UNIVERSIDADE FEDERAL DE LAVRAS

KELLY TATIANA BOCANEGRA GONZÁLEZ

GENETIC DIVERSITY AND FUNCTIONAL TRAITS VARIABILITY OF *Ceiba pentandra* (L.) Gaertn. IN THE TROPICAL DRY FORESTS OF COLOMBIA

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia Florestal, área de concentração em Silvicultura e Genética Florestal, para a obtenção do título de Mestre**.**

Orientadora
Dra. Dulcinéia de Carvalho

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DEDICO

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RESUMO GERAL

Ceiba pentandra (L.) Gaertn é uma espécie típica da floresta decídua tropical (FDT) da Colômbia. Este ecossistema na atualidade encontra-se altamente fragmentado, o que compromete a perpetuação e os bens e seviços que a sociedade recebe dele. A carência de informação primária do nível genético e ecológico das espécies que habitam o FDT dificulta o estabelecimento de planos de manejo e recuperação de seus fragmentos. Este trabalho teve por objetivo determinar a diversidade genética e a variabilidade dos traços funcionais de *C. Pentandra*, através de sítios representativos do FDT na Colômbia. Marcadores microssatélites (SSR's) foram utilizados para a obtenção da diversidade genética, e quatro traços funcionais para as análises de variabilidade. As populações apresentaram alta diversidade genética, com média de H_e 0,85. A maior riqueza alélica encontra-se nas populações de *La Pintada* y *Sogamoso*, sendo sítios prioritários para a conservação *in-situ* e fontes de sementes potenciais para atividades de reflorestação no futuro. O coeficente de endogamia entre as populações foi baixo (-0,024; -0,28). As análises das variações dos traços funcionais indicaram alta variabilidade para os traços: área foliar específica (AFE), contida foliar de matéria seca (CFMS), e densidade de madeira do caule (DM). Nas avaliações de componentes de variança, esta variabilidade foi principalmente representativa dentro das populações e indivíduos, e não representativa, entre populações ou biorregiões. Em termos gerais, *C. pentandra* não apresentou populações com perda de diversidade genética ou processos de depressão endogâmica, o que possibilita uma ampla capacidade de adaptação, evidenciada também no comportamento altamente variável de seus traços funcionais. Os resultados indicam que as populações da espécie encontram-se em bom estado de conservação genética, o que possibilita seu uso nos processos de restauração do FDT da Colômbia.

Palavras-chave: Floresta tropical. Diversidade genética. Marcadores moleculares. Traços funcionais. Fragmentação.

GENERAL ABSTRACT

Ceiba pentandra (L.) Gaertn is a species typical of the tropical dry forest (TDF) of Colombia. This ecosystem is currently highly fragmented, which compromises its perpetuation and the goods and services it offers to the community. The lack of primary information regarding the genetic and ecological levels of the species inhabiting the TDF makes it difficult to establish plans for managing and recovering its fragments. This work had the objective of determining the genetic diversity and the variability of functional traits of *C. pentandra* by means of sites representative of the TDF of Colombia. Microsatellite markers (SSR’s) were used to obtain the genetic diversity and four functional traits for variability analyses. The populations presented high genetic diversity, with average of H_e 0.85. The highest allelic richness was found for *La Pintada y Sogamoso* populations, being priority sites for *in situ* conservation and potential source of seeds for future reforestation activities. The coefficient of inbreeding between populations was low (-0.024; -0.28). The analyses for variation on functional traits indicated high variability for specific leaf area (SLA), dry matter content (DMC) and wood density of the stem (WD). In the evaluation of variance components, this variability was mainly represented within the populations and individuals, and not between populations or bioregions. In general, *C. pentandra* presented no populations with loss of genetic diversity or inbreeding depression processes, which allows wide adaptation capacity, also demonstrated by the highly variable behavior of the functional traits. The results indicate that the populations of this species is in a good state of genetic conservation, allowing its use for restauration processes of the TDF of Colombia.

Keywords: Tropical forest. Molecular markers. Functional traits. Fragmentation.

SUMÁRIO

1 INTRODUÇÃO GERAL

A floresta decídua tropical da Colômbia distribui-se ao longo do país (Figura 1), e de seus recursos, dependem diferentes comunidades rurais. No entanto, da cobertura original deste ecossistema restam somente cerca de 10%, devido a diferentes processos de fragmentação que ainda ameaçam os relictos remanescentes (PIZANO; GARCIA, 2014).

A fragmentação, além de modificar a distribuição espacial das espécies ao longo das gerações, tem efeitos diretos nas características genéticas e nas dinâmicas ecológicas das espécies (CASCANTE; QUESADA; LOBO, 2002; PLISCOFF; FUENTES-CASTILLO, 2011). Entre as principais consequências genéticas estão a diminuição da heterozigosidade, o aumento da endogamia, a diminuição do fluxo gênico, a perda dos processos e serviços ecossistêmicos, e em longo prazo, a extinção das populações locais (AGUILAR et al., 2008; KAGEYAMA; GANDARA, 1998).

A restauração e a conservação desse ecossistema demandam tempo, e dependem diretamente do conhecimento de seus padrões e dinâmicas. Alguns estudos são baseados nas semelhanças da floresta decídua colombiana, como as florestas das ilhas do Caribe, entretanto, as correlações são fracas e existe uma necessidade de gerar estudos que fornecem essa informação para o país (PENINGTON, 2012).

Devido à situação e à necessidade de contribuir na manutenção dos recursos, diferentes instituições nacionais e internacionais, tais como Biodiversity International, International Center for Tropical Agriculture (CIAT), Forestpa, Ecopetrol, Universidade Nacional da Colômbia, Governaciín de Antioquia, Empresas Públicas de Medellín (EPM) e o Laboratório de Conservação Genética de Espécies Florestais da Universidade Federal de Lavras, no Brasil, têm fornecido suportes econômicos, acadêmicos e técnicos para a execução de um plano de recuperação e de conservação nacional desta floresta, que vai ser o roteiro para a restauração e conservação da biodiversidade da floresta decídua da Colômbia.

A presente pesquisa faz parte desta iniciativa e tem por objeto o estudo da espécie *Ceiba pentandra* (L.) Gaerth., da família Malvaceae, representativa deste ecossistema e incluída na estratégia nacional de conservação de plantas da Colômbia (PIZANO;GARCIA, 2014).

O principal objetivo foi avaliar abordagens genéticas e ecológicas para determinar o conjunto genético da espécie no país, e definir a capacidade de adaptação, mediante as características morfológicas (traços funcionais) de *Ceiba pentandra* (L.) Gaerth.

Estas informações são fundamentais para a definição de áreas prioritárias de conservação, áreas de coleta de sementes, banco de germoplasma, áreas geneticamente homogêneas, definir sua prevalência no futuro, segundo as mudanças climáticas e o potencial funcional da espécie. Todas estas informações auxiliarão na elaboração de parâmetros efetivos para a conservação e manejo de *Ceiba pentandra* (L.) Gaerth., e restauração da floresta decídua da Colômbia.

2 REFERENCIAL TEÓRICO

2.1 Floresta decídua na Colômbia

A floresta decídua tropical é um ecossistema caracterizado pela sazonalidade climática, os regimes de fogo e os altos índices de endemismo. Além disso, apresenta baixa diversidade beta, em comparação com as florestas úmidas tropicais (GENTRY, 1995; JANZEM, 1988). É o bioma mais ameaçado dos trópicos, e aproximadamente um milhão de pessoas dependem dos serviços que proporcionam, como medicinas, alimentos, madeira, purificação de água e ar (PIZANO; GARCIA, 2014).

Mais da metade destas formações estão distribuídas desde o norte de México até o Norte de Argentina, sendo a América do Sul a região com maior representação deste ecossistema. Na Colômbia, a floresta decídua está distribuída ao longo do território. No entanto, estima-se que 90% deste ecossistema já se encontram extintos. Na atualidade, resta somente um aproximado de 80.000 Km^2 correspondentes aos 9% da cobertura original, sendo representado da seguinte maneira: 4% de floresta madura e 5% constituídos por remanescentes com diferentes graus de intervenção (Figura 1) (PIZANO; GARCIA, 2014).

A grave situação aumenta a cada dia, pois, somente em 5% da área total existente, encontra-se algum tipo de unidade de conservação, e a área restante, permanece sob pressão antrópica. Além disso, a configuração atual do tipo mosaico com uma conectividade cada vez menor reduz também as possibilidades de conservação em grandes áreas de proteção, reforçando a vulnerabilidade da floresta aos efeitos sinérgicos da fragmentação, sobre-exploração e dos novos cenários de mudanças climáticas globais (PIZANO; GARCIA, 2014; REYNOLDS et al., 2007).

As causas da degradação e a perda de habitat são diversas e históricas. A floresta decídua distribui-se geralmente em terras baixas com condições excelentes para agricultura e bovinocultura. Também são próximas aos maiores assentamentos humanos da Colômbia. Estas atividades remontam-se há 500 anos, desde a época pré-colombiana, e mudaram a composição e a distribuição deste ecossistema, no presente (ETTER; MCPALPINE; POSSINGHAM, 2008).

A transformação da floresta decídua produz diferentes conflitos ambientais, sociais e econômicos. Por um lado, perdem-se os serviços de provisão (alimentos, combustível, madeira, medicina) e regulação (qualidade do ar, regulação do clima, regulação da água) que prestam estas coberturas, e de outra parte, nas áreas com níveis maiores de fragmentação ou desertificação, é impossível manter sistemas produtivos, que facilitem a sobrevivência das populações humanas (ETTER; MCPALPINE; POSSINGHAM, 2008).

Frente ao panorama, são necessárias estratégias para a recuperação e conservação da floresta, que incluam o fornecimento de redes de mosaicos e de reservas naturais, que permitam a conectividade da paisagem e programas de conservação *ex situ* para as espécies mais ameaçadas (KAGEYAMA; GANDARA, 1998).

No entanto, existe um baixo conhecimento genético e ecológico das espécies arbóreas que constituem a floresta decídua no país. Até o momento, não existem relatos de dados biológicos, ecológicos e sociais sólidos que permitam a elaboração de estratégias para a gestão integral deste sistema natural. Devido a esta situação, nos últimos anos, houve um interesse crescente em estudos relacionados à floresta decídua, especialmente, nas consequências da fragmentação (ARCILA-CARDONA, et al., 2012; FERNÉNDEZ-MENDEZ; BERNATE-PEÑA; MELO, 2013; TORRES et al., 2012; VALENCIA-DUARTE; TRUJILLO; ORTIZ; VARGAS RÍOS, 2012; VARGAS, 2012).

Reconhece-se que o processo de recuperação desse ecossistema vai demandar tempo e esforço. Sua distribuição, do tipo relicto isolado, dificulta o entendimento das dinâmicas do ecossistema e de suas espécies. Assim, para sua recuperação, é preciso a formulação de planos de manejo de conservação, baseados no conhecimento profundo da identidade taxonômica, distribuição, interação e biologia dos organismos (PIZANO; GARCIA, 2014).

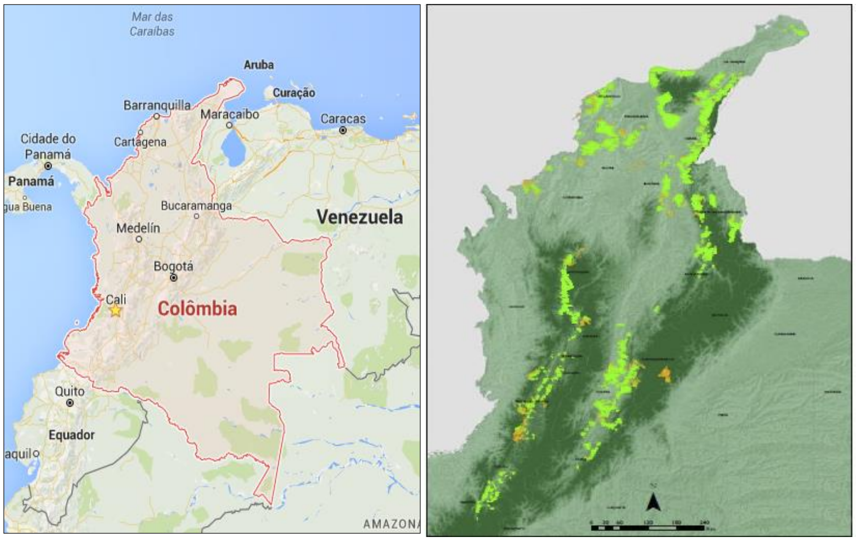

Figura 1 Mapa da Colômbia e dos fragmentos atuais de floresta decídua tropical

Fonte: Google maps (2015) Pizano e Garcia (2008).

