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Characterization of high-oleic peanut natural mutants derived from an intersectional cross

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SUMMARY: As compared with its normal oleate counterpart, high oleate peanuts have better storage quality and several health benefits, and are therefore preferred by peanut shellers and consumers. High oleate has now become one of the main breeding objectives of peanuts. Thus far, over 50 high oleate peanut cultivars have been registered. Yet high oleate peanut breeding relies heavily on a limited number of high oleate genotypes. In this paper, we reported, for the first time, high peanut oleate natural mutants with large seeds derived from an intersectional cross, which were identified with near infra-red spectroscopy and confirmed by gas chromatography. Sequencing of *FAD2* from the high-oleic hybrids along with their normal oleate parents indicated that a 448G >A mutation in *FAD2A* coupled with a 441_442ins A or G in *FAD2B* together caused high oleate phenotypes in these peanut hybrids.

KEYWORDS: *Arachis; FAD2A; FAD2B; GC; High oleate; Intersectional hybrid; NIR*

RESUMEN: ***Caracterización de mutantes naturales de maní alto oleico derivados de un cruce interseccional***. En comparación con su homólogo con contenido normal de oleico, el maní alto oleato mantiene una mejor calidad durante la conservación y tiene beneficios para la salud, y de ahí que sea preferido por desgranadoras de maní y por los consumidores. El alto oleato se ha convertido actualmente en uno de los principales objetivos para la mejora del maní. Hasta el momento, más de 50 cultivares de maní alto oleato han sido registrados. Sin embargo, la reproducción de maní alto oleato se basa principalmente en un número limitado de genotipos alto oleato. En este trabajo se presentan por primera vez mutantes naturales de maní alto oleato con semillas derivadas de un cruce de intersecciones, que fue identificado mediante espectroscopia de infrarrojo cercano y se confirma mediante cromatografía de gases. La secuenciación de *FAD2* de los híbridos de alto oleico junto con sus progenitores oleato normal, indicó que la mutación 448G >A en *FAD2A* unido a un 441_442ins A o G en *FAD2B* juntos da lugar a fenotipos alto oleato en estos híbridos de maní.

PALABRAS CLAVE: *Alto oleato; Cacahuete; FAD2A; FAD2B; GC; Híbrido interseccional; NIR*

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1. INTRODUCTION

With extended shelf life and several health benefits, high-oleic peanuts, i.e., peanuts with an oleate to linoleate ratio (O/L) of no less than 9, are preferred by peanut shellers and consumers (Davis *et al*., 2013; Wang *et al*., 2013). The first high-oleic peanut genotype, called UF435 or later F435, was reported by the University of Florida, USA, by Norden *et al*. (1987). It is a natural mutant with an O/L ratio of over 34. In addition to F435, high-oleic chemical/gamma ray peanut mutants have also been identified by other research groups (Wang *et al*., 2014). Of these high-oleic mutants, most were found to have a G >A base change at the 448 position of the coding region of *FAD2A*, and a 441_442insA mutation in the coding region of *FAD2B* (Wang *et al*., 2013). Two high-oleic chemical mutants, however, were discovered to possess MITE (miniature incerted-repeat transposable element) insertions in *FAD2B* (Wang *et al*., 2014). With the mutants, over fifty high-oleic peanut cultivars have been bred and released worldwide. Since high-oleic peanut cultivars currently available are derived from only a limited number of high-oleic peanut parental lines, additional high-oleic sources are still needed to broaden the narrow gene base of high-oleic peanut cultivars.

Tang *et al*. (2013) found an accession of *A. correntina* PI 331192 with 67.91% oleate. Jiang *et al*. (2009) obtained 4 inter-specific hybrids with >64% oleate, of which yz8913-8, an *A. stenosperma* derivative, had the highest oleate (67.85%). Zhang *et al*. (2009) identified three *A. hypogaea* cv Silihong×*A. pusilla* hybrid derivatives with an O/L ratio of 1.7–1.8. However, inter-specific peanut hybrids or wild *Arachis* species with ≥72% oleate or ≥9 O/L have never been reported.

In this work, we report, for the first time, the identification of high-oleic peanut natural mutants derived from an intersectional cross and a G insertion mutation in *FAD2B* of a mutant.

