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Programa de Pós-Graduação em Fisiologia Vegetal

Tese

CARACTERIZAÇÃO DA BIOLOGIA FLORAL, PERFIL DE ÁCIDOS GRAXOS DO ÓLEO E PRODUÇÃO DE MACADÂMIA

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Caracterização da biologia floral, perfil de ácidos graxos do óleo e produção de macadâmia

Tese apresentada ao Programa de Pós-Graduação em Fisiologia Vegetal da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Fisiologia Vegetal.

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Dedico ao meu marido,

Diego Jaekel Krolow.

"*I believe that everything happens for a reason. People change so that you can learn to let go, things go wrong so that you learn to appreciate them when they're right. You believe lies so you eventually learn to trust no one but yourself...and sometimes good things fall apart so better things can fall together.*"

~ Marilyn Monroe ~

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Resumo

CORRÊA, Elisia Rodrigues. Caracterização da biologia floral, perfil de ácidos graxos do óleo e produção de macadâmia. 2014. 96f. Tese (Doutorado) – Programa de Pós Graduação em Fisiologia Vegetal. Universidade Federal de Pelotas.

Aliado a busca por plantas de macadâmia mais produtivas, o pesquisador possui o desafio constante de tentar associar outras características as quais possam agregar valor a noz e assessorar os mais variados aspectos da produção. Dentre estes se destaca o estudo do caractere polinização, considerando que a autopolinização beneficia a produção, uma vez que plantas com esta característica independem de vetores polinizadores, que estão em declínio populacional atualmente. Junto a essa característica é incessante a busca por um produto (noz) com maior valor nutricional à saúde humana, no caso da macadâmia, um óleo com boa taxa de gorduras monoinsaturadas. Frente a estes desafios o objetivo do trabalho foi de identificar genótipos com maior produção de nozes e que apresentassem alta taxa de autopolinização, além de estabelecer estratégias para melhor a caracterização do perfil dos óleos de macadâmia para a inserção do estudo da composição dos óleos no melhoramento de macadâmia. Para o experimento de autopolinização, racemos florais de cultivares e matérias nativos foram ensacados e acompanhados quanto ao desenvolvimento da noz. Outros racemos florais foram apenas identificados e acompanhados para posterior comparação da polinização aberta em relação a autopolinizada. Três genótipos oriundos do grupo da macadâmia nativa apresentaram autopolinização. Nos estudos com óleo, três ensaios foram conduzidos. O primeiro com o intuito de quantificar o tamanho da amostra de macadâmia para a melhor representatividade referente ao perfil do óleo. Foram colhidas nozes de quatro cultivares, duas plantas por cultivar e vinte nozes por planta. As nozes foram secas e o perfil do óleo extraído determinado por cromatografia gasosa. A variância encontrada entre as nozes provenientes da mesma planta foi maior do que entre os genótipos, conduzindo assim ao objetivo do segundo ensaio. Com o intuito de identificar se o estádio de desenvolvimento da noz interfere no perfil do óleo, vinte nozes de quatro plantas da cultivar A16 foram colhidas e avaliadas. No entanto, como a causa potencial do alto nível de variabilidade é desconhecido ou não pode ser explorada, acredita-se que única outra opção é trabalhar com um grande número de nozes. O terceiro ensaio desenvolvido dentro do âmbito do perfil do óleo foi o da influência da fonte do pólen. Nozes da seleção 11.1 oriundas de três fontes de pólen (polinização aberta, autopolinização, e cruzamento com o genótipo 268) foram colhidas. Foi verificado que a fonte do pólen influencia no perfil do óleo. O objetivo do último experimento foi identificar os melhores genótipos em uma população de plantas de macadâmia, com relação à produção anual de amêndoas, visando estabelecer uma população base para

programa de melhoramento e aprimorar a propagação vegetativa no sistema de produção. Os resultados obtidos pela metodologia de modelos mistos (REML/BLUP) e a correlação entre genótipo e fenótipo demonstram a importância do uso de preditores BLUP como ferramenta para programas de melhoramento relacionados a este estudo de caso.

Palavras chave: Densidade floral, pólen, ácidos graxos, análise multivariada, *Macadamia intergrifolia, Macadamia tetraphylla.*

Abstract

CORRÊA, Elisia Rodrigues. Characterization of floral biology, fatty acids oil profile and macadamia production.2014. 96f. Tese (Doutorado) – Programa de Pós Graduação em Fisiologia Vegetal. Universidade Federal de Pelotas.

Improving the yield is the primary aim for the macadamia breeding program, together with it the breeder has the constant challenge of trying to add others traits which will promote macadamia value. Among these stands out the study of pollination character, a factor that brings benefits to the production, since plants with this feature are independent of pollinating vectors, which are currently declining in population. Along with this feature is the search for a product (nut) with a nutritional value which will bring a beneficial to human health, for macadamia the goal is the development of oil with a good rate of monounsaturated fats. Faced with these challenges, the objective was to identify genotypes with greater production of nuts and to provide a high rate of self-pollination, as well to establish strategies to better characterize the profile of macadamia nut oils. For the self-fertility experiment, racemes from cultivated and wild macadamia tree were tagged and bagged for comparing self-pollination and open pollination. T1002.003, T108.002 and T1023.003 were the only wild genotypes which presents Final Nut Set from self-pollination. In the oil studies, three experiments were carried out. The first aiming to quantify the sample size of macadamia nuts for best characterizes the fatty acids oil profile. Macadamia nuts were harvested, dried, the oil were extracted and after analyzed with gas chromatography. The variance found among the nuts from the same tree is bigger than the variance among the genotypes conducting to the second experiment, aiming to understand the possible source of variability at macadamia oil profile and the better way for controlling it, twenty nuts from four tree of macadamia cultivar, A16, were harvested. However the variance among the nuts was not controlled too. A third experiment was developed for checking if it has a pollen influence at fatty acids profile in the macadamia oil. Nuts from the selection 11.1 from three source of pollen (open pollination, self-pollination and a cross with the genotype 268) were harvested. Overall, the result from the experiments shows that the pollen has an influence in the oil profile**.** The aim of the last experiment was to identify the best genotypes in a population of macadamia plants in relation to annual production of almonds, aimed establishing a base population for a breeding. The results obtained by mixed model methodology (REML / BLUP) and the correlation between genotype and phenotype demonstrate the importance of using BLUP predictors as a tool for breeding programs related to this case study.

Key words: Floral density, pollen, fatty acids, multivariate analyses, *Macadamia intergrifolia, Macadamia tetraphylla.*

Sumário

Introdução Geral

Atualmente, a busca incessante do consumidor por alimentos saudáveis faz com que os agricultores tenham que se adaptar a esta nova tendência, cultivando espécies que atendam a essa demanda do mercado, como verduras e frutas com alta concentração de flavonoides e carotenoides, substâncias que auxiliam na prevenção de certos tipos de doenças, tais como câncer, ou sementes com alto conteúdo de ácidos graxos monoinsaturados, aliados na prevenção de doenças cardiovasculares (Betoret et al., 2011).

A macadâmia é uma das tantas nozes as quais podem contribuir com o produtor no fornecimento de alimentos benéficos à saúde, pois apresenta altas concentrações de ácido e ácidos graxos, que auxiliam na redução do LDL (*Low Density Lipoproteins*). A noz macadâmia é originária da Austrália, considerada a rainha das nozes, com grande potencial de expansão tanto no mercado externo quanto no interno. A Austrália também é o maior produtor mundial, com seis milhões de árvores em uma área plantada de 17.000 ha e produção de aproximadamente 11.500 toneladas de nozes por ano (AMS, 2014). O Brasil ocupa a sétima posição no cenário mundial, sendo a estimativa de produção para 2014 equivalente a 5.500 toneladas (Bonato, 2014).

O crescimento da produção de noz macadâmia no Brasil foi de 15% em 2005, comparado com o ano anterior (ABM, 2005). A macadâmia é cultivada no Brasil por apenas 160 agricultores, sendo utilizada em várias indústrias, tais como as de chocolate e sorvete, porém é principalmente consumida *in natura*, como acompanhamento.

De acordo com a ABM, em 2005, 90% da produção nacional de nozes de macadâmia foram exportadas. Isso ocorre devido ao desconhecimento do produto

no mercado; contribuem também o sistema de distribuição, que ainda é precário, assim como o custo elevado do alimento, cujo quilo é cotado a R$ 120 (produto já processado e embalado).

A produção de nozes para países monitorados pela FAZ (Foreign Agriculture Service) elevou 6%, no presente ano devido ao aumento da produção nos Estados Unidos. A produção de macadâmia no Quênia e na África do Sul aumentou 23%, respectivamente, devido às melhorias nas condições de produção e ao aumento das áreas plantadas (World Horticultural Trade & U.S. Export Opportunities, 2008). Entretanto, a maior produção mundial está localizada na Austrália (40%), EUA (24%), África do Sul (15%), América do Sul e Central (12%) e outras (9%) (AUSTRALIA MACADAMIA SOCIETY, 2008).

Atualmente o principal objetivo do melhoramento de macadâmia é a produção, fortemente influenciada pela polinização, devido à redução de insetos polinizadores provocado pelo mau uso de inseticidas na agricultura. Por esse motivo existe hoje uma busca por genótipos que apresentem alta taxa de autopolinização para que a produtividade não dependa tanto da presença de polinizadores no pomar. Aliado à investigação do pesquisador por genótipos mais produtivos é necessário estar atento às exigências dos consumidores, sendo o desenvolvimento de nozes mais saudáveis uma delas.

Diante do presente exposto, o objetivo deste trabalho é estudar a biologia floral da macadâmia, o perfil do óleo da macadâmia, bem como a utilização de modelos mistos como estratégia para auxiliar na discriminação e seleção de genótipos mais produtivos.

Revisão Bibliográfica

Em 1828, Allan Cunnigham descobriu a macadâmia, quatro anos antes da primeira colonização europeia na Austrália. Essa espécie foi a *M. integrifolia,* encontrada ao sul de Brisbane, Queensland (AMS, 1988). O renomeado explorador australiano Leichhardt foi o primeiro a descrever a espécie botânica de macadâmia nativa, em 1843 (Storey, 1959), na região do Conondale Range, da espécie *M. ternifolia* (Gross e Weston, 1993).

A macadâmia pertence à família Proteaceae, cultivares de macadâmia são oriundas do cruzamento entre *Macadamia integrifolia, Macadamia tetraphylla* e seus híbridos. A expressão *macadâmia nativa* refere-se a todos as formas não cultivadas de qualquer ou de todas as espécies de macadâmia. A terminologia '*integrifolia'* denomina folhas inteiras (Nagao e Hirae, 1992); '*tetraphylla'* designa quatro folhas; '*ternifolia'* institui três folhas e '*jansenii'* originária do nome do seu identificador, R.C. Jansen (1941-) (McConachie, 2009).

Em 1857, Ferdinand von Mueller foi o primeiro a descrever a macadâmia, a partir de uma coleção feita por Walter Hill, obtida no Vale Pine River, norte de Brisbane (McConachie, 1980; Gross e Weston, 1993). Mueller dedicou o gênero macadâmia a John Macadam, secretário (depois presidente) do Instituto de Fisiologia de Vitória (McConachie, 1980). Até 1954, a espécie *M. tetraphylla* era considerada *Macadamia ternifolia* ou *M. integrifolia* (Taylor, 1980; Gross e Weston, 1993), devido a apresentarem espinhos na margem das folhas, sendo essa característica a principal fonte de diferenciação entre as espécies. Em 1956, Smith revisou a taxonomia da macadâmia, coletando material de vários locais e separou as espécies nas três reconhecidas até hoje (*M. integrifolia*, *M. tetraphylla*

e *M. ternifolia*). A quarta espécie adicionada ao gênero foi *Macadamia jansenii*, descoberta mais recentemente (Gross e Weston 1992).

Diferenças morfológicas entre as quarto espécies de macadâmia do Sul da Austrália foram descritas por Storey; Stanley e Ross; Gross, 1957, 1986, 1995 (Tabela 1).

Tabela 1 – Descrição morfológica de caracteres das quatro espécies de macadâmia

	M. integrifolia	*M. tetraphylla*	*M. ternifolia*	*M. jansenii*
Morfologia foliar				
Número de folhas	3	4	3	3
Dimensão da folha (comprimento x largura)	6,5-14 cm x 2-6,5 cm	7-30 cm x1,4-6 cm	9-12,5 cm x 2-3,5 cm	10-17,5 cm x 2,5-5 cm
Razão: comprimento - largura	baixa	Alta	Média-alta	Média
Forma	ovada	Oblonga	Estreitamente ovalada	oblanceolada
Margem	Poucos ou sem espinhos	Sempre e com muitos espinhos	Alguns espinhos	Sem espinhos
Comprimento do pecíolo	6-18 mm	0-4 mm	4-10 mm	2-14 mm
Cor	verde	Rosa a vermelho	Rosa a vermelho	Verde
Morfologia floral				
Cor da flor	Branco creme	Rosa	Rosa	Marrom creme
Morfologia do fruto				
Dimensão da noz (comprimento x largura)	2,5-3,1 cm x 2,4-3,0 cm	2,6-3 cm x 1,6-2,4 cm	1,6 cm x 1,2 cm	1,4-1,8 cm x 1,1-1,6 cm
Espessura da casca	6-10 mm	2-6 mm	1 mm	0,8-1,5 mm

As espécies cultivadas *M. intergrifolia* e *M. tetraphylla* são plantas de porte médio (atingindo altura de 6-18 m e 3-18 m respectivamente) as quais produzem nozes grandes e comestíveis (Janick e Paull, 2008).

No hemisfério sul, a iniciação floral ocorre no final do outono (maio) com a redução da temperatura e o encurtamento dos dias (Trochoulias et al., 1990). A floração ocorre normalmente do final do inverno ao início da primavera (Stanley e Ross, 1986).

As flores da nogueira macadâmia nascem em racemos pendentes de 10 a 30 cm (Figura 1). A floração ocorre normalmente do final do inverno ao início da primavera (Stanley e Ross, 1986), com plantas produzindo aproximadamente 2500 racemos florais com 100-300 flores protândricas (Trueman e Turnbull, 1994). Entretanto, o índice de pega de frutos por racemo é em torno de 0,3%. Estudos demonstram um mecanismo parcial de autoincompatibilidade em flores de cultivares de macadâmia (Sedgley et al., 1990). Por outro lado, pode ocorrer a autopolinização e os grãos de pólen germinam sobre o estigma, mas na maioria das vezes os tubos germinativos são inibidos na parte superior do estilete e não alcançam o ovário para fertilizar o óvulo. Melhor desempenho no crescimento do tubo tem sido obtido com polinização cruzada, com pólen de diferentes cultivares, e aumentos de produção têm sido relatados em pomares contendo mais de uma cultivar (Ito e Hamilton, 1980; Sedgley et al.,1990 apud Sacramento et al., 1999).

Figura 1 – Flores de macadâmia.

Em pomares da espécie *Intergrifolia* as maiores taxas de fertilização foram observadas quando a polinização cruzada foi comparada com autofecundação (Sedgley et al., 1990; Meyers, 1997). Estudos com a espécie *tetraphylla* demonstraram a mesma tendência, Vithanage et al., 2001 demonstrou que a taxa autopolinização é em média de 5-10% e de não mais que 20%.

A floração e polinização são cruciais para o sucesso da produção. O fluxo de pólen de macadâmia é afetado por diversos fatores, tais como: a produção de pólen, a época de floração, os vetores da polinização, o isolamento de indivíduos e populações.

Os principais dispersores do pólen de macadâmia são as abelhas e os insetos nativos assim como certas espécies de aves também participam do processo (Vithanage e Ironside, 1986). A distância à qual o pólen pode ser propagado não foi medida, no entanto, experimentos conduzidos em pomar comercial medindo o fluxo de pólen sugerem que a polinização pode ocorrer ao em um raio de centenas de metros (Vithanage et al., 2001).

O conhecimento da biologia floral da espécie, bem como do desenvolvimento das nozes, é indispensável para o adequado manejo das plantas e para identificar quais genótipos proporcionam melhor frutificação efetiva na polinização natural ou em cruzamentos controlados. Além disso, a caracterização fenológica das cultivares de macadâmia fornece subsídios importantes para aumento da produtividade e qualidade das nozes, visto que o problema associado à autocompatibildiade parcial pode ser atenuado em parte com o plantio intercalado de cultivares compatíveis sob o ponto de vista reprodutivo (Sacramento et. al., 1999). No Havaí, a antese ocorre de novembro a maio, com picos de floração entre janeiro e março, enquanto nas condições da Costa Rica a floração de macadâmia se estende de novembro a fevereiro e a colheita vai geralmente de maio a dezembro (Sacramento, et. al., 2002). No estado de São Paulo, de acordo com os técnicos da empresa Macadâmia Brasilis, a floração ocorre nos meses de junho a setembro e a produção de janeiro a junho (Sacramento e Pereira, 2003).

