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Improvement of Groundnut for Fatty Acids using Marker Assisted Breeding Approaches

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ABSTRACT

The cultivated groundnut (Arachis hypogaea L.), is self-pollinated, allotetraploid (2n = 4x = 40) with a genome size of 2891 Mbp, originated through a single hybridization and polyploidization event. Groundnut oil comprises about 80 % unsaturated fatty acids (UFA) and 20% saturated fatty acid. Consuming oils with high levels of oleic acid is beneficial to human health because it reduces low-density lipoproteins, maintain high-density lipoprotein, slow down atherosclerosis, and reversing the inhibitory effect of insulin production. As a result, peanut oil with a high percentage of oleic acid is preferred, and the oil quality can be determined by the oleic acid and linoleic acid (O/L) ratio. Fatty acid composition of groundnut oil is an important trait from human nutrition point of view as well as oil stability during the storage. Fatty acid desaturase (FAD) enzyme facilitate the conversion of oleic acid to linoleic acid by adding double bond to oleic acid. This enzyme is coded by two homologous genes (ahFAD2A and ahFAD2B) located on A and B sub genomes. Groundnut breeding for foliar disease resistance with desirable fatty acid composition can help in getting improved varieties. In conventional breeding, selection for fatty acid composition is carried out in advance generations, thus requires huge resources to handle and more time. However, it is possible to reject large number of plants in early generations with use of makers associated with ahFAD2 mutant alleles, thus optimizing resources and time.

Key word: *Groundnut, fatty acids, MAS, FAD*

INTRODUCTION

The cultivated groundnut also called peanut (*Arachis hypogaea* L.) is an important oilseed crop grown extensively in tropical and subtropical regions in more than 100 countries. India is second largest producer of groundnut and its oil after China. Groundnuts are valued for their high-quality oil and about 65% of the world's groundnuts are utilized for edible oil. In India, it is cultivated on about 3.7 million ha with the production and productivity of 6.7 million tons and 1810 kg/ha respectively during 2015-16.

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Groundnut kernels contain 40-60% oil, 20-40% protein, and 10-20% carbohydrates and 567 kcal of energy can be gained from 100 g of kernels. Based on the source of origin and varietal group in groundnut, large genetic variability for oil content (36.0–60.3%) was noticed[3]. Peanut seed contains about 44-56% oil, and its seed quality is largely determined by the fatty acid composition. Groundnut oil consists of triglycerides (96.1–96.4%), phospholipids (2.4– 2.9%), sterols (0.69–0.80%), free fatty acids (0.1–0.4%) and glycolipids (0.10–0.14%). Eight fatty acids can be routinely detected in peanut seeds; however, two major fatty acids, oleic acid (C18:1, D9) and linoleic acid (C18:2, D9, D12), account for approximately 80% of the fatty acid composition[14,13]. Major fatty acids in groundnut oil are palmitic acid (8–11%), oleic acid (36–52%) and linoleic acid (24–43%). Fatty acid composition of groundnut oil is an important trait from human nutrition point of view as well as oil stability during the storage. Consuming oils with high levels of oleic acid is preferred because oleic acid may be beneficial to human health by reducing low-density lipoproteins (LDL), maintaining high-density lipoproteins (HDL), slowing down atherosclerosis, and reversing the inhibitory effect of insulin production[19]. To facilitate marker-assisted selection for the high-oleate trait, different types of DNA markers from these two genes have been developed, including cleaved amplified polymorphic sequence markers for FAD2A and FAD2B[7] real-time PCR marker for FAD2B[4] and allele-specific PCR markers for both FAD2A and FAD2B[6]. A genotyping assay for detecting FAD2A mutation by real-time PCR and an efficient method for profiling peanut fatty acids by gas chromatography (GC) analysis have been developed and established[4,21]. Conventional breeding methods in combination with molecular markers are currently being applied in several crops, including groundnut to get much faster results, with more accuracy. In current review, we have tried to give overview of work done to improve desirable fatty acid composition in groundnut by applying molecular techniques.

Molecular Breeding for desirable fatty acid composition:

Peanut seeds contain about 44-56% oil compost of up to 12 fatty acids but two major fatty acids oleic acid (C18:1, Δ9) and linoleic acid (C18:2, Δ9, Δ12) account for approximately 80% of the oil composition[13]. The biochemical difference between these two fatty acids is that linoleic acid contains one more double bond at the Δ12 position of the hydrocarbon chain than oleic acid. Fatty acid desaturase (FAD) enzyme facilitate the conversion of oleic acid to linoleic acid by adding double bond to oleic acid[17,18]. This enzyme is coded by two homologous genes (ahFAD2A and ahFAD2B) located on A and B subgenomes of groundnut. Peanut oils with high percentage of linoleic acid are prone to oxidation, off-flavours, leading to rancidity and short shelf-life during seed storage. Consuming oils with high levels of oleic acid is beneficial to human health because it reduces low-density lipoproteins, maintain high-density lipoprotein, slow down atherosclerosis, and reversing the inhibitory effect of insulin production[19]. As a result, peanut oil with a high percentage of oleic acid is preferred, and the oil quality can be determined by the oleic acid and linoleic acid (O/L) ratio. Getting improved lined of groundnut having foliar disease resistance and high fatty acid content is one of the important aspect for groundnut breeding and it can be achieved in less time and with more accuracy using molecular breeding approaches. First peanut, mutant line, F435 with 80% oleic acid and 2% linoleic acid was reported in 1987 by Norden *et al.*[14]. By applying conventional breeding methods, the first ever high oleate peanut line, SunOleic 95R (80% oeic acid) was bred in USA[8]. This genotype carries mutation in both ahFAD2A and ahFAD2B genes. After development of associated markers to the mutant alleles marker-assisted backcrossing (MABC) was used to improve oleic acid content of a nematode resistant variety, 'Tifguard' with high O/L[7]. In