2.2 Diversidade genética

A diversidade genética é definida como o material bruto sobre o qual a seleção natural atua para permitir a adaptação e a evolução dos organismos, e sua adequação às mudanças ambientais (FRANKHAM; BRASDSHAM; BROOK, 2014). Portanto, é uma das propriedades mais importantes dos ecossistemas, pois tem influência direta na capacidade de perpetuação das espécies (CASANOVES; PLA; DI RENZO, 2011; GUARANTÃ et al., 2000).

Em curto prazo, a perda de diversidade genética pode reduzir a aptidão individual das espécies, inviabilizando o remanescente populacional. Em longo prazo, a redução da riqueza alélica pode limitar a habilidade das espécies a responderem às mudanças devido à ação de forças seletivas, promovendo o processo de extinção (AGUILAR et al., 2008).

Segundo Dias e Kageyama (1991) a variabilidade genética manifesta-se em três níveis: *i*) indivíduos dentro de população, *ii*) populações dentro de espécie e, *iii*) espécies dentro do ecossistema, e é o resultado direto dos processos de mutação, migração, seleção e deriva genética (SILVA et al., 2012).

A distribuição desta variabilidade genética entre e dentro de populações está associada à dinâmica de movimentação dos alelos nas populações, ou fluxo gênico (*Nm*), influenciado pelo sistema reprodutivo da espécie, forma de cruzamento e síndromes de dispersão do pólen e das sementes, dentre outras interações bióticas e abióticas. Por exemplo, populações que são separadas por barreiras naturais ou antrópicas podem se tornar total ou parcialmente isoladas geneticamente, promovendo a endogamia. Além do fluxo de alelos, o tamanho populacional e o ciclo de vida das espécies também influenciam diretamente na diversidade e estrutura genética das populações (JEONG et al., 2010; KAGEYAMA et al., 2003; MARTINS, 1987).

Algumas árvores tropicais têm mecanismos eficazes de dispersão de alelos que permitem a manutenção de altos níveis de variabilidade genética em suas populações, e esses fatores também estão ligados à distribuição geográfica e ao tipo de ecossistema onde as espécies ocorrem naturalmente (HAMRICK; GODT, 1989,1996). No entanto, desconhece-se o comportamento da maior parte das espécies destas coberturas vegetais e, além disso, também se desconhece seu estado de ameaça (PIZANO; GARCIA, 2014).

Desta forma, o estudo e a compreensão da estrutura e variabilidade genética das populações existentes em remanescentes de florestas tropicais, são importantes para estabelecer critérios adequados em ações de recomposição dos ecossistemas degradados. É necessário conhecer a diversidade genética de uma espécie e também como esta variabilidade se encontra distribuída dentro e entre as populações presentes em áreas degradadas (KAGEYAMA, 1987).

Para a análise da diversidade genética é necessário o uso de técnicas moleculares que permitem a avaliação de parâmetros genéticos, como a heterozigosidade, polimorfismo, riqueza alélica, número efetivo de alelos, dentre outros. Estes dados são obtidos por meio de marcadores moleculares, sendo que os mais usados para este tipo de avaliação são os marcadores microssatélites, também chamados SSRs (*Simple Sequence Repeats*), e os marcadores ISSRs (*Inter Simple Sequence Repeats*) (VICENTE et al., 2004).

A mensuração dos níveis de diversidade genética é essencial para aperfeiçoar estratégias de conservação no panorama atual de desflorestamento e mudanças climáticas, pois auxilia a identificação de espécies e áreas prioritárias para a conservação e o conhecimento da sustentabilidade dos organismos em longo prazo (FLORIAN et al., 2013; GEBUREK; KONRAD, 2008).

2.3 Marcadores Microssatélites

Também chamados SSRs (*Simple Sequence Repeats*) por suas siglas em inglês, os marcadores microssatélites são sequências de DNA compostas de um a seis nucleotídeos repetidos em tandem. Este tipo de sequência encontra-se em genomas de eucariotos e procariotos (CAIXETA et al., 2006). Os microssatélites estão distribuídos em regiões codificantes e não codificantes, e constituem uma das classes mais polimórficas de marcadores disponíveis hoje e, deste modo, são regiões úteis para o estudo a nível populacional. Também, são muito informativos em plantas, requerem pouca quantidade de DNA, e podem ser analisados pelo processo de PCR (*Polymerase Chain Reaction*) (ORTH; ANTIQUERA, 2013).

O alto nível de polimorfismo é atribuído a dois mecanismos de mutação: ao deslizamento no anelamento das fitas do DNA (*Slipped Mispairing*), e ao *crossing over* desigual (*Unequal crossing over*) (VÁZQUEZ-LOBO; MORALES-GARCÍA, 2014). No processo de replicação do DNA pode ocorrer um erro no reanelamento das fitas devido a um deslizamento no alinhamento que ocorre durante a síntese da nova fita de DNA. Uma das fitas se dissocia momentaneamente da outra e pode voltar a associar-se em uma posição diferente, e com a continuação da elongação da síntese resulta em um aumento no comprimento da sequência microssatélite, ao passo que se o erro ocorre na fita molde, haverá uma deleção de *repeats*. No caso do processo de *crossing-over* desigual, durante a recombinação ocorre a perda de repetições em uma cromátide e o incremento na outra. No entanto, sabe-se que a ocorrência de mutações em microssatélites na ausência de recombinação, é uma evidência de que o mecanismo de *crossing-over* desigual não é o mecanismo predominante na origem dos polimorfismos dos microssatélites (VÁZQUEZ-LOBO; MORALES-GARCÍA, 2014).

Todas essas características fazem dos microssatélites um marcador eficiente para o mapeamento genômico, estudos de ligação, identificação de genótipos, proteção de variedades, avaliação de purezas de sementes, utilização e conservação de germoplasma, estudos de diversidade, análises genéticas de locos quantitativos, seleção assistida por marcadores, e análises de bibliotecas para clonagem de genes. O alto nível de variação detectado com os marcadores microssatélites aumenta a resolução do estudo de genealogia e diversidade genética do germoplasma e reduz o número de marcadores requeridos para distinguir os genótipos.

A grande desvantagem destes marcadores é a necessidade de serem isolados e desenvolvidos especificamente para cada espécie, não sendo possível utilizar a estratégia de desenho de ‘*primers universais*’, embora alguns microssatélites tenham demonstrado sucesso em sua utilização entre espécies dentro de um mesmo gênero botânico. O desenvolvimento destes marcadores envolve um processo demorado, trabalhoso e de alto custo. No entanto, os marcadores microssatélites vêm sendo utilizados em espécies arbóreas nos ecossistemas tropicais (CAIXETA et al., 2006). Alguns trabalhos desenvolvidos com SSR’s em florestas decíduas incluem as espécies *Calyptranthes clusiifolia* (FIDELIS, 2015), *Caryocar brasiliense* (MELO JUNIOR et al., 2012), *Samanea saman* (CASCANTE; QUESADA; LOBO, 2002). Na Colômbia, existem alguns projetos em andamento, no entanto, existe informação reportada somente para a espécie *Enterolobium ciclocarpum* (GIL-TOBON, 2015).

2.4 Variabilidade de traços funcionais

Os traços funcionais (TF) são definidos como os componentes morfológicos, fisiológicos, bioquímicos, estruturais, fenológicos ou ainda, as características comportamentais das plantas ou de partes da planta, que são expressas em fenótipos, e são a resposta às propriedades e aos processos ecossistêmicos (DÍAZ et al., 2013; KATTGE et al., 2011; PETCHEY; GASTON 2006).

As variações nos valores dos traços são o resultado das propriedades genéticas da população, o estresse a que está submetida a planta, ou à plasticidade fenotípica (JUNG et al., 2010; PEDROSO; ROCHA-FILHO; LOMÔNACO, 2010; SHOB et al., 2013; VALLADARES et al., 2002). A plasticidade fenotípica é descrita como a capacidade de um genótipo de modificar a expressão do fenótipo em resposta às condições ambientais adversas, tais como a temperatura, seca, pressão por predação e disponibilidade de recursos (BERGS; ELLERS, 2010; GIANOLI, 2004; PEDROSO; ROCHA-FILHO; LOMÔNACO, 2010; VALLADARES et al., 2002).

A variabilidade fenotípica (medida geralmente pelo coeficiente de variação CV) é expressa ao nível intraespecífico (dentro das espécies) e ao nível interespecífico (entre as espécies) (CARDOSO; LOMÔNACO, 2003; REICH et al., 2003). Entretanto, nos estudos de traços funcionais, a variabilidade fenotípica tem recebido pouca importância (SALGADO-NEGRET et al., 2015) .

Nas avaliações de traços funcionais é comum realizar os trabalhos com poucos indivíduos por população, com poucas populações, ou análises com o valor médio dos traços da população ou da espécie (ALBERT et al., 2010a, 2010b). Isso tem como consequência, a perda na informação que corresponde à detecção dos processos de filtragem, que controlam a montagem das comunidades ecológicas e suas repostas às mudanças climáticas (ALBERT et al., 2010b).

Considerando o que foi mencionado anteriormente, nos últimos anos, diversos estudos têm trabalhado com a variabilidade de traços funcionais, alguns têm definido traços mais variáveis que outros, por exemplo, os traços de folha tais como, Área foliar (AF), Área foliar especifica (AFE), Conteúdo de matéria seca na folha, Conteúdo de água na folha e conteúdo de N, F, traços de raiz (comprimento), e traços com menor variação como a densidade de madeira (caule e galho) (ALBERT et al., 2010b; CARDOSO; LOMÔNACO, 2003; HULSHOF; SWENSON, 2010; JUNG et al., 2010; REICH et al., 2003; SALGADO-NEGRET et al., 2015; SANCHES et al., 2010; SHOB et al., 2013).

Por outro lado, estudos como o de Albert et al. (2010a), focam-se na comparação da variabilidade interespecífica e intraespecífica em comunidades, sua amplitude e a importância de cada uma destas características.

Assim, a avaliação da variabilidade nos traços funcionais é fundamental para a compreensão de como a população é estruturada no tempo e no espaço (PEDROSO; ROCHA-FILHO; LOMÔNACO, 2010). Além disso, essa avaliação é importante para a previsão e a compreensão dos padrões ecológicos e evolutivos (ALBERT et al., 2010a; 2010b; PÉREZ-HARGUINDEGUY et al., 2013). Os traços tornam-se uma ferramenta importante para a elaboração de estratégias de conservação da biodiversidade (BOCANEGRA-GONZÁLEZ et al., 2015).

2.5 Integridade fenotípica

O interesse principal dos biólogos evolucionistas é entender como a seleção natural impulsiona os padrões de evolução dos traços, e como estas mudanças adaptativas podem também ser limitadas por restrições individuais dos traços por causa de mudanças intensas e rápidas no entorno, isto se pode quantificar por meio da avaliação da integridade fenotípica (TORICES; MUÑOZ-PAJARES, 2014; VALIDO; SCHAEFER; JORDANO, 2011).

O termo faz referência ao estudo dos complexos padrões de covariação entre traços funcionais que descrevem um espécime (variação coordenada) (PIGLIUCCI, 2003; SALGADO-NEGRET et al., 2015). Esta integração dos fenótipos é o resultado de diferentes processos genéticos, pleiotropia, fatores de desenvolvimento ou associações fisiológicas e funcionais (GODOY; VALLADRES; CASTRO-DIAZ, 2012; SALGADO-NEGRET et al., 2015).

A integração dos traços tem profundo impacto no rendimento da planta, pois, um fenótipo integrado pode dar uma resposta mais adaptativa à variação ambiental que fenótipos com menor integridade (GODOY; VALLADARES; CASTRO-DIAZ, 2012). Além disso, pode dar origem a evolução dos fenótipos (TORICES; MUÑOZ-PAJARES, 2014). Assim, o conceito converte-se em mecanismo importante para vincular os diferentes aspectos que têm efeitos na aptidão das plantas (GODOY; VALLADARES; CASRTO-DIAS, 2012).

Alguns pesquisadores propõem que a IF aumenta com o estresse ambiental, no entanto, não se sabe se é um padrão funcional ou se é um sintoma de estresse. Matesanz, Gianoli e Valladares (2010) propõe a realização de estudos que consigam elucidar esta hipótese.

Por outro lado, as análises de IF são geralmente estimadas por meio de procedimentos de covariância ou correlações significativas entre os diferentes traços. Estas correlações de traços se dão entre indivíduos dentro de uma população ou entre espécies, ou em diferentes etapas ontogênicas (MATESANZ; GIANOLI; VALLADARES, 2010; PIGLIUCCI, 2003).

