CHARACTERIZATION OF GROUNDNUT CULTIVARS FOR AhFAD2 ALLELE POLYMORPHISM

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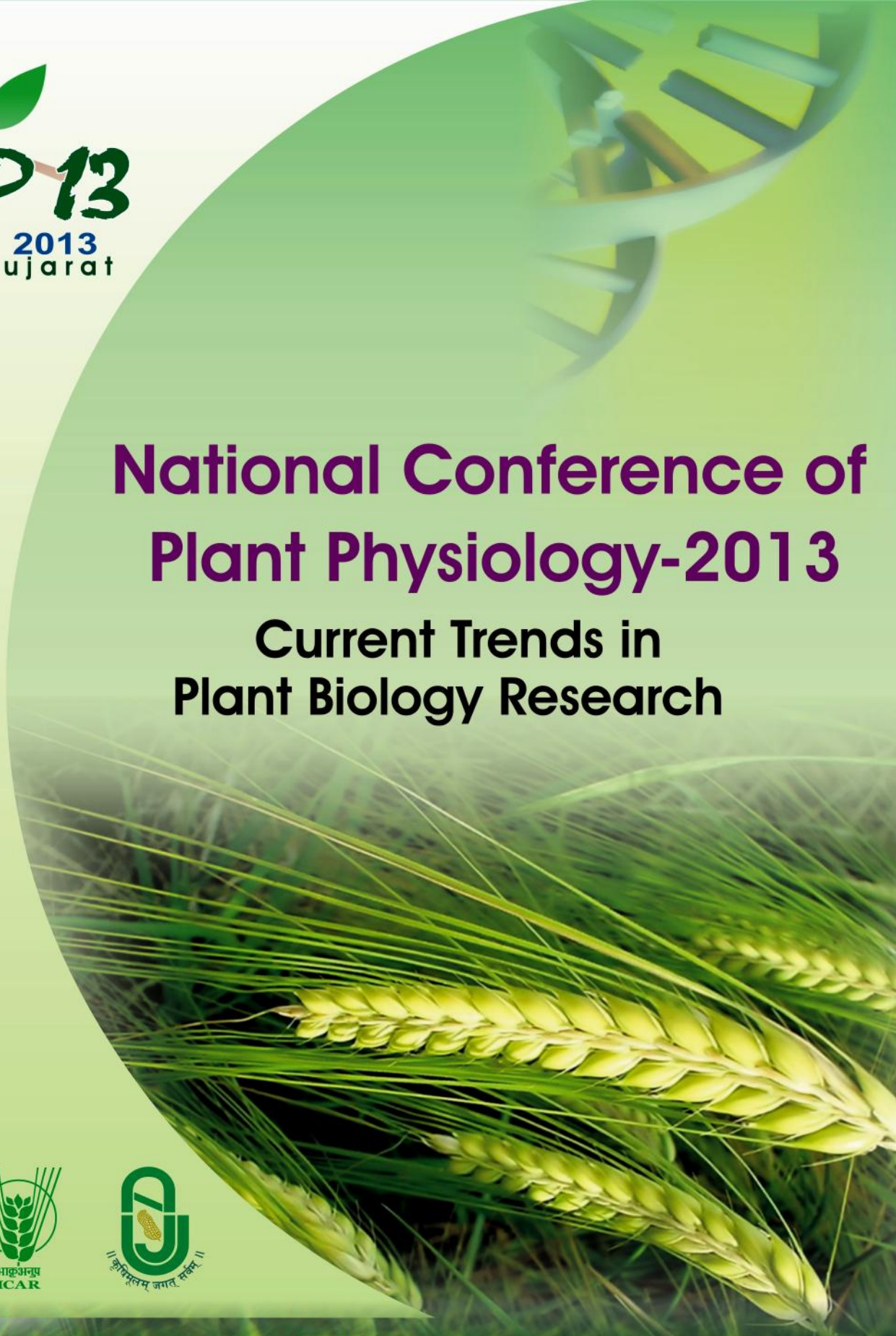

