DANIELE APARECIDA ALVARENGA ARRIEL

CARACTERIZAÇÃO MOLECULAR DE HÍBRIDOS OBTIDOS VIA CRUZAMENTOS NATURAIS E CONTROLE GENÉTICO DA RESISTÊNCIA À MURCHA-DE-CERATOCYSTIS EM *Mangifera indica*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de Doctor Scientiae.

VIÇOSA

MINAS GERAIS - BRASIL

2015

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
A775c
2015

Arriel, Daniele Aparecida Alvarenga, 1986-
Caracterização molecular de híbridos obtidos via cruzamentos naturais e controle genético da resistência à murcha-de-ceratocystis em Mangiferaindica / Daniele Aparecida Alvarenga Arriel. – Viçosa, MG, 2015.
ix, 52 f. : il. (algumas color.) ; 29 cm.

Orientador: Acelino Couto Alfenas.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Manga - Doenças e pragas. 2. Manga - Melhoramento genético. 3. Manga - Resistência a doenças e pragas.
4. Mangifera indica. 5. Ceratocystis fimbriata. I. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-graduação em Genética e Melhoramento. II. Título.

CDD 22. ed. 632.46

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APROVADA: 29 de outubro de 2015.

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À minha família, em especial a minha avó Corina (in memorian) que sempre rezou para São Judas Tadeu iluminar os meus estudos,

DEDICO

AGRADECIMENTOS

A Deus, em primeiro lugar, por sempre guiar a minha vida.

À toda a minha família, especialmente aos meus pais, Edson e Nice, à minha irmã Maria Clara, e ao meu noivo Alvaro, por todo amor, carinho, confiança e por serem a minha maior alegria.

À Universidade Federal de Viçosa, em especial ao Programa de Pós-Graduação em Genética e Melhoramento pela oportunidade.

Ao CNPq, à CAPES e à Vale pelo apoio financeiro para o desenvolvimento deste trabalho.

Ao professor Acelino Couto Alfenas pela oportunidade, confiança, orientação e pelos ensinamentos profissionais e pessoais.

À Embrapa Semiárido por ceder as plantas dos cruzamentos utilizados neste estudo, e a todos os seus funcionários, estagiários e pesquisadores que auxiliaram nas coletas do material para propagação das mudas. Agradeço especialmente ao pesquisador Dr. Francisco Pinheiro Lima Neto por todo auxílio e motivação.

Ao professor Dalmo Lopes de Siqueira pela coorientação, pelo auxílio na coleta dos garfos e por toda ajuda prestada no desenvolvimento do trabalho.

Ao Dr. Lúcio Mauro da Silva Guimarães pela coorientação, pelas inúmeras ajudas prestadas, pela paciência, amizade e por acompanhar todas as etapas deste trabalho.

Ao setor de Fruticultura do Departamento de Fitotecnia, em especial ao funcionários Romário e ao Hugo.

A toda família do Viveiro Água limpa em Astolfo Dutra que me recebeu com muito carinho em sua casa. Obrigada Flávio, Elisângela, Eduarda, Reinaldo, Ronaldo e Marli e João.

Ao Departamento de Fitopatologia da UFV pelo apoio.

Ao professor Louis Bernier e a Erika Sayuri Naruzawa e a Université Laval, Quebec, Canadá, que embora não façam parte especificamente deste trabalho, muito contribuíram para meu crescimento profissional e pessoal durante o meu doutorado.

Ao pesquisador Marcos Deon Vilela de Resende pela paciência e por toda ajuda nas análises dos dados.

Aos membros da banca Professor Dr. Leonardo Lopes Bhering, Dr. Leonardo Sarno Soares Oliveira, Dr. Lúcio Mauro da Silva Guimarães e Pesquisador Dr. Francisco Pinheiro Lima Neto.

Aos colegas do Laboratório de Patologia Florestal - PATOMOL - pela amizade, pelo carinho e pela ótima convivência. Em especial agradeço à Daniella Schettini Silva por me ajudar em todas as etapas deste trabalho, à Nathália pela ajuda na genotipagem e à Angélica Nunes e ao Paulo Juscinei Moreira sempre presentes nas inoculações e avaliações. Agradeço também à Jéssica e à Tamilis, e ao Lucas e Gustavo, Raul pela ajuda nas inoculações e nas avaliações.

Aos funcionários do PATOMOL, principalmente à Márcia Maria Brandão e à Mara Souza pelo carinho e pela pronta disponibilidade em ajudar.

Ao BIOAGRO e a todos os seus funcionários, especialmente ao Sr. Paulo por toda atenção.

A todos os funcionários da Clonar Resistência a Doenças Florestais pelo intenso auxílio durante todo o meu experimento. Muito obrigada mesmo Eduardo, Andinho, Real, Solange, Pedro, Ricardo, Marcelo, Talyta e Josnei. Eu sei que dei muito trabalho para vocês!

A todos os meus amigos de Viçosa e da vida pelos bons momentos.

E por fim a todos que contribuíram direta ou indiretamente para a realização deste trabalho.

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RESUMO

ARRIEL, Daniele Aparecida Alvarenga, D.Sc., Universidade Federal de Viçosa, outubro de 2015**. Caracterização molecular de híbridos obtidos via cruzamentos naturais e controle genético da resistência à murcha-de-Ceratocystis em Mangifera indica.** Orientador: Acelino Couto Alfenas. Coorientadores: Dalmo Lopes de Siqueira e Lúcio Mauro da Silva Guimarães.

A manga (Mangifera indica L.) é uma das frutas tropicais mais consumidas no mundo e a murcha-de-Ceratocystis causada por Ceratocystis fimbriata Ellis & Halsted é um dos fatores limitantes para sua produção. Embora esforços têm sido empregados na seleção e identificação de materiais resistentes à doença, a base genética da resistência à murcha-de-Ceratocystis em mangueira, informação essencial para direcionar programas de melhoramento que visem desenvolver cultivares resistentes, não é conhecida. Nos estudos sobre o controle genético da resistência, devem-se usar preferencialmente famílias segregantes oriundas de cruzamentos controlados. No entanto, em mangueira, o pequeno tamanho das flores associado à alta abscisão dos frutos torna os cruzamentos manuais muito trabalhosos e com baixo rendimento. Assim, tem-se adotado o uso de polinização aberta a qual pode apresentar indivíduos contaminantes ou oriundos de autofecundação. Portanto, o presente estudo teve como objetivos: 1) confirmar por meio de marcadores microssatélites a origem híbrida de seis progênies obtidas via cruzamentos naturais; 2) estudar o controle genético da resistência à murcha-de-Ceratocystis em mangueira. Na primeira parte, plantas isoladas das cultivares Coquinho, Espada, Haden, Keitt, Palmer e Van Dyke foram identificadas dentro de pomares comerciais estabelecidos com a cultivar Tommy Atkins. Frutos de cada uma destas seis cultivares foram colhidos para a produção das mudas que posteriormente foram levadas para o campo. Folhas das progênies e dos genitores foram coletadas, o DNA foi extraído e a caracterização dos híbridos foi feita utilizando seis marcadores microssatélites. A percentagem de híbridos confirmados

nos cruzamentos (33 a 89 %) foi maior que a relatada na literatura usando polinização manual e a estratégia adotada mostrou-se eficiente na obtenção e caracterização dos híbridos. Na segunda parte do estudo, avaliou-se a resistência à murcha-de-Ceratocystis nas cultivares Coquinho, Espada, Haden, Keitt, Palmer, Van Dyke e Tommy Atkins e estudou-se o controle genético da resistência à doença em 197 plantas derivadas de cruzamentos de cada uma dessas cultivares com a "Tommy Atkins". As cultivares Keitt, Palmer, Tommy Atkins e Van Dyke foram mais resistentes e as cultivares Coquinho, Espada e Haden mais suscetíveis à doença. Os resultados deste estudo mostram que a herança da resistência à murcha-de-Ceratocystis em mangueira é poligênica com uma prevalência de genes com efeitos de dominância e epistasia. O ganho genético obtido com a seleção das 10 melhores plantas, ou seja, as mais resistentes, foi de 46%, o que significa uma redução de 46% na severidade da doença. Em geral, observou-se baixa frequência de alelos favoráveis à resistência a doença na população estudada o que indica a necessidade de introdução de novos materiais genéticos.

ABSTRACT

ARRIEL, Daniele Aparecida Alvarenga, D.Sc., Universidade Federal de Viçosa, October, 2015**. Molecular characterization of hybrids obtained by natural crossings and genetic control of resistance to Ceratocystis wilt in Mangifera indica.** Adviser: Acelino Couto Alfenas. Co-advisers: Dalmo Lopes de Siqueira and Lúcio Mauro da Silva Guimarães.