Alguns estudos em plantas que têm trabalhado com IF são os (MARTINEZ-CABRERA et al., 2011; SALGADO-NEGRET et al., 2015; VALIDO; SCHAEFER; JORDANO, 2011). Neste último, por exemplo, se avalia os níveis de IF para a espécie *Aextoxicon punctatum* em fragmentos de florestas nebulares no Chile, no interior dos fragmentos e nas bordas. A maior IF nas bordas é explicada pela pouca disponibilidade de recursos nestas áreas.

2.6 *Ceiba pentandra* (L.) Gaertn.

Ceiba pentandra (L.) Gaertn. é uma espécie da família Malvaceae, que está distribuída desde o México até a Bacia Amazônica, e no paleotrópico ao oeste de África. Também existem alguns registros de ocorrência na China, no entanto, sabe-se que foram árvores introduzidas (HARTSHORN, 1983, 1990; WU; REVEN; HONG, 2007) (Figura 2). Na Colômbia, é conhecida popularmente como Ceiba, e encontra-se entre 0 e 1500 m do nível do mar. É uma espécie pioneira, típica da floresta decídua do país, apresenta-se geralmente como árvore isolada e é descrita como espécie rara pelos estudos de abundância (FERNÁNDEZ-MENDEZ; BERNATE-PEÑA. MELO, 2013).

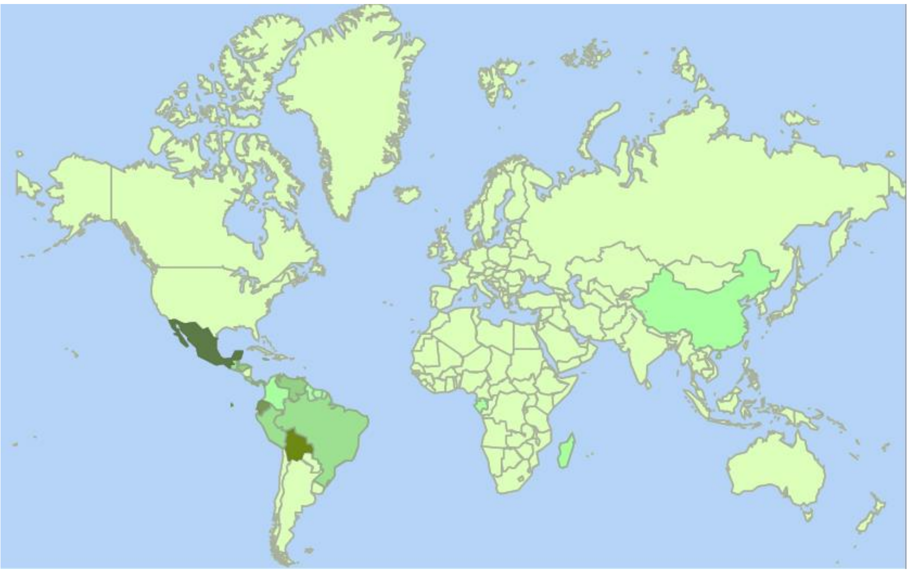

Figura 2 Mapa da distribuição de *Ceiba pentandra* (L.) Gaertn. no mundo
Fonte: Tropicos Missouri Botanical Garden (2015).

Ceiba pentandra (L.) Gaertn encontra-se categorizada em um estado de conservação de preocupação menor (LC), de acordo com The IUCN Red list of threatened species (2013), e está incluída no plano estratégico para a conservação das plantas da Colômbia (PIZANO; GARCIA, 2014). No entanto, no país, não existem relatos de estudos genéticos e ecológicos de suas populações.

Com relação às características da árvore, esta pode apresentar até 70 m de altura e um diâmetro de 3 m acima da raiz tubular. Suas folhas são alternas, palmadas, compostas com 7 ou 8 folíolos. A copa pode ter uma cobertura de 50 m, e o caule é cilíndrico e coberto por espinhos. É hermafrodita e tem flores axilares com pétalas brancas, rosas e amarelas; seus frutos são cápsulas que têm entre 120 e 175 sementes por fruto (GAERTNER, 1791; GRIBEL; GIBBS; QUEIROZ, 1999).

A espécie perde as folhas no início da época seca, antes da floração, entre janeiro e março, no entanto, não florescem anualmente. É uma espécie polinizada por morcegos, abelhas e vento. Seu sistema de reprodução é variável e depende de sua distribuição, variando de autoincompatível até sistema misto, com diferentes graus de autofecundação (LOBO; QUESADA; STONER, 2005).

Figura 3 Árvore, caule e folias de *Ceiba pentandra* (L.) Gaertn.

Ceiba pentandra tem grande valor social e cultural, além de diversos usos, como por exemplo, a fabricação de barcas, devido a sua madeira leve; uso medicinal; fabricação de carvão vegetal; cercas; barreira para ventos; uso ornamental para embelezar a paisagem e; largamente utilizada para reflorestamento e recuperação de solos. Além disso, tem sido usada para a fabricação de bioetanol, biodiesel e polpa para papel (EUMALAI et al., 2012; SILITONGA et al., 2013).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 Genetic diversity of *Ceiba pentandra* (L.) Gaertn. in the tropical dry forest of Colombia using SSR's markers

Artigo formatado de acordo com a NBR 6022 (ABNT, 2003).

ABSTRACT

High degradation of the Tropical Dry Forest (TDF) in Colombia and in some of its species generates the necessity of research to promote its conservation and protection. *Ceiba pentandra* (L.) Gaertn is characteristic tree of this ecosystem with a high potential for its restoration. We genotiped 88 individuals of this specie from 9 populations across the TDF in Colombia by mean of 8 SSR's markers. Were found high levels of genetic diversity (H_e) in the specie with an average of 0.85. Populations in the municipalities of *Sogamoso* (CHI) in the department of Santander and *La Pintada* (PIN) in the department of Antioquia were identified with the highest number of unique alleles (A_{ex}) with an average 0.375 per population, highlighting them as potential sources of germplasm for future planting activities. The analysis of molecular variance indicates that 98% of genetic variability is contained within the populations. Values in the inbreeding coefficient (F_{IS}) were predominantly negatives, suggesting panmictic populations. Finally, Nei's genetic analysis reveals little differentiation among populations; however, the differences are associated with geographic patterns suggesting a low gene flow among distant populations, probably due to natural barriers.

Keywords: Genetic diversity. Fragmentation. SSR's Markers. Tropical Dry Forest.

RESUMEN

La alta degradación del Bosque Seco Tropical (BST) en Colombia y en algunas de sus especies genera la necesidad de investigaciones que promuevan su conservación y protección. *Ceiba pentandra* (L.) Gaertn es un árbol característico de este ecosistema con elevado potencial para su restauración. Genotipamos 88 individuos de esta especie en 9 poblaciones a través del BST en Colombia por medio de 8 marcadores SSR's. Se encontraron altos niveles de diversidad genética H_e en la especie con un promedio de 0.85. Las poblaciones de los municipios de *Sogamoso* (CHI) en el departamento de Santander y *La Pintada* (PIN) en el departamento de Antioquia se identificaron con el mayor número de alelos únicos (A_{EX}) con un promedio de 0,375 por población, destacándose como fuentes potenciales de germoplasma para futuras actividades de plantación. El análisis de varianza molecular indica que 98% de la variabilidad genética está contenida dentro de las poblaciones. Los valores en el coeficiente de endogamia (F_{IS}) fueron predominantemente negativos, sugiriendo poblaciones panmícticas. Por último, el análisis de distancia genética de Nei revela poca diferenciación entre las poblaciones, sin embargo, las diferencias están asociadas a los patrones geográficos lo que sugiere un bajo flujo génico entre las poblaciones más distantes, probablemente debido a las barreras naturales.

Palabras clave: Diversidad genética. Fragmentación. Marcadores SSR's. Bosque seco Tropical.

1 INTRODUCTION

Colombian Tropical Dry Forest (TDF) is an ecosystem that is home of a rich biodiversity. However, fragmentation processes threaten the future existence of TDF and the its organisms (PIZANO; GARCIA, 2014). Fragmentation is been linked to decreasing heterozygosity, reduction of effective size population, decreases in gene flow and long-term extinction of local populations (AGUILAR et al., 2008; KAGEYAMA; BERTIN, 1998) and with this, the loss of ecological processes and ecosystem services. Understanding the patterns of genetic variation between and within populations has a fundamental importance for the development of conservation strategies to ensure resources in the future (KAGEYAMA et al., 2003; VIEIRA-BLANCO, 2007).

The genetic structure of populations is quantified through analysis of variability of gene frequencies (WEIR, 1996), which provides the distribution of genetic diversity in the different hierarchical levels in which the study sample has been structured (KAGEYAMA et al., 2003). Genetic diversity is a parameter of this structure that is defined as the raw material on which natural selection acts and allows the evolution of organisms and their adaptation to environmental changes (FRANKHAM; BRADSHAW; BROOK, 2014). One, the technique to estimate the genetic diversity in natural populations and the changes arising from human action is the use of highly polymorphic molecular markers as SSR's (*Simple Sequence Repeats*) or also called microsatellites markers (RAPOSO et al., 2007; ORTH; ANTIQUERA, 2013). SSR's are short repetitions (1 to 6 pairs) of bases repeated in tandem (side by side)

(CAIXETA et al., 2006). They represent unstable regions of the genome, randomly distributed, forming polymorphic genetic loci that are under mutational changes at higher rates than those observed in other sequences (ORTH; ANTIQUERA, 2013). SSR’s are the closest markers to the ideal of marker for genetic studies of populations. This is because they have a number of alleles and heterozygosity much higher than other markers (RAFALSKI et al., 1996).

Among the many plants species with special characteristics that exist in tropical dry forest is *Ceiba pentandra* (L.) Gaertn, locally known as “*Ceiba*”. This species has many uses, from medical to biodiesel; and has become popular for its potential for reforestation of degraded areas. Is a light-demanding pioneer tree, native populations presented in tropical America and West Africa (BAKER 1965; ZOTS; WINTER, 1994). Is categorized as a minor concern condition (LC) according to The IUCN Red list of threatened species (2013) and is included in the strategic plan for the conservation of plants in Colombia due to, its representativity in dry ecosystems (PIZANO; GARCIA, 2014). However, there are no known genetic studies in the country that back up this categorization. Nevertheless genetic characterization data of the species does exist in dry ecosystems in Mesoamerica (LOBO; QUESADA; STORNER, 2005).

The aim of this study was to analyze the magnitude and distribution of genetic diversity in natural populations of *Ceiba pentandra* (L.) Gaertn across TDF of Colombia using 8 SSR’s markers to define in-site conservation areas for future planting restauration efforts.

2 METHODS

2.1 Study area and sampling design

This study was conducted in seven patches of tropical dry forests located in five departments of Colombia (Figure 1). Between the months November 2014 and June 2015 we collected young and healthy leafs of adult trees of *Ceiba pentandra* (L.) Gaertn separated by distances of at least 50 meters between individuals. For each population we collected between four to fourteen trees and this number depended on the number of individuals available in each site (Table 1). In this study, populations are defined as sets of individuals that are grouped and are spatially distant from other sets of individuals of the same species. Later, all vegetal material was analyzed in the Yucca Genetic Laboratory of International Center for tropical agriculture (CIAT) in Colombia.

