM $MgCl_2$ and 0.1% gelatin] (Sigma-Aldrich, USA), 0.1 µl RedTaq Polymerase (Sigma-Aldrich, USA) and 0.5 µl of DNA after restriction and ligation The product was visualized on 1.8% TBE agarose gel. After amplification the product was diluted by the addition of 15 µl of ddH_2O.

The selective amplification reactions with a slight modifications were performed following the protocol described in Mikulášková et al. (2012), with a total volume of 9.8 µl, comprising 2.3 µl of preamplified DNA, 5.1 µl ddH_2O, 1 µl 10× polymerase buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 11 nM MgCl2 and 0.1% gelatin] (Sigma-Aldrich, USA), 0.2 mM dNTP (Thermo Scientific, USA), 0.5 pmol fluorescent dye-labelled EcoRI primer (Applied Biosystems, USA), 0.5 pmol MseI primer (Generi Biotech, Czech Republic) and 0.2 U RedTaq DNA polymerase (Sigma Aldrich, USA). Selective PCR amplifications were carry out using the followed cycle profile; started at 92°C for 2 min, 65°C for 30 s and 72°C for 2 min. A

touchdown protocol was applied in the following eight cycles of 94°C for 1 s, at 64°C (1°C decrease each cycle) for 30 s, and at 72°C for 60 s. This was followed by 23 cycles of 94°C for 1 s, at 56°C for 30 s and at 72°C for 2 min. Final elongation at 60°C for 30 min.

Eleven primer combinations were tested but only seven primer combinations were selected for final analysis because they produced polymorphic bands. The primer combinations and their sequences are listed in Table 5.

Table 5 List of selected AFLP primer combinations for final analysis with 58 samples

Primers code	Sequence 5´- 3´
6 FAM-*Eco*RI	GAC TGC GTA CCA ATT CAC G
*Mse*I- CTT	GAT GAG TCC TGA GTA ACT T
*Mse*I - CTG	GAT GAG TCC TGA GTA ACT G
*Mse*I - CTA	GAT GAG TCC TGA GTA ACT A
*Mse*I - CAT	GAT GAG TCC TGA GTA ACA T
*Mse*I - CAG	GAT GAG TCC TGA GTA ACA G
*Mse*I - CAC	GAT GAG TCC TGA GTA ACA C
*Mse*I - CAA	GAT GAG TCC TGA GTA ACA A

For all PCR amplifications T100^{TM} Thermal Cycler (Bio-Rad Laboratories, USA) was used. The final products after selective amplification were visualized on 1.8% agarose gels buffered in 1x TBE. Following a successful amplification, the AFLP products were prepared for analysis on 3500 Genetic Analyser, automated sequencer (Applied Biosystems, USA). Ten percente of the samples were analysed twice for error rate estimation.

AFLP fragments analysis was performed using GeneMarker v 2.0.2 (SoftGenetics, USA), polymorphic peaks were scored as presence or absence, then and converted into a binary matrix. In weak samples it was required automatic detection manually. The data was used to calculate percentage of polymorphic fragments, gene diversity (*He*), Shannon's information index (I, LogBase Ľ e), and genetic differentiation (G_{st}) among types of population using POPGENE v1.32 (Yeh et al., 1997). A dendrograms was constructed based on Jaccard's dissimilarity index with unweighted pair group method with arithmetic mean (UPGMA), also principal coordinate analysis (PCoA). These analyses were performed using the DARwin5 software (Perrier and Jacquemoud-Collet, 2006). Analysis of molecular variance (AMOVA) and Mantel test were

performed in GenAlEx v6 (Peakall and Smouse, 2012) AFLP percentage of reproducibility was calculated following Bonin et al. (2004).

5. Results

5.1 Variation in wood physical properties among six *G. crinita* provenances

There was found significant variation ($p \leq 0.05$) in all physical properties due to provenances except for green density (Table 6). Mean basic density (BD) of all trees in the six *G. crinita* provenances was 433 kg/m^3. Tournavista road (TR) provenance had the highest BD, and the Aguaytia river (AR) provenance the lowest BD and the difference was significant. The mean specific gravity (SPG) was 0.45 g/cm^3, and it showed the same trend as BD for TR and AR provenances. Moisture content of the wood (MC) also differed significantly ($p \leq 0.05$) between TR and AR provenances, but in this case AR provenance had a higher value than TR provenance (Mean MC = 70.0%). The mean ratio of tangential to radial shrinkage (coefficient of anisotropy T/R) was 1.59. Tangential shrinkage was higher (4.96%) than radial shrinkage (3.25%). The coefficient of variance (CV) for the coefficient of anisotropy was highest among the physical properties of the wood evaluated, followed by radial shrinkage, oven dry density, tangential shrinkage, air dry density, SPG, green density, BD, MC and volumetric shrinkage. Comparing the average CV for all physical properties for each provenance, Puerto Inca (PI) provenance had highest variation (22%), followed by AR provenance (18%), TR provenance (16%), and the other three provenances (16%).

All physical properties evaluated varied significantly ($p \leq 0.05$) due to tree stem level, with the higher values for the stem's base (Table 7). Physical properties in the middle and top levels were statistically similar. The average coefficient of variation for all evaluated wood traits was higher for the middle section of the stem (18%) compared with the base (15%) and top (17%) sections of the stem.

Table 6 Physical properties for six provenances of *G. crinita* at eight years after establishment. Mean, standard deviation (SD) and coefficient of variance (CV) are given for all wood samples across provenances and for each provenance.

	Physical properties	Across provenances		Provenances											
				Puerto Inca		Nueva Requena		Curimana River		San Alejandro		Aguaytia River		Tournavista Road	
		Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
	BD	433 ± 66	15	443^{ab} ± 78	18	434^{ab} ± 60	14	424^{ab} ± 66	16	424^{ab} ± 62	15	406^{a} ± 56	14	466^{b} ± 59	13
	SPG	0.45 ± 0.07	16	0.46^{ab} ± 0.09	19	0.45^{ab} ± 0.07	15	0.44^{ab} ± 0.07	16	0.45^{ab} ± 0.07	15	0.42^{a} ± 0.06	14	0.49^{b} ± 0.07	15
	MC (%)	70.0 ± 9.4	13	66.9^{a} ± 6.7	10	77.9^{bc} ± 9.0	12	66.6^{a} ± 5.3	8	69.3^{ab} ± 7.4	11	78.7^{c} ± 10.9	14	63.1^{a} ± 6.6	10
Density	Green	728 ± 117	16	736^{a} ± 109	15	757^{a} ± 95	13	704^{a} ± 102	15	717^{a} ± 99	14	727^{a} ± 94	13	728^{a} ± 187	26
Density	Air dry	520 ± 85	16	535^{ab} ± 102	19	533^{ab} ± 78	15	506^{ab} ± 84	16	507^{ab} ± 78	16	483^{a} ± 68	14	559^{b} ± 79	14
Density	Oven dry	471 ± 89	18	469^{ab} ± 137	29	476^{bc} ± 75	16	461^{ab} ± 78	17	465^{ab} ± 75	16	443^{a} ± 65	14	514^{c} ± 74	14
Shrinkage	R %	3.25 ± 0.71	22	3.80^{b} ± 0.96	25	3.20^{ab} ± 0.60	19	2.86^{ab} ± 0.48	17	3.20^{ab} ± 0.66	21	2.81^{ab} ± 0.73	26	3.60^{b} ± 0.84	23
Shrinkage	T%	4.96 ± 0.84	17	4.43^{a} ± 1.40	31	5.00^{ab} ± 0.69	14	4.84^{ab} ± 0.85	18	5.21^{ab} ± 0.84	16	5.06^{ab} ± 0.81	16	5.24^{b} ± 0.46	9
Shrinkage	V%	8.21 ± 1.09	13	8.18^{ab} ± 1.65	20	8.22^{ab} ± 0.96	12	7.72^{a} ± 0.95	12	8.45^{ab} ± 0.87	10	7.88^{ab} ± 1.14	15	8.83^{b} ± 0.96	11
Shrinkage	T/R	1.59 ± 0.50	31	1.25^{a} ± 0.43	35	1.67^{b} ± 0.45	27	1.74^{b} ± 0.43	24	1.68^{b} ± 0.38	22	1.71^{b} ± 0.73	43	1.52^{ab} ± 0.41	27

Note: BD= basic density reported as the ratio of oven dry mass to saturated volume (kg/m^3); SPG= specific gravity (g/cm^3); MC= moisture content; green, air-dry and oven-dry densities (kg/m^3); R=radial; T=tangential; V= volumetric. Values with different superscript within a row are significantly different. Tukey test $p \leq 0.05$.