Estudos têm demonstrado que plantas nativas apresentam pouca floração (McConachie, 1980), embora o número de flores aumente significativamente quando a luz não é um fator limitante. O período e a duração da floração são determinados de acordo com o genótipo (Boyton e Hardner, 2002). No entanto, a quantidade de água disponível, assim como a incidência de luz solar pode provocar variação dentro de cada população de plantas nativas e até mesmo entre os racemos florais das mesmas árvores (Boyton e Hardner, 2002). Tem sido observado que cada espécie tem sua própria programação para florescer e esta se baseia na adaptação à latitude. Flores de *M. jansenii* florescem primeiro, normalmente no final do inverno seguido por *M. ternifolia*, *M. intergripholia* e *M.*

tetraphylla (informação verbal)[1]. Essa variação dentro de cada espécie permite a hibridação interespecífica, se as plantas estão próximas.

Apesar de originária de clima de floresta tropical, plantas de macadâmia se desenvolvem melhor em clima subtropical. Segundo Stepheson (1993), a iniciação floral pode ocorrer sob uma ampla gama de condições climáticas e as gemas florais permanecem dormentes por um período bastante variável (50-96 dias). A transição para o florescimento envolve um sistema complexo de fatores que interagem, incluindo carboidratos e hormônios vegetais, tais como giberilinas, citocininas e etileno (Taiz e Zeiger, 2009).

M. intergrifolia e M. tetraphylla foram descritas por McConachie (1980) crescendo em seu habitat natural, como sendo plantas perenes, de pequeno a médio porte, geralmente em forma compacta. As populações nativas de macadâmia estão dispersas ao longo de riachos, na orla de florestas tropicais na Austrália e em áreas parcialmente abertas, acima de 400 m de altitude é muito raro encontrar árvores da espécie (McConachie, 1980).

Essas populações nativas são encontradas apenas em associação com florestas tropicais, sendo sua sobrevivência fortemente influenciada pelo fogo, herbivoria e competição com outras floras. A dispersão da semente ocorre por meio de pequenos animais e pelo fluxo natural da água, enquanto a dispersão do pólen ocorre principalmente através de pequenos insetos. Acredita-se que os aborígines podem ter ajudado a distribuir sementes a grandes distâncias, devido às populações nativas de macadâmia aparecerem esparsamente distribuídas sobre uma estreita faixa costeira de vales e pequenos intervalos. A *Macadamia*

[1] Informação obtida por Jodi O'Neal do Department of Agriculture and Fishery and Florest (DAFF), na Austrália, em 2013.

tetraphylla está mais ao sul, *M. jansenii* mais distante ao norte, com *M. integrifolia* e *M. ternifolia* no centro, sendo que a *M. integrifolia* ocorre de norte a sul.

Em 1858 Walter Hill, do Jardim Botânico de Brisbane, fez o primeiro plantio de macadâmia e depois começou a disseminar sementes a seus colegas e no mundo (McConachie, 1980). Não demorou muito para que os produtores rurais da região se interessassem pela cultura e começassem a explorar e cultivar macadâmia (McConachie, 1980), em 1893 um informativo foi publicado pela New South Wales Agricultural Gazette (McConachie, 1980) e em 1932 a Sociedade Australiana de Macadâmia foi formada (Cheel e Morrison, 1935).

O primeiro passo na domesticação da macadâmia foi um pomar composto por plantas da espécie M. *intergrifolia* e *M. tetraphylla*, oriundas da população nativa (McConachie, 1980). O cultivo iniciou na década de 1860, com ambas as espécies sendo cultivadas por produtores da região (Bell, 1993). Acredita-se que quatro grandes regiões australianas deram origem às cultivares do mundo, são elas: Monte Bauple (*M. integrifolia*), Brisbane (*M. integrifolia*), Murwillumbah (*M. tetraphylla*) e Lismore (*M. tetraphylla*) (McConachie, 1994). A origem das cultivares híbridas não é conhecida, podem ter sido amostradas da zona de híbridos natural ou podem ser resultado da hibridação dirigida pelo homem.

Antes que qualquer indústria se desenvolvesse, a macadâmia foi disseminada pelo mundo, sendo Havaí e Califórnia os locais de início da comercialização (McConachie, 1980). Em 1948, o Queensland Department of Agriculture and Stock e o New South Wales Department of Agriculture selecionaram 64 genótipos de 1952 plantas (Ross e Wills, 1952).

No Havaí os pesquisadores da Hawaii Agricultural Experiment Station (HAES) deram início ao programa de melhoramento, registrando o desempenho

fenotípico do máximo de indivíduos possíveis em diferentes localidades, para identificar genótipos superiores (Wagner-Wright, 1995). Em 1948, foi lançada a primeira cultivar de macadâmia, pela HAES, tornando-se a principal cultivar plantada em meados de 1950 (Wagner-Wright, 1995).

Em 1930, os primeiros pés de macadâmia foram plantados na África, com sementes obtidas do Havaí e de viveiros australianos (Reim, 1991), porém somente em 1966 foi criada a Sociedade Sul-Africana de Macadâmia (SAMAC) (Lee, 2000).

A macadâmia cultivada compõe duas espécies, *M. intergrifolia* Maiden e Betche e *M. tetraphylla* LAS Johnson e seus híbridos (Nagao, 1992). *M. ternifolia* F. Muell. e *M. jansenii* C.L. Gross e P.H. Weston são duas espécies estreitamente relacionadas às anteriores, porém não são cultivadas devido à presença de glicosídeos cianogênicos (Dahler et al., 1995). Estas são substâncias tóxicas que proporcionam gosto amargo às nozes, quando ingeridas podem causar lesão no sistema nervoso e tireoide (Amorim, et al., 2006).

As primeiras experiências com o plantio de macâdamia no Brasil surgiram em 1935, mas o aquecimento do setor ocorreu na década de 90. De acordo com os produtores, a longevidade das árvores e o baixo investimento inicial que é de R$ 8 mil reais por hectare para implantação da cultura mais R$ 1,6 mil reais de manutenção anual são os atrativos do negócio. O ponto negativo é o início de retorno do capital, que acontece a partir do oitavo ano de cultivo (ABM, 2005).

No Brasil, o melhoramento genético teve início no Instituto Agronômico de Campinas, na década de 70, com a introdução de sementes procedentes do Havaí (EUA). Durante os anos seguintes, foram desenvolvidas 16 variedades.

O número de cromossomos básico da macadâmia é n=14, sendo esta uma espécie diploide (2n=28) (Stace et al., 1998). Existem muitas características de interesse para o melhoramento genético em macadâmia, tais como estrutura da planta, produção, resistência a doenças e qualidade da noz (Hardner et al., 2009). Desde que o Programa de Melhoramento Genético de Macadâmia australiano iniciou, seu objetivo principal é o aumento de rendimento de macadâmia. Como o melhorista precisa estar sempre com os olhos no futuro e como as exigências dos consumidores foram mudando ao longo do tempo, é necessário saber mais informações sobre o perfil do óleo de macadâmia, uma vez que é o maior componente de macadâmia, 59.2g/100g (Maguire et al., 2004). A macadâmia é rica em óleo (80%) sendo grande parte deste constituído por gorduras monoinsaturadas (Curb et al., 2000). No Brasil, há uma pequena produção de óleo de noz macadâmia. Para essa finalidade, as nozes não precisam ter boa aparência, no entanto, não podem ter depreciações, como mofo, ranço e umidade, sendo utilizado para fins farmacêuticos e alimentares. As nozes que se quebram durante o beneficiamento não podem ser exportadas, devido à exigência de um padrão de qualidade.

O produto comercial da macadâmia são seus frutos, os quais são arredondados, verdes, de 2,5 a 3,5 cm de largura com uma casca marrom resistente, a qual protege a noz.

O óleo de macadâmia é rico em ácidos graxos monoinsaturados, sendo encontrado em maior quantidade o ácido oleico, que auxilia na redução dos níveis de triglicerídeos e de colesterol, diminuindo, dessa forma, os riscos de doenças cardiovasculares (Borompichaichartkul et al., 2009).

Os ácidos graxos monoinsaturados presentes em óleo de azeitona e canola têm demonstrado serem neutralizadores ou até mesmo agentes redutores do colesterol. Eles reduzem também a demanda por antioxidantes, sendo relacionados à atenuação do estresse oxidativo. A macadâmia contém aproximadamente 75g de gordura/100g de noz e contém os maiores níveis de MUFA já encontrado em qualquer outra fonte de alimento (>60g/100g noz inteira). Uma dieta rica em alimentos contendo altos níveis de MUFA reduz os níveis de colesterol LDL (Garg et. al., 2003).

Wall (2010) destacou a presença de tocoferol, tocotrienol e esqualeno em macadâmia, constituintes bioativos que possuem propriedades antioxidantes. Tanto os tocoferóis quanto os tocotrienóis são poderosos antioxidantes, evitando ou reduzindo a oxidação lipídica durante o armazenamento. O ranço oxidativo confere mau cheiro e aroma as nozes comprometendo assim a qualidade nutricional das mesmas. Portanto, a identificação de cultivares que apresentam uma melhor estabilidade oxidativa é a chave para programas de melhoramento.

O perfil do óleo nas plantas é determinado primeiramente pela tioesterase, enzimas condensadas e dessaturases. A manipulação das enzimas tioesterase edessaturases em plantas transgênicas tem sido muito bem sucedida com o intuito de produzir modificações no comprimento da cadeia e/ou no grau de instauração dos ácidos graxos presentes nos óleos de sementes (Ohlrogge, 1995).

A biossíntese via plastidial dos ácidos graxos consiste de dois sistemas enzimáticos: acetil-CoA carboxilase (ACCase) e a sintase de ácidos graxos (FAS). ACCase catalisa a formação de malonil-CoA a partir de acetil-CoA, FAS transfere o radical malonil para a proteína transportadora de acil (ACP) e catalisa a

extensão da crescente cadeia acil com malonil-ACP. Os produtos finais da FAS são geralmente C16:0 - C18:0, e a composição final de ácidos graxos de uma célula vegetal é em grande parte determinada pelas atividades de várias enzimas que utilizam estes acil-ACPs na fase de terminação da síntese dos ácido graxo. Dessaturase de estearoil-ACP modifica o produto final da FAS, por inserção de uma ligação dupla cis na posição 9 do C18:0. Reações de síntese de ácidos graxos são terminadas por hidrólise ou transferência da cadeia acil do ACP (Ohlrogge e Jaworski, 1997).

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MACADAMIA FATTY ACIDS OIL PROFILE: WHAT ARE THE SOURCES OF VARIATION?

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ABSTRACT: Here we report the fatty acids presented in macadamia oil, with particular emphasis on the sources which could influence the macadamia oil profile. The findings of this study will be used to determine optimal allocation of sampling effort for an experiment comparing oil content/quality of different macadamia varieties. These data will allow selecting macadamia genotypes with a good ratio of monounsaturated and polyunsaturated fatty acids. Three experiments were carried aiming to understand the possible source of variability at macadamia oil profile and the better way for controlling it. Macadamia nuts were harvest, dried, the oil were extracted and after analyzed with gas chromatography. Overall, the result from the experiments shows that the pollen has an influence in the oil profile.

KEYWORDS: saturated, monounsaturated, polyunsaturated, pollen, gas chromatography

1. INTRODUCTION

Currently the development of functional foods has a great interest from consumers and governments, because food is an approach to achieve innovative products that satisfy the taste, appearance, price and healthier, which are factors for the commercial success of a food products [1]. Today's challenging is to fulfill the consumer's expectancy for products that are relish and healthy simultaneously, so that all kind of business needs to continue innovating for survival to the world complains [2].

Breeders need to be always watchful in the future because the consumers and industry requirements has been changing over the time, increasing the demand for new products. In the case of macadamia, the characterization of content and oil profile in the nuts are important characters to be consider for the breeders, because oils is the best and largest component of macadamia nut, 59.2g/100g [3]. It is well known that a diet with macadamia had positive effects on cholesterol and low-density lipoprotein cholesterol levels when compared with a typical American diet [4]. Lipid/lipoprotein reduction and cardio vascular disease risk factor was reduced adding macadamia nuts in a heart-healthy dietary pattern [5].

The lipids define a set of chemistry substance that are not characterized for showing a common functional group but their high solubility in organic solvents and low solubility in water [10]. The lipids presents in plants are classified into two classes based on their physiological role, functional lipids and storage lipids. Functional lipids participate in vital and metabolic processes; storage lipids (main form of storage as triacylglycerol) serve as a source of energy for growth during seeds germination, deposited as oil bodies in fruit or seeds [11](NELSON, 2008). Most of the lipids have fatty acids in the structure (long chain with more than 12

carbons). Macadamia nuts are a rich source of Monounsaturated Fatty Acids (MUFA) and contain a high percentage of palmitoleic acid. Macadamia oil profile is characterized by the presence of: palmitic acid – C16:0 (2.13 - 32.31%), palmitoleic acid – C16:1w7 (0 – 29.64%), estaric acid – C18:0 (0 – 7.13%), oleic acid – C18:1w9 (45.09 - 84.35%), linoleic acid – C18:2w6 (0 – 13.19%) and eurucic acid – C22:1w9 (0 – 10.32%) [12].[13]Anon (2000) highlithed the species (*M. intergrifolia* and *M. tetraphylla*) as the primary source of macadamia oil content, as well as location, cultivar, horticultural practices, maturity and climate.

Palmitoleate acid can contribute to the pathogenesis of insulin resistance and type-2 diabetes [6], macadamia is the richest known source of palmitoleate, and it has been advise the consume of macadamia nuts to diabetic people [7]. The oil profile is different according with the cultivar, the A16 cultivar contained significantly higher levels of the major saturated fatty acids, palmitic (C16:0), stearic (C18:0) and arachidic (C20:0) as well as the minor saturated fatty acids behenic (C22:0) and lignoceric (C24:0) than cultivar HAES 344 kernels [8].

Changes of fatty acid compositions or oil content determine the quality of macadamia nut. Generally, higher ratio between unsaturated to saturated fatty acids is less susceptible to oxidation due to increased oxidation potential of unsaturated fatty acids [9]. However, the higher ratio more nutritionally beneficial, as this indicates a higher proportion of unsaturated fatty acids which have been shown to reduce the risk of some cancers and heart disease.

The fatty acids presented in macadamia oil, with particular emphasis on the sources which could influence the macadamia oil profile was reported in the present study. The findings of this study will be used to determine optimal allocation of sampling effort for an experiment comparing oil content/quality of

different macadamia varieties. These data will allow selecting macadamia genotypes with a good ration of monounsaturated and polyunsaturated fatty acids.

2. MATERIALS AND METHODS

Macadamia nuts grow in Queensland, Australia, has been harvested in 2012 and 2013. The nuts were dried immediately after harvest to a moisture content of 2-6%, and then used for oil extraction and fatty acid characterization. A general oil extraction and gas chromatography analyses used in the experiments will be described below.

2.1*Oil extraction*

It has been used a hydraulic pressure oil extraction thus been possible to obtain crude oil without chemical contaminants. Samples composed by 20 kernels were squashed at 2,000 Psi (pound force per square inch) and the oil collected in a tube. The oil was storage at near by 10°C until the gas chromatography analysis.

2.2*Gas chromatography (GC) analysis*

The fatty acid composition of the selected samples has been analyzed by gas chromatography. They were prepared following the procedure ISO 5509:20000, and analyzed Varian in 3800 GC system using a Varian auto sampler CP-8400 equipped with a J&W capillary column (DB-23; 30m in length; 0.25 μm ID) with flame ionization detection. The oven, detector, and injector temperature were 170°C, 250°C and 240°C, respectively. The carrier gas was nitrogen, at a pressure of 500 kpa, column pressure 15 psi. FID- Air (instrument grade) 500KPa; Hydrogen (high purity) 300KPa. One microliter of each sample was injected in the GC, and individual fatty acids were expressed as % of total fatty acids.

Experiment 1 *– Study of sources of oil profile variability among cultivars, trees within cultivars, nuts and half nuts within trees within cultivars*

With the aim of studying the oil profile variation among the kernels, four genotypes of macadamia nuts (A4, A16, HAES849 and HAES 791) have been harvested randomly on October of 2012. Twenty nuts, from two trees of each genotype, were harvested randomized, dried, and the oil extracted to be analyzed using GC, which methods was described before.