The mango (Mangifera indica L.) is one of the most consumed tropical fruit in the world and Ceratocystis wilt caused by Ceratocystis fimbriata Ellis & Halsted is a limiting factor for its production. Although efforts have been employed in the selection and identification of materials resistant to Ceratocystis wilt, the genetic basis of resistance to the disease in mango is unknown. This information is essential to guide breeding programs aiming the development of resistant cultivars. In studies on the genetic control of resistance, segregating families, derived from controlled crossings, should be used, preferably. However, the small size of mango flowers associated with a high fruit abscission make artificial crossings laborious and with low yield. Therefore, this study aimed to: 1) to confirm by microsatellite markers the hybrid origin of six progenies obtained via natural crossings; 2) study the genetic control of resistance to Ceratocystis wilt in mango. In the first part of this work, isolated plants of cultivars Coquinho, Espada, Haden, Keitt, Palmer and Van Dyke were identified in commercial orchards established with the cultivar Tommy Atkins. Open pollinated progenies from each cultivar were planted in the field. Leaves from these progenies and parents were collected, DNA was extracted and the characterization of the hybrids was done using six microsatellite markers. The percentage of confirmed hybrids for the crossings (33-89%) was higher than expected by manual pollination, which indicates that the adopted strategy is efficient in obtaining and characterization of the hybrids. In the second part of the work, we evaluated the resistance to Ceratocystis wilt in the cultivars Coquinho, Espada, Haden, Keitt, Palmer, Van Dyke and Tommy

Atkins. Subsequently, we studied the genetic control of resistance to the pathogen on 197 plants derived from crossings between the remaining cultivars and “Tommy Atkins”. The cultivars Keitt, Palmer, Tommy Atkins and Van Dyke were more resistant and the cultivars Coquinho, Espada and Haden were more susceptible. The results of this study show that the inheritance of resistance in mango is polygenic with a prevalence of genes with effects of dominance and epistasis. The genetic gain by selecting the ten more resistant plants was 46%, which means a 46% reduction in disease severity. In general, there was a low frequency of favorable alleles for resistance to the disease in the population studied indicating the need to introduce new sources of genetic materials.

INTRODUÇÃO GERAL

A mangueira (Mangifera indica L.) é uma espécie arbórea frutífera da família Anacardiaceae cuja distribuição natural ocorre na Ásia tropical, desde o leste da Índia até as Filipinas, com centro de origem localizado na Península Malaia. Nestas regiões a planta pode chegar a 40 m de altura e sobreviver por centenas de anos (Mukherjee e Litz, 2009).

A mangueira caracteriza-se por possuir folhas simples e alternadas dispostas em espiral com morfologia variada de acordo com a variedade, apresentando-se roxeadas quando novas e verde escura à medida que envelhecem. O fruto, que é o produto comercial da planta, é uma grande drupa carnosa, contendo um mesocarpo comestível cuja casca pode ser verde, amarela e avermelhada. O tamanho do fruto pode variar de acordo com a variedade, assim como o teor de fibras e sabor. A manga é uma fruta climatérica, com altos teores de vitamina C antes do amadurecimento e altos teores de vitamina A, B1 e B2 quando madura (Mukherjee e Litz, 2009). A inflorescência em mangueira é terminal com a emergência de múltiplas panículas axilares (de 1000 a 6000) que podem conter flores masculinas e hermafroditas (Usman et al., 2001; Pinto, 2004). A floração espontânea ocorre de janeiro a março, no hemisfério norte, e de junho a setembro, no hemisfério sul (Mouco, 2008). A polinização ocorre principalmente por insetos como abelhas, moscas, formigas e trips (Usman et al., 2001; Iyer e Schnell, 2009). Menos de 35% das flores presentes na panícula são polinizadas e apenas 0.01% geram frutos aptos para a colheita (Iyer e Schnell, 2009).

As mangueiras podem ser classificadas em dois grupos distintos, monoembriônicas e poliembriônicas, baseados em seu modo de reprodução seminal e nos seus centros de diversidade (Viruel et al., 2005). De um modo geral, as variedades

monoembriônicas são provenientes da Índia (grupo subtropical), enquanto que as poliembriônicas vêm do Sudeste da Ásia (grupo tropical) (Iyer e Schnell, 2009; Viruel et al., 2005). Cultivares monoembriônicas caracterizam-se por possuírem sementes que geram um único embrião zigótico resultante, em sua maior parte de fecundação cruzada, ou ainda, em uma menor proporção, de autofecundação. Já variedades poliembriônicas caracterizam-se pelo desenvolvimento de mais de um embrião em uma mesma semente, dos quais um pode ser zigótico, sendo a maioria, ou até todos, nucelares (Iyer e Schnell, 2009). O tecido nucelar compõe-se por um tecido materno que envolve o saco embrionário de forma que as plântulas provindas deste tecido são geneticamente idênticas à mãe (Aron et al., 1998).

Conhecida na Ásia como a "rainha das frutas" a manga é a segunda fruta tropical mais consumida no mundo atrás apenas da banana (FAO, 2013). A Índia é o maior produtor mundial da fruta como uma produção anual de cerca de 18 milhões de toneladas, seguida da China, Tailândia, Indonésia, Paquistão, México e Brasil (FAO, 2013).

No Brasil, as primeiras variedades cultivadas de mangueira foram introduzidas pelos navegantes portugueses no século XVII. Essas pertenciam ao grupo tropical, como a "Ubá", "Bourbon", "Espada", "Coquinho", "Rosa" e eram cultivadas na maioria das vezes em pequenas propriedades e de forma extrativista. Somente na década de 1960, com a introdução de cultivares do grupo subtropical, desenvolvidas na Flórida, foi que a mangicultura brasileira teve um grande impulso. Tais cultivares como a "Haden", "Keitt", "Kent", "Palmer", "Van Dyke" e, principalmente, a "Tommy Atkins" (Carvalho et al., 2004) apresentavam características como adaptabilidade, regularidade de produção, aparência e sabor agradáveis (Mukherjee e

Litz, 2009) o que permitiu um maior desenvolvimento da cultura e uma maior aceitação no mercado interno e externo.

Atualmente, a manga destaca-se como uma das principais frutas exportadas no país, ocupa cerca de 73 mil ha e possui uma produção anual de mais de 1.200.000 toneladas. O Brasil possui também a maior produtividade da fruta com cerca de 16 t/ha (FAO, 2013; IBGE 2012). As regiões do Nordeste, sobretudo no Vale do São Francisco, nos estados de Pernambuco e da Bahia, e do Sudeste, no estado de Minas Gerais, nas cidades de Jaíba e Janaúba, e no estado de São Paulo, nos municípios de Monte Alto e Taquaritinga são os principais polos produtores da fruta (Anuário Brasileiro da Fruticultra, 2014).

Dentre os diversos fatores que podem afetar negativamente, ou mesmo inviabilizar, a produção de manga no Brasil estão as doenças. A murcha-de-Ceratocystis, também denominada seca-da-mangueira, mal-do-Recife ou Sudden decline é considerada atualmente uma das principais enfermidades da cultura. Causada pelo fungo Ascomiceto Ceratocystis fimbriata Ellis & Halsted essa doença foi relatada pela primeira vez na cidade de Recife, Pernambuco, no final da década de 1930 (Carvalho, 1938; Pyenson, 1938). Pouco tempo depois foi também detectada em Jardinópolis, São Paulo, dizimando um plantio da variedade 'Bourbon' (Arruda, 1940) sendo em ambos os relatos o agente causal atribuído a Diploidia recifenses. Posteriormente Viegas(1960) descreveu Ceratocystis fimbriata como o verdadeiro agente causal da doença. Atualmente a murcha-de-Ceratocystis ocorre nos estados da Bahia, Rio de Janeiro, Minas Gerais, Goiás, Paraíba, Ceará, Pernambuco, Alagoas, Mato Grosso do Sul e Distrito Federal (Viegas, 1960; Ribeiro, 1997; Batista et al., 2008; Oliveira et al., 2015).

Até o início década de 1990 a murcha-de-Ceratocystis em mangueira era restrita ao Brasil. No entanto, a partir de 1998 a doença foi relatada em plantios de mangueira em Omã (Al-Adawi et al., 2003; Van Wyk et al., 2005) e, em 2005 no Paquistão (Malik et al., 2005). Nestes países a doença tem ocorrido de forma severa com um impacto muito significativo na produção da cultura (Van Wyk et al., 2005). No leste de Omã, no distrito de Barka, a doença chegou a atingir 76% das plantas (Al Adawi et al., 2006) e em 2001 o governo chegou a tomar uma medida de controle de emergência onde em algumas regiões mais de 13% das mangueiras foram queimadas na tentativa de conter a doença (Al Adawi et al., 2006).