The interaction between stem level and provenance was significant ($p \leq 0.05$) for all physical properties with the exception of air-dry density and tangential shrinkage. Although there was significant variation among most of the wood traits among provenances, in general there was little differentiation among provenances means. For example, the provenances with the best basic density, was only 10% denser than the mean of the six provenances tested. Moreover the comparison of trees within provenances was not significant.

Table 7 Variation in physical properties of bolaina blanca based on stem levels. Descriptive statistics are given for 56, 48 and 39 wood samples from base, middle and top stem level respectively.

	Physical properties	Longitudinal stem levels					
		Base		Middle		Top	
		Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
	BD	$496.5^{b} \pm 40$	8	$391^{a} \pm 48$	12	$394^{a} \pm 34$	9
	SPG	$0.52^{b} \pm 0.04$	8	$0.41^{a} \pm 0.05$	13	$0.41^{a} \pm 0.04$	9
	MC (%)	$69.7^{ab} \pm 11.1$	16	$67.8^{a} \pm 7.6$	11	$73.2^{b} \pm 7.8$	11
Density	Green	$839^{b} \pm 39$	5	$653^{a} \pm 62$	9	$662^{a} \pm 122$	18
Density	Air dry	$602^{b} \pm 48$	8	$467^{a} \pm 64$	13	$470^{a} \pm 49$	10
Density	Oven dry	$553^{b} \pm 46$	8	$414^{a} \pm 83$	20	$423^{a} \pm 39$	9
Shrinkage	R %	$3.93^{b} \pm 0.73$	19	$2.96^{a} \pm 0.74$	25	$2.85^{a} \pm 0.67$	23
Shrinkage	T%	$6.48^{b} \pm 1.14$	18	$4.33^{a} \pm 0.71$	16	$4.08^{a} \pm 0.68$	17
Shrinkage	V%	$10.26^{b} \pm 0.99$	10	$7.33^{a} \pm 1.21$	16	$7.05^{a} \pm 1.07$	15
Shrinkage	T/R	$1.80^{c} \pm 0.52$	29	$1.56^{b} \pm 0.45$	28	$1.35^{a} \pm 0.44$	32

Note: BD=basic density (kg/m^3); SPG= specific gravity (g/cm^3); MC= moisture content; green, air-dry and oven-dry densities (kg/m^3); R=radial; T=tangential; V= volumetric. Values with different superscript within a row are significantly different. Tukey test $p \leq 0.05$.

Pearson correlations were positive and highly significant between specific gravity and density at all moisture content levels (i.e., green, air-dry and oven-dry; Table 8). In contrast, MC was negatively correlated with SPG and all densities evaluated. The ratio of tangential to radial shrinkage had a weak positive correlation with green density (GD).

Table 8 Correlation analyses among physical properties of six *G. crinita* provenances.

Physical properties	BD	SPG	MC	GD	ADD	ODD	SHR
BD	1						
SPG	0.951**	1					
MC	-0.415**	-0.394**	1				
GD	0.781**	0.810**	NS	1			
ADD	0.937**	0.903**	-0.350**	0.751**	1		
ODD	0.856**	0.823**	-0.314**	0.686**	0.824**	1	
SHR	NS	NS	NS	0.150*	NS	NS	1

Note: BD=basic density; SPG= specific gravity; MC=moisture content; GD= green density; ADD= air dry density; ODD= oven dry density; SHR= shrinkage (T/R); NS= no significant; * significant at the 0.05 level; **significant at the 0.01 level

5.2 Genetic diversity in *Guazuma crinita* from eleven provenances revealed by ISSR markers

5.2.1 Genetic diversity

The DNA amplified using ISSR markers generated a total number of 65 fragments by the ten primers; the amplified fragments were within a range of 260 to 2,200 bp. The total number of fragments amplified per primer ranged from 1 (UBC 834) to 11 (UBC 841). A total of 61 fragments were polymorphic (93.8 %) (Table 4).

The average number of loci and polymorphic loci generated per primer were 6.5 and 6.1, respectively. The overall analyses of genetic variation at watershed and provenance level are shown in Table 9

Table 9 Analysis of genetic variation generated by ISSR markers in *Guazuma crinita* provenances / watershed from 44 genotypes

Provenances/Watersheds	(PPB %)	Nei's gene diversity (*He*)	Shannon index (*I*)
Nueva Requena (ANR)	41.5	0.15	0.22
Neshuya - stream (ANS)	30.8	0.13	0.19
Tahuayo - stream (ATS)	47.7	0.21	0.30
Curimana - river (ACR)	40.0	0.16	0.23
Aguaytia - river (AAR)	24.6	0.12	0.17
Von Humboldt (AVH)	18.0	0.09	0.13
San Alejandro (ASA)	47.7	0.19	0.28
Curimana (ACU)	56.9	0.23	0.34
Tournavista - road (ATR)	6.15	0.03	0.04
Puerto Inca (PPI)	52.3	0.20	0.30
Macuya (PMA)	67.7	0.24	0.35
Aguaytia watershed (A)	83.1	0.20	0.33
Pachitea watershed (P)	78.5	0.24	0.37
Average (prov. level)	39.4	0.16	0.23
Species level	93.8	0.22	0.36

The percentage of polymorphic bands was greater in Aguaytia watershed, while Nei's gene diversity (*He*) and Shannon index (*I*) were greater in the Pachitea watershed. Among the provenances, the percentage of polymorphic bands was greatest in Macuya (PMA) and lowest in Tournavista-road (ATR). Tournavista-road also had the lowest Nei's gene diversity (*He*) and Shannon index (*I*).

5.2.2 Genetic structure

Overall coefficient of genetic differentiation (G_{st}) was 0.03, which indicates that 3 % of the genetic variability was distributed among provenances. Gene flow (*Nm*) was estimated to be 12.9 migrants per generation contributing alleles between provenances. Analysis of Nei's genetic identities of the pair-wise comparison between provenances varied from 0.84 to 0.96. Genetic distances among provenances ranged from 0.025 to 0.155 with a mean of 0.061 (Table 10). The lowest genetic distance was between Nueva Requena (ANR) and Curimana river (ACR) provenances which are both located along the Curimana river (Figure 8), suggesting that downstream dispersal of seeds reduces the genetic distance. Puerto Inca (PPI) from the Pachitea watershed and Aguaytia river (AAR) from the Aguaytia watershed had the highest genetic distance (0.155), suggesting that genetic distance was higher between provenances from different watersheds. However, the genetic distance was relatively low between most provenances in the Aguaytia watershed and Macuya, which is in the Pachitea watershed (Figure 8). It may reflect the exchange of genes by the influence of human activity along closer watersheds.