Analysis of variance was performed for estimating the variability among the genotypes, trees and nuts in each tree, for all the fatty acids previously describe in the introduction of the present study. The repetibility coefficients were estimated by the Principal Components based on the Covariances (PCC) and Correlation Matrix (PCCM). It was estimated the number of assessment needed for predicting the coefficient of determination (R^2). The analyses were conducted using the GENES software (Cruz, 1998).

Experiment 2 *- Pilot study of macadamia oil content assessment*

This pilot study has been designed to explore the sources of variation in oil content of macadamia kernels observed in the first experiment. Measurements of oil content were undertaken using GS, as described in the experiment 1.

Four trees of the cultivar A16 were selected in a commercial orchard, and 10 kernels were harvested from each tree. Of the ten kernels taken for each tree, five were chosen at random, and two oil samples were taken per each kernel. Only one oil sample was taken for the other five kernels from each tree. However, for these five kernels, two aliquots were mixed from the single oil sample providing two samples for the gas chromatography process. These two levels of partial

duplication, one at the oil extraction stage, and another at the aliquot formation stage, resulted in a total of 80 samples (from 40 kernels) to be assessed using GC analysis. The GC machine processes batches of up to 36 samples over a 24 hour period, in sequence from 1 to 26. Hence the randomization was restricted to duplicates of 40 samples per block to guard against any potential trends occurring over this time frame. A total of eight oil measurements were made on these samples.

The experimental design determines the baseline model to be used for analysis. The sampling strategy was structured across two factors, of Tree and Kernel. In addition, two samples of oil were taken from some kernels, and then two aliquots were made for some oil samples. This forms the complete hierarchy of Tree/Kernel/Oil/Aliquot. A linear mixed model was fitted including a random term for each factor in the hierarchy given above. In addition, a random blocking factor with 2 levels was fitted, as the randomization of the partially replicated design split the 80 samples into two groups of 40 samples each. An autoregressive term for order of processing was also included in the model, due to the sequential nature of the GS process. Estimates of variance parameters were obtained using residual maximum likelihood (REML) (Patterson and Thompson 1977). The program ASReml-R (Butler et al., 1999) was used to fit the linear model and estimate variance parameters.

Experiment 3 *- Evaluation of possible pollen influence in macadamia-nut oil profile*

The vegetal material used in this experiment was the selection 11.1 localized at Astonville, which was possible to have different source of pollen from the same mother plant. During the bloom time 20 raceme were bagged at the “looping stage” (when the style pushes out from the perianth to create a visible loop) for self-pollination, after one week the bag is took off for allowing the developing of the nuts. When is possible to see the nut lets the racemes are bagged for not lose the nuts falling on the ground. For characterize nuts from open pollination 20 raceme were tagged at the looping stage and the raceme were bagged when presented nut lets. As macadamia florets are protandrous it was bagged 20 raceme, the paper bags were left on the tree about three weeks until flowering is finished. It was removed the bags when the florets were receptive and applied pollen from the selection 268. Then re-bag and label with parent names and date. For not lose the nut falling on the ground the raceme are bagged again when the nut lets are developing.

After three months the nuts were harvest, 44 nuts from cross pollination, 39 nuts from opened pollination and eight nuts from self-pollination. The procedures for drying the nuts and extracting the oil were the same as previous described as either the use of gas chromatography for fatty acid analyses.

3. RESULTS/DISCUSSION

Experiment 1 *– Study of sources of oil profile variability among cultivars, trees within cultivars, nuts and half nuts within trees within cultivars*

The fatty acids esterified to triacilglicerol were divided in two groups: (18:3, 22:1, and 18:1-HO) needs post-plastidial modification, while the other (10:0, 12:0,

and 18:1) does not[14]. Macadamia oil is rich in unsaturated fatty acids as Palmitoleic acid (16:1), Oleic acid (18:1) and Erucic acid (22:1).

MUFAs were the major group of fatty acids present in macadamia oils, ranging from 79.45% to 88.68%, followed by SFAs which ranged from 15.43% to 18.29% and PUFA which were the least abundant group (1.44 - 2.62) (Table 1); these low percentage of PUFA confirm the results reported for macadamia oil by [15] Kaijser (2000). In date seed, oleicacid were the most significant fatty acid (47.47%) after myristic acid (14.52%), palmitic acid (12.41%) and linoleic acid (10.23%) [16].

It was identified eight fatty acids in macadamia oil in the present study, being four saturated (myristic acid, palmitic acid, stearic acid, behenic acid), three unsaturated (palmitoleic acid, oleic acid, erucic acid) and one polyunsaturated (linoleic acid) whose entire contents within each class was 15%, 84% and 1% respectively. The oleic acid and palmitoleic acid were predominantly in the macadamia oil with 52.57% and 27.41% respectively, (table 1) being very close to the average values reported by Ebrahen et al., (1994)[17]. However, analyzing the fatty acids of macadamia oil, [18] registered 9.7%palmitic acid, 24.1%palmitoleic acid, 3.3%stearic acid, 58.2%oleic acid,3.7% linoleic acid, 0.9% arachidonic acid (not found in the present study here reported), which percentage are different in relationship to the present study.

Linoleic acid stimulates LDL receptor-mediated LDL removal in animal experiments (Fernandez et al., 1997; Fuentes, 1998) [33, 34]. The only countries which recommend the consume of nuts with a rate of linoleic acid and linolenic acid are Canada and USA, establishing with a proportion of 4:1 and 10:1, respectively [19].

In the present study the content of linoleic acid was low (1.44 - 2.62) and lower compared to the average established for macadamia. Low concentrations of polyunsaturated fatty acids could explain why the level of peroxides in the oil is quite low [15, 20] once low levels of polyunsaturated fatty acids will reduce rancidity, increasing the shelf life.

Table 1 – Fatty acid composition (%) of macadamia oil samples

Fatty acid	A4		A16		HAES 849		HAES 791	
	Tree 1	Tree 2	Tree 1	Tree 2	Tree 1	Tree 2	Tree 1	Tree 2
Myristic acid (C14:0)	1.08	2.55	1.62	1.88	1.94	1.70	1.66	1.60
Palmitic acid (C16:0)	11.26	12.43	12.37	12.01	12.46	9.83	10.28	10.71
Stearic acid (C18:0)	2.38	2.48	2.77	2.35	2.97	3.02	2.25	2.19
Behenic acid (C22:0)	1.26	0.84	1.22	1.06	1.40	3.63	1.26	1.10
Oleic acid(C18:1)	55.05	49.72	52.80	48.96	50.45	62.72	53.68	49.20
Palmitoleic acid (C16:1)	27.24	29.35	25.55	30.59	27.14	24.49	27.62	31.66
Erucic acid (C22:1)	1.08	0.87	1.11	1.09	1.15	1.47	1.50	1.22
Linoleic acid (C18:2)	1.78	2.08	2.62	2.09	1.60	1.44	1.76	2.43
Σ SFA	15.98	18.29	17.98	17.30	18.77	18.19	15.43	15.60
Σ MUFA	83.37	79.95	79.45	80.63	78.73	88.68	82.80	82.08
Σ PUFA	1.78	2.08	2.62	2.09	1.60	1.44	1.76	2.43

Σ – Sum

It has been desirable a low percentage of saturated fatty acids and a high percentage of unsaturated and polyunsaturated fatty acids once it reduces 70% the mortality in cardiovascular patients, reduces inflammation caused by rheumatoid arthristis and the reduction of asthma symptoms [21] . The four cultivars showed high concentration of monounsaturated fatty acids and low of saturated, trait aimed by the breeders.

In the table 2, we can analyze how large is the variations between kernels within a tree for all fatty acids tested (look at the relative sizes of variance components for the three levels: cultivar (Genotype), Tree within cultivar (Genotype:Tree), and Kernel within tree (the residual (R) which is Genotype:Tree:Kernel). This may be due to large actual differences between kernels or large variation in the measurement process. As the nut has been harvested from the ground and as macadamia nuts starts falling on March, nuts from the present study have been harvested on October thus the nuts maturity could be different for each nut used in this experiment.

A variation of both percentage for linoleic and palmitic acids among different samples or effect of the plant and nut was checked out, what can be related to ontogeny maturation of the nuts. This variation was detected in embryos quiescentof *Dalbergia miscolobium* with 20 to 50 mm of rootlet showed an increase in palmitic acid and decrease of linoleic acid [22]. Kernel oil content and maturity are directly proportional, the increase of oil content indicate a greater level of maturity [23], it could be a reason for the high variability found on macadamia oil profile.

There was also large variation among trees (Genotype:Tree) within a cultivar for most acids. Following macadamia nut developing of five trees it was found two with different total oil per embryo and the author nominated this variance due to the germination of the embryo [23]. There was little variation between cultivars (Genotype). This oil variation between kernel within the tree will mask any cultivar differences, consequently, it will be very difficult to show any difference between cultivars. As the study of macadamia oil profile is for contribute to the macadamia breeding program is necessary to control the variance found among the nuts within the tree otherwise it will not possible to select a genotype among the others (Table 1).

Table 2 - Attribution Analysis of Gamma for fatty acids in macadamia oil

Fatty acids		Genotype source	Tree|Genotype	Residual
Myristic acid C14:0	Gamma	1.01×10^{-7}	1.36×10^{-1}	1
	Component	8.35×10^{-8}	1.13×10^{-1}	8.25×10^{-1}
	Std.error	1.02×10^{-8}	8.69×10^{-2}	1.01×10^{-1}
Palmitic acid C16:0	Gamma	1.66×10^{-2}	1.85×10^{-1}	1
	Component	7.23×10^{-2}	8.09×10^{-1}	4.35
	Std.error	6.07×10^{-1}	7.4×10^{-1}	5.15×10^{-1}
Palmitoleic acid C16:1w7	Gamma	1.01x10-7	9.45×10^{-2}	1
	Component	4.73×10^{-6}	4.42×10^{-2}	$4.68 \times 10^{+1}$
	Std.error	5.56×10^{-7}	$3.70 \times 10^{+00}$	$5.49 \times 10^{+00}$
Stearic acid C18:0	Gamma	1.07×10^{-1}	1.01×10^{-7}	1
	Component	8.42×10^{-2}	7.95×10^{-8}	7.85×10^{-1}
	Std.error	8.63×10^{-2}	9.37×10^{-9}	9.25×10^{-2}
Oleic acid C18:1w9	Gamma	5.71×10^{-7}	2.34×10^{-1}	1
	Component	4.57×10^{-5}	$1.87 \times 10^{+1}$	$8.00 \times 10^{+1}$
	Std.error	5.32×10^{-6}	$1.22 \times 10^{+1}$	$9.30 \times 10^{+00}$
Linoleic acid C18:2w6	Gamma	0.13	0.10	1
	Component	0.08	0.06	0.67
	Std.error	0.12	0.07	0.08
Behenic acid C22:0	Gamma	2.73×10^{-2}	1.01×10^{-7}	1
	Component	2.21×10^{-2}	8.19×10^{-8}	8.09×10^{-1}
	Std.error	3.71×10^{-2}	9.90×10^{-9}	9.78×10^{-2}
Erucic acid C22:1w9	Gamma	0.040	0.00	1
	Component	0.02	0.00	0.51
	Std.error	0.03	0.02	0.06

Evaluation of agronomic, phisyogical and biochemical traits in fruit breeding programs, like for Macadamia, is a tedious process because of the long juvenile period of trees, the reproductive system, and the existence of climatic factors affecting the expression of the traits. For these reasons, one of the factors for the success in Macadamia breeding program is to know the minimum number of replicates (nuts) of each genetic material to conduced evaluation of oil content and profile. Common greens [24], açai [25] and oil palm [26] have been using repeatability

coefficient estimation for the study of characters, which is helping to define the number of genotypes for efficiency improvement programs.

Considering that the aim of this study was selecting interesting genotypes, the estimation based on oil profile evaluation indicated the necessity to work with at least at least 5 to 19 nuts (PCC- Principal Components based on Covariance) or 15 to 26 nuts (PCCM - Principal Components based Correlations Matrix) (Table 3). It has been found an agreement in the repeatability coefficients magnitude of each traits acquired by the different methods, grating more accuracy (Table 1), however, PCR is not a good method for estimates the coefficient of repeatability for macadamia nut oil profile because the values were low (~ 0.3). It is considered difficult for the breeder identify the best genotypes when the estimates of the coefficient of repeatability is lower than 0.4 [27], proving dissimilarity in the traits replications among one gas chromatography injection and other [28].

Table 3 - Estimates of the coefficient of repeatability ($\hat{r}$), coefficient of determination (R^2) and number of measurements (η) calculated for the fatty acids obtained by Principal Components based on Covariance (PCC) and Correlations Matrix (PCCM) in macadamia

FATTY ACIDS		METHOD	
	R^2 (%)	PCC	PCCM
$\hat{r}$		0.256	0.325
	η para R^2 = 0.8	8 (8.28)	12 (11.61)
Myristic acid	η para R^2 = 0.9	19 (18.64)	26 (26.13)
	η para R^2 = 0.99	205 (204.99)	287 (287.45)
$\hat{r}$		0.372	0.644
	η para R^2 = 0.8	2 (2.21)	7 (6.75)
Palmitic acid	η para R^2 = 0.9	5 (4.97)	15 (15.18)
	η para R^2 = 0.99	55 (54.62)	167 (167.00)
$\hat{r}$		0.281	0.479
	η para R^2 = 0.8	4 (4.35)	10 (10.21)
Palmitoleic acid	η para R^2 = 0.9	10 (9.8)	23 (22.96)
	η para R^2 = 0.99	108 (107.69)	253 (252.58)
$\hat{r}$		0.365	0.549
	η para R^2 = 0.8	3 (3.28)	7 (6.97)
Stearic acid	η para R^2 = 0.9	7 (7.39)	16 (15.68)
	η para R^2 = 0.99	81 (81.26)	172 (172.42)
$\hat{r}$		0.294	0.512
	η para R^2 = 0.8	4 (3.80)	10 (9.63)
Oleic acid	η para R^2 = 0.9	9 (8.55)	22 (21.67)
	η para R^2 = 0.99	94 (94.01)	238 (238.35)
$\hat{r}$		0.289	0.48
	η para R^2 = 0.8	4 (4.34)	10 (9.86)
Linoleic acid	η para R^2 = 0.9	10 (9.77)	22 (22.20)
	η para R^2 = 0.99	107 (107.39)	244 (244.14)
r		0.324	0.595
	η para R^2 = 0.8	3 (2.72)	8 (8.34)
Behenic acid	η para R^2 = 0.9	6 (6.13)	19 (18.77)
	η para R^2 = 0.99	67 (67.41)	206 (206.39)
r		0.34	037
	η para R^2 = 0.8	7 (6.73)	8 (7.76)
Erucic acid	η para R^2 = 0.9	15 (15.15)	17 (17.45)
	η para R^2 = 0.99	167 (166.66)	192 (191.992)

(*) Near number (number calculated)

Considering the macadamia kernel oil content increase with maturity-nut post-harvest, which is followed by oil profile changes [23], this condition can be the factor of variance identified in the experiment. This findings indicates the necessity of others experiment for controlling the variance found among the nuts from the same tree within the same genotype. Thus, this hypothesis can only be proven by monitoring maturation, from flowering to harvest fruits in the same equal and different stages, once the variance within the tree is higher among the genotypes it is not possible to conduct experiments aiming to find the best genotype according with the oil profile.

Experiment 2 *– Pilot study of macadamia oil profile assessment*

According to experiment 1, the greatest source of variation was due to kernels, ranging from 60-90% of the total variance for all acids measured. Variability between duplicate oil samples formed a smaller proportion of overall variance, relative to kernels. This was greatest for Myristic, Palmitic, Oleic and Linoleic Acids and was of the order of 10-15%. Furthermore, there was evidence of variability between repeat aliquot measures, particularly for Behenic and Erucic acids (15-30% of total variation, respectively). The variance components for each term in the sampling hierarchy are shown for the eight oil measurements, as a percentage of total variance, and the residual variance component is formed from variation between duplicate aliquot measures (Table 4).

Table 4: Percentage of variance for the factors Tree and Kernel and the duplicate measurements of oil and aliquots for Gas Chromatography of 8 Acid measurements

	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Behenic	Erucic
Tree	0	8	18	10	12	0	15	0
Kernel	83	75	69	88	66	88	61	69
Oil	15	12	10	2	15	9	0	0
Residual	2	6	4	1	6	4	24	31

The advantage of including the duplicate oil and aliquot samples is that they enable identification of suspected erroneous measurements. The residual plots for two of the acids (Figure 1) show the clear separation of outlying data values, and these could not be detected without the duplicate samples. Outliers were evident for most acid measurements, and often the same two sets of duplicate samples showed up as consistent outliers.