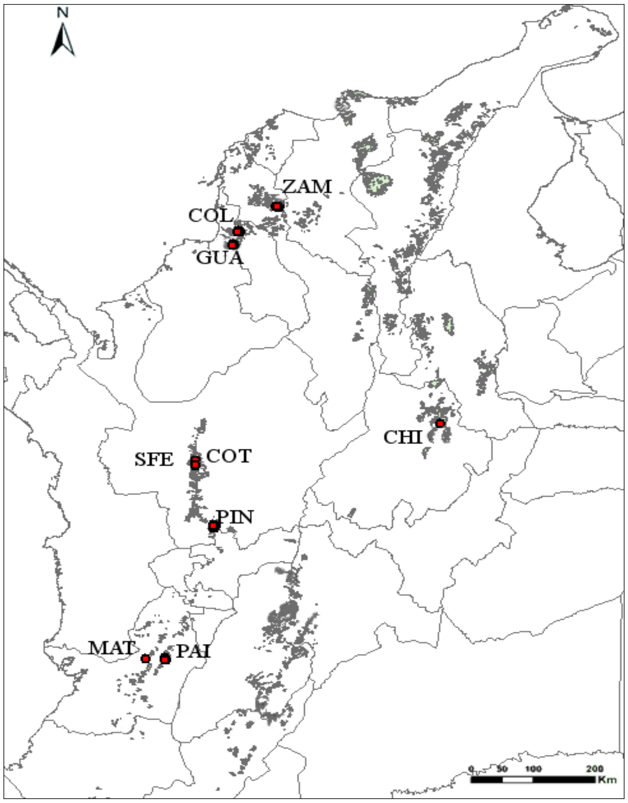

Figure 1 Populations of *Ceiba pentandra* (L.) Gaertn in the different departments of Colombia

Table 1 Location of *Ceiba pentandra* (L.) Gaertn populations. Cod: Code, N: Individuals sample, Lat: Latitude, Lon: Longitude, Alt: Altitude

Departament	Municipality	Cod	N	Lat	Lon	Alt
Santander	Sogamoso	CHI	10	6.83841	-72.9803	754.8
Sucre	Colosó	COL	8	9.53855	-75.3438	222.58
Antioquia	Cotové	COT	5	6.52788	-75.8316	494.87
Sucre	Guamo	GUA	14	10.0193	-74.8900	47.38
Valle del Cauca	Mutate	MAT	4	4.02905	-76.1694	1110.13
Valle del Cauca	La Paila	PAI	12	4.28372	-76.0924	945
Antioquia	La Pintada	PIN	11	5.72588	-75.6162	646.67
Antioquia	Sta fe de Antioquia	SFE	5	6.52537	-75.8043	443.75
Bolívar	Zambrano	ZAM	11	9.89483	-74.89483	33.94

2.2 Study species

Ceiba pentandra (L.) Gaertn. It is found from 0 to 1500 m.a.l.s. It can reach up to 70 m and a diameter of 3 m above the buttress. Its leaves are alternate, palmate, compound with 7 or 8 elliptic leaflets. The canopy have coverage of 50 m and the trunk is characterized by having a cylindrical straight shape covered by numerous spines (GAERTNER, 1791). It is a hermaphrodite tree with axillary flowers petals (white, pink and yellow); its fruits are capsules containing from 120 to 175 seeds per fruit between woolly fibers (GRIBEL; GIBBS; QUEIROZ, 1999). Regarding the habitat and ecology, it develops on riverbanks, in a variety of soil conditions, from very sandy soils with strong drainage to clay soils and flooding. It is a aggressive pioneer specie that grows in abandoned fields and cleared lands. The trees lose their leaves at the beginning of the dry season, before flowering between January and March and immediately after blooms. It is pollinated by bats, bees and wind. Its reproduction system varies along its distribution, ranging from totally self-incompatible to a mixed system with varying degrees of inbreeding (LOBO; QUESADA; STORNER, 2005).

2.3 Genetic analysis

We used Doyle and Doyle (1987) protocol for extraction of DNA with some modifications described by Novaes, Rodrigues e Lovato (2009). Thereafter we selected eight SSR's loci (CP1, CP12, CP13, CP15, CP18, CP19, CP20, CP22) developed by Brondani et al. (2003) for *Ceiba* *pentandra* (L.) Gaertn. To carry out detailed genetic analysis, NED, FAM, PET and VIC fluorescent dyes were used to allow allele scoring. The polymerase chain reaction (PCR) amplification was performed in a total volume of 12 µL containing 0. 5 U of Platinum® Taq (Invitrogen®, USA), 1X PCR Buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl) (Invitrogen®, USA), 0.2 mM of each dNTP (Promega Corp., USA), 8.0 mg /mL BSA, 3.0 mM MgCl2 (Invitrogen®, USA), 0,05 pmol/µL Labelled M13 primer, 0,01 pmol/µL M13 tagged-Forward primer, 0,05 pmol/µL reverse primer and 40 ng of DNA. PCR was conducted in a Mastercycler® pro (Eppendorf, Germany) for 1 cycle 95 °C, 2 min, followed by 15 cycles (30s at 94°C, 30s at 65°C and 30s at 72°C), followed by 35 cycles (15s at 94°C, 15s at 50°C and 45s at 72°C). The PCR products were separated in 2% agarose gels to check if the loci were polymorphic or not, PCR products were run on an ABI PRISM 3730 DNA Analyzers sequencer and sized with GeneScan-500LIZ (Applied Biosystems) standard size. Allele sizes were determined using GeneMapper version 4.0 (Applied Biosystems) software.

2.4 Statistical analysis

Detection of null alleles

In genetic studies where SSR's markers are used, usually are found null alleles. The no detection of these alleles causes genotyping errors that can lead to deviations from the proportion equilibrium due to a deficiency of heterozygotes (ALVARES, 2014). For this analysis was used the Micro Checker software. The frequency of each locus was estimated using the estimator Brookfield nº 1: $r = (H_e - H_o) / (1-H_e)$ (VAN OOSTERHOUT et al., 2004).

Genetic diversity in populations

We analyzed the genetic diversity of populations through measuring the following parameters: average number of alleles observed (A_o) and effective (A_e), average of exclusive alleles (A_{ex}), expected heterozygosity or gene diversity (H_e), observed heterozygosity (H_o) and inbreeding coefficient (F_i). All parameters were determined with GenAlEx 6.5 software (PEAKALL; SMOUSE, 2006, 2012). Beside, we assessed deviations from the expected proportions by the Hardy - Weinberg equilibrium model tested from Fisher's exact test. The exact probabilities lower than the significance level (0,05 or 0.01) indicate significant deviations. This analysis was performed in GENEPOP software on- line (RAYMOND; ROUSSET, 1995; ROUSSET, 2008). Finally, analysis of molecular variance (AMOVA) was used to determine how the genetic variability of *Ceiba pentandra* (L.) Gaertn is distributed among and within populations located in different patches of tropical dry forest. Statistical significance was achieved with 1000 permutations using the GeneAlEx 6.5 software (PEAKALL; SMOUSE, 2006, 2012).

Other structural parameters of populations

The genetic distance and genetic similarity (NEI, 1978) between populations were estimated at GeneAlEx 6.5 software (PEAKALL; SMOUSE, 2006, 2012) and were used for the construction of a dendrogram, through SAHN (Sequential Agglomerative, Hierarchical and Nested Clustering) method in NTSYS software.

3 RESULTS

3.1 Detection of null alleles

Among the 88 assessed individuals with eight microsatellite loci, the analysis indicated null alleles in the locus CP1 and CP15 with significant estimates <0.025 and <0.001 respectively. Presence of null alleles was suggested due to excess of homozygotes present in all classes of alleles size. However, the correction of these alleles by indication of the program was not necessary.

3.2 Genetic Diversity in the populations

The analysis of genetic diversity was estimated across the nine sampled areas and within each of them. The A_o mean value was 6.68 and A_e mean value was 4.94. Both parameters in CHI population had a higher value A_o= 8,37; A_e=5,70 (Table 2). COL and PAI populations revealed any values in A_{ex} while CHI and PIN present higher values than other populations studied (Table 2). Average of genetic diversity (H_e) was 0.85 and H_o=0.78. In general, all populations showed high values of these indices (Table 2). F_i was insignificant in all populations (Table 2).

On the other hand, only GUA population showed a significant value of deviation from Hardy-Weinberg equilibrium, in the remaining populations we do not find significant values. Finally, by analyzing the distribution of genetic variation of *Ceiba pentandra* (L.) Gaertn between and within nine populations of tropical dry forest was obtained that most of the variation is found 98% within populations and just 2% among populations (Table 3).

Table 2 Genetic characterization of populations of *Ceiba pentandra* (L.) Gaertn. A_o= average number of alleles observed; A_e= average number of effectives alleles; A_{ex}= average of exclusive alleles; H_e= expected heterozygosity; H_o= observed heterozygosity; F_{IS}= inbreeding coefficient

Populations	A_o	A_e	A_{ex}	H_e	H_o	F_i
PIN	7.875	5.627	**0.375**	0.830	0.816	-0.024
COT	6.250	5.280	0.125	0.925	0.798	-0.171
SFE	5.875	4.918	0.250	0.875	0.785	-0.112
GUA	7.500	5.136	0.125	0.916	0.797	-0.155
COL	6.000	4.657	0.000	0.719	0.773	0.074
ZAM	7.125	5.123	0.125	0.875	0.793	-0.113
MAT	4.500	3.781	0.250	0.906	0.719	-0.288
CHI	8.375	5.708	**0.375**	0.825	0.808	-0.033
PAI	6.625	4.270	0.000	0.781	0.746	-0.042
Mean	6.68	4.94		0.85	0.78	

Table 3 Analysis of molecular variance in nine populations of *Ceiba pentandra* (L.) Gaertn. in the tropical dry forest

	df	SS	MS	Est. Var.	%
Among Pops	8	38.381	4.798	0.085	2%
Among Indiv	71	235.544	3.318	0.000	0%
Within Pops	80	268.500	3.356	3.356	98%
Total	159	542.425		3.441	100%

3.3 Other structural parameters of populations

The average of Nei’s genetic distance between populations was 0.43. The lowest distance was found between populations MAT and PAI with value of 0.209 and the high distance was between MAT and COT populations with a value of 0.824 (Table 4). Nei's genetic similarity exposes a low differentiation among populations. (Table 4).

Table 4 Genetic distance (below diagonal) and similarity estimates (above diagonal) between populations of *Ceiba pentandra* (L.) Gaertn. PIN: La Pintada, COT: Cotové, SFE: Sta Fe de Antioquia, GUA: Guamo, COL: Colosó, MAT: Mutate, CHI: Chicamocha, PAI: La Paila

	PIN	COT	SFE	GUA	COL	ZAM	MAT	CHI	PAI
PIN	---	0.598	0.657	0.752	0.710	0.697	0.671	0.735	0.673
COT	0.515	---	0.557	0.689	0.540	0.585	0.439	0.538	0.554
SFE	0.420	0.585	---	0.664	0.619	0.606	0.604	0.584	0.602
GUA	0.285	0.372	0.410	---	0.777	0.751	0.647	0.747	0.666
COL	0.343	0.617	0.479	0.253	---	0.705	0.584	0.718	0.620
ZAM	0.360	0.535	0.501	0.286	0.350	---	0.606	0.701	0.723
MAT	0.398	0.824	0.505	0.436	0.538	0.501	---	0.708	0.811
CHI	0.307	0.620	0.538	0.291	0.331	0.355	0.345	---	0.757
PAI	0.396	0.591	0.508	0.406	0.478	0.325	0.209	0.278	---

Dendrogram analysis allowed discriminate between two clusters. The first cluster is integrated by PIN, SFE, GUA, COL, MAT, CHI and PAI populations, while second cluster with the COT population. The population genetically closer to the second cluster is known as SFE and is also the most geographically nearby COT population (Figure 2).

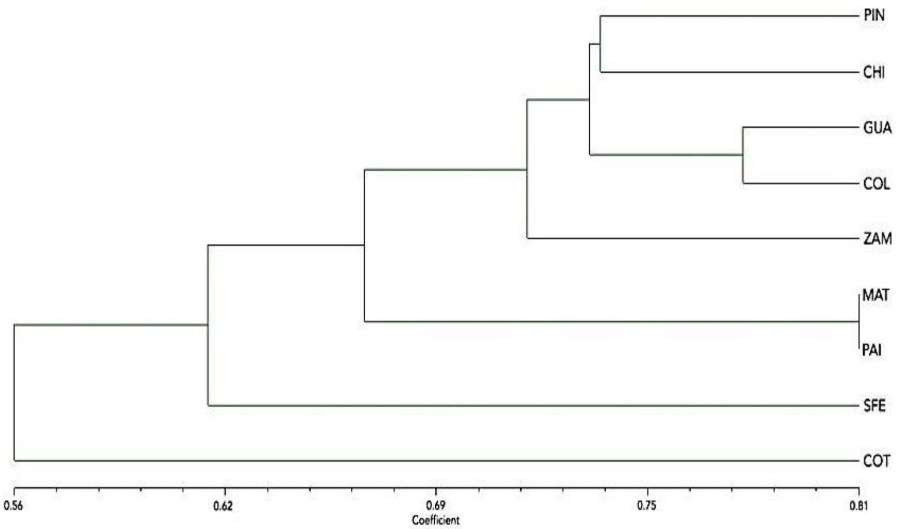

Figure 2 Dendrogram of populations of *Ceiba pentandra* (L.) Gaertn calculated according to the genetic similarity (NEI, 1978). PIN: La Pintada, COT: Cotové, SFE: Santa Fe de Antioquia, GUA: Guamo, COL: Colosó, MAT: Mutate, CHI: Chicamocha, PAI: La Paila

4 DISCUSSION

We found a high genetic diversity in all populations evaluated, this correspond to good state of genetic conservation of *Ceiba pentandra* (L.) Gaertn. Also, the number of unique alleles differ in each population, being an important parameter for definition of populations like potential banks of seed. Finally, we found relation between the genetic similarity and geographic distance.