In order to assess relationships among provenances, cluster analysis was used to generate a dendrogram based on Jaccard's dissimilarity among the 44 genotypes (Figure 9). The dendrogram showed that the genotypes were grouped into three distinct clusters. The first cluster included 22 genotypes [five from the PMA and ASA provenances (PMA24, PMA23, PMA44, PMA11, PMA36, ASA11, ASA10, ASA14, ASA01 and ASA15), two from PPI, ATS, ACU and ANR provenances (PPI01, PPI13, ATS09, ATS01, ACU07, ACU06, ANR13 and ANR07), one from ACR, AAR, ANS and AVH provenances (ACR16, AAR01, ANS12 and AVH01)]. The second cluster included 21 genotypes [five from PMA provenance (PMA16, PMA46, PMA42, PMA33, and PMA01), three from ANR and ACR provenances (ANR08, ANR12, ANR11, ACR17, ACR20, and ACR14), two from ANS, ATR and ACU provenances (ANS08, ANS10, ATR04, ATR01, ACU03, and ACU04), and one from PPI, AAR, AVH, and ATS provenances (PPI03, AAR13, AVH11, and ATS02)]. The third cluster included only one

genotype from ANS provenance (ANS09). Topology of the dendrogram showed that clusters were not related to the geographic origins of the genotypes.

Table 10 Nei's genetic distance and geographic distance among the provenances of *G. crinita*.

	ANR	ANS	ATS	ACR	AAR	PPI	AVH	PMA	ASA	ACU	ATR
ANR	***	39.3	36.7	34.2	90.4	137.2	76.7	118.3	80.6	47.4	62.9
ANS	0.037	***	2.8	16.8	52.4	101.8	37.9	81.9	41.5	11.8	24.8
ATS	0.068	0.065	***	16.4	55.2	103.7	40.4	84.0	44.1	13.0	27.0
ACR	0.025	0.036	0.068	***	57.8	116.6	51.0	96.5	53.7	28.5	39.6
AAR	0.081	0.099	0.112	0.086	***	78.9	29.3	59.8	25.4	50.1	38.1
PPI	0.135	0.114	0.090	0.106	0.155	***	66.4	20.4	64.9	91.0	77.2
AVH	0.053	0.042	0.122	0.047	0.109	0.151	***	45.9	4.8	29.8	14.1
PMA	0.029	0.036	0.058	0.033	0.081	0.081	0.043	***	44.4	71.5	57.2
ASA	0.063	0.058	0.063	0.057	0.077	0.087	0.071	0.037	***	34.0	18.5
ACU	0.039	0.048	0.070	0.055	0.106	0.138	0.083	0.044	0.058	***	15.7
ATR	0.046	0.039	0.095	0.052	0.104	0.148	0.067	0.057	0.089	0.076	***

Geographic distance in kilometers above diagonal and Nei's genetic distance below diagonal. See Table 2 for provenance abbreviations.

There was a weak positive relationship ($r = 0.27$, $p < 0.05$) between genetic and geographic distance for the 11 provenances based on the Mantel test. This suggests that geographic distance had a small effect on genetic differentiation among provenances.

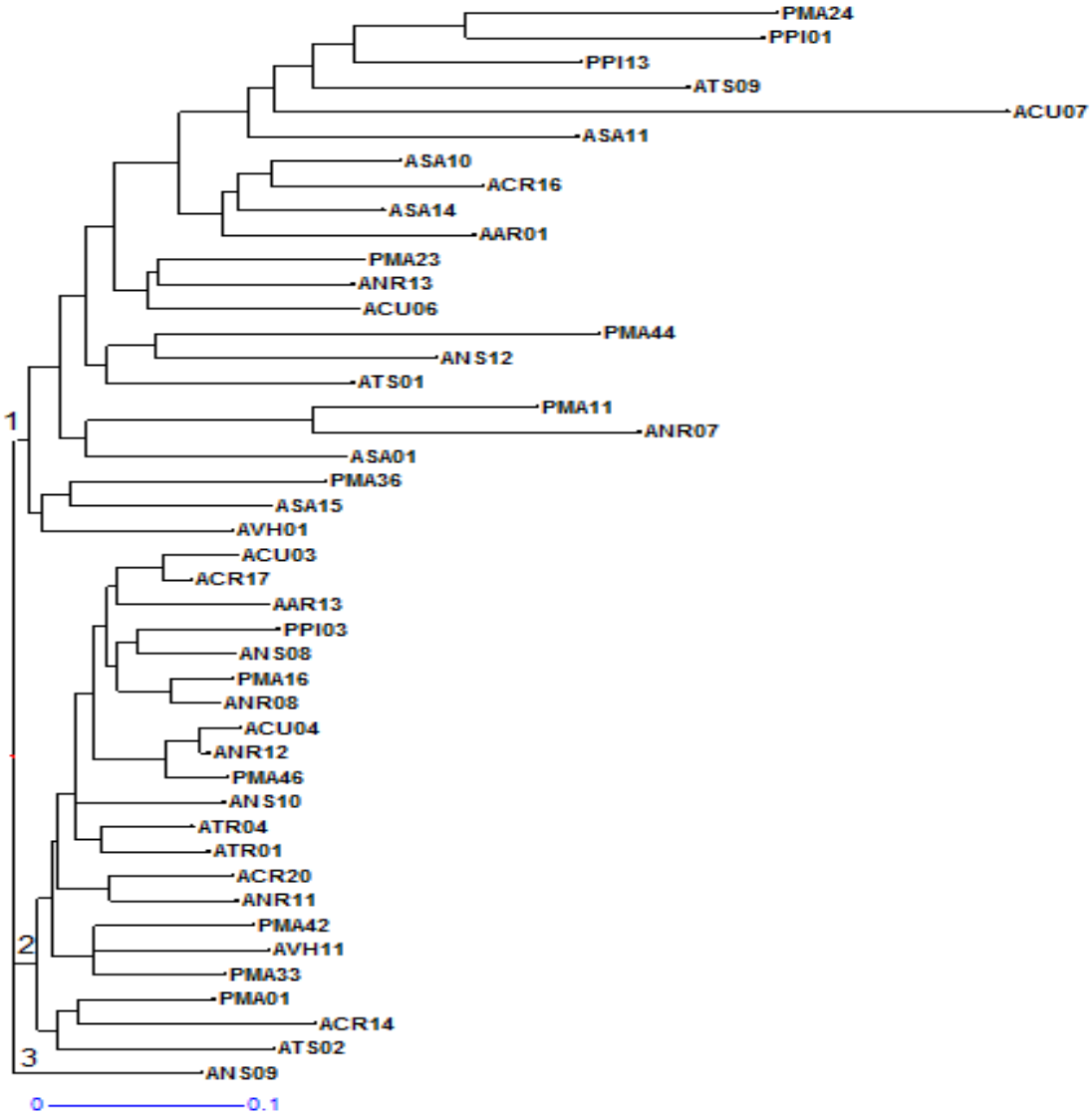

Figure 9 Dendrogram of 44 *Guazuma crinita* genotypes based on Jaccard's dissimilarity index

5.3 Genetic diversity of *G. crinita* among three types of populations

5.2.3 AFLP fingerprint

Seven primer combinations, selected for the analysis revealed between 10 (5th primer combination) to 35 (1th primer combination) fragments in 58 *G. crinita* samples, with the mean of 24 fragments. Of the total 170 fragments, 99.42% were polymorphic. The fragments were in a size range of 52 to 336 bp (Table 11). The first primer combinations (EcoRI-ACG/ *Mse*I-CTT) was the most successful with a polymorphic rate of 20.6% of all, the least was EcoRI-ACG/ *Mse*I- CAG (5.9%).