Em ambos os países do oriente, inicialmente a doença foi atribuída ao fungo C. fimbriata, assim como no Brasil. No entanto, estudos posteriores baseados em análises filogenéticas utilizando a região ITS, indicaram que duas novas espécies C. omanenis (Al-Subhi et al., 2005) e, principalmente, C. manginecans (Van Wyk et al., 2007) eram os agentes etiológicos da doença em Omã. Com base em comparações de sequências gênicas o mesmo grupo de pesquisa descreveu duas novas espécies, C. mangicola e C. mangivora associadas à doença no Brasil (Van Wyk et al., 2011). No entanto, recentes trabalhos desenvolvidos por Harrington et al. (2014) e Oliveira et al. (2015) demonstraram que C. manginecans, C. mangicola e C. mangivora são, na verdade, haplótipos de C. fimbriata e não novas espécies.

Os sintomas da doença podem variar de acordo com o local de infecção do patógeno. Se a infecção é via parte aérea os principais sintomas são murcha do galho afetado, seguida de seca e presença de gomose. Se a infecção ocorre pelas raízes o sintoma típico é a murcha da planta como um todo, que morre rapidamente. Em ambos os casos é observada a descoloração do xilema da árvore (Viegas, 1960; Ploetz e Freeman, 2009). A doença é disseminada por mudas, ferramentas de poda,

implementos agrícolas contaminados e por insetos como Hypocryphalus mangiferae e coleobrocas do gênero Xyleborus também conhecidos como besouros de Ambrosia (Rossetto e Ribeiro 1983; Al Adawi et al., 2006; Van Wyk et al., 2007; Masood et al., 2008; Souza et al., 2013).

A principal estratégia de melhoramento que vem sendo utilizada no que se refere à murcha-de-Ceratocystis tem sido a seleção de cultivares e/ou porta-enxertos resistentes. O procedimento básico deste método consiste na inoculação de isolados de C. fimbriata em diferentes cultivares de mangueira, já aceitas no mercado, em busca daquelas resistentes ou com diferentes graus de resistência à doença (Ribeiro et al., 1984; Ribeiro et al., 1986; Rossetto et al.,1996; Galli et al., 2011). No entanto, não existem na literatura trabalhos que busquem entender a base genética da resistência à doença, informação essencial para direcionar os métodos de melhoramento e facilitar as decisões de escolha dos processos de seleção mais eficientes a serem empregados. Desta forma, devido à importância da doença para a cultura da manga e à falta de estudos relevantes nesta linha de pesquisa, estudar a herança da resistência em mangueira é fundamental para o desenvolvimento e seleção de genótipos resistentes visando mitigar as perdas advindas pela doença.

Entretanto, para que estudos do controle genético de caracteres de interesse possam ser delineados é fundamental a realização de cruzamentos controlados visando a geração de híbridos e de famílias segregantes. Em mangueira o pequeno tamanho das flores associado a alta abscisão de frutos faz com que os cruzamentos controlados manuais, além de trabalhosos, apresentem rendimentos muito baixos. Por essa razão, técnicas de polinização aberta tem sido preferidas para obtenção de híbridos na cultura (Pinto et al**.,** 1995; Honsho et al**.,** 2012; Bally et al**.,** 2009). Assim, pode-se explorar a natureza alógama da mangueira associadas a medidas que favoreçam a polinização

cruzada a partir de pólen proveniente de uma fonte desejada. Apesar dos maiores rendimentos obtidos via cruzamentos naturais, indivíduos provenientes de autofecundação e ou contaminação por pólen indesejável podem ocorrer nas progênies obtidas. A presença desses 'contaminantes' pode atrasar o programa de melhoramento, torná-lo mais dispendioso e levar a conclusões errôneas em estudos de herança. Assim, é fundamental que os programas de melhoramento da mangueira utilizem ferramentas disponíveis, como os marcadores de DNA, para confirmar se a planta obtida do cruzamento é verdadeiramente um híbrido intervarietal desejado.

Diversos tipos de marcadores moleculares estão disponíveis, os quais se diferenciam pela tecnologia utilizada para revelar variabilidade ao nível de DNA. Os marcadores moleculares microssatélites ou SSRs (Simple Sequence Repeats) têm sido bastante utilizados na confirmação da paternidade e identificação de híbridos, por ter um preço acessível, possuir bom conteúdo de informação polimórfica devido à expressão codominante e pelo fato de ser multialélico (Borém e Caixeta, 2006). Atualmente, diversos marcadores SSR estão descritos para M. indica (Duval et al., 2005; Ravinskar et al., 2011; Schnell et al., 2005; Viruel et al., 2005) o que torna o seu uso de fácil aplicação.

Dessa forma, esta tese está dividida em dois capítulos. No primeiro, avaliou-se por meio de marcadores microssatélites a porcentagem de híbridos intervarietais obtidos por aproveitamento de cruzamentos naturais. No segundo capítulo, em razão da importância da mangicultura no atual cenário nacional e das crescentes perdas que o fungo C. fimbriata têm proporcionado à cultura, objetivou-se, a partir de seis progênies intervarietais, compreender o controle genético da resistência à murcha-de-Ceratocystis em M. indica, de modo a auxiliar no melhoramento da cultura. Espera-se

que os resultados desses estudos possam gerar informações para subsidiar o controle da doença e o melhoramento da mangueira como um todo.

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CAPÍTULO 1

ARTICLE 1

To be submitted to the journal Revista Brasileira de Fruticultura

IDENTIFICATION OF MANGO HYBRIDS BY MICROSATELLITE MARKERS

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ABSTRACT - In Brazil, mango production is based mainly in the cultivar Tommy Atkins. However, the use of one or few cultivars increases the risks of losses caused by pests and disease. To mitigate these losses and diversify the market, it is necessary to develop new cultivars throughout intervarietal hybridization. However, the technique of hybridization based on artificial pollination is laborious and frequently results in fruit abortion. Thus, it is generally made by open pollination, which not always guarantee the origin of the male parent. In this study we evaluated the paternity of open pollinated progenies using microsatellite markers in an orchard, formed by "Tommy Atkins" putatively crossed with "Coquinho", "Espada",'Haden", "Keitt", "Palmer" and 'Van Dyke'. Leaves of seedlings and parents of each cultivar were collected, the DNA was extracted and the confirmation of hybridization was made by microsatellite markers. We found high levels of hybridization, even in crossings with polyembryonic cultivars as female genitors. Our results indicate that open pollination is efficient and useful to develop new mango cultivars.

Index terms: Mangifera indica, hybridization, molecular markers, breeding.

IDENTIFICAÇÃO DE HÍBRIDOS DE MANGA POR MARCADORES MICROSSATÉLITES

RESUMO - No Brasil, a produção de manga é baseada principalmente na cultivar Tommy Atkins. No entanto, a utilização de uma ou poucas cultivares aumenta os riscos de prejuízos causados por pragas e doenças. Para atenuar essas perdas e diversificar o mercado, é necessário o desenvolvimento de novas cultivares por meio de hibridação intervarietal. No entanto, a técnica de hibridização com base na polinização artificial é trabalhoso e muitas vezes resulta em aborto de frutas. Assim, ela é geralmente feita por meio de polinização aberta, o que nem sempre garante a origem do progenitor masculino. Neste estudo avaliou-se a paternidade de progênies de polinização aberta utilizando marcadores microssatélites em um pomar, formada pela "Tommy Atkins" supostamente cruzado com "Coquinho", "Espada", "Haden", "Keitt", "Palmer" e "Van Dyke". Folhas das progênies e de cada cultivar foram colhidas, o DNA foi extraído e a confirmação da hibridação foi feita por meio de marcadores microssatélites. Foram encontrados níveis elevados de hibridação, mesmo em cruzamentos com cultivares poliembriônicas como genitores femininos. Nossos resultados indicam que a polinização aberta é eficiente e útil para desenvolver novas cultivares de mangueira.

Termos para indexação: Mangifera indica, hibridação, marcadores moleculares, melhoramento.

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INTRODUCTION

Currently Brazil is the seventh world's largest mango producer (FAO, 2013) with an area of approximately 73,000 ha and annual production of 1,200,000 tons (BRAZILIAN FRUIT YEARBOOK, 2014) based mainly on the Tommy Atkins cultivar. Although this cultivar has good skin color, moderate resistance to some diseases, good shelf life and good response to flower induction, it presents some problems as internal fruit breakdown, flower malformation, lower level of Brix (<17%) and high latex oozing (PINTO et al., 2011; PINTO, 2004). In addition, the use of one cultivar on a large scale increases the risks of losses by pests and diseases and limits the market expansion of mango with different flavors. Thus, it is necessary to develop new cultivars to diversify the market and expand the area of mango in Brazil.

The intervarietal hybridization has been an important method to obtain new cultivars of mango (PINTO et al**.,** 2011). However, the small size of the flowers and the high fruit abscission make the manual-controlled crossings laborious and with very low yield. Therefore, open pollination has been preferred for hybrid production (PINTO**,** 1995; HONSHO et al**.,** 2012; BALLY et al**.,** 2009). Thus, it is possible explore the outcrossing mating system predominant in mango associated with measures to promote cross-pollination with pollen from a desired source. This approach has been used for crossings in cages and poly-cross fields (PINTO**,** 1995; HONSHO et al**.,** 2012; BALLY et al**.,** 2009).