4.1 Detection of null alleles

The use of SSRs markers generates some limitations, usually has null or silent alleles, not amplified during the PCR reaction and also not detected in the genotypage. This can lead to a wrongly detection of homozygotes, raising its quantity and influence of the estimates of genetic diversity. For this reason, it is necessary that these alleles are differentiated (BRUFORD; WAYNE, 1993; GUICHOU et al., 2011). In this study, some locus indicated the presence of null alleles; however there wasn't a significant presence that could amend our data and results. The primers used in this study were high efficiency.

4.2 Genetic diversity in the populations

The seven assessed populations of *Ceiba pentandra* (L.) Gaertn had high levels of genetic diversity, this means that the specie is in good state of genetic conservation. This values matches with other studies in fragmented dry ecosystems with species like *Ceiba pubiflora* (MALVEIRA, 2012) and *Enterolobium cyclocarpum* (GIL-TOMBO, 2015). Schoen and Brown (2001) suggesting that a small amount of positive genetic variation can allow population a better adaptation to environmental pressures. Because of this characteristic, we considered the number of unique alleles parameter more significant that genetic diversity index like representative parameter for definition of seed banks. In this context we defined "*La Pintada*" and "*Sogamoso*" populations like potential sites for extractions of seeds and seedling for restauration process in fragments of tropical dry forest in Colombia.

Respect to inbreeding coefficient (F_i), all populations showed negative values. These means that the crossing of related individuals is low and that the evaluated populations are panmictic and in a long term, they tend to conserve the genetic diversity of this specie. Studies in the semideciduous forest and Cerrado ecosystems by the species *Esenbeckia leiocarpa* (KAYEHAMA et al., 2003) and *Calyptranthes clusiifolia* (FIDELIS, 2015) showed a F_i with a tendency to be positive, generally because the decrease of habitat that affects mainly conservative species with complex reproduction system due to decline of pollinators, seed dispersers and promotes pioneer or heliophilous species with more variables reproduction systems. So that, considering that *Ceiba pentandra* (L.) Gaertn is a pionner especie, this tendency can be present in our ecosystem of study, influencing in a positive way the reproductive system of this specie. Explaining why is not affected for fragmentation process in the evaluated relicts.

4.3 Other structural parameters of populations

In the analysis of the dendrogram is noted that all populations are more clustered in relation to the COT population. However, between all populations doesn't exist high genetic distances. Also, the dendrogram shows that genetic distances between populations preserve geographic patterns, that is the smaller genetic distances correspond to geographically close populations, except in in the cases of municipalities populations of "S*ogamoso*" y "*La Pintada*". Being the same populations sites, with an addition in its genetic pool.

The relationship between genetic distance and geographical distance can obey probably to geographical barriers and landscape such as the Andes mountain ranges, great rivers and at the same time by fragmentation processes that occur in them and prevent gene flow between populations.

Finally, *Ceiba pentandra* presents a good state of genetic conservation for the country. Populations are able to perpetuate in the future and the constant fragmentation of tropical dry forest has no implications for their gene pool. On the other hand, although all the evaluated populations are possible seed banks and the seedling stand located in the municipalities of *Sogamoso* in the department of Santander and *La pintada* in the department of Antioquia. Ceiba is a potential specie in the recovery of degraded areas of tropical dry forest both as be fast growing specie and because state of its genetic characteristics.

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ARTIGO 2 Functional traits variation of *Ceiba pentandra* (L.) Gaertn across Colombian tropical dry forest

Artigo formatado de acordo com a NBR 6022 (ABNT, 2003).

ABSTRACT

The variability of functional attributes in an intraspecific level determines the response of species to environmental conditions and their effects on ecosystem processes. The aim of the research was to determine the behavior of traits of *Ceiba pentandra* (L.) Gaertn at different levels and define where the greater variation is located. At the same time, we evaluated the integration of the traits. Four functional traits were analyzed in this study: specific leaf area (SLA), leaf dry matter content (LDMC), stem wood density (SWD) and branch wood density (BWD) of the specie through Tropical Dry Forest (TDF) fragments located in different bioregions of Colombia. Significant variations were found in the attributes of LDMC and SWD at the bioregions. Attributes of SLA, SWD and LDMC presented a significant variation among populations. The analysis of variance components reveals that in the SLA and LDMC traits more of the 45% of the variation is within individuals, in the case of SWD and BWD traits over 65% of the variance is located within populations. Finally, the evaluated traits for *Ceiba pentandra* (L.) Gaertn showed a representative phenotypic integrity

Keywords: Functional traits. Variability. Phenotypic integration. Tropical dry forest.

RESUMEN

La variabilidad de los atributos funcionales a nivel intraespecífico permite determinar la respuesta de las especies a las condiciones ambientales y su efecto en los procesos ecosistémicos. El objetivo de la investigación fue determinar el comportamiento de los rasgos de *Ceiba pentandra* (L.) Gaertn en diferentes niveles y definir donde está ubicada la mayor variación. Al mismo tiempo, evaluamos la integración de sus rasgos. Cuatro rasgos funcionales fueron analizados, área foliar específica (AFE), contenido foliar de materia seca (CFMS), densidad de madera del fuste (DM) y densidad de madera de rama (DR) de la especie a través de diferentes fragmentos de Bosque Seco Tropical (BST) ubicados en diferentes bioregiones de Colombia. Se encontraron variaciones significativas en los atributos de los rasgos CFMS y DM en las bioregiones. Los atributos de AFE, CFMS y DM presentaron variación significativa entre las poblaciones, por otro lado los atributos de AFE, CFMS presentaron variación significativa dentro de las poblaciones y dentro de los individuos. El análisis de componentes de la varianza mostró que en los rasgos AFE y CFMS más 45% de la variación se encuentra dentro de los individuos, en el caso de los rasgos DM y DR más del 65% de la varianza está ubicada dentro de las poblaciones. Finalmente, los rasgos evaluados presentaron integridad fenotípica representativa.

Palabras clave: Rasgos funcionales. Variabilidad. Integración fenotípica. Bosque seco Tropical.

1 INTRODUCTION

Functional traits are the features of plants that represent ecological strategies and determine how they respond to environmental factors and are the answer to the properties and ecosystem processes (DÍAZ et al., 2013; KATTGE et al., 2011). Attributes of functional traits are variables between and within species (ALBERT et al., 2010a) these variation results of evolutionary properties of population, environmental stress or phenotypic plasticity (JUNG et al., 2010; NEGREIROS et al., 2014; PEDROSO; ROCHA-FILHO; LOMÔNACO, 2010; REICH et al., 2006; SHOB et al., 2013; VALLADARES et al., 2002). The value of the variability is important because it allows filtering processes detection that control ecosystems and their responses to climate change and fragmentation processes (ALBERT et al., 2010a, 2010b; VIOLLE et al., 2007). Usually, species are commonly described by functional trait measurements collected from a few individuals, from one or few populations and average of the population or species, disregarding the intraspecific variability (ALBERT et al., 2010b; HULSHOF; SWENSON, 2010; SALGADO-NEGRET et al., 2015). Due to is, little is known about the role of intraspecific variability on traits and the non-random assembly mechanisms that control the coexistence of species, including habitat filtering and niche differentiation (JUNG et al., 2010). Intraspecific variability can occur at different levels: (*i*) differences between mean traits of populations occurring in contrasted environmental conditions, (*ii*) differences between individuals of a population or (*iii*) differences between traits of an individual, all this associated to direct gradients such as temperature, rainfall and nutrients availability (ALBERT et al., 2010a; MATESANZ; GIANOLI; VALLADARES, 2010). Also, the intraspecific variability is an important resource because express the range of possible values of traits and explains the functional experience of individuals from the same species that occurring in different environmental conditions (ALBERT et al., 2010b). At the same time, traits of species adapted to environments with low resource availability tends to show a stress resistance syndrome characterized by low phenotypic variation (NEGREIROS et al., 2014). In addition, comparative studies in traits often ignore the phenotypic integration (GODOY; VALLADARES, CASTRO-DIAS, 2012), this feature refers to pattern and magnitude of functional correlation among different plant traits (PIGLIUCCI, 2003). Integrated phenotypes increase the survival chances compared to those of less integrated phenotypes (VAN HAVERMAET, 2015). At the same time, some studies suggest that phenotypic integration in plants increases with environmental stress (GIANOLI, 2004; MARTINEZ-CABRERA et al., 2011; SCHLICHTING 1989; WAITT; LEVIN 1993; VAN HAVERMAET, 2015). Therefore, environmental gradients comprising stress factors provide an excellent system to analysis the behavior of functional traits and its consequences for plant–plant interactions and overall community dynamics (JUNG et al. 2010; MATESANZ; GIANOLI; VALLADARES, 2010; SHOB et al.; 2013).

Tropical dry forest (TDF) is a complex and fragile ecosystem (PORTILLO-QUINTERO; SÁNCHEZ-AZOFEIFA, 2010) considered one of the most threatened in the tropics by climate change and fragmentation (JANZEN, 1988). The variability in its plants functional traits has been associated with adaptation to light and water availability and a coordinated tolerance of plants to shortage of resources (CASTELLANOS-CASTRO; NEWTON, 2015). In Colombia it is found in small isolated fragments in six biogeographical regions (PIZANO; GARCIA, 2014; FERNÁNDEZ-MÉNDEZ; BERNATE-PEÑA; MELO, 2013). Some patches are under desertification process; due to these characteristics are a good ecosystem of study for gain an understanding of species behavior in response to environmental change. In this study, we investigated the extent of intraspecific variability of leaf traits because it plays a particularly important role over primary production (photosynthesis), water relations and energy balance and other physiological attributes (ASNER; SCURLOCK; HICKE, 2003; CASANOVES; PLA; DI RIENZO, 2011) and wood traits because this has important implications in carbon cycles (WIEMANNF; WILLIAMSON, 2002) of *Ceiba pentandra* (L.) Gaertn specie of Malvaceae family in seven forest patches in three bioregions. This specie is typical of Colombian TDF and is included in the strategic plan for the conservation of plants in the country (PIZANO; GARCIA, 2014). We want to respond i) how is the variability of selected traits between bioregions, between populations, within populations and within individual ii) if there exists phenotypic integration between traits of this specie. These responses are important because give knowledge of autecology of the species and its potential contribution to the ecosystem processes and services, on the other hand are basic information to build predictive models of the effect of climate change and fragmentation on populations of the species and its adaptability

2 METHODS

2.1 Study area and sampling design

We evaluated seven populations located in three bioregions of tropical dry forest in Colombia. For each site we considered the access to fragment, size, geographical and climatic contrasts for the definition of zones. All climate data were extracted from Worldclim.org. The study sites are located between 253 and 1110 m above sea level, rainfall ranges between 849 and 2069 mm per year and average annual temperatures are between 24ºC and 28° C (Table 1). Between November of 2014 and June of 2015 adult plants of *Ceiba pentandra* (L.) Gaertn were measured. We select the maximum number of trees found with a minimum distance of 50 m between each and we measured leaf and wood functional traits.

Bioregions

Cauca river valley

The patches of tropical dry forest that still exist in the Cauca valley bioregion; probably doesn’t reach more than 1.900 ha. This dry sub-humid region extends along a close parallel to the river stretch which borders the departments of Cauca and Valle del Cauca and in the south of Antioquia department. These forests are very close to the transition to submontane forests. Its rainfall, ranging between 1,500 and 1,800 mm is divided into two periods, from April to May and October to November, in which 70% of the total annual rainfall falls; drought in these forests does not seem as severe as in other regions, for the period when the vegetation loses foliage usually lasts no more than a few weeks (ARCILA-CARDONA, 2012; DÍAZ, 2006; TORRES et al., 2012; VARGAS, 2012). There, we measured three natural populations of *Ceiba pentandra* (L.) Gaertn, one in the Valle del Cauca department and two in Antioquia department (Figure A).

Caribbean coast

Tropical dry forests of this area are the largest and best preserved of Colombia. This is a secondary forest formation, totaling some 133,500 ha, but considerable portions of them, around 70,000 ha, have high degree of transformation. In this region, a greater variety of vegetation cover types due to climatic and soil conditions. Many areas have azonal vegetation, ie, floristic elements with xerophytic features like cacti and thorny bushes and its dominant trees that have smaller sizes than elsewhere and are leafless more than half the year. One cause of this phenomenon is the strong influence of the trade winds (DÍAZ, 2006). There, we measured three natural populations of the same specie, one in Bolivar department and two in Sucre department (Figure A).