Ten percentage of the sample size was independently replicated with the same primer combinations, resulting in 85% of the fragment reproducibility among replicated samples

Table 11 Description of the primer combination fragmentation

N	Primer combination	Number of Fragment	Fragment range size (pb)	Polymorphism (%)
1	EcoRI-ACG/ *Mse*I- CTT	35	58-231	20.6
2	EcoRI-ACG/ *Mse*I- CTG	23	60-215	13.5
3	EcoRI-ACG/ *Mse*I- CTA	19	69-198	11.1
4	EcoRI-ACG/ *Mse*I- CAT	27	52-176	15.9
5	EcoRI-ACG/ *Mse*I- CAG	10	71-140	5.9
6	EcoRI-ACG/ *Mse*I- CAC	29	64-336	17.0
7	EcoRI-ACG/ *Mse*I- CAA	28	52-228	16.4
	Total	171		

5.2.4 Genetic diversity and population structure

In a single measurement of intrapopulation variation, *e.g.*, the percentage of polymorphic fragments, samples from a natural population exhibited highest variation (72.51%), followed by the samples cultivated population (49.12%). There was lower variation from the different provenances with semi-domesticated background (20.66% in average). However the semi-domesticated population as one population, showed 54.39% of polymorphic fragments (Table 12). Suggesting such a collection of genotypes as a good source of genetic variation.

Genetic analysis of 58 *G. crinita* samples with 170 polymorphic fragments generated Nei's genetic values from 0.09 to 0.10 (Table 12), the Shannon index information (*I*) ranged from

0.15 to 0.19 and average value of 0.16. In comparing three types of population, all measure (PF, PPF, *He*, *I*) were higher from the population of natural origin.

Table 12 Measurements of genetic diversity in three types of populations; Natural regenerated, Cultivated in home nursery, Semi-domesticated.

Type of population	N° of samples	N° of PF	PPF (%)	Nei's gene diversity (*He*)	Shannon index (*I*)
Natural	19	124	72.51	0.10	0.19
Cultivated	15	84	49.12	0.09	0.15
Semi-domesticated	24	93	54.39	0.09	0.16
Species level	58	170	99.42	0.11	0.20

PF…Polymorphic Fragments
PPF…Percentage of Polymorphic Fragments

Genetic differentiation value from *Gst* coefficient estimated among three types of populations in eight provenances was 0.10, which indicates that 10% of the genetic variability was distributed among types of population. According to Nei's genetic identity comparison between population types, suggested that the highest identity (0.011) existed between natural and cultivated populations and the lowest (0.022) cultivated and semi-domesticated populations. Considering the pairwise genetic distance between provenances, the value ranged from 0.011 to 0.063. Nueva Piura and Tingo Maria where the most similar with the minimum distance value of 0.011, while the highest value of genetic distance (0.063) was between Nuevo Piura - Puerto Inca and Tahuayo - San Alejandro (Table 13).

Table 13 Nei's genetic identity (above diagonal) and distance (below diagonal) from eight *G. crinita* provenances analysed by AFLP

Provenances	NR	TS	SA	CR	NP	TM	PI	MA
NR	****	0.960	0.960	0.972	0.967	0.962	0.959	0.972
TS	0.040	****	0.939	0.948	0.950	0.951	0.943	0.951
SA	0.041	0.063	****	0.978	0.947	0.952	0.972	0.971
CR	0.028	0.054	0.023	****	0.971	0.975	0.971	0.979
NP	0.033	0.051	0.054	0.029	****	0.989	0.939	0.963
TM	0.038	0.050	0.049	0.026	0.011	****	0.942	0.968
PI	0.042	0.059	0.029	0.030	0.063	0.059	****	0.972
MA	0.029	0.050	0.030	0.022	0.037	0.032	0.029	****

NR= Nueva Requena, TS= Tahuayo Stream, SA= San Alejandro, CR= Curimana River, NP= Nuevo Piura, TM= Tingo Maria, PI= Puerto Inca, MA= Macuya

For assessing the overall genetic diversity the analysis of molecular variation (AMOVA) was performed, which showed that 84% of variation was associated within provenances, while among provenances the variation was only 4% (Table 14). The level of differentiation among provenances was higher (PhiPT =0.16) than among type of population (PhiRT=0.12) at $p < 0.001$.

Table 14 Results of the analysis of molecular variation (AMOVA) of 58 *G. crinita* individuals representing three types of population within eight provenances

Source of variance	Degree of freedom (*df*)	Sum of Square (SS)	Variance component	Variance (%)	*p* value [a]
Among Type of populations	2	99.43	1.60	12	< 0.001
Among Provenances	5	69.95	0.58	4	< 0.001
Within Provenances	50	583.56	11.67	84	< 0.001
Total	57	752.95	13.85	100	

[a] Significance tests after 999 permutations

A dendrogram base on Nei's genetic distance (Figure 10) grouped the provenances into three main clusters, the first cluster with Tahuayo provenance, different among the other semi-domesticated provenances. The second cluster joined together Tingo Maria y Nuevo Piura provenances, they are provenances with higher genetic variability. The third cluster is homogeneous as it comprises provenances from semi-domesticated background although it was subdivided into two sub-clusters with Macuya, Curimana, Puerto Inca and San Alejandro fused as sub-cluster 3A, while Nueva Requena alone remained as sub-cluster 3B. The dendrogram

based on Jaccard's dissimilarity grouped the 58 samples into two main clusters with seven sub-clusters (Figure 11). The first cluster (A) grouped 48 samples, this cluster comprises 3 sub-clusters with TM05 as a first sub-cluster, and 9 samples from natural population (NP 07, 08, 05, 06, 03, 12, 19, 14, and 15) grouped together as a second sub-cluster. The third sub-cluster grouped 37 samples, all samples from semi-domesticated population (24 samples), it suggested similarity between the samples from the semi-domesticated population even when they were originated from six different provenances, probably because they were submitted to the same parameters of selection. This is congruent with the first dendrogram (Figure 10). In addition 15 and 8 samples from population natural and cultivated respectively. The second group (B) comprises 10 samples, it was subdivided into four sub-clusters of 1, 1, 2 and 4 samples respectively [(TM07); (TM06); (NP09, 18) and (NP10, 11, TM14, TM15)]. The patterns of genetic relationship were also visualized based on principal coordinates analysis (PCoA) (Figure 12), it was consistent with the dendrograms (Figure 10 and 11) where it was possible to distinguish close relationship between some samples from natural and cultivated populations. There was no found significant correlation between geographic and genetic distances, done by Mantel test.

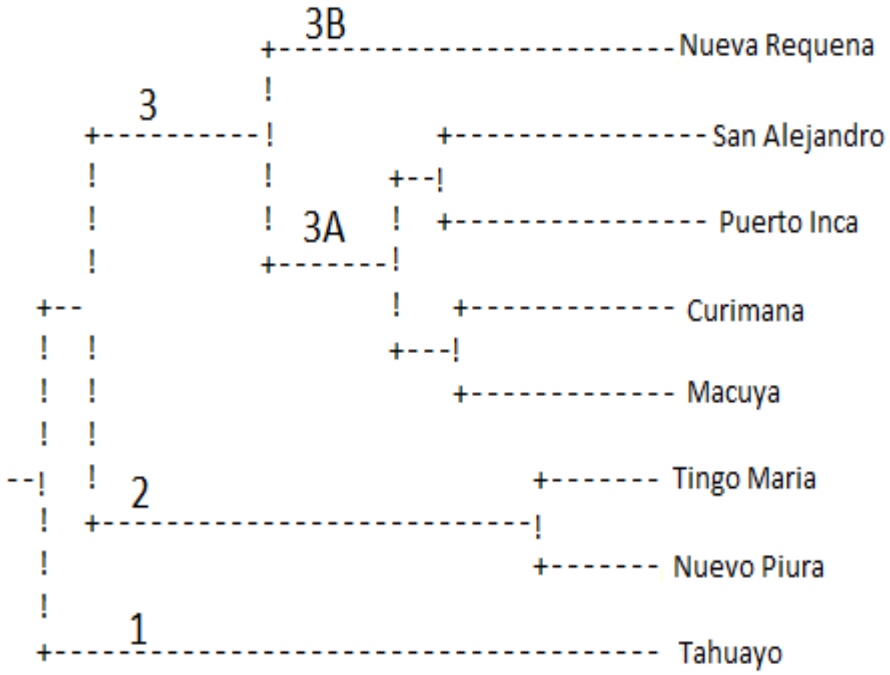

Figure 10 Dendrogram base on Nei's genetic distance among eight *Guazuma crinita* provenances. Nuevo Piura- Natural population. Tingo Maria- Cultivated population. Nueva Requena, San Alejandro, Puerto Inca, Curimana, Macuya and Tahuayo- semi-domesticated populations.