conventional breeding, selection for fatty acid composition is carried out in advance generations, thus requires huge resources to handle and more time. However, it is possible to reject large number of plants in early generations with use of makers associated with ahFAD2 mutant alleles, thus optimizing resources and time. A peanut breeding line containing about 80% oleic acid and 2% linoleic acid was first identified by Norden *et al.*[14]. The incorporation of high-oleic genes into new peanut breeding lines resulted in the SunOleic cultivar, a high-oleic variety released by the Florida Agricultural Experiment Station in 1995[8]. The SunOleic peanut variety has a favourable high oleic acid content and consequently extended shelf life.

Fatty acid composition of groundnut oil is an important trait from human nutrition point of view as well as oil stability during the storage. The modified profiles of the fatty acids provide an opportunity to choose a particular brand of groundnut oil for individual requirement and preference. The first two peanut high-oleate mutants (with about 80% oleic acid and 2% linoleic acid) were identified in F435 experimental lines (F435-2-1 and F435-2-2) by screening fatty acid composition[14]. The first plant gene characterized for Δ12 fatty acid desaturase (FAD2) was cloned in Arabidopsis by a forward genetics approach[15]. Successful introgression of the FAD mutant alleles from SunOleic 95R with increase oleic acid has already been reported using marker assisted breeding approaches[4,7,10]. Fatty acids can be esterified by saponification-transesterification method[11] and can be identified by comparing the retention time of standard fatty acid methyl ester mixture under same temperature condition and gas flow rate in Gas Liquid Chromatography. Wang *et al.*[23] has done real-time PCR (RT-PCR) to determine the FAD2 genotype and determine the fatty acid composition by gas chromatography analysis. The linked allele-specific[6] and cleaved amplified polymorphic sequences (CAPS)[7] markers for both the ahFAD2 genes (ahFAD2A and ahFAD2B) are available for use in molecular breeding. Currently, there are multiple methods i.e., real-time PCR[4], AS-PCR[6] and CAPS markers[7] for marker assisted breeding to employ to determine high oleic versus normal oleic peanuts. Further, the deployment of marker assisted selection increase breeding efficiency of conventional breeding approaches leading to the rapid development of improved cultivars[9,20]. In this context, Pandey *et al.*[16] reported the relationship of FAD2 genes with peanut oil quality and Wang *et al.*[22]has done quantitative trait locus (QTL) study for the minor fatty acids except oleic (C18:1) and linoleic (C18:2) acids in peanuts. Wang *et al.*[22] have reported correlations among different saturated and unsaturated fatty acids, QTLs controlling these fatty acids, identification of consistent QTLs for these fatty acids, and effect of FAD2A and FAD2B mutant alleles on these fatty acids. Recently Janilla *et al.*[10] has applied molecular breeding approach for introgression of fatty acid desaturase mutant alleles (ahFAD2A and ahFAD2B) to enhance oil quality in high and low oil containing peanut genotypes.

Summary:

Cultivated peanut is an allotetraploid and typically contains about 50% oil in the seeds. The majority (~80-90%) of the extracted oil is composed of three primary fatty acids: palmitic (C16:0), oleic (C18:1), and linoleic (C18:2). The flavour, stability, shelf life, and nutritional quality of peanut and peanut products are dependent on the fatty acid composition (ratio of saturated, monounsaturated, and polyunsaturated lipids) of the extracted oil[1,13]. Oleic, linoleic and linolenic acids have been reported to lower the plasma cholesterol levels and low-density lipoproteins but, higher proportion of polyunsaturated fatty acids (PUFA) in oil increases the chances of oxidation, which leads to unpleasant odours and tastes, thus oil cannot be stored for long time. Replacing partially hydrogenated oil by high oleate groundnut oil can minimize the consumption of Trans fatty acids and/or problems of PUFA which are not healthy. Saturated fatty acids, on the contrary, are stable, but they are associated

with heart problems[5]. The ratio of oleic to linoleic acid (O/L) is a determinant of oil stability. Oils with higher O/L ratio are less prone to oxidation and off flavours and also extend the shelf life by delaying the development of rancidity, also reduce the risk of cardiovascular disease, prevent cancer, increase insulin sensitivity and ameliorate some inflammatory diseases. Mondal *et al.*[12]has report isolation of high oleate groundnut mutant through gamma ray and sodium azide mutagenesis and also confirm high oleate trait in this mutant is due to a point mutation from guanine to adenine. Understanding of fatty acid metabolism pathway in peanut and pyramiding favourite alleles from different fatty acids by marker-assisted selection for improving peanut fatty acid composition. Groundnut varieties with desirable fatty acid content can be useful in breeding programme in combination with high yield, short duration, drought tolerance or foliar diseases resistance for getting improved groundnut variety and it can be achieved faster by applying molecular breeding approaches.

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