2. MATERIALS AND METHODS

2.1. Peanut materials

The peanut hybrid seeds (F_3) used for fatty acid analysis were from the cross Rihua 1×Rosy Red. Rihua 1 and Rosy Red seeds were used for comparison. Rosy Red is an intersectional hybrid between *A. hypogaea* L. cv Silihong (a Valencia type cultivar) and *A. rigonii* (a section Procumbentes species) produced following a post-pollination hormone treatment of flower bases (Wang *et al*., 2012). Rihua 1 is a peanut variety derived from the cross Luhua 3×Huayu 16. All peanut materials were planted and harvested at the SPRI Laixi Experiment Station.

2.2. Methods

Spectral data of sundried bulk peanut seeds from individual single plants were collected using a near infra-red (NIR) machine (Matrix-I, Bruker Optics, Germany) with a 5cm-diameter rotating sample cup. Each sample was scanned 3 times. The calibration equation for bulk seed samples was used to predict the oleate content of peanut seeds from single plants as previously reported by Wang *et al*. (2011, 2014).

Individual single seeds from a single plant with ≥72% oleate were then scanned with the same NIR machine equipped with a small cup for single seed use purpose. The oleate content was predicted by near infrared spectroscopy (NIRS) calibration equation for single intact peanut seeds (Wang *et al*., 2011; Wang *et al*., 2014).

The fatty acid composition of single peanut seeds was determined by gas chromatography (GC) as described by Yang *et al*. (2012).

DNA was extracted from a slice of cotyledonary tissue distal to the embryo end of a single peanut seed weighing 3–5 mg using the method previously reported from our laboratory (Yu *et al*., 2010). Gene-specific PCR primer pairs, aF19/R1 and bF19/R1 (Patel *et al*., 2004), were used to amplify *FAD2A* and *FAD2B* in peanuts. The PCR mixture was made up of 75 mg peanut DNA template, 0.4 μmol·L^{-1} upstream and downstream primer each, 2.5 mmol·L^{-1}dNTPs, 5 μL of 10×Trans *Taq* HiFi (High Fidelity) Buffer I and 0.6 μL of Trans *Taq* DNA Polymerase High Fidelity (Trans Gen Biotech, Beijing). The thermal cycling program consisted of a pre-denaturation of 3 min at 94 °C, followed by 32 cycles of 94 °C for 40 sec., 53 °C for 40 sec. and 72 °C for 1 min, and a final extension of 5 min. at 72 °C, and was run on a Dongshenglong EDC-810 PCR machine. PCR products were checked on a 1% agarose gel, recovered and purified using a Tiangen Gel Midi Purification Kit (Tiangen, Beijing), and ligated into pGEM-T vectors (Promega, Beijing). Chemically competent DH5α cells of *Escherichia coli* were used in the heat shock transformation. Well isolated white colonies, after PCR screening with the above mentioned primers, were sent for DNA sequencing by Shanghai Sunny Biotechnology Co., Ltd.

The high-oleic peanut seeds (F_3) identified in this study were sown in the field in spring. Conventional agronomic practices were followed. Pods were harvested and sundried in autumn. The number of pods/seeds per plant, pod/seed weight per plant and 100-seed mass for each plant were counted, measured and recorded.

3. RESULTS

3.1. Identification of a peanut plant with ≥72% oleate content

A total of 180 single (Rihua 1×Rosy Red) F_2 plants were harvested and analyzed with the NIRS calibration equations for bulk seed samples predicative of main fatty acid contents. Of them, however, only one plant, F2-420-425-29, was identified as having at least 72% oleate. Individual single seeds (F_3) from the plant were then analyzed with the NIRS calibration equation for single seeds. Of the 20 well-filled single seeds suitable for NIRS analysis, 12 with no less than 72% oleate were first found with the help of NIRS, and then confirmed by GC as with >77% oleate content and >25 O/L (Table 1), whereas the parents, Rihua 1 and Rosy Red, only had less than 50% oleate and their O/L was no more than 2 (Table 1).