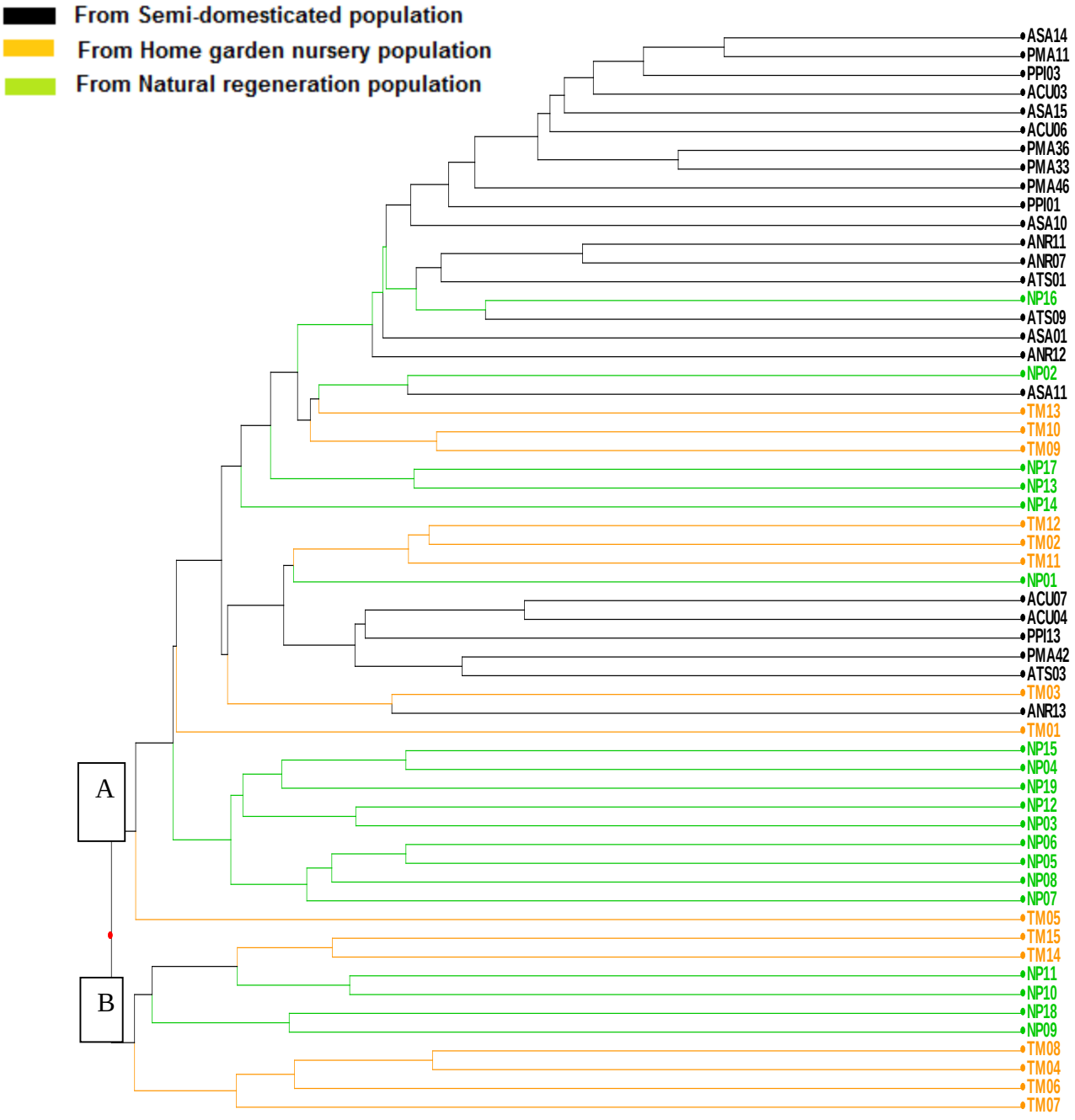

Figure 11 Dendrogram based on 171 AFLP loci for 58 samples of *G. crinita.*

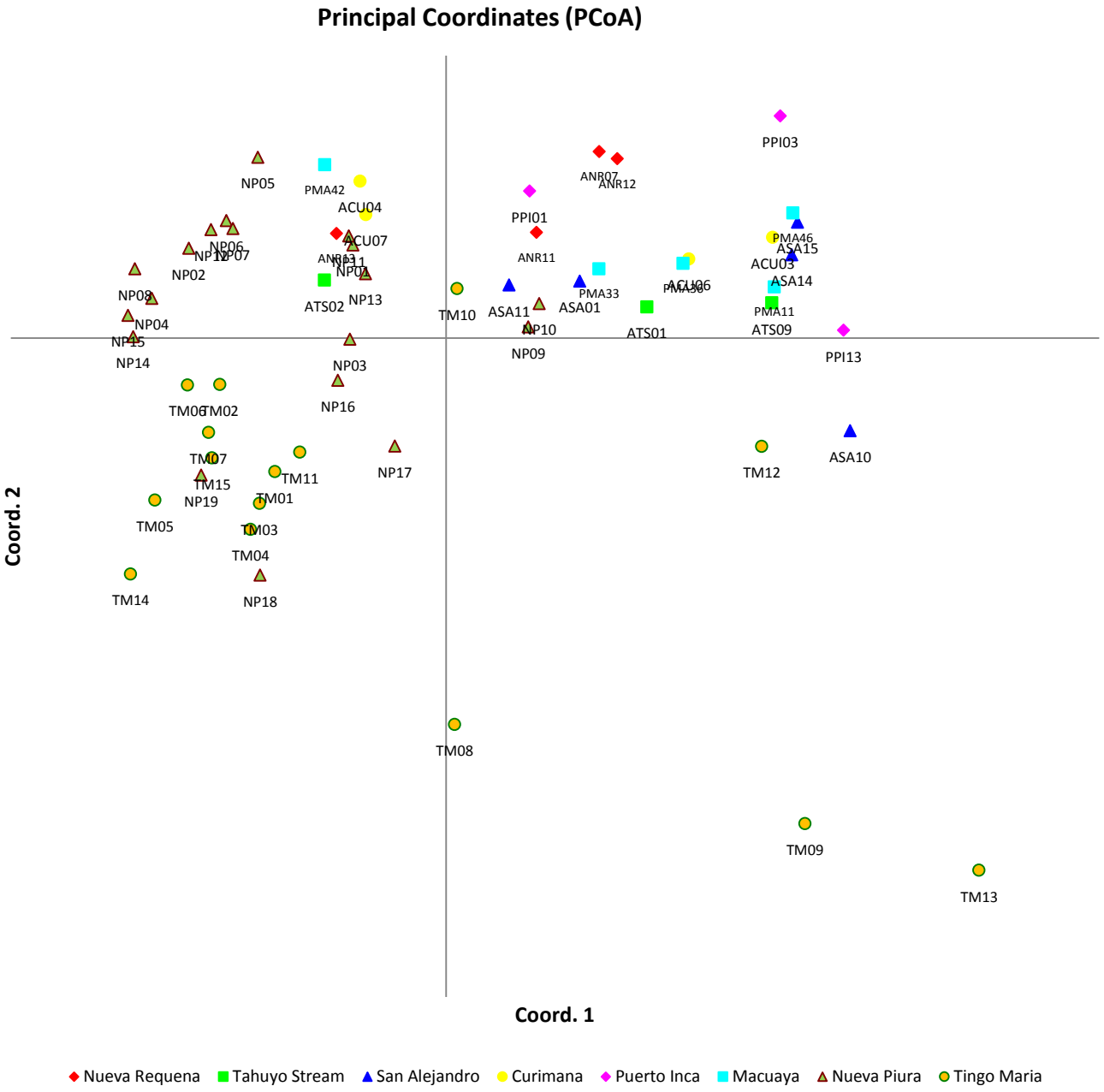

Figure 12 Principal component analysis of 58 samples belonging to eight provenances, based on AFLP markers analysis.