Despite the higher yields obtained via open pollinated crossings, individuals from selfing and or contamination by unwanted pollen may occur in the progenies. Since mango is a perennial plant, with long juvenile period, high costs and time are demanded to obtain the hybrid offspring and release a new cultivar. The presence of these “contaminants” can delay the breeding program, making it more costly and

leading to erroneous conclusions in inheritance studies. Thus, the use of available tools such as DNA markers may help in the confirmation of hybridization.

Among the molecular markers, the microsatellites (simple sequence repeat or SSRs) stand out for their high degree of polymorphism, the codominance and the Mendelian inheritance. These characteristics make them suitable for studies of diversity, paternity determination, conservation and movement of germplasm (VIRUELL et al., 2005). Currently, several SSRs markers are available for mango (DUVAL et al**.,** 2005; SCHNELL et al**.,** 2005; VIRUELL et al**.,** 2005; RAVISHANKAR et al**.,** 2011), making their use of easy application.

Thus, in this study we evaluated the hybridization efficiency in six mango progenies derived from natural pollination in orchards located in the "Vale do São Francisco" through SSRs markers.

MATERIAL AND METHODS

The putative hybrids evaluated in this study were obtained from open pollinated crossings between "Tommy Atkins" and other six mango cultivars. In a commercial orchard of the cultivar Tommy Atkins, isolated plants (at approximately 200 m distance) of "Coquinho", "Espada", "Haden", "Keitt", "Palmer" and "Van Dyke" were identified and utilized as female parents. Fruits of each of these six cultivars were harvested and their seeds were sown for seedling production. Six months old seedlings were planted in the Experimental Station of Mandacaru of Embrapa Semiárido, located in Juazeiro, Bahia, northeastern Brazil, at a spacing of 4 x 4 m. The plants were irrigated by microaspersion and fertilized according to soil analysis and chemical leaf analysis based in technical recommendations for the crop.

For extraction of genomic DNA, leaves from each of the parents and progenies were collected, identified, placed in paper bags and taken to the Laboratory of Forest

Pathology (BIOAGRO) at the University Federal de Viçosa, Minas Gerais, Brazil. The CTAB extraction protocol as previously described by Khan et al. (2007) with additional modifications was used. About 400 mg of leaf tissue were placed in microcentrifuge tubes (2 mL) and then 800 uL of preheated extraction buffer at 65 °C were added (CTAB 3% 2M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8 0.2% PVP-40 and 0.6% β-mercaptoethanol added at the time of extraction). Two beads of 5 mm diameter were added to the microcentrifuge tubes and samples were macerated in the macerator to disruption TissueLyser II (QIAGEN) for 5 min at 30 Hz. Subsequently, the samples were maitaned in a water bath at 65 °C for 50 min and homogenized each 10 min. After this, 600 uL of CIA (24 mL of chloroform to 1 mL of isoamyl alcohol) were added, followed by homogenization of the samples by vortexing and centrifugation at 12,000 rpm (Eppendorf 5415R) for 5 min. The supernatant was transferred to a microcentrifuge tubes (1.5 mL), containing 200 uL of Protein Precipitation Solution (Wizard® Genomic DNA Purification Kit, Promega), homogenized and centrifuged as described. The supernatant was transferred to new microcentrifuge tube (1.5 mL) containing 600 uL of ice isopropanol, homogenized and incubated for at least one hour at -20 °C. Afterwards, the samples were centrifuged for 10 min at 12,000 rpm and the supernatant was discarded. The precipitate was washed twice with 70% ethanol and once with 100% ethanol by applying a centrifugation at 12,000 rpm for 3 min each wash. The pellet was dried at room temperature for an hour and, then, it was resuspended in 50 uL of TE buffer (10 mM Tris HCl and 1 mM EDTA, pH 8.0) plus 1 uL of RNase Solution (Promega). The DNA was kept overnight at 37 °C and subsequently quantified in Nanodrop® (Thermo Scientific) The DNA concentration was adjusted to 10 ng uL^{-1} and it was stored at -20 °C.

An initial screening with 17 microsatellite markers was performed (Table 1). However, only primers that showed a good quality of amplification and genotyping and / or exclusive alleles of the male parent were selected for analysis. For all SSR's tested the forward primers were labeled with 6-FAM fluorescence at 5 'end. PCR reactions were conducted with a solution containing 30 ng DNA, 1.33 mM of each primer (forward and reverse) and 1 X Go Taq ® Master Mix 2X (Promega) and ultrapure water to make a final volume of 15 uL.

Amplifications consisted of an initial denaturation at 95 °C for 5 min followed by 40 cycles at 94 °C for 1 min, melting temperature (TM) according to each primer (Table 1) for 1 min and 72 °C for 1 min, with a final elongation at 72 °C for 30 min. After amplification, the PCR products were diluted at a ratio of 1:40 using sterile water. For analysis of amplified fragments in automated DNA sequencer, in 3 uL of diluted PCR (1:40) were added 6.8 uL Hi-DITM formamide (Applied Biosystems), 0.2 uL of GeneScanTM LIZ®-120 Size Standard (Applied Biosystems). The mixture was denatured for 3 min at 95 °C and the genotyping carried out in the Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems) of the Forest Pathology Laboratory/Bioagro/UFV. The genotypes were determined using GeneMapper® v.5.0 software (Applied Biosystems).

The size of the progenies evaluated varied from 35 to 54 (Table 2). For each progeny, the criterium to consider as hybrids was individuals that had, in at least one of the loci evaluated, one allele of the male parent (“T. Atkins”) and one allele of the female parent. Plants that presented alleles of only female parent for all loci analyzed were considered selfings. Plants that had one allele of maternal origin and one allele different from those found in the male parent (“T. Atkins”), in at least one of the evaluated loci, were considered hybrid of unknown male parent.

RESULTS AND DISCUSSION

Among the 17 tested microsatellite primers, six were selected (Table 3) because they showed clear and well-defined peaks. For the crossing between "Coquinho" and "T. Atkins" (CxT), all six primers allowed the hybrid identification once they presented alleles exclusives from the male parent. For the crossing between "Espada" and "Tommy Atkins" (ExT), there was no amplification for the primer MiCIR022 and all the others primers selected showed exclusive alleles from the male parent. Exclusive alleles from the male parent were found for the primers mMiCIR028, MiIIHR-28, MiIIHR-29 and MiIIHR-36 in the crossing between "Haden" and "Tommy Atkins" (HxT); MiCIR022, mMiCIR028, MiIIHR-28 and MiIIHR-29 in the crossing between the cultivars "Keitt" and "T. Atkins" (KxT); MiCIR022, mMiCIR028, MiIIHR-29 and MiIIHR-36 in the crossing between the cultivars "Palmer" and "T. Atkins" (PxT); and MiCIR022 and MiIIHR-36 in the crossing between the cultivars "Van Dyke" and "T. Atkins" (VxT) (Table 3). Common alleles were found among "Haden, "Palmer" "Van Dyke" and "T. Atkins" (Table 3) for all primers studied. This was expected since "Haden" is likely the genitor of these three cultivars (SCHNELL et al., 2006).

In three of the six families evaluated (HxT, KxT and PxT) the percentage of hybridization was higher than 83% (Table 2). In the VxT family, the hybrid origin was confirmed for only 40.5% of the individuals. The genealogy of 47% of the plants of this family could not be determined because they did not showed exclusive alleles of the male parent for both primers used for genotyping. This is because these two cultivars share many alleles for the two primers studied. For this reason, these individuals were also genotyped by using the primer MiIIHR24 in order to prove if they were originated by selfing or cross-pollination. If by selfing, they would have the

genotype 262/262. However, this result was not observed and these plants were classified as putative hybrids. Therefore, for the VxT family, the number of hybrids obtained may have varied from 40.5 to 87.25 %. Thus, specifically for this crossing, new primers should be tested to find different alleles between the two cultivars. In the remaining families, a greater number of primers presented exclusive alleles of the male parent. Thus, their plants were genotyped for more loci and the identification of the hybrids could be performed.

The lower hybridization found in CxT (33%) and ExT (37%) crossings may be atributted to polyembryony of “Coquinho” and “Espada”. The polyembriony produces a large number of nucellar individuals, i.e. clones of the mother plant, and hinders the identification of zygotic seedlings that are resulting from crossings. The presence of only exclusive alleles from the female parent in most of the plants in the CxT and ExT crossings for the primers MiIIHR24, MiIIHR29, MiIIHR36 (data not shown) is an indication that they are nucellar. These results corroborate the findings of Santos et al. (2010) that evaluated the open pollinated crossings between “Espada” x “T. Atkins” (ExT) and “Haden” x “T. Atkins” (HxT). According to these authors, the reduced number of hybrids in the ExT crossing compared to the HxT crossing is explained by polyembryony of “Espada” and the lack of synchronized flowering of the two parents. Thus, a solution to increase the frequency of hybridization of these cultivars is the application of practices that provided the synchronized flowering between these two groups such as the application of paclobutrazol (PBZ), nitrates and water stress. Another alternative is the use of monoembryonic cultivars as female progenitors, which would prevent the presence of nucellar seedlings in the progeny.