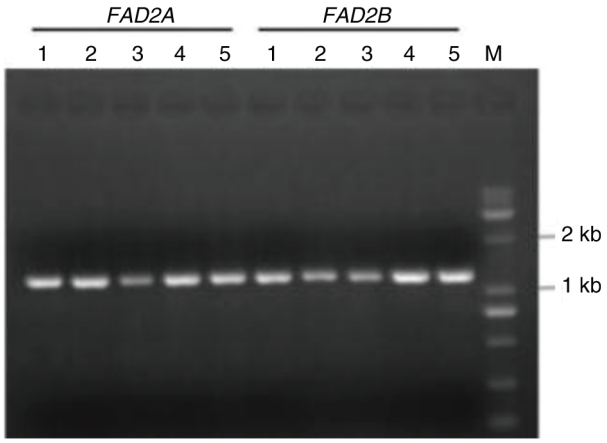

FIGURE 1. PCR products of *FAD2A* and *FAD2B*.1–3: Three Hybrids with >77% oleate (F2-420-425-29-3, F2-420-425-29-7 and F2-420-425-29-13). 4: Rihua 1. 5: Rosy Red. M: Biomiga D2000 Plus DNA Ladder.

3.2. Cloning and sequencing of *FAD2A* and *FAD2B*

Three high-oleic peanut seeds (see the footnote of Table 1) along with their parents were used to prepare DNA templates for the amplification of *FAD2A* and *FAD2B* with high fidelity DNA polymerase. PCR products of expected size were obtained (Figure 1), recovered and cloned. For each seed, 10 well isolated colonies were recovered and sent for sequencing.

All of the *FAD2A* sequences from the 3 high-oleic seeds and from the Rosy Red seed had a G to A base substitution at position 448 of the coding region (448G >A). *FAD2A* from Rihua 1 had a G at this position. The results suggested that the mutated type *FAD2A* in the 3 high-oleic seeds were inherited from their male parent, Rosy Red.

Two of the 3 high-oleic seeds only possessed *FAD2B* with an A insertion in its coding sequence (441_442insA). One seed, F2-420-425-29-3, however,

TABLE 1. Single peanut seeds (F_3) with higher than 72% oleate as confirmed by GC and their parents

	Fatty acids (as percentage of total)								
Seed no./Identity	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C20:0**	**C20:1**	**C22:0**	**C24:0**	**O/L**
Hybrids									
F_2-420-425-29-2	5.55	4.34	79.61	2.03	1.92	1.27	2.58	2.70	39.14
F_2-420-425-29-3[a]	4.98	4.45	80.19	1.38	2.00	1.25	2.70	3.05	58.05
F_2-420-425-29-6	5.08	4.56	80.74	1.85	1.90	1.20	2.40	2.27	43.76
F_2-420-425-29-7[a]	5.51	3.92	80.12	2.01	1.85	1.44	2.70	2.45	39.85
F_2-420-425-29-9	5.07	4.05	81.65	2.00	1.93	1.37	3.06	0.87	40.85
F_2-420-425-29-10	5.32	3.68	81.24	1.90	1.80	1.39	2.82	1.85	42.75
F_2-420-425-29-11	5.84	4.02	79.85	1.98	1.83	1.33	2.72	2.42	40.29
F_2-420-425-29-12	5.25	4.19	80.50	2.09	2.03	1.33	2.91	1.70	38.59
F_2-420-425-29-13[a]	5.15	3.93	81.40	1.73	1.90	1.39	2.94	1.56	47.10
F_2-420-425-29-18	5.41	4.31	80.17	1.65	2.03	1.40	3.06	1.97	48.46
F_2-420-425-29-20	6.16	4.09	77.76	3.08	1.92	1.56	2.95	2.49	25.26
F_2-420-425-29-21	5.07	5.54	79.86	1.62	2.12	1.11	2.34	2.35	49.32
Parents									
Rihua 1	10.84	4.65	45.66	33.32	1.67	0.73	2.02	1.12	1.37
Rosy Red	11.40	2.93	48.93	30.82	1.41	0.98	2.52	1.03	1.59

[a]Three hybrid seeds were randomly selected for cloning of *FAD2A*/*FAD2B*.