6. Discussion

6.1 Variation in wood physical properties

The study reported the first to assess variation in the main physical properties of the wood among six provenances of *G. crinita* stands from the Peruvian Amazon. Although limited number of individual trees was sampled in this study, there were found statistically significant differences in all physical properties among provenances except for green density, suggesting that more extensive sampling would reveal considerable variation in wood properties among provenances.

Mean basic density of *G. crinita* wood at eight years of age in our study (433 kg/m^3) was slightly greater than the value reported by Weber and Sotelo-Montes (2008) at 32 months (417 kg/m^3), suggesting that variation in basic density of this species due to environment and age factors is not very significant, this is consistent with the affirmation that wood basic density is under strong genetic control (Zobel and Jett, 1995). This value reaffirms that *G. crinita* wood is moderately dense (~410 kg/m^3 to ~600 kg/m^3, Sebille-Martina, 2006). This represents an advantage because of the lower transport and transformation costs; furthermore this species can be used to build small structures, musical instruments, and interior finish items (Reynel et al., 2003).

In this study, wood basic density and specific gravity (SPG) differed significantly between two provenances in the Aguaytia watershed (Aguaytia River and Tournavista road provenances). Differences in basic density were also reported among a larger set of provenances of *G. crinita* from different watersheds in the Peruvian Amazon (Weber and Sotelo-Montes, 2008). These results confirm that there is potential to select provenances with greater wood density, but there may be a trade-off with wood volume. This is because tree growth and basic wood density are negatively correlated in some provenances and planting environments (Weber and Sotelo-Montes, 2008). In addition, denser wood of some species have higher values for shrinkage during drying (Sotelo-Montes et al., 2007), which could result in boards that are warped and unusable for certain products. In our study, there was a weak positive correlation between green wood density and shrinkage.

Wood from the lower stem of *G. crinita* at eight years had significantly greater basic density and SPG than wood from the middle and top of the stem. Similar results were reported for *G. crinita* and *C. spruceanum* at younger ages (Weber and Sotelo-Montes, 2005, 2008). This is a general tendency since wood density is usually greater at the base due to the higher

compaction of the stump tissues exerted by overlapping cells along the stem and tree crown (Ali et al., 2010).

The average moisture content (MC) of wood of *G. crinita* at eight years in this study was 70%. This was lower than the value (85.6%) reported by Rivera-Samaniego (2014) for *G. crinita* trees from a higher altitudinal zone (660 m.a.s.l.). The MC in the base of the stem was much lower in this study than in the study at the higher altitudinal zone (69.7% versus 98.5%). Altitude appears to be an important source of variation in MC for *G. crinita* and for other species (Zobel and Jett, 1995). The value found in this study indicated a relatively high amount of free water in the wood structure; therefore the wood would probably have low mechanical resistance and internal stress for drying operations (Ali et al., 2010). However, Agostegui (1974) reported that the wood of *G. crinita* performed well during the drying process.

There was no significant difference in shrinkage among provenances of *G. crinita* in this study. Tangential shrinkage was higher than radial shrinkage (4.96 versus 3.25%), as reported for many tropical tree species (*e.g.*, *C. sciadophylla*, *Laetia procera* (Poepp) Eichl, *Ocotea guyanensis* Aubl, *Eschweilera decolorens* Sandw, *Miconia fragilis* Naud, *Carapa procera* A. D.C, *Virola surinamensis* (Rolander) Warb, *Simarouba amara* Aubl, *Qualea rosea* Aubl (Ruelle et al., 2007); *C. spreuceanum* (Sotelo-Montes et al., 2007)). Furthermore there was a significant difference in shrinkage among stem levels, with higher values in the base of the stem. The ratio of mean tangential to radial shrinkage (1.6) indicated that the wood of *G. crinita* is dimensionally stable and homogeneous for primary and secondary transformation such as narrow boards, ceiling, housing liners (Sebille-Martina, 2006). A similar value was reported by Agostegui (1974).

The physical properties of wood depend upon the moisture content of the material (Pliura et al. 2005; Sebille-Martina 2006). Correlation between density, specific gravity and moisture content indicated that wood with higher density and specific gravity has lower water content. Green density was positively correlated with the ratio of tangential to radial shrinkage; this has practical implications because many smallholder producers and sell green timber (Sebille-Martina 2006).

Despite the significant variation in wood physical properties, the interaction between tree and provenance indicated close relationship between provenances, corresponding to the structure revealed by neutral marker (ISSR), although the patters of variation in biophysical measures are strongly influence by natural selection within specific edaphoclimatic conditions (Millar and Westfall, 1992).

6.2 Genetic variation among eleven semi-domesticated *G. crinita* provenances

In this study of 44 genotypes from 11 provenances of *G. crinita* in two watersheds in the Peruvian Amazon, we characterized genetic diversity within the species at the molecular level using 10 ISSR markers. Although we were aware that our sample size was not extensive (limitation caused by small number of individual trees in the clonal orchard), other researchers have investigated genetic diversity in tree species using even smaller sample sizes (*e.g.* Hernández et al., 2006, Thangjam, 2014).

This study revealed 93.8 % polymorphic loci. Levels of genetic diversity within provenances estimated by Nei's gene diversity and Shannon genetic index (*He* = from 0.03 to 0.24; *I* = from 0.04 to 0.35) were similar of those reported by other researchers for other tropical tree species. For example, Russell et al. (1999) used amplified fragment length polymorphism (AFLP) markers to analyse genetic diversity in provenances of *C. spruceanum*, which has a similar distribution pattern as *G. crinita* in the Peruvian Amazon, and reported that *He* ranged from 0.26 to 0.35. Gillies et al. (1997) used random amplified polymorphic DNA (RAPD) analysis to study populations of *Cedrela odorata* L. in Costa Rica, and reported that within-population diversity levels (*I*) ranged from 1.18 to 1.89. Hollingsworth et al. (2005) evaluated diversity of natural and cultivated stands of *I. edulis: He* was 0.59 to 0.70, which are higher than *G. crinita* in this study. However *He* of *G. crinita* is moderately high for woody and perennial species based on the analyses of Loveless and Hamrick (1987), indicating the presence of several alleles at some loci and an equitable distribution of allelic frequencies at most loci.

Overall genetic differentiation (G_{st} = 0.03) and gene flow (*Nm* = 12.9) indicate that 97 % of genetic variability was due to differences within provenances, and the provenances were not subject to genetic isolation. The high value for gene flow reflects both pollen dispersal and seed dispersal. Seeds can be dispersed over long distances by wind, and also downstream by water so we expected little differentiation among provenances. This is similar to the case of *C. spruceanum*, which showed much higher genetic variability within than among populations in the Peruvian Amazon (Russell et al., 1999). The results were also consistent with results from provenance and provenance/progeny tests of *G. crinita*, which showed considerable genetic variation in tree growth and wood density within provenances (Rochon et al., 2007, Weber and Sotelo-Montes 2008, Weber et al., 2011).