Chicamocha canyon

Between the valleys of Eastern and Western Cordilleras of the Andes, particular local conditions are created regarding to air humidity and rainfall. These valleys constitute enclaves or dry forest fragments and riparian, mostly in an advanced state of degradation. The dry forests of the Chicamocha Canyon region in the department of Norte de Santander are part of these areas and due to geographical isolation and have higher rates of endemism compared to other bioregions (DÍAZ, 2006; VALENCIA-DUARTE; TRUJILLO ORTIZ; VARGAS RÍOS, 2012). There, we measured a natural population of *Ceiba pentandra* (L.) Gaertn specie in Santander department (Figure A).

2.2 Study species

Ceiba pentandra (L.) Gaertn is a tree of Malvaceae family, naturally from Mexico to Amazon basin and paleotropical in West Africa (BAKER, 1965; GAERTNER 1791; HARTSHORN, 1983). It is known for its rapid growth and persistence in climax forests, where it is often one of the tallest emergent trees and is common in the riverbanks. *Ceiba pentandra* (L.) Gaertn has alternate leafs, clustered at the tips of the branches, palmate compound from 11 to 40 cm long, including the petiole, composed of 7-8 leaflets, from 1.5 to 5 x 15 x 4 cm, narrowly elliptic. Its stem is solid, thick, straight, with large and well-developed buttresses, has numerous strong conical spines and its wood have low density (GAERTNER, 1791; TAMARIT-URIAS; FUENTES-SALINAS, 2003). Is pollinated by bats, bees and wind. Its breeding system is variable and depends on its distribution, from fully self-incompatible until mixed system with varying degrees of self-fertilization (GRIBEL; GIBBS; QUEIROZ, 1999; LOBO; QUESADA; STONER, 2005).

In Colombia *C. pentandra* is distributed between 0 and 1500 m above level of sea. The specie is categorized as a minor concern condition

(LC) according to The IUCN Red list of threatened (2013). However, there are unknown genetic and ecological studies of the actual state of their population in the country.

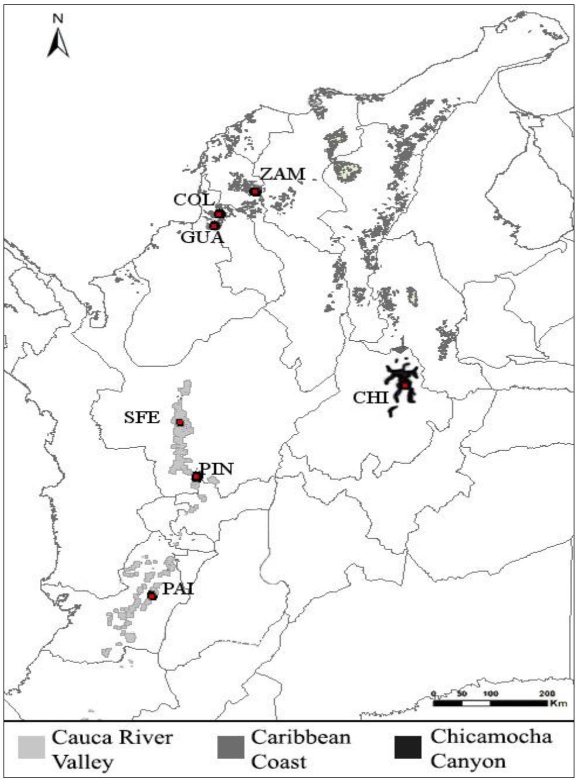

Figure 1 A. Populations of *Ceiba pentandra* Gaertn in the different bioregions of Colombia.

Table 1 Location and climatic characterization of *Ceiba pentandra* Gaertn populations. Population values of annual rainfall and annual temperature are means (WORLDCLIM GLOBAL CLIMAE DATA, 2015). N: Individual sample, Lat: Latitude, Lon: Longitude, Alt: Altitude (m.a.s.l), AR: Annual rainfall (mm), TºC: Annual mean temperature.

Bioregion	Department	N	Code	Lat	Lon	Alt	AR	T °C
Cauca river valley	Valle del Cauca	12	PAI	4.2837	-76.0924	945	1333	23.8
	Antioquia	11	PIN	5.7258	-75.6162	646.67	2069.96	24.9
	Antioquia	5	SFE	6.5253	-75.8043	443.75	1403	26.3
Caribbean coast	Sucre	8	COL	9.5385	-75.3438	222.58	1353.81	26.5
	Sucre	14	GUA	10.019	-74.8900	47.38	1118.58	28
	Bolívar	11	ZAM	9.8948	-74.8948	33.94	964	28
Chicamocha canyon	Santander	10	CHI	6.8384	-72.9801	754.8	889.49	24.2

2.3 Measurement of functional traits

Leaf traits

From each individual we collected five mature and non-senescent leaves of multiple branches with different sun exposures and without herbivore damage. We measured through photographs and the ImageJ software (IMAGE PROCESSING AND ANALYSIS IN JAVA, 2015) to determine leaf area after we dried leaves for 48 hours at 65-70º C to obtain leaf dry mass (g) and then calculate specific leaf area (SLA) (mm^2 mg^{-1}). We included petiole in the analysis. Leaf dry-matter content (LDMC) (mg g^{-1}) was measured with leaf dry mass and was divided for its fresh mass (CORNELISSEN et al., 2003; PÉREZ-HARDENGUY et al., 2013).

Wood traits

We measure stem wood density (SWD) (g cm$^{-3)}$ for each tree with diameter ≥ 9 cm, first we remove the loose bark of stem, after we get the wood samples using a forest borer with a 0.50 cm diameter to extract cores from tree to 1.3 m above the ground (CHAVE et al., 2006). Cores were dried between 65 to 70ºC in a drying oven for two days. Trait was measured with dry mass divided by fresh volume of wood per core; we used water-displacement method (CORNELISSEN et al., 2003; PÉREZ-HARDENGUY et al., 2013). In addition, we measured the branch wood density (BWD) (g cm-3) using the same method that SWD, but instead of cores we use discs of branch with a diameter ≥ 2.5 cm.

2.4 Statistical analysis

It was initially conducted an analysis of normality for all data, then general descriptive statistics were calculated to get an overview of traits in bioregions, between and within populations and within individual. After that, we realized an ANOVA to 5% and Tukey comparison analysis for each trait according to level (between bioregion, between populations, within populations and within individuals). Then, to assess the sources of data variability and its patterns, we performed a variance component analysis through a nested ANOVA between bioregions, between populations, within populations and within individuals. In the last two cases, the analysis only made by leaf traits (SLA, LDMC) due to number of samples by tree. All data analyses were performed in Infostat (DI RIENZO et al., 2014) and Manitab 17 (MANITAB, 2010) softwares.

We assessed the phenotypic integration of specie through Pearson’s correlation coefficients matrix, to test for significance in all possible pairwise comparisons between the measured traits.

3 RESULTS

3.1 General descriptive statistics

The number of samples tested for the SLA trait was between 19-50, for LDMC was between 20 and 50. In the case of traits wood (SWD and BWD) samples collected were between 4-11 for each one. The bioregion level averages of traits were SLA: 210.26; LDMC: 0.38; SWD: 0.33; BWD: 0.38 in Cauca river valley, SLA: 216.54; LDMC: 0.43; SWD: 0.28; BWD: 0.40 in Caribbean coast and SLA: 274.42; LDMC: 0.45; SWD: 0.36; BWD: 0.45 in Chicamocha canyon and the CV behave the same way that in the level of population where leaf traits have higher values (Table 2). In other hand, the averages values evaluated at a population level for leaf traits (SLA and LDMC) were between 222.12 and 0.41 in each case and the wood traits (SWD and BWD) values correspond to 0.31 to 0.40 respectively. In the case of CV values they were higher in leaf traits with a values of 30.95% to 44.44% for SLA and 10.31% to 40.80% for LDMC meanwhile wood traits showed lower values ranging from 15.08% to 25.12 % for SWD and 5.15% to 27.655 for BWD (Table 2). Finally, within individuals exists a large range of values, in the case of SLA trait. Each one of the individual of CHI population has values between 84.72 to 621.71, for COL population each individual has values between 99.76 to 316.06, GUA individuals population have values between 64.85 to 345.34, in PAI individuals population had values between 79.54 to 282.96, PIN individuals population has values between 75.9 to 505.85, SFE individuals population have between 91.05 to 377.48 and ZAM individuals population have values between 83.55 to 475.24; for LDMC trait the values have a large range too, in each one of the previous populations an individual has values between 0.24 to 0.91; 0.36 to 0.52; 0.28 to 0.57; 0.16 to 0.71; 0.02 to 0.48; 0.21 to 0.46 and 0.37 to 1.01 respectively (Annexed 1).

Table 2 Descriptive Statistics analysis of functional leaf and wood traits of *Ceiba pentandra* Gaertn. SLA (Specific leaf area); LDMC (Leaf dry matter content); SWD (Steam wood density); BWD (Branch wood density); n (number of individuals observed); SD (Standard desviation); CV(Coefficient of variation)

Bioregion	Population	Trait	n	Mean ± SD	CV
Cauca river valley	PAI	SLA	37	160.49±63.26	39.42
	PAI	LDMC	50	0.42±0.17	40.38
	PAI	SWD	11	0.35±0.05	15.08
	PAI	BWD	9	0.41±0.09	22.17
	PIN	SLA	50	234.49±97.8	41.71
	PIN	LDMC	50	0.38±0.07	18.5
	PIN	SWD	10	0.33±0.07	21.48
	PIN	BWD	10	0.37±0.06	16.19
	SFE	SLA	19	235.83±89.4	37.91
	SFE	LDMC	20	0.34±0.05	14.83
	SFE	SWD	4	0.32±0.05	16.9
	SFE	BWD	4	0.37±0.02	5.15
Caribbean coast	COL	SLA	35	242.27±91.66	37.83
	COL	LDMC	35	0.44±0.05	10.31
	COL	SWD	7	0.33±0.08	23.07
	COL	BWD	7	0.42±0.1	24.06
	GUA	SLA	50	173.07±53.57	30.95
	GUA	LDMC	50	0.39±0.06	16.12
	GUA	SWD	9	0.26±0.05	19.99
	GUA	BWD	10	0.38±0.11	27.65
	ZAM	SLA	50	234.3±96.14	41.03

	ZAM	LDMC	50	0.47±0.11	23.93
	ZAM	SWD	11	0.25±0.06	25.12
	ZAM	BWD	11	0.42±0.07	17.87
Chicamocha canyon	CHI	SLA	50	274.42±121.94	44.44
	CHI	LDMC	50	0.45±0.13	28.25
	CHI	SWD	10	0.36±0.06	17.37
	CHI	BWD	10	0.45±0.07	15.58

3.2 Analysis of variance in traits between the bioregions and between populations

During bioregion analysis it was found that in LDMC and SWD traits significant differ between the regions of Cauca river valley and Caribbean coast, the SLA and BWD traits did not present any significant variation (Figure B). In the analysis among seven populations we observed for SLA trait difference between PAI and CHI populations, for LDMC trait the difference was between SFE and ZAM population, SWD trait showed difference between ZAM and CHI population and BWD trait do not showed difference. The tendency is that CHI population is more variable (Figure C). However, this population is not dominant in the bioregion analysis.