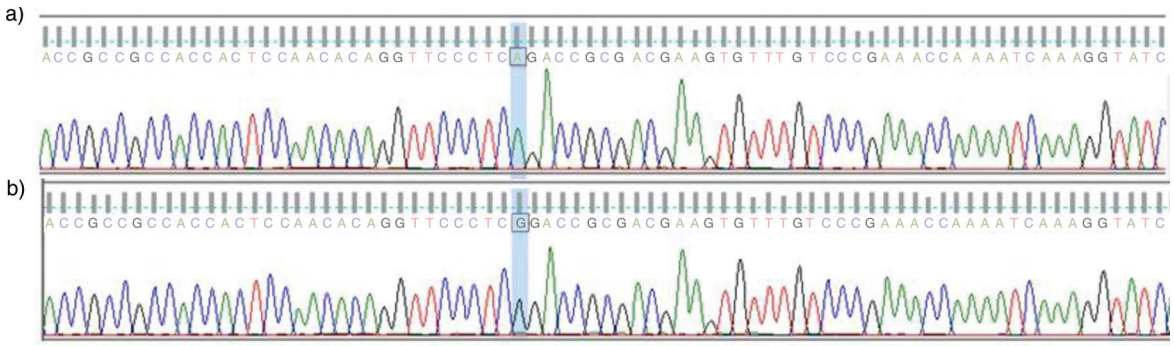

FIGURE 2. Insertion mutations in the coding region of *FAD2B* identified in a high-oleic seeds (F2-420-425-29-3). a) 441_442insA. b) 441_442insG.

had both *FAD2B* with an A insertion (441_442insA) (Figure 2a) and *FAD2B* with a G insertion (441_442insG) (Figure 2b). For the seed, 8 of the 10 colonies sent for sequencing were found to have a 441_442insA in *FAD2B*, whereas the other two possessed a 441_442insG in *FAD2B*. Neither of the insertions could be found in the parents, Rihua 1 or Rosy Red.

3.3. Performance of the high-oleic peanut mutants

Twelve high-oleic peanut F_3 seeds from the present study were sown in May, 2014. The plants developed normally and seeds were harvested in Sept 2014. The number of pods per plant varied from 29–51, and the number of seeds per plant ranged from 49–89. The 100-seed mass was 82.64–119.44 g. Pod weight and seed weights per plant were 44.56–82.61 g and 32.97–60.98 g, respectively. The figures could be transformed into yields of 3226.06–5980.81 kg per hectare for pods and 2386.97–4414.84 kg per hectare for seeds.

4. DISCUSSION AND CONCLUSIONS

4.1. Origin of the high oleate phenotype

Only the peanuts with no less than 9 O/L ratios can be termed as high-oleic peanuts (Davis *et al.*, 2013). As such, the peanut hybrid seeds in Table 1 lived up to the high-oleic standard.

Since in the fields of the SPRI Laixi Experiment Station, the intersectional hybrids in the study were well isolated from the high oleate lines/varieties/derivatives of other origin, the possibility of out crossing can be fully excluded, which was supported by the low frequency of high oleate plants/seeds in F_2/F_3 generations. A novel mutation, G insertion in the coding sequence (441_442insG) in *FAD2B*, further confirmed this hypothesis, suggesting that the high oleate intersectional hybrids were natural mutants.

4.2. Molecular basis of the high oleate phenotype

Earlier studies showed that a 448G > A mutation in *FAD2A* caused an amino acid change (D150N), and severely reduced oleoyl-PC (phosphatidychloline) desaturase activity (Bruner *et al.*, 2001). Reportedly, 441_442insA, a frame shift in *FAD2B*, resulted in a truncated, inactive protein and the loss of one of the histidine boxes believed to be important to the enzyme activity of oleoyl-PC desaturase (López *et al.*, 2001; Yu *et al.*, 2008). The 441_442insG in the *FAD2B* of peanuts has not been reported previously. Frequent occurrence of a base insertion between the 441 and 442 positions in the coding region of *FAD2B* in our studies and other authors' reports indicated that this site is likely to be a hot spot of mutation in peanuts (Yu *et al.*, 2008; Wang *et al.*, 2014). Similar to the 441_442insA, the G insertion in the *FAD2B* coding region may also lead to a shortened oleoyl-PC desaturase with reduced enzyme activity.

To summarize, 448G >A mutation in *FAD2A* and 441_442insA and/or 441_442insG in *FAD2B* together contributed to the high oleate phenotype of the three peanut intersectional hybrid derivatives in this study.

4.3. Usefulness of the high-oleic mutants

Thus far, no high-oleic peanut intersectional hybrids or wild *Arachis* species have been reported. Presently available high-oleic sources were all mutants of the cultivated peanut with small- or medium- sized seeds. The high-oleic peanut mutants identified in the study, however, were intersectional hybrid derivatives with larger seeds. Moreover, some of them exhibited high productivity. Utilization of the high-oleic sources from the present study in peanut breeding programs may help breed high-oleic peanut cultivars with high yield potential and high genetic diversity.

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