There was a weak positive relationship between geographic and genetic distance of the provenances. However, the cluster analysis did not reveal any clear geographical pattern among the genotypes from the different provenances. Cluster groups, contained genotypes from several

different provenances. These results probably reflect the high gene flow in the sample region or limitation of the ISSR markers to reveal the existing variation among provenances.

Results suggest that the provenances (populations) of *G. crinita* in the sample region form a metapopulation of slightly differentiated subpopulations that are not genetically isolated because of gene flow (Lowe et al., 2005; White et al., 2007). The dynamic of gene flow via rivers within and among watersheds in the Peruvian Amazon Basin (Janský, 2008) and forest fragmentation due to shifting cultivation could contribute to the formation of subpopulations (Dourojeanni, 1987). A report of genetic differentiation of *S. macrophylla* using microsatellites markers indicated moderate but significant degree of differentiation among populations in the Brazilian Amazon (F_{st} = 0.097), presumably due to the larger sample region compared with our study (Lemes et al., 2003). Kelly et al. (2004) studied temporal and spatial genetic variation of *V. paradoxa* in Africa where little variation was found at microsatellite loci between sampled stands with no evidence for genetic bottleneck events in agroforests.

Special effort may be focused on a strategy that involves maintaining the high level of genetic diversity already found within semi-domesticated genotypes, furthermore, they could be used for tree improvement programs.

6.3 Genetic variability among natural, cultivated and semi-domesticate population of *G. crinita*

A number of authors have examined both natural stands of tropical trees and cultivated samples (*e.g*. Hollingsworth et al., 2005; Dawson et al., 2008) in order to characterize levels of genetic variability and population structure.

The genetic variability among three populations in different domestication stage was characterized based on amplified fragment length polymorphism (AFLP) markers, with 99.42% of polymorphism at species level, whereas the ISSR analysis at species level revealed 93.8% of polymorphism among eleven *G. crinita* provenances in the Peruvian Amazon. Although the methods were different, they both confirmed high level of genetic variation in *G. crinita*. Some biological patterns in the species might contributes to its high levels of variation as Hamrick and Godt (1989) observed in long-lived perennial species which generally maintain relatively higher levels of variation than annuals, in addition the same authors indicated that high genetic diversity is associated with the species' colonizing success, such is the case of *G. crinita* as a pioneer tree species. Moreover our study analysed this variation among three types of population in different stages of domestication within eight provenances, comparing the genetic diversity parameters, such as PPF, *He*, *I*. Natural population was more genetically variable than

semi-domesticated populations. Thus domestication process via artificial selection has reduced the levels of *G. crinita* genetic diversity, other studies also confirm that wild populations usually maintain high levels of polymorphism compared to cultivated populations (*e.g.*, Abo-Elwafa and Shimada, 1995; Hollingsworth et al., 2005). Although high variability levels of the wild populations are expected because they were not subject to any of the selection pressures of domestication, and the preservation of higher genetic variability would favour their survival under natural conditions (Simons et al., 1994; O'Neill et al., 2001). However the overall genetic parameters among provenances from semi-domesticated background showed to be even higher than those cultivated in the home garden nursery. It may be caused by the genetic composition of the semi-domesticated tree that were essentially dependent on the characteristics of theirs initial seeds, collected from mother trees selected based on phenotypic traits from natural populations. This likely led to an increase of genetic diversity in the rising early semi-domesticated collection along the Aguaytía watershed in the Peruvian Amazon that started in 1998, the details of initial of such selection were reported by Rochon et al. (2007).

The relatively low level of genetic variation among eight *G. crinita* provenances, can be explain by the high gene flow value (*Nm*=12.9) revealed by ISSR markers, which is directly associated to dispersal system of its small seed through wind and water over long distances (Weber et al., 2011). Thus the high level of variation within provenances was congruent with different genetic reports based on phenotypic traits (Rochon et al., 2007, Weber et al., 2011). Ruseell et al. (1999) reported 9 % of variation among population in *C. spruceanum*, from the Peruvian Amazon Basin supported by seven AFLP primer combinations. Nassar et al. (2011), found also very small variation among populations of three species native from the Amazon Basin based on allozymes as genetic marker, 8%, 6% and 7% of variation among *Samanea saman* (Jack.) Merr., (Fabaceae), *Guazuma ulmifolia* Lam., (Malvaceae) and *Hura crepitans* L., (Euphorbiaceae) populations respectively. This pattern was also expected due to the sampling across close geographic scale (Hanrick et al., 1992); however, in another species within extended geographic sampling there was found little but significant population differentiation [*e.g.*, *S. macrophylla* (Lemes et al., 2003); *V. paradoxa* (Kelly et al., 2004)]. Although regarding the genetic differentiation among types of populations, the three parameters tested were corresponding; [(AMOVA =12%), (G_{st} coefficient = 0.10) and (PhiRT=0.12) at $p < 0.001$)] which indicated 12% of variation due to the domestication stage suggesting slight genetic bottleneck. Therefore, the proper management of natural regenerated populations should be considered to be adopted for conservation of genetic resources of *G. crinita*.

For further understanding of relationship among provenances and samples, three analysis were performed: first the dendrogram based on Nei's genetic distances which grouped five of the semi-domesticated provenances within one cluster suggested relatively close relationship between them as they were subjected to same parameters of selection for domestication program (Soudre, 2012). However Tahuayo provenance seemed to be genetically different among the semi-domesticated populations. Furthermore, the trees were propagated asexually, which is usually associated with lower genetic polymorphism (Clapham, 1978). However, Hamrick and Godt (1989) in studies of allozyme variation in clonal plants concluded, that clonal populations may have high genetic variability, which corresponded to this analysis where semi-domesticated population had relatively high polymorphism (54.39%). Nuevo Piura and Tingo Maria provenances with less human intervention were grouped in one cluster, in addition both presented higher number of polymorphic fragments (124 and 84 respectively). The cluster analysis did not reveal clear relationship between genetic and geographic distance, but reflected significant differentiation due to population type ($p < 0.001$). Second and third dendrogram based on Jaccard's dissimilarity and principal component analysis also distinguished individuals from Nuevo Piura and Tingo Maria genetically different from semi-domesticated individuals and a low genetic structure was reflected in coherence with the genetic differentiation obtained with ISSR analysis. Moreover, in other Amazonian tree species, even within extended areas, low levels of population structure were observed (Nassar et al., 2011; Lemes et al., 2003). Such pattern of diversity is important for understanding macroecology, the impacts of climate changes and domestication, and areas of high conservation priority (Moritz, 2002).

6.4 Management of the genetic variability of *G. crinita*

Forest plantations with fast-growing tree species have become an important source of wood in the tropics because commercial dimensions are reached in relatively short rotation periods (Weber and Sotelo-Montes, 2008; Leakey et al., 2012). Tree domestication has evolved over the last two decades to become an important global programme, phenotypic measurements and molecular techniques have been used to analyse genetic diversity in several agroforestry tree species (Rochon et al., 2007, Motamayor et al., 2008; Jamnadass et al., 2012, Leakey et al., 2012). Certainly the use of molecular marker analysis should be complementary to the evaluation of phenotypic measures for better discrimination of genetic variability of a species (White et al., 2007).

Several studies regarding genetic variation of *G. crinita* based on phenotypic traits have been published by Rochon et al. (2007); Weber and Sotelo-Montes (2008), Weber et al. (2011), however this was the first research that combined phenotypic measurement (wood properties) with molecular analyses (ISSR and AFLP markers) to assess the genetic variability of the species as base for genetic improvement, conservation and management. Although this research was conducted in a relatively small geographical area in the Amazon the results presents significant insight of the genetic variation in *G. crinita*.