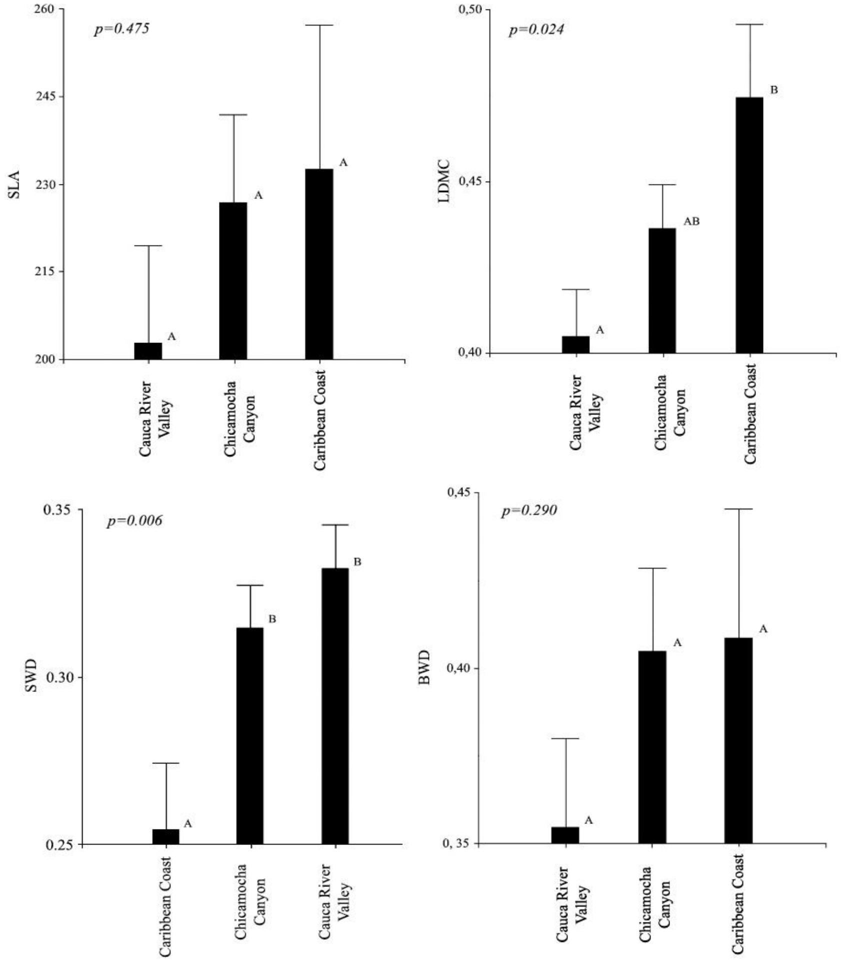

Figure 2 ANOVA and Tukey comparison of leaf and wood functional traits of *Ceiba pentandra* Gaertn in three tropical dry forest bioregions in Colombia (Cauca river valley, Chicamocha canyon and Caribbean coast). Means with common letter are not significantly different ($p \leq 0.05$). SLA (Specific leaf area); LDMC (Leaf dry matter content); SWD (Steam wood density); BWD (Branch wood density)

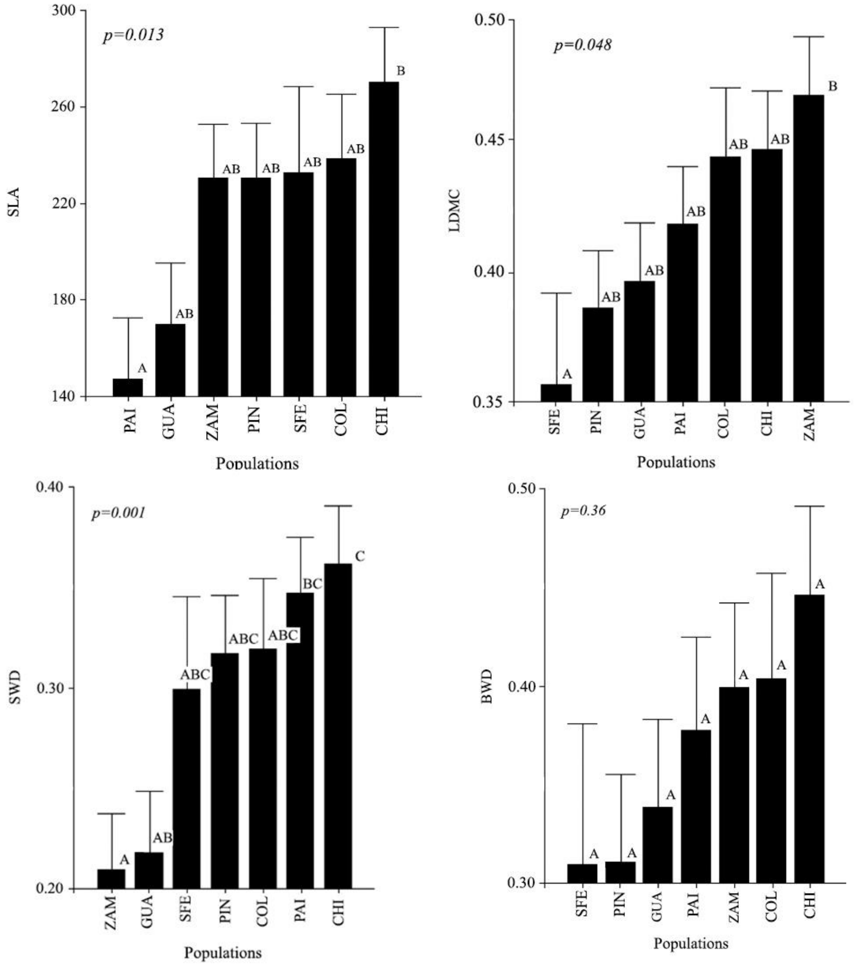

Figure 3 ANOVA and Tukey comparison of functional traits of seven populations of *Ceiba pentandra* (L.) Gaertn. Means with common letter are not significantly different ($p \leq 0.05$). SLA (Specific leaf area); LDMC (Leaf dry matter content); SWD (Steam wood density); BWD (Branch wood density)

3.3 Analysis of variance in traits within populations and within individual

Within population we found that all populations, except SFE, presented significant variability within them. In relation with LDMC trait, SFE, CHI and ZAM populations do not showed a significant variability. In general, we can note that these traits have a significant variability within populations (Table 3). In other place, analysis of intraindividual level showed that in each population the SLA and LDMC traits vary considerably in each individual, for CHI population each individual presented a CV between 14.6% to 47.06% and 4.8% to 55.68% in each trait, in COL population each individual showed a CV between 13.03% to 28.39% and 4.41% to 11.52%, in GUA population each individual showed a CV between 13.06% to 33.54% and 1.93% to 17.54%, in PAI population each individual showed a CV between 6.61% to 66.56% and 2.56% to 25.2%, in PIN population each individual showed a CV between 10.46% to 44.95% and 2.13% to 11.33%, in SFE population each individual showed a CV between 19.9% to 49.08% and 6.11% to 17.81% and ZAM population each individual showed a CV between 13.36% to 42.92% and 2.63% and 48.78%. (Annexed 1).

Table 3 ANOVA and Tukey comparison of leaf functional traits within population of *Ceiba pentandra* (L.) Gaertn. SLA (Specific leaf area); LDMC (Leaf dry matter content); CV(Coeffiecient of variation); p-value*(significant)

Bioregion	Population	Trait	CV	p-value
Cauca river valley	PAI	SLA	22.44	<0.0001*
		LDMC	18.47	<0.0001*
	PIN	SLA	33.52	0.0011*
		LDMC	14.35	0.0003*
	SFE	SLA	35.98	0.218
		LDMC	13.63	0.1372
Chicamocha canyon	CHI	SLA	32.24	0.0042*
		LDMC	28.51	0.544
Caribbean coast	COL	SLA	23.44	<0.0001*
		LDMC	8.02	0.0034*
	GUA	SLA	23.85	0.0003*
		LDMC	9.83	<0.0001*
	ZAM	SLA	24.46	<0.0001*
		LDMC	22.91	0.2015

3.4 Components of variance

The variance component analysis indicates that within populations the variability is higher. BWD and SWD traits have the most representative values 66.52% and 92.13% respectively. In the case of SLA and LDMC traits, the highest percentage of variance is within individuals; this may be due to high location leaves on the tree and lack of protocol to obtain them. The variance components at bioregion level were 22.77% for the SWD trait and indifferent to the rest of traits. Finally, between population level, SLA and LDMC traits showed values among 10.6% and 5.78%, for SWD trait was 10.71% and BWD do not showed any values. (Table 4).

Table 4 Variance components for each trait of *Ceiba pentandra* Gaertn. BP (between populations); WP (within populations); WI (within individuals); SLA (Specific leaf area); LDMC (Leaf dry matter content); SWD (Steam wood density); BWD (Branch wood density)

Components	SLA (%)	LDMC (%)	SWD (%)	BWD (%)
Bioregion	1.3	2.49	**22.77**	7.87
BP	10.6	5.78	10.71	0
WP	**40.74**	**39.05**	**66.52**	**92.13**
WI	**47.36**	**52.68**		
∑	100	100	100	100

3.5 Phenotypic integration of functional traits

The analysis showed very significant relationships between BWD and SLA and SWD traits. Also, we found significant associations between SLA and LDMC. SWD did not show a relationship with any leaf trait. This result reveals that exist a significant phenotypic integration of the specie (Table 5).

Table 5 Pearson correlation coefficients between leaf and wood traits. SLA (Specific leaf area); LDMC (Leaf dry matter content); SWD (Steam wood density); BWD (Branch wood density)

Traits	LDMC	SWD	BWD
SLA	**0.12(0.04)**	0.12(0.38)	**0.28(0.04)**
LDMC		0.06(0.64)	0.50(8.5E-05)
SWD			**0.34(0.01)**

4 DISCUSSION

4.1 General descriptive statistics

The determination of the general descriptive statistics of the considered traits in individuals, population and bioregion levels led to the identification of several intraspecific patterns for *Ceiba pentandra*. For the SLA functional trait, it was expected that like pioneer specie had high values and so it was. At the same time, respect to high values of SLA trait expected a positive relationship between LDMC and SLA traits, this behavior was presented too.

Ceiba pentandra Gaertn (L.) shown that the mean value for SWD clearly coincided with the successional type to belonging the specie. On other hand, looking at the mean values of SWD and BWD across the all levels evaluated, the mean values of BWD differed to 80% of the respective mean value of SWD. This values is very important, if is considered that some research is remplaced the SWD by BWD.

4.2 Analysis of variance in traits between the bioregions and between populations

Each one of the bioregions evaluated (Cauca river valley, Caribbean coast and Chicamocha canyon) have differences in their environmental characteristics, average temperatures ranging between 24 ° C to 28 ° C and annual rainfall goes from 800 mm to 1,600 mm. These variations generate different conditions in each case for the specie, which can be observed through dissimilarity in some functional traits LDMC, SWD between bioregions and for SLA, LDMC and SWD among populations. In both cases, higher values of traits were presented in the populations located in bioregion of Chicamocha canyon, which has the least rainfall and temperatures, contrary to population located in Cauca river valley were presented low values of traits but this bioregion presented high rainfall and temperatures. *Ceiba pentandra* (L.) Gaertn is then a specie with highly variable functional traits that allows an adaptation to different types of environments. This explains the ability of specie to inhabit in climax forests, disturbances forest (GRIBEL; GIBBS; QUEIROZ, 1999) and the same time, in a variety of ecosystems ranging from dry forests in Central America (LOBO; QUESADA; STONER, 2005) to humid Amazonian forests (BRONDANI et al., 2003). Some studies showing this variability of traits in different ecosystem are: Zots and Winter (1994) assessed SLA trait of *Ceiba pentandra* (L.) Gaertn in Panama rainforest and scored an average of 201.6 cm2 and Camargo-Roa (2014) assessed the SLA and LDMC in a fragment of dry forest in Colombia that scored an average of 30.22 and 0.68 by each trait. In the first study the value differs 20.52 cm2 less than the average of the specific leaf area recorded in our populations and in the second study the difference is 191.9 cm2. As for wood density, Barcenas (1985) and Tamarit-Urias and Fuentes-Salinas (1996) assessed the specie in dry ecosystem of Mexico and found a density of 0.23 and 0.29. Moreover, a record described by Camargo-Roa (2014) for a fragment of dry forest in Colombia was 0.76. These studies vary from 0.08, 0.02 and 0.45 with respect to the average density in our study 0.31. These relations drives the importance of intraspecific analysis at functional level, because, besides the loss of biological information, is underestimated the functional potential of the specie and its interaction in ecosystem.

4.3 Analysis of variance in traits within populations and within individuals

Within populations and within individuals was found the most significant values of variability for SLA and LDMC functional traits. According to Albert et al. (2011) the variability in these levels can be due to the co-existence of different genotypes and also to different plasticity of these different genotypes to environmental conditions. At the same time, Poorter (2009) described that the values in these leaf traits are variable due to as leaf's size, influenced by the amount of light or shadow receiving. In functional studies to mediate this situation are used protocols like the ones described in our methodology for collection of samples according exposure to light and shadow of leaf.

Intraindividual level differences in the values of the traits that may also result from the development stage of the leaf level, individual history, and current trait values within the individual being influenced by former disease or disturbance.

4.4 Components of variance

Components of variance analysis indicate that within populations and within individual are the higher values of variability of functional traits of study. This behavior is influenced by different factors around each tree like shade, light and resource availability. Hulshof and Swenson (2010) made a similar study where evaluated components of variance of leaf functional traits of ten species of seasonally dry tropical forest and found that the majority of variation in traits was often explained by between species differences, at the same time, they found that leaf trait variation was very high within species. On other hand, Van Havermaet (2015) study the functional traits of species *Bursera simaruba* and *Enterolobium cyclocarpum* in tropical dry forest in Colombia, finding in the analysis of variance components also the highest values within populations and suggest that trait data variability for the species arises from external conditions rather than inherent characteristics. The values found at the individual level were low compared with our study. So, the high variation within populations and individuals is factor that only depends on environmental characteristics where the plant is found.