The samples were for Tree 3 Kernel 3, (3-3), and Tree 3 Kernel 9, (3-9). For kernel 3-3, duplicate oil samples were taken, and for kernel 3-9 duplicate aliquot samples were taken. Due to the consistent detection of outlying values for four kernels across all measured traits, these values were removed from the data and the variance components were recalculated (Table 4). The greatest change in percentage variance explained on the exclusion of these data points is in the oil variance component for Palmitoleic and Oleic acids, and in the residual (aliquot) variance component for Behenic acid. However, the same overall trends and relativities between variance components are evident for the analysis with these data points included and excluded.

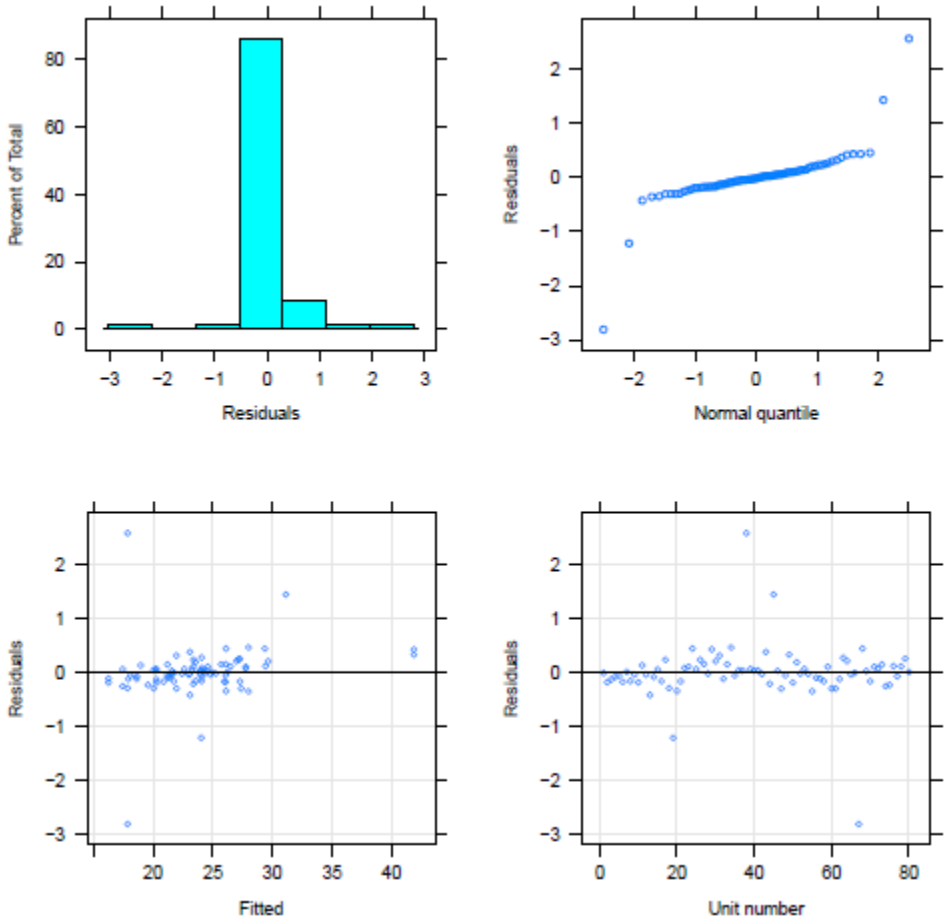

Figure 1: Plots of residuals from the fitted statistical model for Palmitoleic acid – trait C16:1W7. This plot shows the high/low residual pairs for sets of duplicate mesurements on a kernel.

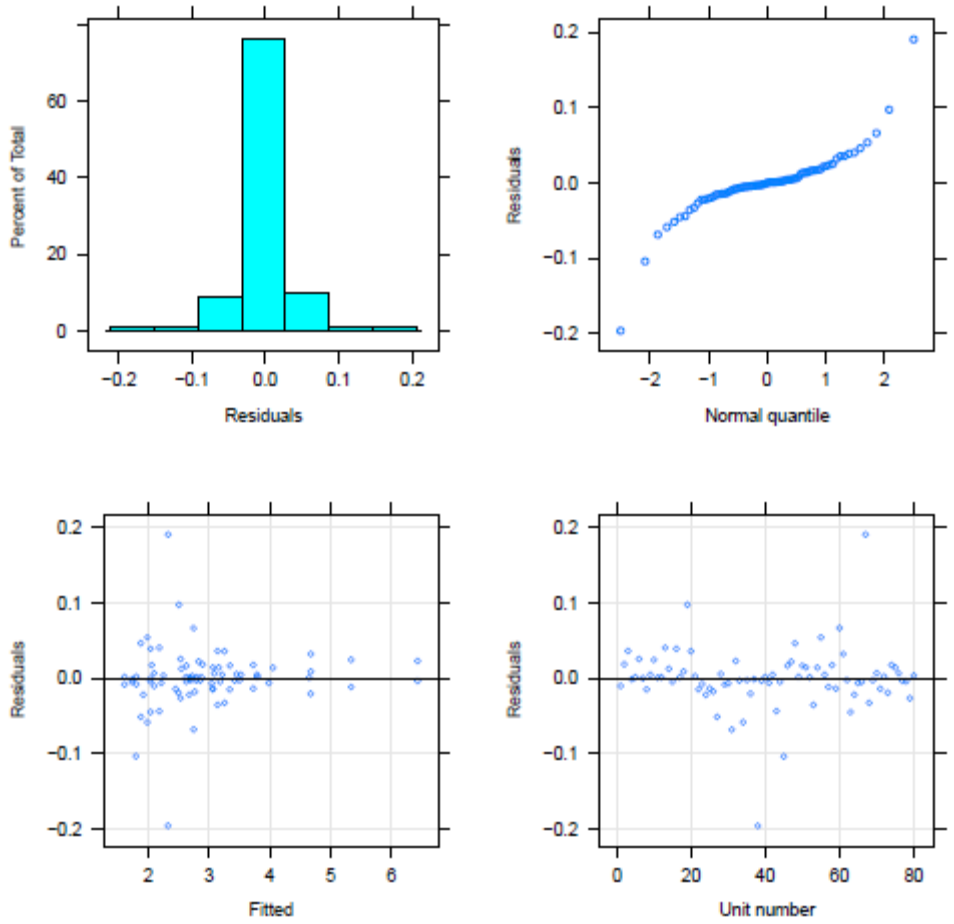

Figure 2: Plots of residuals from the fitted statistical model for Stearic acid - trait C18.0. This plot shows the high/low residual pairs for sets of duplicate mesurements on a kernel.

Table 5: Percentage of variance for the factors Tree and Kernel and the duplicate measurements of oil and aliquots for Gas Chromatography of 8 Acid measurements – outliers have been excluded from the data

	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Behenic	Erucic
Tree	0	16	25	8	6	0	12	0
Kernel	82	65	73	91	85	88	72	69
Oil	17	16	1	1	7	9	1	0
Residual	1	2	1	1	2	4	15	31

The results of this pilot study highlight the difficulties of developing a sampling strategy to detect varietal differences, because a dominant source of variation is between kernels within a tree. One strategy for reducing this high level

of variation between kernels is to consider possible factors that may be causing this variation and then to stratify sampling across levels of this factor. As in fruits the light can increase the sugar contents [29], in rapseed it was demonstrated when light is provided the embryo, carbon metabolism can be extremely efficient, converting up to 95% of carbon into storage material as well as oil [30]. Even though there is no evidence that macadamia orchard crowding reduced nut quality [31], however further experiments needs to be handle it for checking if light interception can influence macadamia nut oil profile.

However, if the potential cause of this high level of variability is unknown or cannot be explored, then the only other statistical option is to sample a large number of kernels. There is also evidence of a lesser proportion of variance within each kernel, as measured by the variance component for oil, and also for variation between aliquots of the same oil, as measured by residual variance. Therefore, it is desirable to retain a low level of duplication for oil and/or aliquots in any subsequent experimental design. An additional benefit, over and above variance estimation, lies in the ability to detect outlying or suspected aberrant values, which is only achievable through duplicate measures.

Experiment 3 *- Pollen influence in macadamia-nut oil profile*

It has been identified the same fatty acids from the experiment 1 and 2, and the fatty acids composition of the nut samples from cross pollination ranged from 0.34% to 18.81% for saturated fatty acids, from 0.30% to 54.54% MUFA and from 0.50 to 1.92 for polyunsaturated fatty acids. The nuts samples from open pollination ranged from 1.03% to 15.48% for saturated fatty acids, 0.57% to 56.95% for monounsaturated fatty acids and 0.42 to 2.00% for polyunsaturated fatty acids. The nuts samples from self-pollination ranged from 0.67% to 13.43%

for saturated fatty acids, 0.64% to 54.26% for monounsaturated fatty acids and 1.13% to 1.47 for polyunsaturated fatty acids (Figure 3).

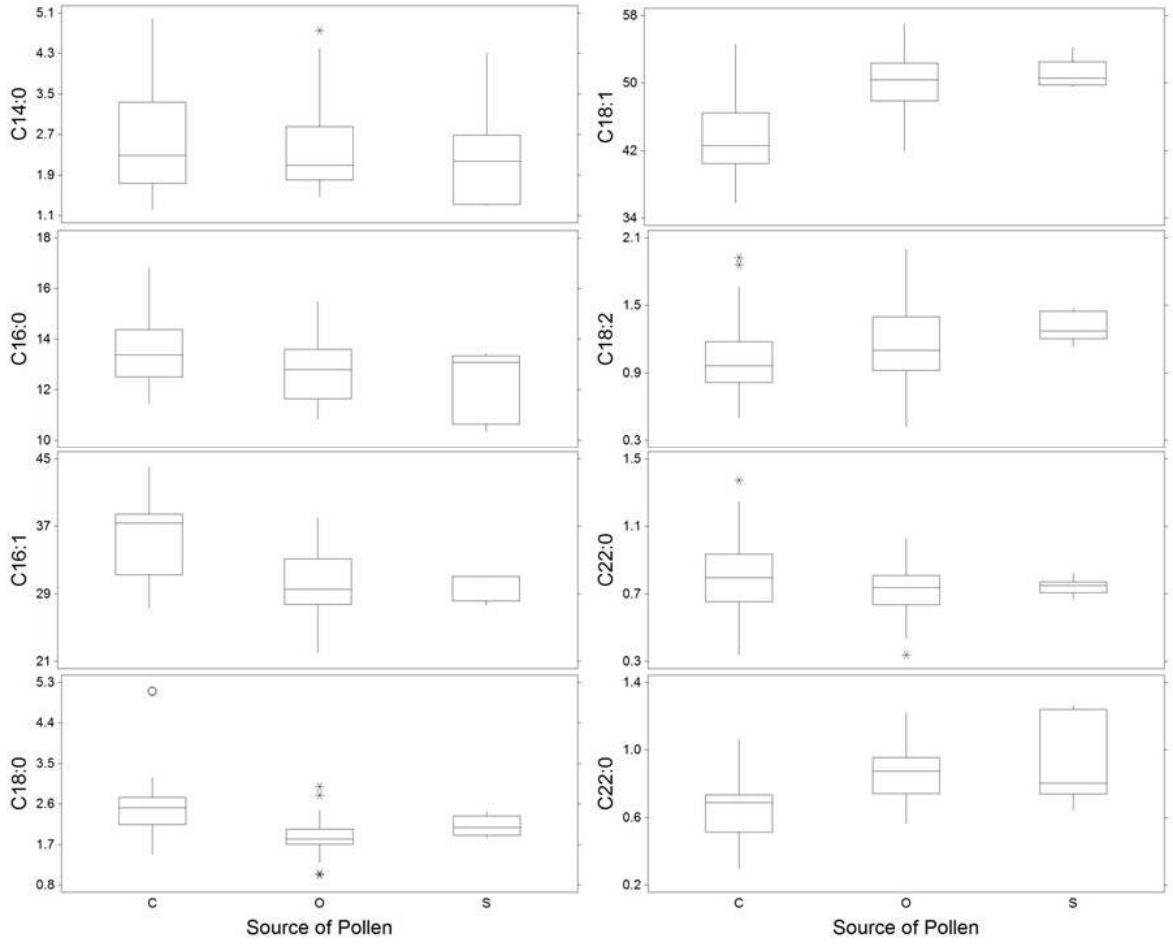

Figure 3 – Percentage of fatty acids according with the source of pollen, (C) – Pollen from Astonville 11.1 (O) – Pollen from open pollination, (S) – Pollen from self-pollination

It was identified eight fatty acids in macadamia nuts C16:0, C16:1, C18:0, C18:1, C18:2, C20:0 C20:1 and C22:0 [32], but the percentage of C16:0 (ranged from 3.38 to 15.48%), C16:1(ranged from 0.00 to 17.84%), C18:0 (ranged from 1.33 to 9.59%)was lower than it registered in the present experiment on the other hand the content of C18:1 (ranged from 29.01 to 77.67%), C18:2 (ranged from 2.45 to 61.15%) was higher in relation that verified in our determination. Rodrigues et al. (2013) [35] found the same fatty acids identified by the present study as well, and lower percentage of C16:0 (5.05%), C16:1w7 (11.19%), C18:0 (%2.01) and C18:2w6 (1.06%).

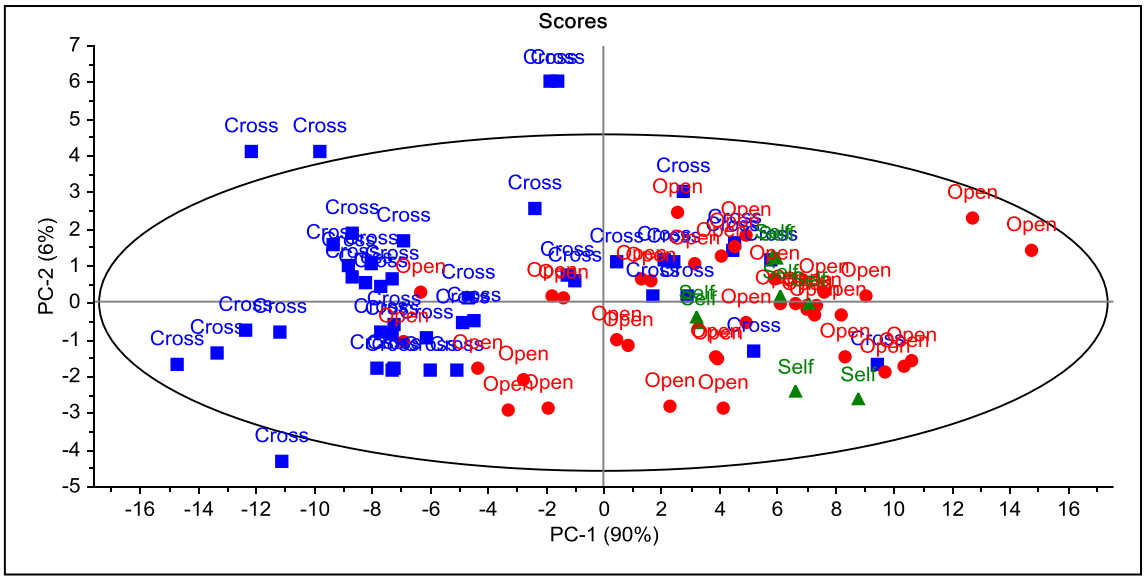

Figure 3 – Two-dimensional scatter plot of scores for two components (PC1 and PC2) from Principal Components Analyses of macadamia fatty acids profile.