Although the percentage of hybrids was high, in all crossings there were individuals with a common female parent allele, but with different alleles from those

of the putative male parent (“T. Atkins “). This indicates that there was pollen from other cultivars and the distance of 200 m between the orchards was not sufficient to prevent contamination of other parents. In a study on the effect of the distance of the pollen donor to produce hybrids between “Maya” and “T. Atkins”, Degani et al., (1997) Dag et al., (2009) observed a gradual decrease in hybrization rates with increasing distance from the pollinator. Dag et al., (2009) found hybridization between distant pollinators up to 165 m from the mother plant. Santos et al., (2011) observed that the distance of 200 m was sufficient to prevent contamination of a mother plant of “Haden” pollinated by “T. Atkins”. Our results suggest that pollen dispersal in mango can occur between areas further than 200 m apart from each other. This is an important information to design future crossing orchards, in order to avoid contamination by undesirable pollen. Although all the procedure for the establishment of this orchard had been thoroughly planned, we cannot exclude the possibility of genotypes admixing during the production or planting of the seedlings. A more detailed and specific experiment should be undertaken to prove this hypothesis.

The high outcrossing rate found in this study can be observed directly by the high percentage of hybrids found (Table 2). Individuals from self-pollination were identified only for the HxT, KxT and VxT crossings at rates lower than 8.5%. Although outcrossing is predominant in mango, incompatibility mechanisms have been reported among specific crossings (BALLY et al., 2009), like “T. Atkins” x “Mallika” “(PINTO, 2004). This phenomenon can limit the generation of progenies from cultivars with complementary agronomic traits. The success in obtaining hybrids between “T. Atkins” and monoembryonic cultivars found in this study indicates the feasibility in planning controlled crossings and the general combining ability of the “T. Atkins”, considered promising cultivar for breeding programs.

Hand pollination in mango is laborious, very time-consuming, and of very low yield. Mukherjee et al., (1961) and Singh et al., (1980) reported yields of 3.85% even with the application of technical improvements in the crossing such as limited number of pollinated flowers per panicle and pollination of a larger number of panicles due to the high abscission index. Furthermore, Pinto (1995) by combining the technique of Mukherjee et al., (1961) and the use of fungicides reached maximum values of 8.2% of hybridization. However, they did not confirm the hybrid nature of the plants either by phenotypic or molecular markers.

In our study, the hybridization method was adopted after identifying individuals of other cultivars in orchards of "T. Atkins". However, the percentage of hybrids confirmed was higher than that obtained by hand pollination, even in crossings with polyembryonic cultivars as female genitors. This reinforces the feasibility of this technique. Thus, replacement of the crown of a single plant in an orchard with a particular cultivar could be applied in breeding programs of mango in order to generate hybrids (SANTOS et al., 2010). This procedure would take advantage of the natural mango outcrossing to obtain a higher number of hybrids from desired parents, as in other open pollination techniques like polycross fields and "crossing cages" (HONSHO et al., 2012).

In conclusion, the hybridization rate found for all families evaluated in this study was satisfactory. The technique used to achieve the desired hybrids, in association with the application of molecular markers to distinguish individuals derived from self-fertilization or contamination, is promising in mango breeding programs. The hybrids generated in the crossings here studied can be used in various studies such as evaluation of the matrices productivity, resistance to diseases and pests, post-harvest studies, and market acceptance. We expect that, among the evaluated

individuals, new potential cultivars can be identified bearing the favorable traits of “T. Atkins” and other complementary features of interest found in other parents.

Furthermore, the confirmation of both parents will improve future studies, facilitating the statistical analysis of the segregation patterns in the breeding population and will provide a better understanding of genetic studies in mango.

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TABLES

TABLE 1: SSR primers tested for genotyping of mango cultivars

Primer	Repeat motif	Melting temperature (°C)	Reference
MiSHRS-1	$(CT/AG)_{14}$	50 °C	Schnell et al., 2005
MiSHRS-4	$(CT/AG)_{11}$	57 °C	
MiSHRS-29	$(TG/CA)_{9}$	46 °C	
MiSHRS-32	$(CA/TG)_{9}$	53 °C	
mMiCIR003	$(TG)_{10}$	51 °C	Duval et al**., (**2005**)**
mMiCIR010	$(TG)_{13}$	51°C	
mMiCIR022	$(AC)_{16}$	51 °C	
mMiCIR028	$(CA)_{7}$	57 °C	
mMiCIR030	$(TG)_{12}$	51 °C	
mMiCIR032	$(TG)_{19}$	51 °C	
mMiCIR036	$(TG)_{11}$	51 °C	
MiIIHR19	$(AC)_{11}$	55 °C	Ravishankar et al., (2011)
MiIIHR24	$(CA)_{9}$ TACC$(CATA)_{6}$	57 °C	
MiIIHR28	$(GA)_{12}$	57 °C	
MiIIHR29	$(GT)_{10}$	51 °C	
MiIIHR34	$(GGT)_{9}$ $(GAT)_{5}$	55 °C	
MiIIHR36	$(TC)_{17}$	57 °C	

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TABLE 2: Number of plants, hybrids, selfing and putative nucellar clones of the female genitor.

Cultivar (Female)	Crossing	Number of plants	Hybrids *	Selfing	Hybrids of unknown male parent **	Nucellar clones
Coquinho	CxT	49	16 (33%)	0	1(2%)	32 (65%)
Espada	ExT	35	13 (37%)	0	2(6%)	20 (57%)
Haden	HxT	54	47 (87%)	4 (7%)	3 (6%)	-
Keitt	KxT	53	47 (89%)	1 (2%)	5 (9%)	-
Palmer	PxT	41	34 (83%)	0	7 (17%)	-
Van Dyke[1]	VxT	47	41 (87.25%)	4 (8.5%)	2 (4.25%)	-
Van Dyke[2]	VxT	47	19 (40.5%)	26 (55.5%)	2 (4.0%)	-

* Hybrids derived from “Tommy Atkins” as the male parent
** Hybrids of unknown male parent
[1] Hybrids confirmed for the crossing between “Van Dyke” and Tommy Atkins.
[2] Potencial hybrids for the crossing between “Van Dyke” and Tommy Atkins.
CxT: Crossing between “Coquinho” and “Tommy Atkins”; ExT: Crossing between “Espada” and “Tommy Atkins”; HT: Crossing between “Haden” and “Tommy Atkins”; KxT: Crossing between “Keitt” and “Tommy Atkins”; PxT: Crossing between “Palmer” and “Tommy Atkins”, VxT: Crossing between “Van Dyke” and “Tommy Atkins”.

TABLE 3: Microsatellite genotypes of the parent cultivars.

Loci	Alelles (bp*)						
	"Coquinho"	"Espada"	"Haden"	"Keitt"	"Palmer"	"Van Dyke"	"Tommy Atkins"
mMiCIR022	156/166**	-	150/166	144/166	142/166	160/166	150/166
mMiCIR028	184/190	184/184	184/186	184/190	184/190	186/190	186/190
MiIIHR24	242/256	240/256	240/246	240/246	244/246	246/262	246/246
MiIIHR28	106/110	104/112	104/112	106/112	104/106	104/106	104/106
MiIIHR29	150/152	150/152	150/154	145/150	152/154	145/154	145/154
MiIIHR36	222/242	229/231	222/226	226/229	222/229	222/229	226/229

* base pairs

**Allele 1 and allele 2

CAPÍTULO 2

ARTICLE 2

To be submitted to the journal Science Horticulturae

Genetic control of resistance to Ceratocystis wilt on Mangifera indica

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Abstract

Ceratocystis wilt, caused by Ceratocystis fimbriata Ellis & Halsted is one of the most serious limiting factors for mango production in Brazil. Despite efforts in selecting and identifying resistant mango cultivars to Ceratocystis wilt, the genetic basis of resistance is yet unknown. In this study, by artificial inoculations of the pathogen in different cultivars and in their progenies, we found that "Keitt", "Palmer", "Tommy Atkins" and "Van Dyke" were more resistant and "Coquinho", "Espada" and Haden were more susceptible. Results of the inoculation on the progenies of these cultivars using "Tommy" as male parental showed that the resistance in mango is polygenic with prevalence of genes with effects of dominance and epistasis. The genetic gain with the selection of the 10 more resistant plants was 46%, which means a reduction of 46 % in disease severity. In general, there was a low frequency of alleles favorable to the disease resistance in the population studied, which indicates the need of introduction of new sources of genetic materials, carrying genes for resistance.