4.5 Phenotypic integration of functional traits

We found relationship between foliar traits (SLA, LDMC) and between wood traits (SWD, BWD), due to the nature of features of each type of trait. Relation between leaf traits and SWA was not found. SLA, SWD traits had a significant correlation with BWD trait. In general, these results suggest that functional traits of *Ceiba pentandra* (L.) Gaertn are integrated and this represents a greater ability to adapt to climate change on the specie and tolerance of climatic extremes. Van Havermaet (2015) identified some tropical dry forest species like *Bursera simaruba*, *Hura* *crepitans* that have similar values of integrity between their functional traits.

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CONSIDERAÇÕES FINAIS

As populações de *Ceiba pentandra* Gaertn (L) avaliadas possuem adequado estado de conservação genética para conservação *in situ* e *ex situ*. São potenciais fontes de sementes e mudas para atividades de restauração ambiental na floresta decídua da Colômbia. Ao mesmo tempo, a alta variabilidade de seus traços e sua integridade permite sua adaptabilidade a diferentes tipos de ambiente, sendo uma espécie chave nas áreas afetadas pelas mudanças ambientais.

Estudos genéticos e funcionais são pioneiros, pois oferecem pautas para o conhecimento da autoecologia das espécies, seu potencial aporte nos processos e serviços dos ecossistemas e, além disso, constituem um novo campo de pesquisa científica na Colômbia, já que é uma pesquisa exploratória em componentes da biodiversidade que nunca foram trabalhados.

ANEXOS

ANEXO A - Analysis of leaf traits within individual in each population

Sitio	ind	Variable	n	Media	D.E.	E.E.	CV	Mín	Máx
CHI	1	SLA	5	136.08	35.27	15.77	25.92	84.72	182.24
CHI	2	SLA	5	202.41	54.93	24.56	27.14	152.84	290.61
CHI	3	SLA	5	280.38	84.33	37.71	30.08	202.95	388.03
CHI	4	SLA	5	289.34	136.15	60.89	47.06	132.79	448.33
CHI	5	SLA	5	267.61	91.07	40.73	34.03	181.87	422.81
CHI	6	SLA	5	186.68	27.25	12.19	14.6	163.65	231.55
CHI	7	SLA	5	307.41	187.82	84	61.1	160.23	621.71
CHI	8	SLA	5	426.1	130.71	58.46	30.68	277	593.61
CHI	9	SLA	5	303.97	65.41	29.25	21.52	236.61	376.61
CHI	10	SLA	5	344.2	94.18	42.12	27.36	246.64	471.48
COL	1	SLA	5	263.01	34.28	15.33	13.03	225.19	316.06
COL	3	SLA	5	225.71	64.07	28.65	28.39	138.42	319.2
COL	4	SLA	5	132.53	30.04	13.44	22.67	99.76	167.76
COL	5	SLA	5	394.38	104.07	46.54	26.39	309.01	567.45
COL	6	SLA	5	201.82	50.73	22.69	25.14	155.2	279.28
COL	7	SLA	5	211.49	43.25	19.34	20.45	167.04	276.69
COL	8	SLA	5	266.98	33.29	14.89	12.47	218.26	311.45
GUA	1	SLA	5	219.47	28.66	12.82	13.06	187.23	249.48
GUA	2	SLA	5	237.82	69.91	31.26	29.4	152.63	345.34
GUA	3	SLA	5	142.54	46.37	20.74	32.54	68.77	191.34
GUA	4	SLA	5	159.44	20.52	9.18	12.87	129.71	185.75
GUA	5	SLA	5	131.55	40.98	18.33	31.15	64.85	165.62
GUA	6	SLA	5	138.2	29.71	13.29	21.5	102.4	182.84
GUA	8	SLA	5	139.4	36.4	16.28	26.11	87.45	175.51
GUA	9	SLA	5	192.69	34.7	15.52	18.01	137.04	227.91
GUA	10	SLA	5	218.69	33.2	14.85	15.18	178.36	253.16
GUA	11	SLA	5	150.89	50.6	22.63	33.54	91.63	228.36
PAI	1	SLA	5	107.12	28.42	12.71	26.53	85.54	156.86
PAI	2	SLA	5	98.21	20	8.94	20.37	79.54	128.07
PAI	3	SLA	5	242.87	16.06	7.18	6.61	225.57	262.24
PAI	6	SLA	5	208.36	19.51	8.72	9.36	188.75	230.94
PAI	7	SLA	5	206.52	47.78	21.37	23.14	162.89	282.96
PAI	8	SLA	5	107.9	36.42	16.29	33.75	60.83	156.18
PAI	9	SLA	5	167.32	47.62	21.3	28.46	116.57	230.19
PAI	10	SLA	2	123.24	82.02	58	66.56	65.24	181.24
PIN	1	SLA	5	340.74	128.83	57.62	37.81	164.86	505.85
PIN	3	SLA	5	162.64	23.27	10.41	14.31	136.82	189.93
PIN	4	SLA	5	246.1	110.62	49.47	44.95	75.9	362.35
PIN	5	SLA	5	295.3	114.96	51.41	38.93	183.04	437.96
PIN	6	SLA	5	325.68	77.46	34.64	23.78	213.36	424.78
PIN	7	SLA	5	165.61	17.33	7.75	10.46	146.07	187.19
PIN	8	SLA	5	137.23	24.25	10.85	17.67	109.19	166.6
PIN	9	SLA	5	210.44	77.71	34.75	36.93	125.81	294.57
PIN	10	SLA	5	209.24	31.06	13.89	14.84	161.33	237.57
PIN	11	SLA	5	251.93	72.71	32.52	28.86	155.73	358.36
SFE	7	SLA	5	179.19	87.95	39.33	49.08	91.05	312.45
SFE	8	SLA	4	248.83	99.4	49.7	39.95	153.25	377.48
SFE	9	SLA	5	221.82	44.15	19.74	19.9	181.65	286.03
SFE	10	SLA	5	296.11	99.54	44.52	33.62	171.22	415.67
ZAM	1	SLA	5	360.79	48.19	21.55	13.36	306.62	416.37

Sitio	ind	Variable	n	Media	D.E.	E.E.	CV	Mín	Máx
ZAM	2	SLA	5	321.23	98.6	44.1	30.7	248.5	475.24
ZAM	4	SLA	5	181.04	55.27	24.72	30.53	83.55	220.78
ZAM	5	SLA	5	188.74	32.97	14.74	17.47	141.58	231.6
ZAM	6	SLA	5	153.75	27.55	12.32	17.92	127.6	199.1
ZAM	7	SLA	5	174.67	25.86	11.57	14.81	142.41	203.92
ZAM	8	SLA	5	360.67	63.48	28.39	17.6	281.05	422.01
ZAM	9	SLA	5	266.16	70.05	31.33	26.32	207.37	387.74
ZAM	10	SLA	5	170.22	73.05	32.67	42.92	101.99	277.37
ZAM	11	SLA	5	165.71	31.06	13.89	18.74	118.34	202.62

Sitio	ind	Variable	n	Media	D.E.	E.E.	CV	Mín	Máx
CHI	1	LCDM	5	0.51	0.02	0.01	4.8	0.48	0.54
CHI	2	LCDM	5	0.51	0.07	0.03	14.34	0.45	0.64
CHI	3	LCDM	5	0.45	0.02	0.01	4.29	0.42	0.47
CHI	4	LCDM	5	0.41	0.01	0.01	2.75	0.4	0.43
CHI	5	LCDM	5	0.4	0.04	0.02	8.68	0.35	0.44
CHI	6	LCDM	5	0.4	0.02	0.01	4.83	0.37	0.42
CHI	7	LCDM	5	0.39	0.03	0.01	7.69	0.34	0.42
CHI	8	LCDM	5	0.49	0.2	0.09	39.79	0.24	0.79
CHI	9	LCDM	5	0.51	0.28	0.13	55.68	0.24	0.91
CHI	10	LCDM	5	0.39	0.19	0.08	47.2	0.25	0.66
COL	1	LCDM	5	0.48	0.04	0.02	7.39	0.43	0.51
COL	3	LCDM	5	0.41	0.03	0.01	6.74	0.38	0.44
COL	4	LCDM	5	0.45	0.05	0.02	10.54	0.38	0.51
COL	5	LCDM	5	0.44	0.04	0.02	9.09	0.39	0.49
COL	6	LCDM	5	0.49	0.02	0.01	4.41	0.47	0.52
COL	7	LCDM	5	0.41	0.01	0.01	3.16	0.4	0.43
COL	8	LCDM	5	0.42	0.05	0.02	11.52	0.36	0.47
GUA	1	LCDM	5	0.39	0.04	0.02	9.07	0.36	0.45
GUA	2	LCDM	5	0.4	0.05	0.02	13.46	0.36	0.49
GUA	3	LCDM	5	0.4	0.04	0.02	10.16	0.36	0.46
GUA	4	LCDM	5	0.37	0.02	0.01	5.67	0.34	0.39
GUA	5	LCDM	5	0.28	0.01	2.40E-03	1.93	0.28	0.29
GUA	6	LCDM	5	0.45	0.03	0.02	7.8	0.43	0.51
GUA	8	LCDM	5	0.36	0.01	0.01	3.64	0.34	0.37
GUA	9	LCDM	5	0.46	0.08	0.04	17.74	0.34	0.57
GUA	10	LCDM	5	0.33	0.01	3.70E-03	2.52	0.32	0.34
GUA	11	LCDM	5	0.44	0.01	4.00E-03	2.05	0.42	0.44
PAI	1	LCDM	5	0.33	0.02	0.01	4.95	0.32	0.36
PAI	2	LCDM	5	0.36	0.02	0.01	4.6	0.35	0.39
PAI	3	LCDM	5	0.56	0.09	0.04	15.59	0.49	0.71
PAI	6	LCDM	5	0.43	0.01	4.90E-03	2.56	0.41	0.44
PAI	7	LCDM	5	0.33	0.01	0.01	4.02	0.32	0.35
PAI	8	LCDM	5	0.36	0.03	0.01	8.15	0.32	0.4
PAI	9	LCDM	5	0.29	0.07	0.03	25.21	0.16	0.34
PAI	10	LCDM	2	0.36	0.02	0.02	5.98	0.34	0.37
PIN	1	LCDM	5	0.38	0.02	0.01	6.52	0.34	0.4
PIN	3	LCDM	5	0.38	0.04	0.02	11.14	0.34	0.45
PIN	4	LCDM	5	0.3	0.03	0.02	11.33	0.27	0.36
PIN	5	LCDM	5	0.39	0.02	0.01	4.91	0.37	0.42
PIN	6	LCDM	5	0.43	0.02	0.01	3.93	0.4	0.44
PIN	7	LCDM	5	0.39	0.01	3.70E-03	2.13	0.38	0.4
PIN	8	LCDM	5	0.37	0.02	0.01	6.77	0.33	0.39
PIN	9	LCDM	5	0.29	0.15	0.07	52.3	0.02	0.38
PIN	10	LCDM	5	0.36	0.03	0.01	8.78	0.31	0.39
PIN	11	LCDM	5	0.47	0.01	0.01	2.88	0.45	0.48

Sitio	**ind**	**Variable**	**n**	**Media**	**D.E.**	**E.E.**	**CV**	**Mín**	**Máx**
SFE	7	LCDM	5	0.35	0.04	0.02	12.41	0.31	0.41
SFE	8	LCDM	4	0.34	0.02	0.01	6.11	0.31	0.36
SFE	9	LCDM	5	0.38	0.06	0.03	15.8	0.31	0.46
SFE	10	LCDM	5	0.3	0.05	0.02	17.81	0.21	0.34
ZAM	1	LCDM	5	0.48	0.03	0.01	5.89	0.46	0.53
ZAM	2	LCDM	5	0.45	0.04	0.02	8.11	0.41	0.49
ZAM	4	LCDM	5	0.47	0.03	0.01	5.53	0.43	0.5
ZAM	5	LCDM	5	0.43	0.01	0.01	2.63	0.42	0.45
ZAM	6	LCDM	5	0.41	0.01	0.01	3.24	0.4	0.43
ZAM	7	LCDM	5	0.39	0.02	0.01	4.96	0.37	0.42
ZAM	8	LCDM	5	0.53	0.21	0.09	40.14	0.4	0.9
ZAM	9	LCDM	5	0.43	0.04	0.02	8.86	0.4	0.48
ZAM	10	LCDM	5	0.54	0.26	0.12	48.78	0.39	1.01
ZAM	11	LCDM	5	0.57	0.04	0.02	6.63	0.53	0.63