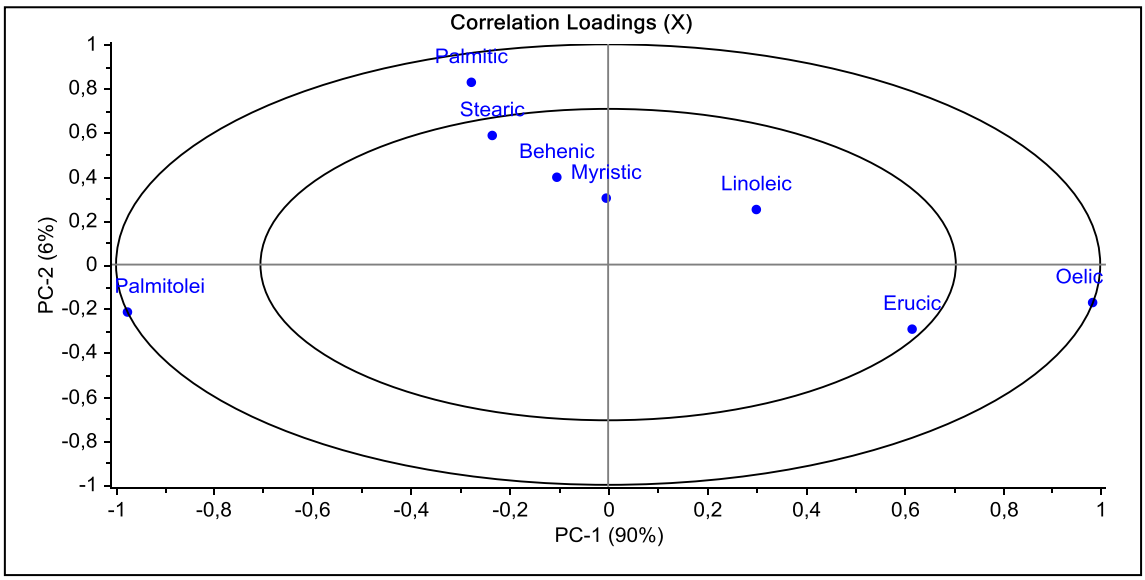

Figure 4 – Correlation loadings of fatty acids presented at macadamia oil along (PC1 and PC2).

Nuts from cross pollination were clustered opposite to the nuts from open pollination indicating a pollen influence in oil profile(Figure 3), loadings shown large contributions from oleic acid and palmitoleic acid, showing that open pollination nuts were positively correlated with C18:1w9 and cross pollination with C16:1w7(Figure 4). The self-pollination nuts had the same profile as the nuts from

open pollination indicating that some open pollination could be actually from self-pollination, once it was not controlled in the present study.

Studying the maternal effect on fatty acid composition of soybeans, [33] found that oleic and linoleic acids had little influence of pollen parent, but in certain crosses the genotype of the male parent influenced the linolenic acid fraction. PC1 described 90% of the variance (Figure 3), and PC2 only 6, allowing to stratify in at least two dimensions with 96% variation.

Overall, the result from the experiment shows that the pollen has an influence in the oil profile. Hence, if the goal of the breeder is to develop a genotype with a good ration of monounsaturated and saturated it is necessary to analyze the genotype which will be used as a pollen source. Once identified the combination, the crosses between genotypes that produce good rate of monounsaturated and saturated it can be advice for the growers with the aim of produce a healthier nut and with a longer shelf life.

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Evaluation of some flower characters in wild and cultivated macadamia tree

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Key words:*Macadamia integrifolia* Maiden and Betche*, Macadamia tetraphylla* L. A. S. Johnson*,* nut set; pollen; Proteaceae; raceme; self-fertility.

Abstract

The aim of the present study was to identify self-fertile genotypes as well as to study the variability in floret density, raceme length and pollen viability of wild and cultivated macadamia genotypes. Eleven plants of cultivated macadamia and ten plants of wild macadamia were used in the study. During the flowering period 30 racemes from each plant were enclosed in paper bags, for self-pollination, and another 30 racemes were tagged for opened pollination. Four months later, the Final-Nut-Set (FNS) was counted. The raceme length and florets density mm^{-1} of raceme was recorded in the lab and in the field to estimate the number of florets by the raceme during the final bloom. T1002.003, T108.002 and T1023.003 were the only wild genotypes which just presents FNS from self-pollination (0.01). Correlation found between the count in the field and in the lab (r=0.53) evidence that the raceme samples collected during the final bloom is a good way for estimating the number of florets in racemes of the tree under field conditions. Hang drop technique showed the lowest percentage of pollen germination, whereas the fluorochromatic and the TTC had similar results. The present results indicated that the macadamia florets can coordinate the male and female maturity resulting in self-pollination, breaking away the protandry bound. More experiments are needed in order to confirm the self-fertility rate found for some wild genotypes as well as experiments with cultivated genotypes in which a good caterpillar control is achieved, allowing to verify if self-pollination occurs in the cultivars too.

Introduction

Macadamia is a member of the Proteaceae family and it is the only Australian native tree which was developed into a commercial food crop. Nowadays, the macadamia genus was reduced to four species (McConachie, 2009): *Macadamia tetraphylla* (Johnson), *Macadamia integrifolia* (Maiden and Betche), *Macadamia ternifolia* (Mueller) and *Macadamia jansenii*. Designed for producing edible kernels, *Macadamia integrifolia* and *Macadamia tetraphylla* are the economic members of the family (Nagao, 1992).

Macadamia nut has a great potential to increase its world production, because over the last decade, more than half North American consumers are changing their habits, trying to eat foods in agreement with existing health conditions and almost half of the consumers consider snack as an important part of a healthy eating habit throughout the day (Simonis, 2012). Macadamia is considering the world's finest gourmet nut crops, being part of snacks (Janick and Paull, 2008), furthermore it was indicated as an important nut to be used in the diet due its health nutritional attributes (Curb et al., 2000).

The largest growers of macadamia are represented by the Australian Macadamia Society (AMS), which contributes with 40% of world production, Hawaii Macadamia Nut Association (HMNA) participates with 20% of production and 40% of consumers market and Southern African Macadamia Association (SAMAC) with 15% of total production. In Hawaii the bloom period occurs between November and May (Nagao et al, 1998), in Australia begins on May (Moncur et al., 1985) and in the end of August is the period when all florets are opened (Boyton and Hardner, 2002). The flowers are borne in pairs or groups of 3 or 4 (Kermond, 1996) on long racemes of 100-300 protandrous, hermaphroditic florets, white or cream-colored flowers (Nagao, 1992). The raceme is pedant with 100 mm to 300 mm in length. Even forming up to 2,500 raceme per tree, less than 0.3% produces nuts (Ito, 1980; Moncur et al., 1985).

Macadamia has plentiful hermaphroditic flowers with low nut set (Trueman, 2013). Low fruit set in many plant species is a consequence of pollinator limitation (Cunningham, 2000), and self-incompatibility which occurs in several species as *Macadmia* spp. (Sedgley, 1998; Ayre, 1994), *Malus spp*. (Iuchi, 2006), *Pyrus spp*. (Webster, 2002), *Tectona grandis* Linn. F. (Tangmitcharoen and Owens, 1997), in

Prunus salicina Lindl. (Bandeira et al., 2011; De Conti et at., 2013), and in several other plants species. Self-incompatibility is supposed to be a mechanism to promote cross-pollination (Marshall and Folson, 1991). On the other hand, Shivaramu and Sakthivel (2012) reported fifty-five species of insects visiting macadamia florets being *Apis mellifera* and *Trigona carbonaria* the most frequent visitors of macadamia florets, contributing to the pollination.

Flowers of plants have adopted a broad range of reproductive strategies which promotes out breeding. Self-incompatibility is one of the most significant groups of strategies, which is defined as 'the inability of a fertile hermaphrodite flower plant to set seed upon self-pollination' (Ito, 1980).

Self-incompatibility is widespread in flowering plants, which is a biochemical recognition and rejection process preventing self-fertilization. Pollen tube growth can be inhibited on the stigma or in the style (Shivana, 2003).

The most common reproductive barrier in flowering plants is gametophytic self-incompatibility, controlled by S-locus that prevents self-fertilization (De Nettancourt 2001). The S-locus comprises at two linked genes: S-RNase (specifically expressed in the style) and S-locus F-box proteins (essential to reject self-pollen by their cytotoxic activity) (Zuriaga et al., 2012).

As many tropical trees, macadamia is mass flowering, producing much more flowers than nuts. According to Sedgley et al. (1985) macadamia flowers are protandorus. Protandry is a dichogamy mechanism; which anther dehiscence and stigma receptivity are temporally separated (Shivanna, 2003).

Sedgley et al. (1985) observed that the pollen is removed from the style by insects and appears to be a pollen-attractiveness mechanism rather than an adaptation for self-pollination and even if the pollen continues within the style it will germinate 2 days post-anthesis when the stigma is fully receptive.

Sedgley (1983) pointed out that incompatibility in self-pollinated macadamia flowers results from arrest of pollen tube growth within the style. For Matthews and Sedgley (1998) *M. integrifolia*, exhibit partial pre-zygotic self-incompatibility which operates in the upper style, a region just beyond the area of maximum cell complexity.

As macadamia is a native Australia tree, self-incompatibility has been a mechanism employed by the trees in the florets for prevents self-fertilization, promoting out-cross and maintaining genetic variability (Ayre, 1989). Macadamia

cultivars have a tendency to be self-sterile, hence nut set on self-pollinated racemes are low, and cross-pollination between cultivars results in the initial nut set increase (Trueman and Turnbull, 1994b). Self-incompatibility mechanism characterizes the breeding systems of many proteaceous species, despite some species have reported significant levels of self-compatibility (Ayre, 1994).

Planting different cultivars in or among the rows for improving cross-pollination is recommended to obtain a profitable yield (Macadamia grower's handbook, 2004). The stigma of an individual flower is receptive for several days enhancing the opportunities for cross-pollination. On the other hand, the small amount of pollen available from an early flowering cultivar might not be enough to pollinate a late flowering cultivar by natural means, if pollination success is limited by pollen viability.

Knowledge of the reproductive mode of a species is important for several reasons but mainly for the management and preservation of plant genetic resources. It is also important for genetic improvement as the key to better succeed on utilization of wild germplasm in the breeding programs.

The kernel quality and yield have been identified as a priority characters for selection in the breeding macadamia programs. As pollination is a factor which influences production if we could identified a genotype with a high-medium rate of self-fertility it could increase yield. Moreover, there is evidence that pollinators are declining as a result of local and global environmental degradation (Biesmeijer, 2007). Larson and Barrett (1999) supported the view that self-fertility can enhance plant fertility, minimizing the dependence of pollinators. Autogamous species depend less on pollen vectors, and are therefore less likely to be pollen limited than non-autogamous self-compatible species. Therefore, autogamy is a means of reproductive assurance when pollination is uncertain.

Thus the aim of this study was to identify genotypes with a medium to high degree of self-fertility for using in the macadamia breeding program. Another objective was to compare flower biology in wild and commercial varieties of macadamia.

RESULTS

The traits assessed in the present study show variability of the characters associated to floral biology in the wild and cultivated groups of *Macadamia* spp. However for assessing the Final Nut Set (FNS) from self-fertility it was necessary to know how many florets each genotype produces. As macadamia raceme has nearly 300 florets, counting all the florets per raceme for estimating FNS is a difficult and time consuming task. For the purpose of minimize the time spent in counting macadamia florets a correlation between the florets counting in the field and the estimation made in the lab from the samples collected of each genotype (number of florets per raceme length in mm) was calculated. An independent-samples t-test has been used to check the effectiveness of the correlation, r=0.53, (p=0.00000569). This will be a significant tool for a pre-selection aiming self-fertility once it will minimize the work of counting the florets in the field.

Like other crops for having a profitable yield, macadamia also requires pesticides applications. After each raceme had been enclosed in paper bags, it was verified that the cultivar group located at Nambour trial presented the racemes infested with caterpillar (*Cryptoblabes hemigypsa*) (Gallagher et al., 2003), for this reason it was not possible to measured FNS in this cultivar group (Supplementary 1).

Supplementary 1 – Caterpillar damage (*Cryptoblabes hemigypsa*) at macadamia raceme.

At the Tiaro trial, where the wild group is located, it was also observed some infestation and this has to do, at least in part, with the absence of nuts in genotypes WIL_1B and WIL_7A The clones WIL_1A and WIL_7B, however, had yields. WIL_1, WIL_2 and WIL_4 were the only wild genotypes which produced nuts from self-pollination(Figure 1).

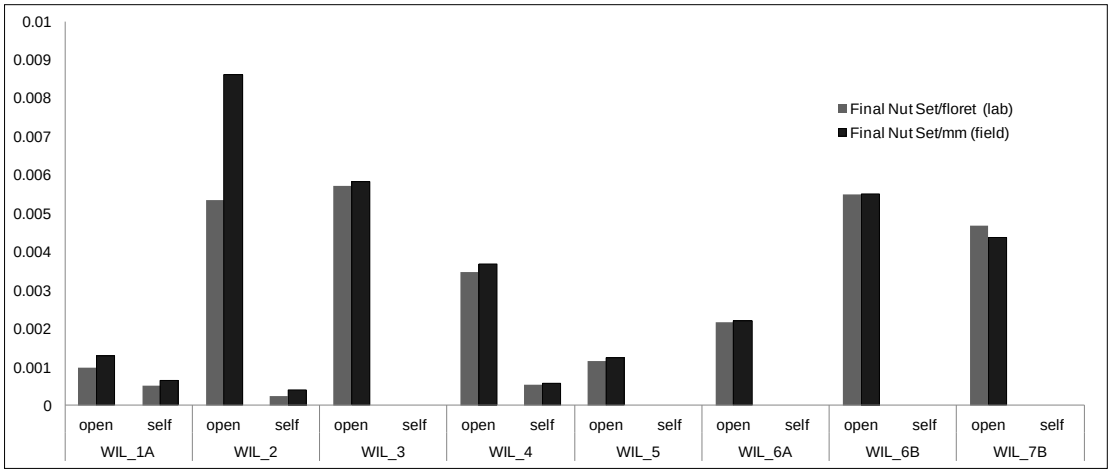


Figure 1- Percentage of final nut set of macadamia racemes wild genotype.

For checking if the raceme measured in the field during the FNS evaluation could be a tool for estimating the number of florets the following correlations were calculated: (1) raceme length measured in the lab with the raceme length measured in the field; (2) raceme length measured in the lab and number of florets; (3) raceme length measured in the field and number of florets.

The correlations estimated for the cultivar group was high in the three cases, with r=0.8, although the correlations for the wild group was moderate in the three cases, r=0.5. But even with a high or moderate correlation developed none of them was significative because the degrees of freedom were low. With the aim of establish a tool for the breeders assess the number of florets without the necessity of counting each floret, a regression equation for each genotype where number of florets per inflorescence (*y*) and axis length (*x*) was calculated (Table 3).

Table 3 – Correlation between the number of florets and raceme length, raceme samples collected in the end of bloom for measuring in the lab where number of florets per inflorescence (*y*) and axis length (*x*)

Genotypes	r		Regression Equation
CUL_1	0,82	***	y = 0,65x + 29,98
CUL_2	0,50	**	y = 0,31x + 69,89
CUL_3	0,50	**	y = 0,29x + 74,53
CUL_4	0,53	**	y = 0,36x + 54,36
CUL_5	0,77	***	y = 0,45x + 50,76
CUL_6	0,72	***	y = 0,57x + 53,06
CUL_8	0,54	**	y = 0,38x + 71,98
Média	0,62		Y = 0,43x + 57,87
EPM	0,05		
WIL_1A	0,66	***	y = 0,49x + 50,57
WIL_1B	0,80	***	y = 0,79x + 52,99
WIL_2	0,54	**	y = 0,39x + 54,71
WIL_3	-0,30	*	y = -0,28x + 207,19
WIL_4	0,71	***	y = 0,78x + 21,97
WIL_5	0,70	***	y = 0,49x + 83,99
WIL_6A	0,78	***	y = 0,71x + 48,20
WIL_6B	0,70	***	y = 0,68x + 56,88
WIL_7A	0,81	***	y = 0,86x + 27,47
WIL_7B	0,79	***	y = 0,74x + 47,54
Média	0,68		y = 0,62x + 65,15
EPM	0,049		

*(P<0,05), **(P<0,01) and ****(P<0,001)

The lab racemes from the final flowering presented amplitude from 66 mm to 270 mm, averaging 138.54 mm for the cultivars. The wild group exhibits amplitude of 67-310 mm, with an average of 159.01 mm. The racemes measured

in the field for the cultivars were longer than the samples measured in the lab, with an amplitude of 62-312 mm, and average of 174.47 mm. The wild group had lower amplitude (110-310 mm) as compare with the lab samples and an average of 212.49 mm (Figure 2).