1 Introduction

The mango (Mangifera indica L.) is one of the most consumed tropical fruits in the world (FAO, 2013). Native to Asia, the mango was initially spreads by the Portuguese navigators in 1600 in Africa and subsequently in Brazil (Mukherjee, 1972; Mukherjee and Litz, 2009). Nowadays, the mango is globally cultivated in most tropical and subtropical countries. India is the world's largest producer of mango with about 18 million tons per year (FAO, 2013). Brazil is the seventh world's largest producer and crop cultivation is concentrated in the "Vale do São Francisco" region, in Bahia and Pernambuco states, and in southeast of the country in Minas Gerais and São Paulo (Brazilian Fruit Yearbook, 2014). Currently, cultivars originated from Florida (USA) such as "Haden', "Keitt", "Kent", "Palmer", "Van Dyke" and, especially, "Tommy Atkins", are the most planted in Brazil and in other Western countries (Carvalho et al., 2004; Knight et al., 2009). Brazilian cultivars such as Coquinho, Rosa, Ubá, and Espada also have an expressive cultivated area. "Espada" is widely used for rootstock and "Ubá" for juice production, especially in the Zona da Mata of the Minas Gerais state (Rocha et al., 2014).

Ceratocystis wilt, caused by Ceratocystis fimbriata Ellis & Halsted is one of the main limiting factors for mango production in Brazil. Ceratocystis wilt, also known as "Seca da Mangueira", "Mal do Recife" or "Mango Decline" was first described in Recife, Pernambuco, and the causal agent was attributed to Diplodia recifensis (Carvalho, 1938), and later on as Ceratocystis fimbriata (Viegas, 1960). At the end of the 1990's, this disease was reported for the first time outside Brazil, in the Middle East in Oman and subsequently in Pakistan (Al-Adawi et al., 2003; Malik et al., 2005) becoming a disease of international concern.

Symptoms of the disease can vary according to the local of infection in the plant. If the infection takes place on branches, the main symptoms are wilting of the affected branches, followed by foliage drying and gum exudation in the trunk. If the infection occurs through the

roots, a typical symptom is wilting of the entire plant and a sudden death is observed. In both cases, xylem discoloration is observed as consequence of the pathogen infection (Viegas, 1960; Ploetz and Freeman, 2009). Ceratocystis wilt is disseminated by infected rootstock or grafted plants, contaminated pruning tools and by insects such as Hypocryphalus mangiferae and coleoborers of the Xyleborus genera, also known as Ambrosia beetles (Rossetto and Ribeiro, 1983; Al Adawi et al., 2006; Van Wyk et al., 2007; Masood et al., 2008; Souza et al., 2013). The wide host range of C. fimbriata, the survival of the fungal structures in the soil and the colonization of vascular system of the plants make difficult the management of the disease. Eradication of infected plants and branches with symptoms of the disease and planting of resistant genotypes selected by inoculations of the pathogen under controlled conditions are the most suitable measures of control.

Over the last decades, efforts have been employed in the selection and identification of resistant cultivars (Ribeiro et al., 1984; Rossetto et al., 1996; Galli et al., 2011). However, the genetic basis of resistance to Ceratocystis wilt in mango is unknown. This information is fundamental to support and guide the breeding programs to develop new commercial resistant cultivars. Therefore, in this work, we evaluated the genetic basis of the resistance to Ceratocystis wilt by artificial inoculation of the pathogen in “Tommy Atkins”, “Coquinho”,”Espada”,”Haden”, “Keitt”, “Palmer” and “Van Dyke and their progenies using “Tommy Atkins” as pollen donor.

2 Material and methods

2.1 Plant material

As many as 197 mango genotypes derived from six full-sib families provided by the Breeding Program of Mango of the Empresa Brasileira de Pesquisa Agropecuário (Embrapa)-Semiárido, were evaluated (Table 1). The cultivar Tommy Atkins was the male parent of all crossings. The Brazilian cultivars Coquinho and Espada and the American cultivars Haden, Keitt,

Palmer and Van Dyke were the female parents. All plants of the six families were genotyped by using microsatellite markers to confirm their hybrid nature (Arriel et al., 2015-Article 1).

The progenies were obtained through open pollination, where each female parent was identified in the midst of an orchard of the cultivar T. Atkins. The fruits from the female parents were harvested and taken to the experimental campus of Embrapa-Semiárido for seedling production and subsequent planting in the field at a spacing of 4 x 4 m. The plants were micro-aspersion irrigated and fertilized according to soil analysis.

As the evaluation for resistance to Ceratocystis wilt is destructive, clonal replicates of each genotype were obtained by grafting, using "Imbu" as rootstock.

Due to variation in survival of the grafted plants, the number of individuals per family or progeny as well as the number of clonal replicates per plant varied, which caused the experiment to be unbalanced (Table 1). However, for the vast majority of the analyzed plants (91%), the number of clones was equal to or greater than three. In general, the analysis had at least two replicates for all plants.

2.2 Inoculations

The isolate SPMA3 of C. fimbriata of the culture collection of the Forest Pathology Laboratory (UFV/Viçosa, MG), obtained from infected host tissue of "Palmer" in Monte Alto, São Paulo, was used. This isolate was selected for its abundant sporulation in culture medium and its high aggressiveness in a broad spectrum of mango cultivars (Oliveira et al., 2015a). The identity of the isolate was confirmed by sequencing of the regions ITS, β-tubulin, TEF-1α and mating type genes of DNA and subsequent comparison of its sequences with others of C. fimbriata in an extensive study involving isolates of the pathogen collected in mango on Brazil, Oman and Pakistan (Oliveira et al., 2015b).

For inoculation, the bark replacement method was employed (Alfenas et al., 1983). The fungus was grown in V8-AM medium (Brito et al. 2014), at 28°C in a 12-h photoperiod for ten days. Posteriorly, at about 6 cm above the grafting point, a 6.4 mm disk of bark was removed and replaced by an inoculum plug of the same size taken from an active growing colony. After inoculation, the wound was covered with PVC plastic to prevent contamination and dehydration. Plants of the cultivar Espada were used as susceptible comparator (Ribeiro et al., 1986; Araújo et al., 2014). Ten plants equally treated with medium plug without fungus were used as control. The plants were incubated in a greenhouse where the average daily temperature and humidity was 26 °C (17 to 36 ºC) and 80.80% (46.00 to 95.00%), respectively. At about 60 days later, the inoculated plants were sectioned longitudinally and the length of xylem discoloration above and below the point of inoculation was measured. Disease severity was calculated by dividing the lesion length (cm) by plant height from the grafting point (cm) and then multiplying by 100 (Oliveira et al., (2015c).

2.3 Statistical and genetic analysis

The Dunnett test ($p \leq 0.05$) was used to classify parents as resistant or susceptible to Ceratocystis wilt by using the software STATISTICA 12.0 (StatSoft). The estimates of the variance components and genetic parameters were obtained by the method of mixed models via the REML (Restricted maximum likelihood) / BLUP (Best linear non-biased prediction) procedure (Resende, 2007) using the software Selegen-RemL / Blup (Resende, 2002). The REML/BLUP adjustment was based on the following mixed model:

$$y = Xr + Zf + Sc + e,$$

in which, y is the vector of data, r is the vector of fixed effects (replicates) added to the general average, f is the vector of the effects of families of full sibs (random), c is the vector of individuals effects inside family of full sibs (random), and e is the vector of errors (random). The capital letters represent the incidence matrices for the referred effects.

The variance components associated with the model can be decomposed as follows:

Total genetic variance among full-sib families (Vfam):

$Vfam = (1/2)\sigma_a^2 + (1/4)\sigma_d^2$

Total genetic variance among individuals inside families (Vindividual/fam)

$Vindividual/fam = (1/2)\sigma_a^2 + (3/4)\sigma_d^2 + \sigma_{ie}^2$

The total heritability is given by $h_g^2 = \frac{V\,fam + Vindividual/fam}{Vf}$, which:

σ_a^2: Additive genetic variance

σ_d^2: Dominance genetic variance

σ_{ie}^2: Epistatic interactions genetic variance

Vf : Phenotypic variance

The significance of the model's effects were tested by deviance analysis, using the likelihood ratio test (LTR), which significance was evaluated using the chi-square test with one degree of freedom at 1% and 5% probability of error type I. This type of analysis is indicated in cases of models with unbalanced data (Resende, 2007).

The genetic gain with selection of the top 10 most resistant plants was also estimated. The genotypic values were estimated taking into account the information of individuals and families.

3 Results

Plants of highly susceptible genotypes showed wilt symptons and subsequently died (Figure 1B). The cultivars (Figure 2) and their progenies (Figure 3) varied in resistance (Figure 1A and 1B) and bark lesions and xylem discoloration were observed along the stem (Figure 1C and 1D). The lesions developed, in most plants, mainly towards the plant apex. Non-inoculated plants did not show any disease symptoms and the fungi were re-isolated from infected plants.

