Notas Científicas

Chemical similarity among domesticated and wild genotypes of peanut based on *n*-alkanes profiles

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Abstract – The objective of this work was to analyze the epicuticular *n*-alkane profile of domesticated and wild peanut genotypes. Foliar epicuticular *n*-alkanes of four *Arachis hypogaea* genotypes and two wild species – *A. monticola* and *A. stenosperma* – were analyzed by gas chromatography. Chemical relationships between them were evaluated using the Dice coefficient and UPGMA method. Two clusters were formed: one with four *A. hypogaea* genotypes and the other with the two wild species. There is more similarity between the BR1 and LIGO-PE06 genotypes and between the BRS 151 L-7 and BRS Havana genotypes.

Index terms: *Arachis hypogaea, Arachis monticola*, *Arachis stenosperma*, epicuticular wax, hydric stress, phenetics.

Similaridade química entre genótipos domesticados e selvagens de amendoim baseada no perfil de *n*-alcanos

Resumo – O objetivo deste trabalho foi avaliar os perfis de *n*-alcanos epicuticulares foliares de genótipos de *Arachis hypogaea* domesticados e selvagens. Os perfis de *n*-alcanos epicuticulares foliares de *A. hypogaea* de quatro genótipos domesticados e de duas espécies selvagens – *A. monticola* e *A. stenosperma* – foram analisados por cromatografia gasosa. A similaridade química entre eles foi avaliada pelo coeficiente de Dice e pelo *método* UPGMA. Dois agrupamentos foram formados: um com os quatro genótipos de *A. hypogaea* e o outro com as duas espécies selvagens. Há maior similaridade entre os genótipos BR1 e LIGO PE06 e entre BRS 151 L-7 e BRS Havana.

Termos para indexação: *Arachis hypogaea, Arachis monticola*, *Arachis stenosperma*, cera epicuticular, estresse hídrico, fenética.

Peanut (*Arachis hypogaea* L.) is a major commodity crop, and its origin is in South America. Several species of peanut have been cultivated for their edible seeds, however only *A. hypogaea* has been domesticated and widely distributed (Oliveira et al., 2006). This species is cultivated in different regions of the world, under diverse climate conditions and, in Brazil, it grows, primarily, under rainfed conditions in the northeastern region (Santos et al., 1999). Embrapa has been conducting research on peanut cultivars BR1, BRS 151 L-7 and BRS Havana, among others (Santos et al., 2006).

Different factors are involved in the tolerance to water deficit in peanuts. Thus, some molecular markers have been used to detect genetic variation and the performance of hybrids (Borges et al., 2007). Moreover, genotype selection based on productivity has shown its effectiveness (Hopkins et al., 1999).

Analysis of cuticular constituents, especially the alkanes, has been used to determine taxonomic similarity in native (Medina et al., 2006) and cultivated species (Szafranek & Synak, 2006). The alkanes are universal constituents of the cuticle and, due to their hydrophobic properties, are of crucial importance to reduce water loss. In different peanut genotypes, for example, the amount of alkanes and other constituents of cuticle waxes were found to increase in response to water deficit (Yang et al., 1993; Samdur et al., 2003).

The cuticular lipids have not been studied in any of the peanut genotypes developed by Embrapa. Therefore, the objective of this study was to analyze the epicuticular *n*-alkane profile of four genotypes domesticated and of two wild species of peanut. An additional relationship concerning the tolerance to water stress was hypothesized through a phenotypic analysis based on the distribution of *n*-alkanes.

Seeds of four genotypes of *A. hypogaea* – BR1, BRS 151 L-7, BRS Havana and LIGO-PE06, also known as Goiana – and two wild *Arachis* species – *A. monticola* Krapov. & Rigoni and *A. stenosperma* Krapov. & Gregory – were obtained from Embrapa Algodão, Campina Grande, Brazil. This genotypes was cultivated (January-May 2009) at the Departamento de Botânica of the Universidade Federal de Pernambuco in plastic bags containing 8 kg of soil with the following attributes: sand, loam and silt mixture at 650, 190 and 160 g kg^{-1}, respectively; exchangeable Ca, Mg, and K, in mmol$_c$ dm^{-3}, 0.95, 0.65, and 0.15, respectively; pH (H_2O) 4.7. The soil was not handled and it was irrigated daily to field capacity. The average temperature and relative humidity were 34.6^{o}C and 49.8%, respectively. The plants remained under these conditions for 63 days, and then they were harvested and submitted to chemical assays. The average phenological cycle of these lineages ranges between 80 and 90 days.