Combining patterns of diversity based on wood physical properties and molecular analysis suggested low population structure. The lack of significant interaction between tree and provenances in wood physical properties corresponded to what Rochon et al. (2007) observed in the variation of tree height and stem diameter. These results might be due to the weak genetic structure revealed even by the ISSR markers. Although the genetic structure revealed by molecular analysis would reflect the integrated effects of three evolutionary forces (gene flow, genetic drift, mutation), whereas variation patterns in wood traits would largely reflect natural selection within local environments in addition to the evolutionary forces (Millar and Westfall, 1992; Sotelo-Motes et al., 2003). Particularly, the seed dispersion of *G crinita* seems to play an important role in maintaining high levels of variation within population.

Nevertheless molecular markers used in this study are dominant markers (Zietkiewicz et al., 1994; Vos et al., 1995), both performed high reproducibility, though AFLP markers were able to detect higher number of polymorphic fragments compared to ISSR (171 and 65, respectively). AFLP technique most often estimates high levels of polymorphism and give high genotypic resolution (Isagi et al., 2004; Baldwin and Husband, 2013). The analysis of ISSR and AFLP markers revealed high levels of genetic variability at species level and partition of this variation among populations, higher variation within provenances (populations) than among them, which is also typical for another tropical species (*e.g*., Hamrick et al., 1992; Russell et al., 1999; Thangjam 2014). Moreover higher genetic diversity was expected in natural populations sexually reproduced (Clapham, 1978).

Furthermore, the molecular markers answered different questions regarding bolaina blanca genetic diversity. Therefore in this study it could not be compared the performance of the two markers because the number of populations, samples and approach were different, however, based on the principle of the markers and availability of resources AFLP markers seems to be more accurate for genetic characterization at molecular level (Isagi et al., 2004; Mikulášková et a., 2012; Baldwin and Husband, 2013).

The genetic variability in adaptive traits represents an advantage to the species, as it is a necessary condition for the survival and evolution of a species (Koski, 2000), such was observed in *G. crinita* in this study, in addition to its high genetic variability within provenances/populations revealed by the molecular markers. In order to maintain such variability some genetic management implications are discussed below.

According the evaluation of wood physical properties there is likely potential for selection of provenances with better wood properties, therefore proper training of local people in a range of germplasm collection, selection, propagation, management, harvesting and processing techniques could make an important contribution to the genetic management and wood productivity of *G. crinita* resources (Weber et al., 2001; Dawson et al., 2007).

The results also confirmed that even a small clonal garden, used for ISSR analysis, could possess relatively high genetic diversity and thus could form a good base for further domestication of the species. However this practice is likely to further reduce genetic variation in planted populations, though the time taken to do so will depend on the longevity of the species and interactions between overlapping generations in farmland (Dawson et al., 2009).

In addition to *ex situ* clonal gardens, *in situ* conservation of populations of *G. crinita* with higher genetic diversity should be promoted to maintain a broad genetic base for future tree improvement programs. Since seeds are dispersed downstream by rivers, we might expect higher genetic diversity below the confluence of major rivers (Russell et al., 1999), so targeting conservation in these areas may be an effective conservation strategy.

Significant differentiation observed between natural and semi-domesticated population suggests promotion of conservation and sustainable use of the tree species to prevent genetic depression. Special attention should be on the management of natural regeneration which may result in good stands and large diversity, only if, the regeneration fellings are carry out in appropriate way. For example, to leave a sufficient number of good seed trees, that may represent the dominant trees of the former stands which promising maintenance of genetic adaptations as well as the genetic diversity and to use adequate site preparation (Koski. 2000). Nevertheless the strong demand of trees at young age may force the local farmers to log all valuables stems (Weber et al., 2011), which could lead to maintain small trees as future seed trees, this kind of negative selection could result in genetic erosion if repeated over generations, once again, participatory domestication may play an important role now that there is still available high genetic variability (Weber et al., 2001; Dawson et al., 2009) of *G. crinita* in the Peruvian Amazon.

7. Conclusions

The research revealed significant variation on phenotypic traits and molecular diversity among various populations and provenances of *G. crinita* genetic in the Peruvian Amazon.

The tree origin (provenance) had relatively strong influence in wood physical properties as significant differences in nearly all wood properties among six *G. crinita* provenances from the Peruvian Amazon were found. It suggests high potential to select provenances with desirable wood properties for further domestication and breeding. The observed variation along stem level within individual tree and provenances represents good source for genetic gain, intended for tree improvement that may help to cope with current environmental variation.

Although the results suggested variation in wood physical properties within a limited number of tree samples, it is recommended future studies with higher amount of tree samples from each provenance and different experimental plots among other watersheds, to find provenances with most desirable wood properties.

The molecular markers used in this study of *G. crinita* provenances/populations showed high levels of polymorphism. Higher diversity was found within rather than among provenances. It seems that, there exists extensive gene flow so the provenances were not genetically isolated. There was no clear grouping of genotypes based on the genotype's provenance of origin and proximity to other provenances. Nevertheless there was a weak positive correlation between genetic and geographic distance of the provenances in the case of semi-domesticated provenances.

Higher level of genetic diversity was found in natural population compared to cultivated and semi-domesticated populations. Moreover high genetic variation was observed in overall semi-domesticated population analysis.

Even in small number of samples in our study, a significant diffe1rentiation was observed among population types (natural, cultivated and semi-domesticated), this result suggested slight genetic bottleneck in semi-domesticated populations.

Therefore *in situ, circa situ, ex situ* conservation and appropriate management of the natural regenerated populations are recommended to maintain *G. crinita* genetic resources in order to cope potential inbreeding depression otherwise, and environmental changes.

To maintain wide genetic variation in domesticated stands, further sampling in extent geographic scale from natural and cultivated stands are required for *G. crinita* populations and other multiuse agroforestry species.

This study also indicates that ISSR and AFLP markers are effective in detecting polymorphism and characterization of genetic variation of a tree species.

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Manuscript accepted for publication

Tuisima-Coral L, Hlásná-Čepková P, Lojka B, Weber JC, Filomeno-Alves-Milo S. 2016. Genetic diversity in *Guazuma crinita* from eleven provenances in the Peruvian Amazon revealed by ISSR markers. Bosque 37(1)-2016. (IF 0.39)

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Tuisima-Coral L, Odicio-Guevara JE, Lojka B, Weber JC, Lluncor-Mendoza D. Variation in wood physical properties of timber tree *Guazuma crinita* among six provenances in Peruvian Amazon. Manuscript submitted to Madera y Bosques (IF: 0.29)

Manuscript in preparation

Tuisima-Coral L, Hlásná-Čepková P, Lojka B, Weber JC, Mandák B. Does domestication process reduce genetic diversity of *Guazuma crinita*?

Conference contribution

1. Tuisima-Coral L, Hlásná-Čepková P, Lojka B. 2014. Inter simple sequence repeat (ISSR) markers as reproducible tools for genetic diversity analysis of an agroforestry tree species *Guazuma crinita* from Peruvian Amazon. In. Tielkes E (ed.) Tropentag 2014 - International Research on food security, Natural Resources Management and Rural Development. Step 17-19, 2014. Czech University of Life Sciences Prague. ID 907.
2. Tuisima-Coral L, Odicio-Guevara JE, Lojka B, Weber JC, Lluncor-Mendoza D. 2015. Variation in wood physical properties among six *Guazuma crinita* provenances in the Peruvian Amazon. In Pohanková Z (ed.) Second International Tropical Biodiversity Conservation Conference. Nov 3-4, 2015. Czech University of Life Sciences Prague. 24-25 pp.