Among the parents studied, “Keitt” was the most resistant with an average of 16.2% of disease severity and “Espada” was the most susceptible with 96.12% (Figure 2). Although all cultivars tested were susceptible, the disease was less severe on “Palmer”, “Tommy Atkins”, ”Van Dyke” and “Keitt” and they were grouped together by the Dunnett test at 5%. “Espada”, “Coquinho” and “Haden” were the most susceptible and they were grouped separately from the other cultivars (Figure 2).

All families studied segregated for resistance (Figure 3). However, the genotypic average for none of them was lower than that presented by the most resistant cultivar, Keitt, nor higher than that presented by the most susceptible cultivar, Espada (Table 1 and Figure 2). Overall, the average of all crossings was close to the overall average of the population (Table 1). For all crossings, individuals with genotypic values of severity out of the range of their parents were observed (Figure 3), which indicates the occurrence of transgressive segregation.

The genotypic effects of individuals within families were significant for the deviance analysis ($p \leq 0.01$). For the families, the genotypic effects were smaller, but still significant ($p \leq 0.05$) (Table 2). The relative coefficient of variation of 0.25 (Table 3 and 4) indicated a high environmental variance in the total variation. However, because of the great number of replicates, the estimated values of accuracy were high (>0.70) (Table 3) (Resende and Duarte, 2007) which indicates a good precision and experimental quality. The estimated total heritability (or broad-

sense heritability) was 0.37 (moderate to high magnitude). The heritability of individuals within families was 33%, whereas the heritability between families was 3.7% (Table 3).

The estimation of variance components in full-sib families and individuals within families did not allow separation of the additives effects from effects of dominance and epistasis. However, when assessing the magnitude of heritability values between and within families and the composition of the genetic variances involved in their estimation, we can conclude that effects of dominance and epistasis have a much greater contribution than additive effects in the character expression (see section 2.3: "Statistical and genetic analysis" and the expressions for estimation of variance among full-sib families and variance among individuals inside families). Estimates of genetic variance among full-sib families were much higher than those obtained within full-sib families. The magnitude of the additive variances involved in the estimation of variance between and within families is the same ($1/2\sigma_a^2$). Then they contribute equally to the total variance in both situations (between and within families). In turn, the variances due to the dominance deviations have a much larger contribution between families ($3/4\ \sigma_d^2$) than within families ($1/4\ \sigma_d^2$), and the variances due to the epistatic effects are present only in the estimation of the variance between families. Thus, it can be concluded that larger magnitudes of variance among families compared to within families are associated with the occurrence of epistatic effects and dominance. These results indicates that mango has a complex genetic control for resistance to Ceratocystis wilt.

The genetic gain obtained with the selection of the 10 most resistant plants, was 46%, which means a reduction of 46 % in disease severity (Table 4).

4 Discussion

This study aimed to elucidate the genetic control of the resistance to Ceratocystis wilt on mango. From our results, we can infer that the methods of inoculation and disease evaluation used

in the present study were appropriate to access the resistance to Ceratocystis wilt in different materials once they allowed for the expression of a considerable genotypic variability. This variability associated with high values of accuracy enabled the discrimination between highly susceptible and resistant genotypes. The heritability values found associated with the high environmental influence indicate that the inheritance of resistance in mango to Ceratocystis wilt is polygenic. Additionally, the larger magnitudes of non-additive genetic effects compared to additives effects also indicates that genes with effects of dominance and epistatic interaction are the main responsible for the trait control.

The genetic inheritance of most agronomic traits in mango has not yet been determined. The main reasons for this are long juvenile phase, high heterozygosity, polyembryony, high natural fruit shedding, cultivar incompatibility and small size of the populations (Bally et al., 2009; Iyer and Schnell, 2009). Although we have studied a limited number of families and plants/family, the microsatellite markers allowed the identification of hybrids in the progenies studied and ensured a more accurate statistical analysis of the data (Arriel et al., 2015- Article 1). Furthermore, the high estimated values of selective accuracy (Resende and Duarte, 2007) indicated confidence in the estimation of genetic values and, therefore, security in the selection (Resende, 2002).

This is the first study to understand the inheritance model of Ceratocystis wilt resistance in M. indica. However, the genetic control of resistance has been reported in other pathosystems involving Ceratocystis and tree species. Brawner et al., (2015) evaluated the inheritance of resistance of Acacia mangium to C. fimbriata (sin.C. acaciivora). As in our study, these authors found that non-additive effects prevail in the resistance to the fungus as well as low magnitudes of narrow-sense heritability (0.06). On the other hand, Rosado et al. (2010) studied the genetic parameters involved in the resistance to C. fimbriata in interspecific progenies of Eucalyptus

grandis and E. urophylla and found values of broad and narrow-sense heritability of 0.59 and 0.50, respectively. These authors showed that there is a strong additive genetic control involved in the resistance to the disease in Eucalyptus. The results of these studies showed that, overall, inheritance of resistance to Ceratocystis wilt is a quantitative trait, but the allelic interactions vary with the host species. Furthermore, the results found by Rosado et al. (2010) indicate that, for Eucalyptus, the use of families derived from crossings between different species allowed for an increase in the number of favorable alleles for resistance and greater values of heritability. Identification of new sources of resistance from other Mangifera species should be evaluated and introduced in future crossings to increase the genetic variability and support breeding programs for disease resistance and other traits.

The low heritability values between families, associated with discrete gains, did not allow for the selection of a family whose effects greatly contribute for disease resistance. Similar results were found by Brawner et al. (2015) and Roux et al. (2000) evaluating Acacia spp. for resistance to C. fimbriata (sin.C. acaciivora). In our studies, all families showed individuals highly susceptible indicating the existence of unfavorable alleles for resistance in the parents tested. Actually, all cultivars used in this study were susceptible, but they differ in disease intensity. These results lead us to conclude that the alleles controlling resistance to this disease are relatively rare in the population studied. This associated with allelic interactions of dominance and epistasis hindered the generation of a greater number of resistant individuals in these families. On the other hand, the highest heritability values between individuals within families indicated the feasibility of selection and genetic gains in the selection of more resistant individuals in the population as a whole.

The low frequency of alleles controlling resistance in the progenies studied and the small effect of families are probably related to the parentage among some of the genitors used (Schnell et al., 2006). Pedigree studies by microsatellite markers indicate that the cultivars Tommy Atkins, Palmer and Van Dyke are possibly derivate from "Haden" (Schnell et al., 2006), which is highly susceptible to the Ceratocystis wilt. Thus, the relative resistance found in "Tommy Atkins" "Palmer" and "Van Dyke" is probably originated from an unknown parent, crossed with "Haden". In addition, Coquinho and Espada, although not related with Florida cultivars, are very susceptible to the disease, which not favored the presence of favorable alleles for resistance in their progenies.

With the exception of the cultivar Palmer the lower susceptibility of "Keitt", "T. Atkins" and "Van Dyke" found in this study was also previously reported in others experiments (Ribeiro et al., 1984; Ribeiro et al., 1986; Rossetto et al., 1996; Galli et al., 2011; Araújo et al., 2014). Nevertheless, the high physiological variability in aggressiveness has been reported in the population of C. fimbriata from mango and other crops (Rossetto et al., 1996; Araújo et al., 2014 Nunes, 2015, Oliveira et al., 2015 c). Thus, the fungal isolate used in our study is highly aggressive to different mango cultivars and sporulates very well in culture medium (Oliveira et al., 2015a). However, extrapolating our results to other isolates is uncertain and it should be experimentally proved.

The greatest magnitudes of non-additive effects controlling resistance in M. indica to Ceratocystis wilt suggest that search for resistance should be performed in crossings between genetically divergent cultivars. The resistant genotypes should be multiplied clonally and their resistance validated against different isolates of the pathogen. However, the low frequency of alleles favorable for disease resistance in the materials studied and the low genetic diversity between the main mango cultivars grown in Brazil indicate the need of introduction of new genetic

materials to expand the genetic basis of this crop. Sources from other species or varieties of Mangifera may favor the development of new commercial varieties resistant do Ceratocystis wilt and suitable for the market.

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Figures and tables

Figure 1: Symptoms of Ceratocystis wilt on mango plants at 60 days after inoculation. A: Plant more resistant with small necrotic lesion (arrow) at inoculation point; B: Dead plant of a highly susceptible genotype; C: Bark lesions. The arrows indicate the point of grafting (upper arrow) and inoculation (lower arrow); D: Xylem discoloration.