The waxes from thirty fully expanded, fresh, intact leaflets from six plants of each genotype of *A. hypogaea* and the two wild *Arachis* species were extracted by means of two rapid (30-sec) washes with dichloromethane, fractionated by preparative thin layer chromatography. The *n*-alkane fractions were analyzed in a gas chromatograph (Oliveira et al., 2003). The identification of the compounds was performed through the comparison of retention times with authentic samples *n*-alkane standard solution C21-C40 (Fluka S.A, Costa Rica) and mass spectrometry was used to confirm the *n*-alkane identities when necessary.

Chemical relationships among the four genotypes and wild species were evaluated using the *n*-alkane profiles. The *n*-alkane profiles between C21 (uncosane) and C35 (pentatriacontane) were treated as a unique character and scored as present (1) or absent (0). A dendrogram was constructed using the Dice coefficient and an unweighted pair-group method analysis (UPGMA). These analyses were performed using the NTSYS-pc software, version 2.11X (Rolf, 2000).

The quantitative distribution of epicuticular *n*-alkanes identified in the four genotypes and the two wild species of peanut were used to investigate their chemical similarity by cluster analysis. Although the qualitative profiles of *n*-alkanes were similar, quantitative differences were found. The occurrence of *n*-alkanes in epicuticular wax of different species of *Arachis*, including *A. hypogaea*, was previously determined by Yang et al. (1993).

Generally, there was a predominance of long-chain *n*-alkanes, such as untriacontane (C31) and tritriacontane (C33), in all genotypes. The long chain *n*-alkanes are important constituents of the waxes and are predominant in native species from arid environments such as the Caatinga biome (Oliveira et al., 2003). Long-chain *n*-alkanes are known to be more resistant to the penetration of polar compounds such as water, which plays an important role in the regulation of leaf water status (Oliveira et al., 2003). Thus, the development of genotypes of peanut with a high content of *n*-alkanes, especially those with a strong hydrophobic character, can improve plant tolerance to water stress.

Analysis of similarity (Dice coefficient and UPGMA) showed the occurrence of two clusters (Figure 1). The first is composed by four *A. hypogaea* genotypes and the second by two wild species. From another point of view, the high occurrence of C33 and C31 *n*-alkanes joins the *A. hypogaea* genotypes with the wild species due to the high similarity coefficient score of 0.93 on a scale ranging from 0 to 1. In the first cluster, there is a higher similarity between the BR1 and LIGO-PE06 genotypes and between the BRS 151 L-7 and BRS Havana genotypes. The main feature that separates the two wild species from these genotypes into a second cluster is the absence of long-chain *n*-alkanes (C34 - tetratriancontane and C35 - pentatriacontane).

Cluster analysis is a statistical method widely used to assess the genetic distance between specimens, differing mainly in the choice of the similarity coefficient. However, in some instances, the use of different similarity coefficients does not significantly alter the final result (Meyer et al., 2004). For example, the use of the Jaccard coefficient did not alter the layout of the phenogram shown in Figure 1. Given that the biosynthesis of cuticle lipids may be influenced by environmental factors, the presence of a character (alkanes) may be more important than its absence, because sometimes metabolites are not detected due to their low concentrations. For this reason, the Dice coefficient was chosen. This coefficient gives a high weight to the presence of a character and does not assume that the dual absence of a character is a factor of similarity.

According to Nogueira & Santos (2000), peanut genotypes analyzed showed different physiological responses to water deficit. These authors evaluated the

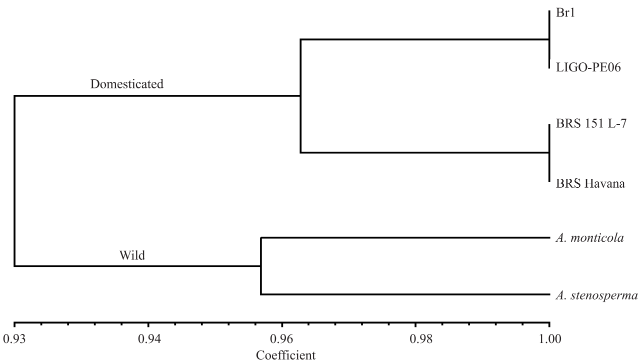

Figure 1. Similarity dendrogram based on epicuticular *n*-alkane distributions in genotypes of *Arachis hypogaea* and wild species of peanut, analyzed by Dice coefficient and UPGMA clustering methods.

performance of the BR1, BRS 151 L-7 and BRS Havana genotypes under water stress, and concluded that the first shows the lowest values of diffusive resistance and negative values of water potential, and for this reason is considered the most appropriate genotype for the northeastern region of Brazil. In the same study, genotypes BRS 151 L-7 and BRS Havana also exhibited tolerance to water stress, with similar transpiration rates. Azevedo Neto et al. (2010) found that genotypes of *A. stenosperma* were more sensitive to water stress than other species.