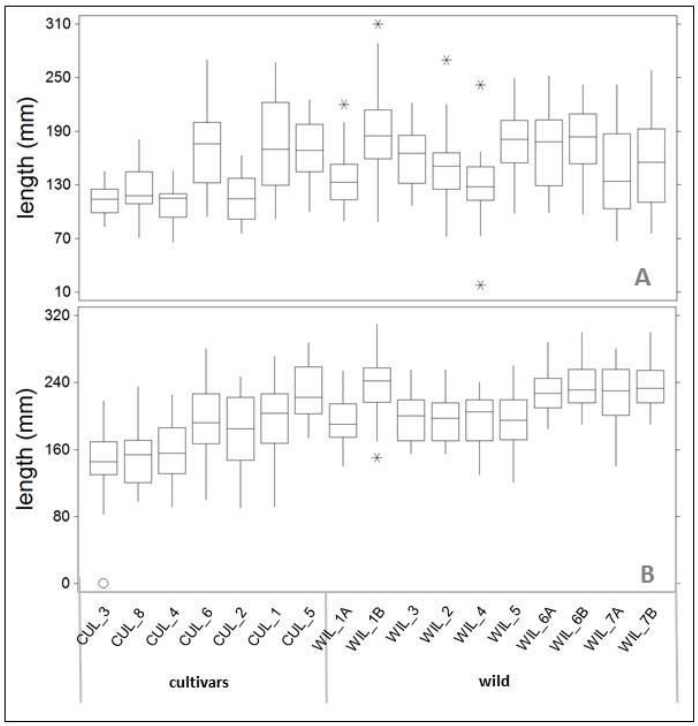


Figure 2 – Boxplot of raceme length. The box is equivalent to quartiles with the median of the data shown as line. Circle represent probable outlier and stars represent extreme values. (A) raceme collected in the field during the final flowering and measured in the lab. (B) raceme measured in the field during the final nut set.

Comparing the two racemes stages (Figure 2A and 2B) is possible to observe that the raceme increase the length during the time. By the end of the flowering the average of length was shorter than the length during the FNS. In average the raceme length increased 50 mm from the final flowering to the FNS.
There was a significant difference for the raceme length among the clones of WIL_1 in both field and lab samples. Clones of WIL_6 and WIL_7 did not show this difference.

The lab samples of CUL_5 and CUL_6 had the longest raceme whereas CUL_3 and CUL_4 had the shortest. The wild genotype racemes are longer than those of cultivated genotypes. Within the wild group the longest raceme measured on the lab samples was from the genotype WIL_1 (188.83 mm) and the shortest

was WIL_4 (127.10 mm). Among the racemes of genotypes of the cultivars group, measured on the lab samples, WIL_6 was the longest, averaging 238.90 mm and WIL_4 (194.33 mm) was again the shortest. For the cultivars group the largest raceme from the lab sampling was the cultivar CUL_6 (169.43 mm) and the shortest CUL_4 (109.20 mm) though in the racemes at the field the lengthiest was the cultivar CUL_5 and the shortest the CUL_3 (Figure 2).

The cultivars show similar floret density whereas the wild material has a different behavior. CUL_4, CUL_5 and CUL_1 are the cultivars with more florets density mm^{-1}. From the wild group it is possible to highlight the genotype WIL_2 which has the double of florets density than the others. The genotype WIL_1 was the only one that showed difference between the clones. WIL_1A showed the double of density when compared to the clone WIL_1B (Figure 3).

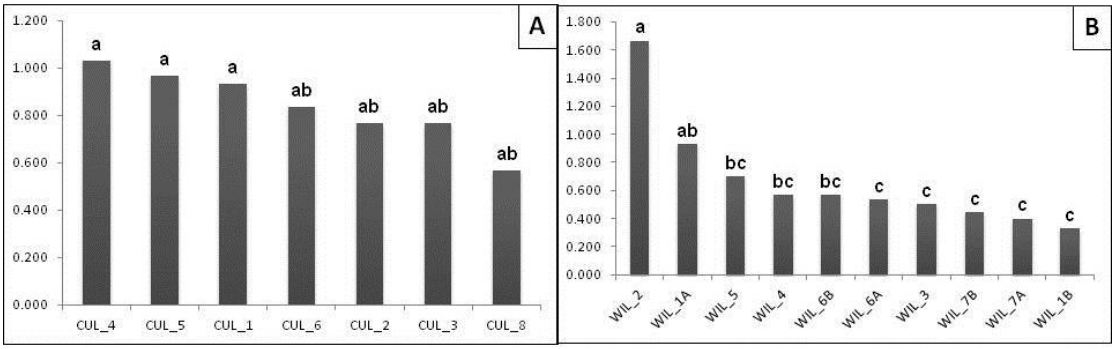


Figure 3 - Macadamia flower density. (A) cultivars. (B) wild genotype. The letters explain the statistical differences.

Wild genotypes showed higher pollen viability than the cultivars. The rate of pollen germination in macadamia was different according to the test carried out (Figure 4). Hang drop technique showed the lowest percentage of pollen germination, whereas the fluorochromatic and the TTC had similar results. The low percentage of pollen viability of the cultivars could have been influenced by the time of pollen collection (end of bloom).

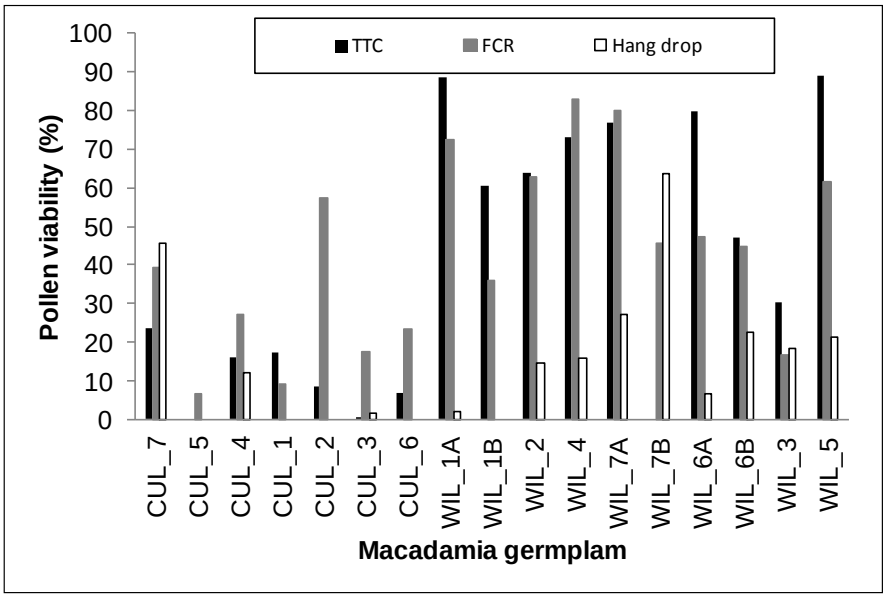


Figure 4 - Macadamia pollen viability test of wild and cultivar germplasm. (FCR) Fluorochromatic test (TTC) (2, 3, 5–triphenly tetrazolium chloride) test (Hang drop) In vitro germination test - Hanging drop technique.

Discussion

The traits evaluated in the present study are part of the primarily goal of the macadamia breeding program which is the yield. Studying the self-fertility by assessing the FNS allowed a better understanding about the floral biology diversity presents in the cultivated and wild macadamia germplasm.

For estimating the rate of nuts from self-fertility it was necessary to count the number of florets, a task consider tedious, difficult and time consuming by the researcher once the florets are very close to each and there are in large number. In this way, the moderate Cohen (1988) correlation index found between the count in the field and in the lab evidence that the raceme samples collected during the final bloom is a good way for estimating the number of florets.

Macadamia FNS was low (0.01%), and the main factor responsible for the lack of in the cultivars was the caterpillar infestation (O'hare et al., 1995).

The wild material has an interesting characteristic besides the self-fertility rate, even with no spraying in the present study it had production, demonstrating that some genotypes could have a tolerance to caterpillar or not been attractive for it (non preference mechanism). Tests with caterpillar and raceme florets should be conducted in the lab for confirm this theory.

The three wild genotypes that produced nut from self-fertility showed a overcoming the protrandy on part of the florets (Nagao, 1992). Future experiments should be carried with more clones from the same three genotypes for confirm the self-fertility rate (0.01%) and for testing the environment influence likewise predict the caterpillars before flowering period.

The FNS was low, however the genotypes which showed self-fertility should be used in future experiments. Working with A4 and HAES246 cultivars Wallace et al. (1996) found a FNS from self-pollination, similar to the obtained cross pollination, and Pisanu (2009) found low levels of nut set from hand self-pollination compared with outcrosses (3.25-6.79) and open (0.04) pollination treatments. The low nut set in the current study could be due the lack of fertilizer in the trials. It has been proposed low soil fertility as another factor that can limit the fruit set (Ayre, 1989), because the nut retention in the branch depend on the available carbohydrate, and represent an adjustment of crop load, aiding to minimize the amount of resources lost by abscission (Trueman and Turnbull, 1994).

Macadamia raceme presents nearly 300 florets (Utara, 1954), but variation of 100 to 300 florets or 30-173 florets has been related, respectively, by Trueman (2013) and Pisanu (2009). The range of variation in the present study for the cultivar group was 109-173 florets, average of 141 while the wild group ranged from 132-188 florets, with an average of 159. The cultivated group showed higher florets density what is expected due to the selection (breeding). The genotypes which can be point out are the CUL_8 which seen to be bottom and WIL_2 which has the biggest florets density. Gross (1995) describes *M. tetraphylla* inflorescences with 70-300 mm long and *M. integrifolia* inflorescences with 65-140 mm long. Kermond and Baumgart (1965) differentiate *M. tetraphylla* to *M. integrifolia* racemes by different characteristics being the racemes length one of them. *M. integrifolia* raceme is 100-300 mm long with 100 to 300 flowers and *M. tetraphylla* is 150-450 mm long with 100-300 flowers.

Due to the large number of florets per raceme to count the number of florets for estimating the fruit set is very challenging. Abbott (1985) calculated a regression equation for *Bankisa grandis* (Proteaceae) between the number of florets per inflorescence (*y*) and axis length (*x*): $y=741.7 + 125.5x$, $r=0.90$. Working with regression equation allows the researcher gain access to the number of florets measuring only the length of the racemes from the final flowering, which is

much easier and less time consuming. The equations developed in the present study will allow the macadamia breeders to access the number of florets without the necessity of counting the number of florets. This can be used as another trait in the pre-selection of the progenies.

It is not well documented the effects of temperature and water on raceme length, however for Moncur et al. (1985) raceme growth can be limited by the stored reserves which are probably influenced by weather conditions. Our data show that the raceme of wild plants from the Tiaro germplasm bank, which were irrigated, were much longer than the raceme from the cultivars plants at Nambour not irrigated. The raceme elongation took between 63 days (Moncur et al., 1985). Long racemes are interesting because allow the good development of the nuts, while a shorter raceme could make a pressure among the nuts inducing abscission affecting the FNS.

The results found in the present study about the florets density are similar with the ones found by Heard and Exley (1994), 0.66 to 1 florets mm^{-1} of raceme. As well as similar to the description of flowering pattern reported by Stephenson (2005) to the varieties A16, HAES 741 and HAES 816 (Table 1).

Table 1 – Flowering pattern description from Stepheson (2005), based on the characteristic of different cultivars

Cultivar	**Flowering pattern**
HAES 246	Extended
HAES 344	Medium in length
HAES 741	Condensed
HAES 816	Light
HAES 842	Heavy flowering
HAES 849	Light, condensed
A16	Moderately intense, condensed
A268	Short
Daddow	Moderately heavy

In relation to the pollen viability estimation, it was observed that coloration of the pollen grains with dyes overestimate the percentage of viable pollens (Einhardt et al., 2006). The TTC and FRC test are supposed to color alive pollen , however sometimes the pollen is dead but colors because of the starch content, enzymes, chromatin, etc. (Galletta, 1983). Several investigators have reported false positive responses with tetrazolium test as well with fluorochromatic. Furthermore, Hang drop technique is better because shows the pollen that are viable and the one that has conditions for germinate. Lincol, 1982 cited by Dafni and Firmage (2000) defined pollen viability as “the capacity to live, grow, germinate or develop”.

Considering just the hang drop test, the studied genotypes showed low pollen viability. As it took some days for testing the pollen viability we can imply that storing the pollen decreases its viability or the fact that it was collected at the final bloom could cause such reduced viability. At this period the plant could be forward its reserves to the development of the nut resulting this low values. The wild genotypes with cloned trees presented different behavior between them in the three tests. According to Dafni and Firmage (2000) when the pollen has low longevity increases the chance of allogamy.

Similarly others variables studied in this paper the wild group of plants had higher pollen viability than the cultivars, and his could have been influenced by the plant conditions once the wild material were irrigate whereas the cultivars were not. The pollen viability can be influenced by different factors as plant species, genotype, season, temperature, humidity, time of day and light (Stanley and Linskens, 1974 cited Galletta, 1983).

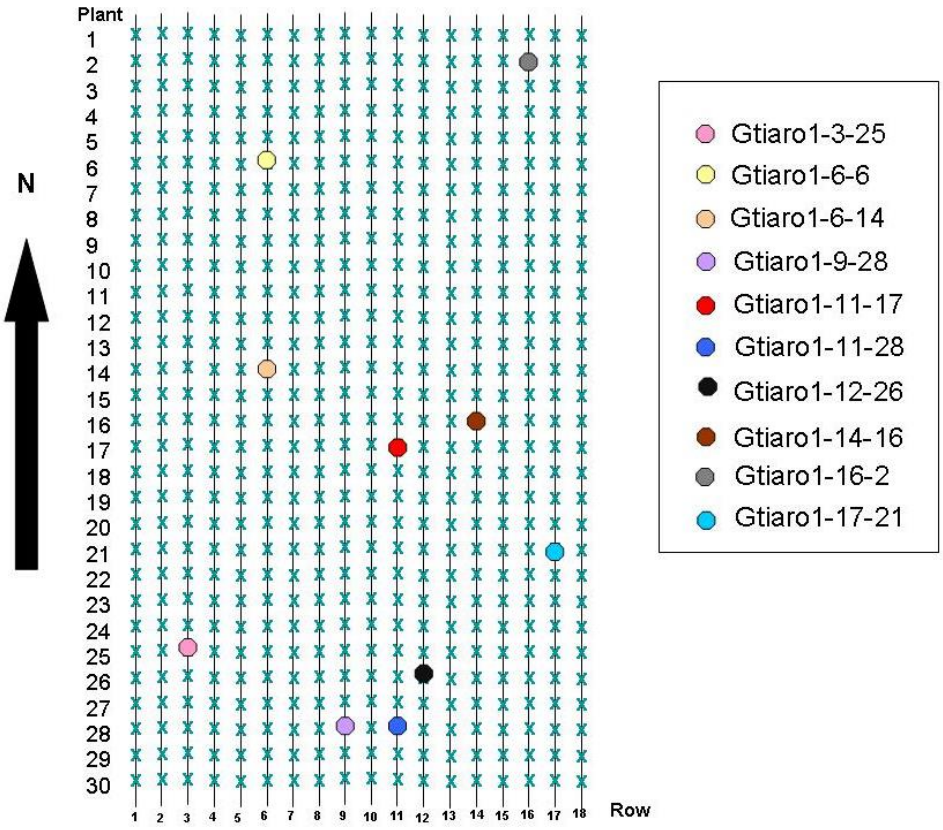
Growing self-compatible macadamia in single cultivar orchards is desirable, once the need for pollinators would be lower. For this reason, self-compatibility is now one of the objectives in macadamia breeding programs.

The present results indicated that the macadamia florets can coordinate the male and female maturity for results a self-pollination, breaking away the protandry bound. More experiments are needed with the aim of confirm the self-fertility rate found for some wild genotypes as well as experiments with cultivars with adequate caterpillar control to allow observing if self-pollination occurs on the cultivars too.

MATERIAL AND METHODS

Study site and plant material

Experiment was conducted in two places, at Maroochy Research Station in Nambour (26° 38'S, 152° 56'E), and at germplasm wild macadamia trial in Tiaro (25°43'S, 152° 35'E) Queensland, Australia. The trees of the cultivars used in this experiment at Nambour were 30 years old and were grafted onto rootstocks. The trees from Tiaro were 13 years old and were from cuttings from different trees of the rain forest (Supplemental 1).



Supplementary 2 - Map of the wild material at Tiaro. The colourful circles represents the plants used in the study.

Both orchards did not receive any cultural treatments as pruning and fertilizers and just the wild macadamia trial was irrigated during the summer.

From September to October, eleven genotypes of cultivated macadamia and 10 genotypes of wild macadamia have been used in the evaluation (Table 2), and each one was represented by two cloned plants.