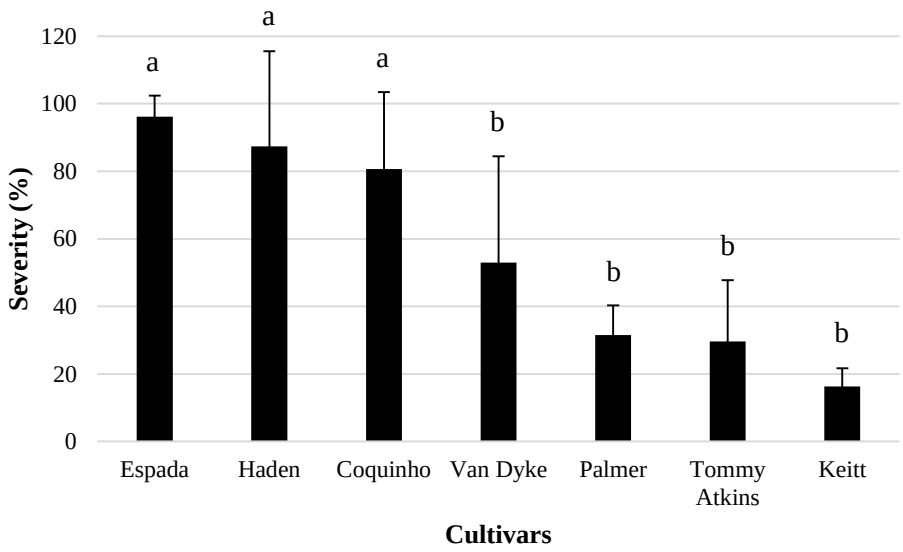

Figure 2: Severity on mango cultivars at 60 days after inoculation with Ceratocystis fimbriata. Cultivars followed by the same letters do not differ by Dunnett test ($p \leq 0.05$). Cultivar "Espada" was used as susceptible control.

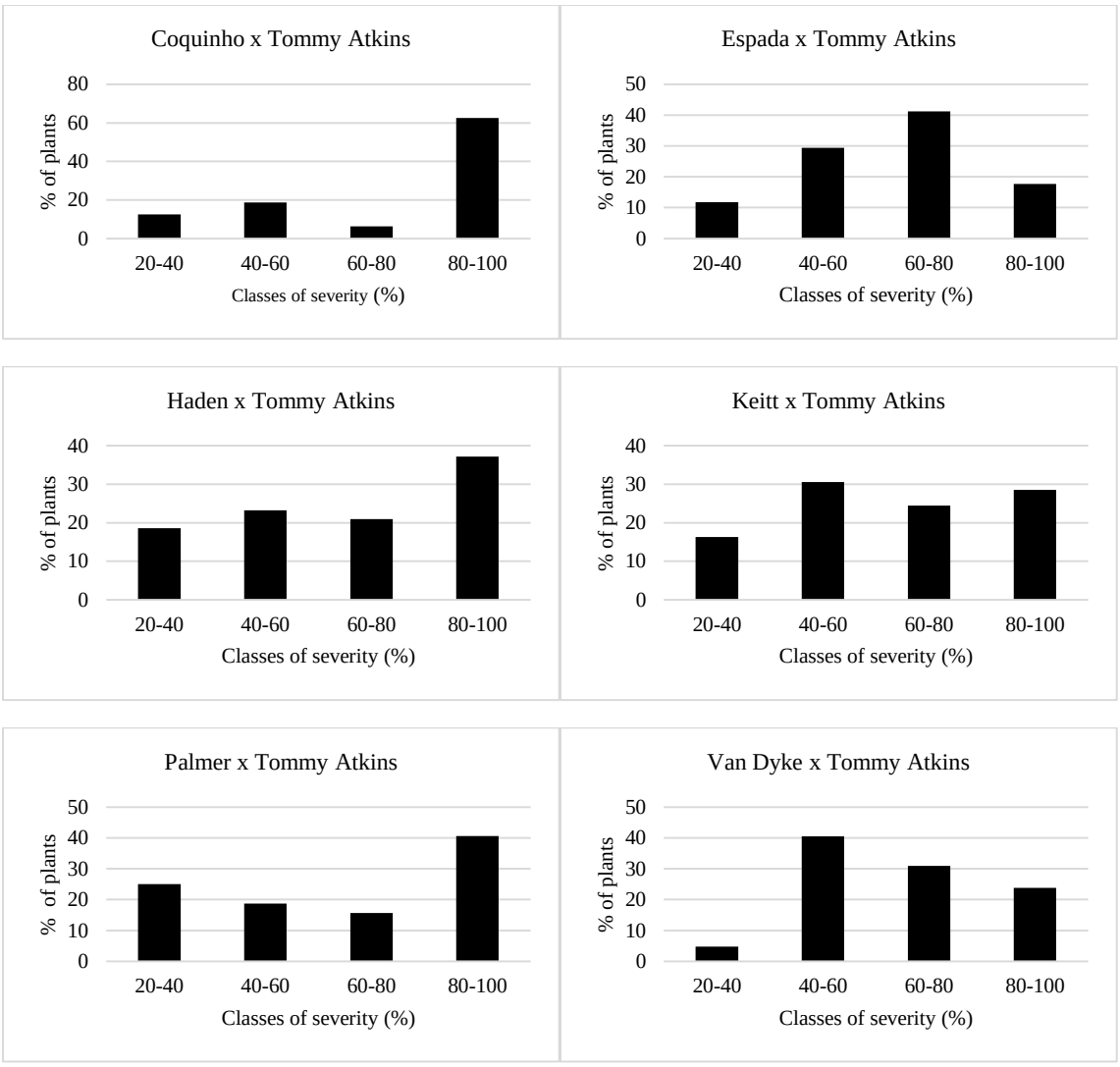

Figure 3: Frequency distribution based on classes of severity at 60 days after inoculation with Ceratocystis fimbriata for the six families of Mangifera indica evaluated.

Table 1: Crossing, number of individuals per progeny (crossing) and genotypic averages of severity for each studied family.

Crossing (♀ x ♂)	Number of evaluated plants by progeny	Genotypic average
Coquinho x Tommy Atkins	16	72.808
Espada x Tommy Atkins	13	60.411
Haden x Tommy Atkins	47	65.791
Keitt x Tommy Atkins	47	56.552
Palmer x Tommy Atkins	33	64.146
Van Dyke x Tommy Atkins	41	61.206
Overall average		63.486

Table 2: Analysis of Deviance

Effect	Deviance +	LTR
Family	7882.85	4.4*
Individual effects inside family	8036.81	164.36**
Full model	7878.45	

+: Deviance the fitted model without the corresponding effect.

* and **: Significant by chi-square test at 5% (3,84) and 1% (6,63) respectively.

Table 3: Estimates of genetic parameters for resistance to Ceratocystis wilt in Mangifera indica based on the lesion size percentage on the branch.

Genetic parameters	Values
Total genetic variance among full-sib families	44.83
Total genetic variance among individual inside families	401.14
Environmental variance	761.37
Individual phenotypic variance	1207.33
Heritability between full-sib families	0.037 ± 0.017
Heritability of individuals within family	0.33
Individual heritability in the broad sense	0.37
Coeficient of genotypic variation (CVg in %)	10.55
Coeficient of residual variation (CVe in %)	43.45
Relative coefficient of variation	0.25
Accuracy of family selection	0.70 a 0.79
Accuracy of potencial cultivars selection	0.71 a 0.91
Average	63.48

Table 4: Genetic gain for resistance to wilt Ceratocystis with the selection of the top 10 potential cultivars.

Family	Plant	Genotypic effect below average	Genotypic value	Accuracy	Genetic gain (%)
Keitt x Tommy Atkins	KT22	-38.8390	24.6467	0.81	-61.18
Keitt x Tommy Atkins	KT52	-38.0126	25.4731	0.81	-59.88
Keitt x Tommy Atkins	KT94	-35.5517	27.9340	0.86	-56.00
Palmer x Tommy Atkins	PT26A4	-34.9163	28.5695	0.82	-55.00
Palmer x Tommy Atkins	PT11A5	-33.9431	29.5427	0.88	-53.47
Keitt x Tommy Atkins	KT31	-32.7547	30.7310	0.77	-51.59
Keitt x Tommy Atkins	KET5A7	-30.8808	32.6050	0.86	-48.64
Van Dyke x Tommy Atkins	VT72	-30.1311	33.3546	0.77	-47.46
Van Dyke x Tommy Atkins	VT59	-30.0833	33.4025	0.86	-47.39
Keitt x Tommy Atkins	KET4A7	-29.0753	34.4104	0.87	-45.80

CONCLUSÕES GERAIS

Os resultados deste trabalho permitem concluir que:

1. Os marcadores microssatélites são eficientes na identificação de híbridos intervarietais em Mangifera indica;
2. A herança para a resistência à murcha-de-Ceratocyistis em Mangifera indica é poligênica com maior magnitude de efeitos de dominância e interações epistáticas;
3. Há baixa frequência de alelos favoráveis a resistência à doença nos materiais estudados;
4. Fontes de outras espécies ou variedades de Mangifera devem ser testadas a fim de favorecer o desenvolvimento de novas variedades comerciais resistentes à murcha-de-Ceratocystis e adequadas para o mercado.