Although the cluster analysis showed that BR1 genotype has a greater similarity to LIGO-PE06 and BRS Havana genotypes is similar to that of BRS 151 L-7 (Figure 1), this analysis does not reflect the reality of these genotypes. For example, BR1 belongs to subspecies *fastigiata*, while LIGO-PE06 is of subspecies *hypogaea*.

The first genotype is physiologically highly tolerant to water stress, while the second is more sensitive. Thus, the total quantitative composition of the cuticular waxes must be investigated in order to better understand the importance of leaf surface wax of peanut in the adaptations to drought. Further studies on the physicochemical and functional properties of the cuticle may point to new directions in researches on Brazilian genotypes more tolerant to semi-arid conditions.

References

AZEVEDO NETO, A.D.; NOGUEIRA, R.J.M.C.; MELO FILHO, P.A.; SANTOS, R. Physiological and biochemical responses of peanut genotypes to water deficit. **Journal of Plant Interactions**, v.5, p.1-10, 2010.

BORGES, W.L.; XAVIER, G.R.; RUMJANEK, N.G. Variabilidade genética entre acessos de amendoim. **Pesquisa Agropecuária Brasileira**, v.42, p.1151-1157, 2007.

HOPKINS, M.S.; CASA, A.M.; WANG, T.; MITCHELL, S.E.; DEAN, R.E.; KOCHERT, G.D.; KRESOVICH, S. Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. **Crop Science**, v.39, p.1243-1247, 1999.

MEDINA, E.; AGUIAR, G.; GÓMEZ, M.; ARANDA, J.; MEDINA, J.D.; WINTER, K. Taxonomic significance of the epicuticular wax composition in species of the genus *Clusia* from Panama. **Biochemical Systematics and Ecology**, v.34, p.319-326, 2006.

MEYER, A. da S.; GARCIA, A.A.F.; SOUZA, A.P. de; SOUZA JUNIOR, C.L. de. Comparison of similarity coefficients used for cluster analysis with dominant markers in maize (*Zea mays* L). **Genetics and Molecular Biology**, v.27, p.83-91, 2004.

NOGUEIRA, R.J.M.C.; SANTOS, R.C. dos. Alterações fisiológicas no amendoim submetido ao estresse hídrico. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.4, p.41-45, 2000.

OLIVEIRA, A.F.M.; MEIRELLES, S.T.; SALATINO, A. Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. **Anais da Academia Brasileira de Ciências**, v.75, p.431-439, 2003.

OLIVEIRA, E.J. de; GODOY, I.G. de; MORAES, A.R.A. de; MARTINS, A.L.M.; PEREIRA, J.C.V.N.A.; BORTOLETTO, N.; KASAI, F.S. Adaptabilidade e estabilidade de genótipos de amendoim de porte rasteiro. **Pesquisa Agropecuária Brasileira**, v.41, p.1253-1260, 2006.

ROLF, J. F. **NTSYSpc**: Numerical Taxonomy and Multivariate Analysis System, Version 2.1, Exeter Software, Setauket, New York, USA, 2000.

SAMDUR, M.Y.; MANIVEL, P.; JAIN, V.K.; CHIKANI, B.M.; GOR, H.K.; DESAI, S.; MISRA, J.B. Genotypic differences and water-deficit induced enhancement in epicuticular wax load in peanut. **Crop Science**, v.43, p.1294-1299, 2003.

SANTOS, R.C. dos; FARIAS, F.J.C.; RÊGO, G.M.; SILVA, A.P.G. da; FERREIRA FILHO, J.R.; VANSCONCELOS, O.L.; COUTINHO, J.L.B. Estabilidade fenotípica de cultivares de amendoim avaliadas na região Nordeste do Brasil. **Ciência e Agrotecnologia**, v.23, p.808-812, 1999.

SANTOS, R.C. dos; FREIRE, R.M.M.; SUASSUNA, T. de M.F.; REGO, G.M. BRS Havana: nova cultivar de amendoim de pele clara. **Pesquisa Agropecuária Brasileir**a, v.41, p.1337-1339, 2006.

SZAFRANEK, B.; SYNAK, E. Cuticular waxes from potato (*Solanum tuberosum*) leaves. **Phytochemistry**, v.67, p.80-90, 2006.

YANG, G.; ESPELIE, K.E.; TODD, J.W.; CULBREATH, A.K.; PITTMAN, R.N.; DEMSKI, J.W. Cuticular lipids from wild and cultivated peanuts and the relative resistance of these peanut species to fall armyworm and thrips. **Journal of Agricultural and Food Chemistry**, v.41, p.814-818, 1993.

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