Table 2 – Genotypes used in the experiment, the three first letters in the abreviation represents the group which the genotype bellows, the number refers to the genotype and the letters A and B to the clones of its

CULTIVAR	ABREVIATION	WILD	ABREVIATION
A4	CUL_1	T1002.003_1	WIL_1A
A16	CUL_2	T1002.003_2	WIL_1B
HAES 741_1	CUL_3A	T1008.002	WIL_2
HAES 741_2	CUL_3B	T1009.002	WIL_3
HAES 816	CUL_4	T10.23.003	WIL_4
BEAUMONT_1	CUL_5A	T1025.004	WIL_5
BEAUMONT_2	CUL_5B	T1055.005_1	WIL_6A
2|18McI_1	CUL_6A	T1055.005_2	WIL_6B
2|18McI_2	CUL_6B	T1090.002_1	WIL_7A
HAES 660	CUL_7	T1090.002_2	WIL_7B
HAES 804	CUL_8		

During flowering, thirty racemes with florets at the looping stage (near 2 days pre-anthesis) from each plant were enclosed in paper bags (15.8 X 39.6cm) and sealed against the branch with a clothes peg, for self-pollination. In addition 30 racemes at the same stage were tagged for checking percentage of opened pollination.

Whereby macadamia raceme has large numbers of florets it becomes difficult to count the number of them on plants in the field in order to estimate the nut set percentage. Thirty racemes from each tree were collected for assessment of the florets density mm^{-1}. The florets were counted and the length of the raceme axis were measured in the lab.

Four months after the racemes had been bagged and tagged the number of fruits were counted to calculate Final-Nut-Set (FNS). The nut set was defined as:

$$Nut\frac{set}{Florets} = \frac{Nut\ Set}{\left\{\left(\frac{average\ florets\ per\ raceme\ in\ lab}{average\ raceme\ length\ in\ lab\ (mm)}\right) \times\ field\ length\right\}}$$

Aiming to verify if the raceme sample collected during the final flowering was a trustful procedure for determining macadamia nut set it was checked in one wild germplasm (T1025.004). The same procedure described above was used but the counting of florets and measurements of racemes length were done in the field and after other raceme sampling were collected for counting in the lab. The Pearson coefficient (Staton, 2001) has been used for comparing the lab and the field scores.

In the present study the major objective has been the FNS, however others variables were captured as average of raceme length, density florets and pollen viability. The pollen viability study was carried out with the same plants. Raceme developed in the end of the flowering season (28/09/2012) from each tree was bagged when the inflorescences were at “looping stage” (approximate 2d pre-anthesis). After two days to dehisce, pollen was collected, inserting the raceme into a plastic tube, which was twisted up and down, until a thin coating of pollen adhered to the tube’s inner surface (Mc Conchie et al., 1997). The plastic tubes containing the pollen were stored in a plastic bag with silica gel in the freezer (-20°C). Two months after pollen collection three viability tests were conducted: Fluorochromatic (FCR) test, *2,3,5–triphenly tetrazolium chloride* (TTC) test and In vitro germination test - Hanging drop technique. The tests were done with two replicates for each individual.

Fluorochromatic (FCR) test

The methodology used was the one described by Heslop-Harrison et al. (1984). Flourescein diactetate was freshly prepared and 0.2mL placed on the slide, then with the aid of a spatula the pollen were dispersed into the drop. The slide was left for 2 minutes and examined by fluorescence within 5 minutes. Pollen grain that shows a fully, rightly fluorescent colour were counted as viable and 100 grains were examined per slide, two replicates per genotypes.

TTC *(2,3,5–triphenly tetrazolium chloride) test*

The procedure adopted was that of Cook and Stanley (1960) with some modification. First 10% stock solution was prepared. From this solution 1 portion was mixed with 9 portion of 60% sucrose solution. Therefore, the amount in the final TTC solution was reached at 1%. Pollen was dispersed in a drop of the medium on a microscope slide, and covered immediately with a coverslip. The pollen on the slides was incubated in the dark at room temperature for up 48h. Pollen grain that shows a dark red colour was counted as viable and 100 grains were examined per slide, two replicates per genotypes.

In vitro germination test - Hanging drop technique

The Van Teigham cell on a slide was made with melted paraffin, a drop of 15% sucrose and 0.1% boric acid solution was placed onto slide, the pollen was dusted on the drop with an aluminium rod (Scorza and Sherman, 1996). Subsequently the slide was quickly inverted and incubated under dark condition at room temperature during 48h. Later a coverslip was placed over the Van Teigham cell making possible to count the pollen germination. One hundred pollen grains were counted in, two replicates per genotypes. The pollen grain which showed a tube longer than its diameter was considered germinated. A descriptive statistical was conducted for the three tests conducted

Statistical analyses

Descriptive analyses were used to analyse raceme length, FNS and pollen viability. The least significative difference was used to compare means for density florets. Pearson correlation was used between the number of florets and raceme length from the samples counted in the lab and t Student test was carried out for testing the significance of each correlation. Simple Linear Regression was performed for estimating the number of florets from the raceme length.

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Evaluation and ranking of Macadamia genotypes using mixed models

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ABSTRACT – It was assessed annual yield of kernels in a population of macadamia (*Macadamia integrifolia*) aiming to identify the best genotypes relative to this trait for two goals: to establish a base population for breeding program and increase yield in production systems based on vegetative propagation. The production from 46 plants of 23 genotypes was analyzed using mixed models with REML/BLUP methodology by WOMBAT software, including effects of genetics, harvesting year, site, pickings frequency and plant age. It was observed that the heritability is low for production and the age of the plant has direct and significant association with the production during the juvenile period. The genotypes IAC-920, HAES 741-MAUKA, HAES 344-KAU and AFRICANA are highlighted by different selection strategies, weighted by genotypic and/or phenotypic value. By the first criterion it was also stand out the genotypes HAES 791, GUARANI I, HAES 816, PALMEIRAS, IAC-412-B, GUARANI II, 772, DOROTHY and FLOR ROSA MG and the genotype IAC-412-B by the second criterion of selection. These results and the correlations obtained demonstrate the importance of using BLUP predictors as a tool for breeding programs in this case study.

Key words: *Macadamia integrifólia*, genotypic value, yield, kernel, REML/BLUP.

Avaliação e ordenação de genótipos de Macadâmia utilizando modelos mistos

RESUMO – A produção anual de amêndoas em uma população de macadâmia foi avaliada para identificar os melhores genótipos com relação a esta característica, visando estabelecer uma população base para programa de melhoramento e incrementar o rendimento do sistema de produção baseado em propagação vegetativa. A produção de 46 plantas representando 23 genótipos foi analisada empregando modelos mistos, com a metodologia REML/BLUP, incluindo os seguintes efeitos: genético, ano de colheita, local, frequência de coletas e idade da planta. Foram observadas uma baixa herdabilidade para

essa característica e uma associação direta e significativa da idade da planta com a produção durante o período juvenil. Quatro genótipos são destacados por diferentes estratégias de seleção, considerando o desempenho genotípico e/ou fenotípico. Os resultados obtidos pelo modelo adotado e a correlação entre genótipo e fenótipo demonstram a importância do uso de preditores BLUP como ferramenta para programas de melhoramento relacionados a este estudo de caso.

Palavras-chave: *Macadamia integrifolia*, valor genotípico, produção, amêndoa, REML/BLUP.

INTRODUCTION

Macadamia (*Macadamia integrifolia*) is an arboreal species belongs to the Proteaceae family. The natural habitat is the east coast of Australia distributed in Queensland and New South Wales (JOHNSON, 1954; SMITH, 1956). The macadamia geographical distribution includes Australia (center of origin), USA, Hawaii (center of diversification), Africa, Guatemala, Costa Rica, Colombia, Paraguay and Brazil (DIERBERGER; MARINO NETTO, 1985).

The main commercial product of macadamia is the kernel, which are classified into different grading for different consuming markets. The refined flavor of kernels has aroused the interest of international markets for consuming *in natura*, roasted as snack or as a fine ingredient in the chocolate industry. The second grade quality kernels from the processing line are destined for oil extraction to be used in the cosmetics and pharmaceutical manageable and its highly valued (STEPHENSON, 2005).

Macadamia was introduced into Brazil in 1931 by Henrique Jacobs (SIMÃO, 1998). Since then it has spread throughout the country to become an important agribusiness commodity due to international demand of the different consuming markets. In the early years of present century, Brazil assumed the 7th position among producers of macadamia in the world, with largest production situated in São Paulo State (POLTRONIERI et al., 2005). Macadamia breeding program began in Brazil in the 1940's as an initiative of Instituto Agronômico de Campinas (IAC) using seed imported from Hawaii and USA (SOBIERJASKI et al., 2006).

Although macadamia breeding began more than 70 years ago there is little information available on the characterization of genetic variability of the genotypes used in Brazil. One of these initial actions in the current breeding program is the evaluation and

characterization of superior genotypes. However this task is slow and arduous when working with a perennial tree crop like macadamia with a long juvenile period. Breeding programs commonly employ repeated cycles of selection and recombination of genotypes from existing breeding populations and germplasm collections (PEACE et al., 2003). Characters of interest for each selection step are evaluated to assign genetic values which in turn are recombined in future generations and propagated and/or cloned.

For development of new cultivars, the knowledge of interest genetic characteristics is extremely important. One of the ways to identify the individuals carrying desirable genes is the genetic evaluation of selection candidates, which must be grounded in their additives genetic values, for use in recombination, and in genotypic values to be cloned. This requires the estimation of additive and non-additive genetic variance, for the sexual and asexual reproduction respectively (CRUZ; CARNEIRO, 2003).

ANOVA (Analysis of Variance) and method REML/BLUP (Restricted Maximum Likelihood/ Best Linear Unbiased Prediction) are the main procedures to estimate genetic parameters in testing and predicting of progenies. When unbalanced data sets are used, such as genetic selection procedure, the classical ANOVA models are inadequate, because estimated differences are biased (RESENDE, 2002; ROCHA et al., 2006).

Henderson (1973) has introduced the mixed models methodology, which includes fixed and random effects, serving both to estimate averages of blocks by the Generalized Least Squares (GLS) method, and to predict additive genetic values, in tests of half-sib progeny. The restricted maximum likelihood method in the mixed models is very important by generate unbiased estimates of parameters (HENDERSON, 1973, 1984).

In perennial plants, BLUP is the best predictor of genetic values. The REML/BLUP method estimates fixed effects as the Best Linear Unbiased Estimator (BLUE) and, simultaneously, predict the value of random genetic effects (BLUP) and random effects uncorrelated included in this model (RESENDE, 2002), without the necessity of a classical statistic design.

Macadamia species have a long (12 years) juvenile period (PIMENTEL, 2007), which together with a lack of genetic information motivates breeders to study the relationship between agronomical and genetic traits for developing breeding strategies.

The objective of this study was identify the best genotypes relative to the annual yield of kernels in a population of macadamia germplasm, using mixed models, for grouping individuals aiming to establish a base population for breeding program and to improve results in the production systems based on vegetative propagation.

MATERIALS AND METHODS

For this study were used the nut tree crops from a commercial orchard managed by the Macadamia Brasilis Company. Registration and data analysis were performed at the Laboratory of Experimental and Computational Statistics Biometrics at Universidade Federal de Pelotas (UFPel). The annual yield for each tree was expressed in kilograms (kg) during the three years of the study.

The harvest data was recorded from an orchard, four kilometers from Itapira in the subtropical region of São Paulo State, Brazil. Average annual temperature is 26°C, 72% relative humidity and 1390 mm rainfall. The trial consisted of 46 individuals of different ages grafted onto selected root stocks, located at six different sites on the orchard, from the germplasm collection, comprising 23 genotypes. Information was recorded from harvests (January to June) of 2009, 2010 and 2011, including plant identification, location (planting site), planting date and, for each picking, date and weight of harvest. The last two items were used for determine the annual kernel production and the number of pickings. Environmental effects on phenotypic expression were reduced by: standardizing tree spacing (6 m between plants and 8 m between rows) in all locations; pruning side branches or inside the canopy for better lighting and aeration (held during the month of June) and spraying in pre-bloom for preventing pests and diseases. The registered data are unbalanced due to several factors: starting year of harvesting for each plant, replication of the experiment subjects, presence of the genotypes at the sites and numbers of pickings per year per individual.

The trait annual production was analyzed by mixed linear model methodology using REML implemented by WOMBAT software (MEYER, 2007), assumed the following model:

$$\mathbf{y} = \mathbf{Xb} + \beta\mathbf{t} + \mathbf{Zg} + \mathbf{Wp} + \varepsilon,$$

where:

y is the vector of annual kernel production in kg;

X is the (design) incidence matrix of fixed effects;

b is the vector of fixed effects (location, number of pickings in the year and grand mean);

t is the vector of values for the fixed covariable (plant age in the year of production);

β represents the linear regression coefficients associated with the covariable t;

g, p are the vectors of genetic and permanent environmental random effects;

Z, W are the design matrices corresponding to random effects;

ε is the vector of random residuals.

It was assumed that the unique trait (annual production) has normal distribution centered in the mean, given fixed parameters (location, age and number of pickings). In addition, the genetic and production year effects and residuals were assumed independently and normally distributed with mean zero and (co)variance matrix equal to $\mathbf{I}\sigma^2_{\mathbf{g}}$, $\mathbf{I}\sigma^2_{\mathbf{pe}}$ and $\mathbf{I}\sigma^2_{\mathbf{e}}$, respectively,where **I** is the identity matrix of corresponding order and $\sigma^2_{\mathbf{g}}$, $\sigma^2_{\mathbf{pe}}$ and $\sigma^2_{\mathbf{e}}$ are the genetic, permanent environmental and residual variances, respectively. For the purpose of this analysis, genotypes were considered unrelated.

The covariable age has been adjusted only with linear effect, due to the age period of the data available coincide with the juvenile period, for all plants used.

Estimates of broad-sense heritability (h^2) and intraclass coefficient (c^2) have been determined according to the following equations:

$$h^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_{pe} + \hat{\sigma}^2_e} \quad \text{and} \quad c^2 = \frac{\hat{\sigma}^2_{pe}}{\hat{\sigma}^2_g + \hat{\sigma}^2_{pe} + \hat{\sigma}^2_e}.$$

The genotypic value was calculated for each genotype by adding the corresponding value of BLUP (genetic effect) to the grand mean of the trait.

An appropriate parameters file was prepared to configure the WOMBAT software for processing the designed model. For complementary calculations and graphics and for data preparing were used the functionalities of Microsoft Excel program. The collected data, including all factors of the model, replications and the three measures of annual production, were recorded in a suitable disposition for analysis using WOMBAT.

RESULTS AND DISCUSSION

In the present study were estimated the variance components, the means of annual individual production and corresponding tree age, the indicators of the Logarithm of the Likelihood function (Log_e L), the Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC), according to Table 1.

Table 1. Means and estimates of variance components

Statistic	Estimate	ASE(1)
Mean of Annual Production (kg)	10.027	-
Mean of Age	7.044	-
Phenotypic variance ($\hat{\sigma}^2_{\mathbf{p}}$)	38.107	18.226
Genotypic variance ($\hat{\sigma}^2_{\mathbf{g}}$)	4.800	2.831
Year variance ($\hat{\sigma}^2_{\mathbf{pe}}$)	13.206	17.959
Residual variance ($\hat{\sigma}^2_{\mathbf{e}}$)	20.101	2.922
Heritability (h^2)	0.126	0.089
Intraclass correlation (c^2)	0.347	0.311
Log_eL(1)	-260.010	-
AIC(1)	526.020	-
BIC(1)	534.204	-

(1) ASE: approximate sampling error (only where it is applicable); Log_eL: Logarithm of the likelihood function; AIC: Akaike's Information Criterion; BIC: Bayesian Information Criterion.

The heritability coefficient estimated by genetic parameter was low (12.60%) which shows that much of the variation of the characteristic among individuals is due to environmental differences and interactions among genotypes and environments. The estimate for the intraclass correlation coefficient (c^2 = 34.6) was relatively moderate (RESENDE, 2002). There is little published works about studies assessing macadamia heritability in relation to production. However studies conducted on characteristics that influence the final factor production, such as fruit size and nut size, reported that the heritability is high for these traits (HARDNER et al. 2001; HARDNER et al. 2009). In these studies, was reported difference among cultivars, confirming the existence of genetic variation which is difficult to assess. This is attributed to the focus which the selection was made, usually on the basis of phenotypic performance, without the control of environmental variation, implying low accuracy in the estimation of the genetic effects, especially for traits of low heritability like kernel production. Hardner et al. (2002) reported low heritability in a broad sense for the production of plants ($6\% < h^2 < 22\%$).

The 23 genotypes are classified in decreasing order of genotypic performance (Table 2) by BLUP of random effects represented in the model.

Table 2. Evaluated genotypes and their performance parameters obtained from the linear model applied using REML/BLUP, in decreasing order of genotypic value

Genotype	Mean of Annual Production (kg)	Genetic effect (BLUP) (kg)	Genotypic value (kg)	Relative Performance (%)	
				Genotypic	Phenotypic
IAC-920	15.626	3.883	13.911	100.0	73.8
HAES 741-MAUKA	19.061	2.098	12.126	87.2	90.1
HAES 344-KAU	15.316	1.652	11.680	84.0	72.4
AFRICANA	11.758	0.876	10.903	78.4	55.6
HAES 791	7.420	0.821	10.849	78.0	35.1
GUARANI I	0.358	0.542	10.569	76.0	1.7
HAES 816	4.109	0.423	10.451	75.1	19.4
PALMEIRAS	0.969	0.354	10.382	74.6	4.6
IAC-412-B	21.162	0.338	10.366	74.5	100.0
GUARANI II	1.093	0.323	10.350	74.4	5.2
772	6.290	0.139	10.166	73.1	29.7
DOROTHY	5.966	0.134	10.162	73.0	28.2
FLOR ROSA MG	7.841	0.034	10.062	72.3	37.1
BEUAMONT (695)	8.680	-0.352	9.676	69.6	41.0
IAC-920 X	9.159	-0.411	9.617	69.1	43.3
CANNON	9.129	-0.516	9.511	68.4	43.1
741 EDSON	4.619	-0.787	9.241	66.4	21.8
HAES 842	5.061	-0.935	9.093	65.4	23.9
IAC-CAMPINAS B	10.851	-0.944	9.083	65.3	51.3
HAES 788-PAHALA	6.589	-1.041	8.987	64.6	31.1
HAES 849	5.291	-1.470	8.557	61.5	25.0
HAES 814	5.458	-1.473	8.555	61.5	25.8
HAES 246-KEAUHOU	11.956	-3.689	6.338	45.6	56.5

Predictors for relative genetic effect are shown in Table 2 and predictors for relative effect of production year match to 2.328 kg in 2009, 1.4 kg in 2010 and -3.728 kg in 2011. The knowledge of the genetic and phenotypic variance has a significant effect in the premature selection (CARVALHO et al, 2008). By adopting this order, each genotype can be checked in relation tophenotypic value (average annual production) and genotypic value (BLUP), along with genotypic and phenotypic relative performance.

A comparative approach between the genotypic and phenotypic values is shown in Figure 1. The first quadrant highlights the five genotypes that simultaneously represent the best performances with respect to genotypic and phenotypic values. The contradictory results between the genotypes 246 (fourth quadrant) and AFRICANA (first quadrant) can be used to emphasize the differences revealed by BLUP usage, given that although both of they presented good phenotypic performance, its genotypic values are opposite. The superior phenotypic value produced by the interaction genotype versus environment does

not guarantee to know the genetic value for these individuals. Thereby, the genotypic value predictor obtained by BLUP allows the breeder to select the superior genotypes which will maximize the possibility of a genetic progress in the selection (SÖLKNER et al., 2008).

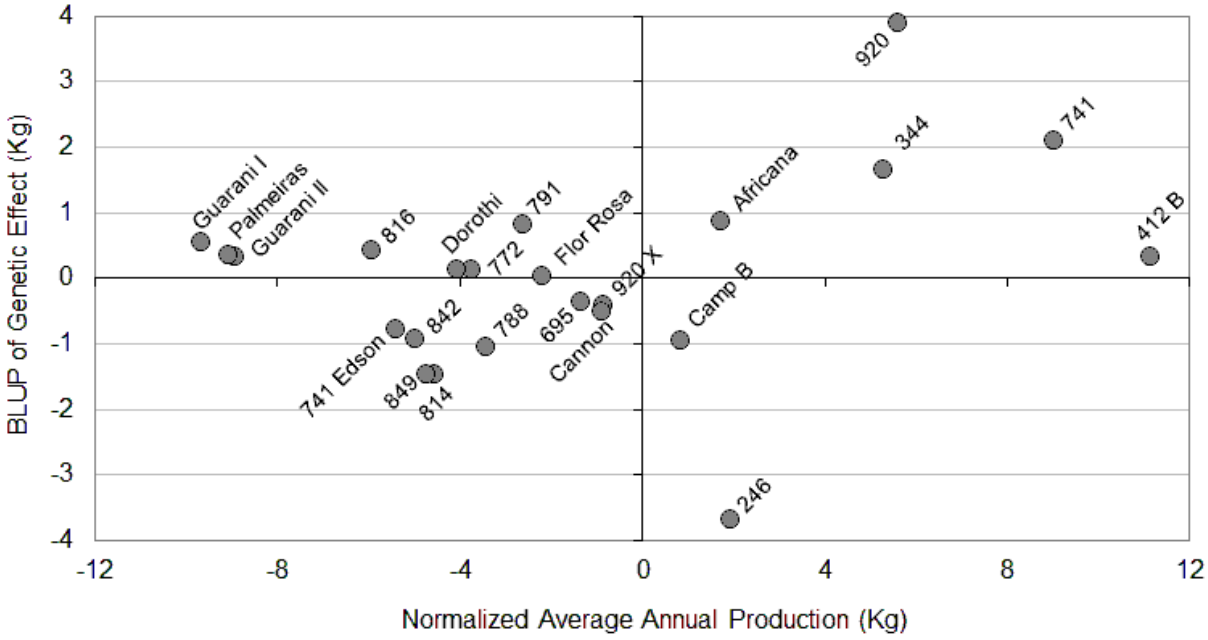

Figure 1. Performances of the genotypes: Genotypic BLUP versus Normalized Phenotypic Mean. The point labels refer to genotype designations (in reduced form, for some cases, in order to clarify the graphic).

An exploratory analysis using the Spearman correlation among the observed means (phenotypic value) and BLUP obtained for genotypes (genotypic value) showed low correlation coefficient (20.65%) and low level of reliability of this association (t test not significant at 5%). This represents the absence of a strong association between the phenotypic and genotypic values in this evaluated population and strongly demonstrates the importance of using the BLUP for the prediction of genetic values. Three groups of interest are presented in Table 3 to explore the best genotypes, correlating them to equivalent groups with highest phenotypic means, by composition and order. In the highlighted central columns are included the elements related to criteria for group formation and correlations among the selection criteria. The resulting classes are shown in two columns left and right, including the group average phenotypic value and the relative degree of performance corresponding to the selection criterion (genotypic or phenotypic). For each criterion, the groups were composed by seven, 10 and 13 genotypes with the best performances, determined by BLUP or average production, respectively. Comparing

equivalent groups between the two selection criteria in terms of composition, result in coincidences of 57%, 50% and 54% of its members, respectively. These degrees of coincidence are obtained both in the overall assessment of the three years, as the separate assessment for each year of production, except for the comparison of groups of seven individuals in the first crop, with only 29% of coincidence. Selection based only on individual phenotypes can lead to discarding genotypes that have a high genotypic value for the trait. In the case of the group of top 10 elements, individuals such as 791, GUARANI I, HAES 816, PALMEIRAS and GUARANI II would be discarded. Analyzing the results for genetic value (Table 3), it can be observed that the groups selected have average rates of relative performance (70-75%) higher than the equivalent rates of selected groups by phenotypic value (30-50%).

Table 3. Grouping genotypes by selection strategy: genotypic classes, phenotypic classes and correlations between the corresponding groups of two strategies

Selection by Genotype						Selection by Phenotype	
Genotypic Class	Average Phenotypic Value	Number of selected	Proportion of selected	Correlation (Spearman)	Coincidence grade(1)	Average Phenotypic Value	Phenotypic Class
>= 75%	13.003	7	30.43%	92.86%	57.10%	14.394	>= 50%
>= 74%	12.286	10	43.48%	44.24%	50.00%	13.577	>= 40%
>= 70%	11.411	13	56.52%	40.66%	53.85%	12.769	>= 30%

(1) Coincidence between members in the genotypic and phenotypic classes in the same level.

Two approaches must be considered to advise the selection of the best genotypes in this study: (a) the selection for vegetative propagation (SelecVP) and (b) the selection for establishment of a base population (SelecBP). Species that can be vegetative propagated allow increasing the efficiency of the selective process (MAIA et al., 2011). This facility factor in the breeding program is essential for accelerate the propagation of superior genotypes and its variance, in special, for species which has a long time to obtain results about aimed characteristics. In the SelecVP case, aimed at formation of production orchards or its improvement, it is recommended the selection of individuals regarding the genotypes whose relative performances were estimated 75% or more (first genotypic class as characterized in Table 3). This strategy represents the selection of approximately 30% of the evaluated genotypes and results in the following top seven genotypes (Table 2): IAC-920, HAES 741-MAUKA, HAES 344-KAU, AFRICANA, HAES 791, GUARANI I and HAES 816. Among these it is found two of the mostly cultivated genotypes: HAES 344 and HAES 741 (WALLACE, 2012). For the SelecBP case, aimed at implementation of a

breeding program, the selected group should be expanded because the genetic variability is highly important for any breeding program, implying in a necessary reduction of the selection differential. Therefore, in the present case study, it is recommended to select genotypes by adopting the relative performance of 70% at least (third genotypic class as characterized in Table 3). With this strategy, the 13 top genotypes are indicated, representing approximately 57% of the evaluated genotypes. This includes those seven already listed and over the following six (Table 2): PALMEIRAS, IAC-412-B, GUARANI II, 772, DOROTHY and FLOR ROSA MG. In a study with a similar purpose and circumstances, but using big number of plants from 312 clones of *Eucalyptus spp*, Garcia (2005) has adopted a greater selection differential for representative clones from the best genotypes. Thus the relative performances were bounded to 80%. The less restrictive selecting factors adopted in this macadamia study were fixed due to the small number of plants assessed.

Considering that the available data have limitations to get a better level of accuracy related to exclusively genotypic selection, a mixed strategy, more parsimonious, is also applicable, taking as criterion the combination of genotypic and phenotypic best performances simultaneously. Using this strategy, as noted earlier (Figure 1), the top five genotypes are: IAC-920, HAES 741-MAUKA, HAES 344-KAU, AFRICANA and IAC-412-B.

According to U.S. International Trade Commission (1998), the macadamia has its first crop economically viable between six to eight years after planting. Topp et al. (2012) emphasizes the high cost for assessing macadamia yield and tried to develop a breeding strategy for reducing it for the macadamia breeding program. All plants used in this study were aged between six and 11 years in the final harvest of the evaluation period, except of the single individual of the genotype PALMEIRAS, three years old. For all plants, the harvest periods coincide with the juvenile period, when it is expected a direct relationship between increasing age and production growth. It is possible clearly infer the occurrence of this relationship in this study (Figure 2a). This finding was confirmed in the applied model by the highly significant (P=0.01) coefficient of 0.737 in the regression between covariable "age" and the trait "production". All plants suitable for group selection SelecVP were aged between seven and 11 years in the last year of harvesting and most aggregated to the formation of group SelecBP were younger, indicating that the results obtained are strongly related with the trait production.

Although the management practices have been identical in all locations (planting sites), the estimators for the influence of this component (Figure 2b) highlighted CHANFLORA and VELHA as the locations in the orchard with best performances for macadamia production. It was observed that both the locations, CHANFLORA and VELHA, had a high incidence of the best individuals, both in relation to the phenotypic mean and in relation to genotypic predictor (BLUP), highlighting the presence of the three best BLUP (IAC-920, HAES 344-KAU e HAES 741-MAUKA). The Figure 2c allows checking the relationship between the effect of number of pickings per harvest in the average of production and in the used model. It is emphasized that, for this fixed effect, the model has presented estimators with production levels higher than the observed means, for nearly all the 18 layers of number of pickings.

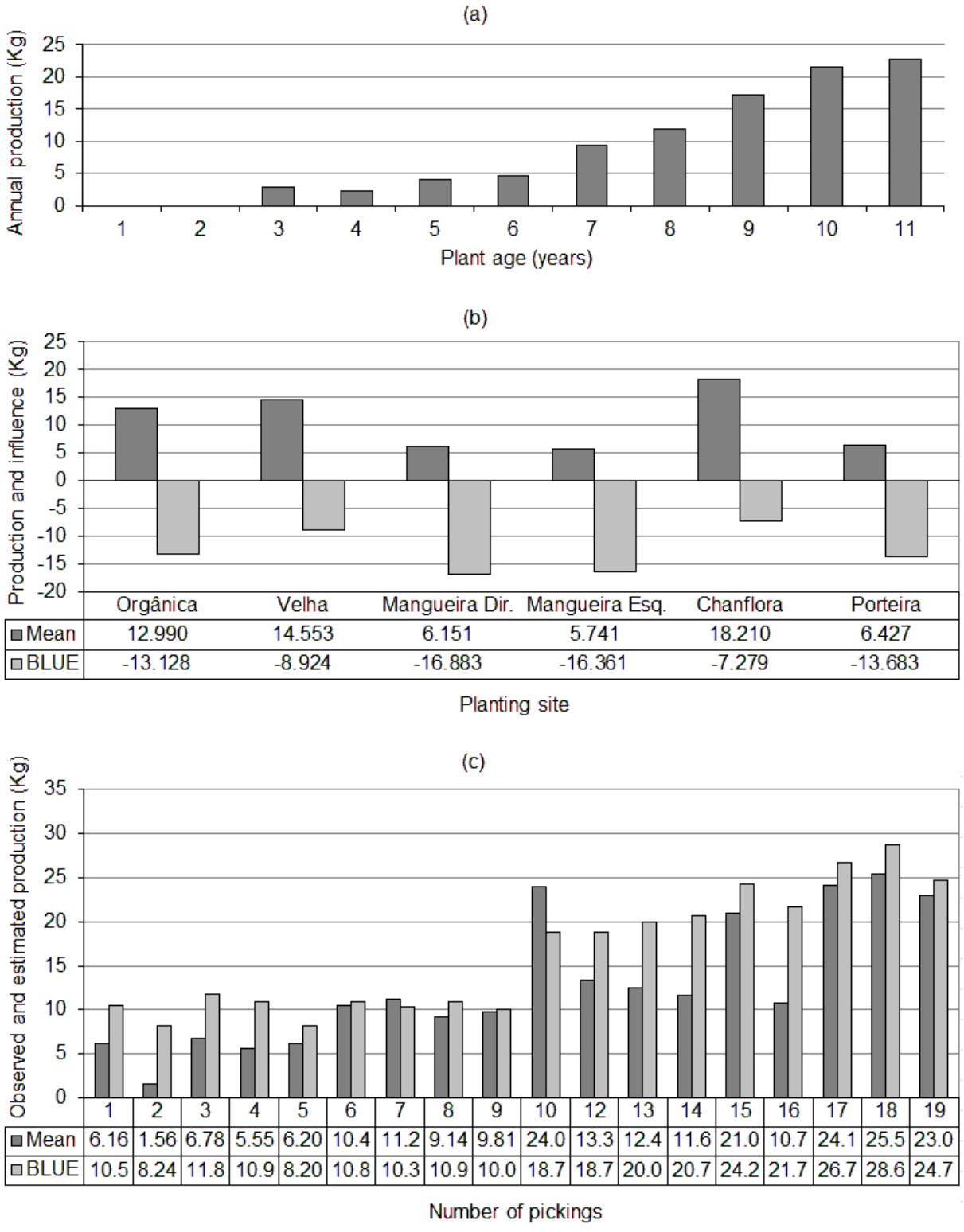

Figure 2. Annual production of macadamia nuts in the study population represented by the evolution during juvenile period (a) and observed means and fixed effects estimators (BLUE) in relation to planting sites (b) and number of pickings per harvest (c). Itapira-SP-Brazil.

CONCLUSIONS

1. The genotypes IAC-920, HAES 741-MAUKA, HAES 344-KAU and AFRICANA are highlighted by different selection strategies, weighted by genotypic and/or phenotypic performance. By the criterion of greater genetic value also stand out, as a result, the genotypes HAES 791, GUARANI I, HAES 816, PALMEIRAS, IAC-412-B, GUARANI II, 772, DOROTHY and FLOR ROSA MG. The genotype IAC-412-B also stands out by the criterion of greater phenotypic value.

2. The age of the plant has direct and significant variation in production during the juvenile period, with an estimated average increase of 0.737 kg per year of age.

3. The results obtained by mixed model methodology (REML/BLUP) and the correlation between genotype and phenotype demonstrate the importance of using BLUP predictors for genetic values in the selection of macadamia genotypes in the population studied.

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