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O 6-8. CHARACTERIZATION OF GROUNDNUT CULTIVARS FOR *AHFAD2* ALLELE POLYMORPHISM

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INTRODUCTION

Cultivated groundnut (*Arachis hypogaea* L.) is one among the five most important oilseed crops. Its seeds contain 45-51% oil which consists of about 12 fatty acids. Among these there are two predominant fatty acids viz., oleic (C18:1, Δ9) and a linoleic (C18:2, Δ9,Δ12) (Dean *et al.,* 2009) which account for approximately 80% of the oil composition. Oleic acid is a highly stable fatty acid compared to linoleic acid; therefore, groundnut oils containing high oleic acid and low linoleic acid levels are strongly demanded since it would improve both oil stability and nutritional quality. Thus, breeding groundnut variety with high oleic acid/linoleic acid (O/L) has become one of the major goals in plant breeding.

Since the cultivated groundnut is an allotetraploid, homoeologous genes exist in the A and B genomes. Loss of function of oleoyl-PC desaturase activity with mutant alleles *ahFAD2A* and *ahFAD2B* results in high oleate trait in groundnut. Two homoeologous genes for Δ12 fatty acid desaturase (i.e. *FAD2A* and *FAD2B*) were isolated and characterized in groundnuts which are responsible for the conversion of oleic acid to linoleic acid (Jung *et al.,* 2000). This study was done to find the mutation(s) in the *ahFAD2* gene(s) of released varieties of India, so as to establish the relationship between normal and high O/L ratio traits for groundnut.

MATERIALS AND METHODS

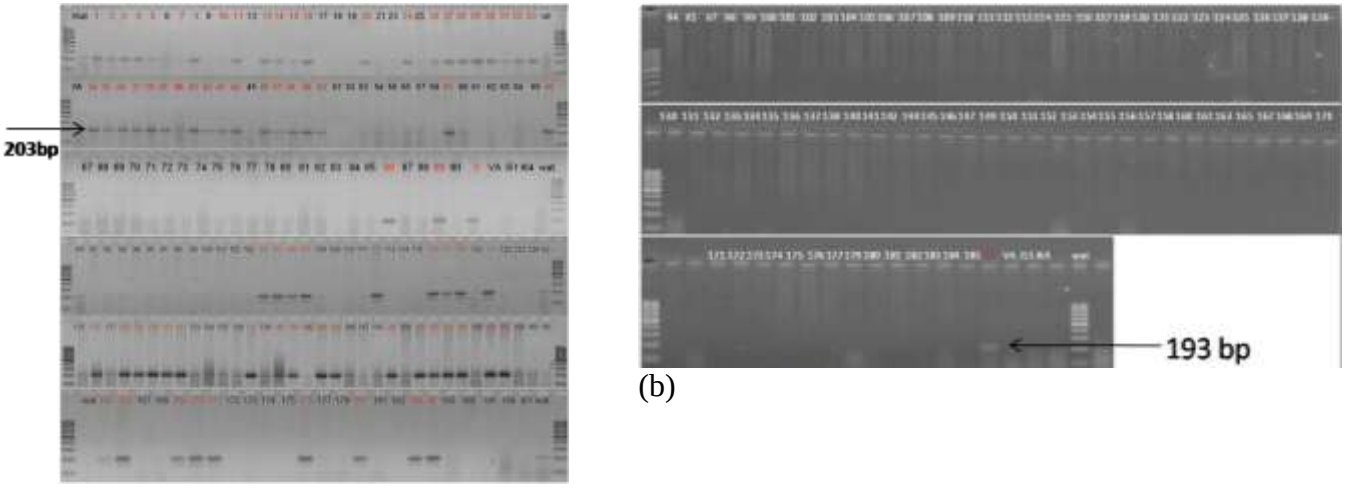
A total of 172 release varieties were used for the genetic characterization using Allele Specific PCR markers. The gene on the A genome was designated as *FAD2A*, and the mutant allele (*fad2A*) had 1-bp substitution (G:C→A:T) at position 448 after the start codon, resulting in a missense amino acid substitution from aspartic acid to asparagine (D150N). The gene on the B genome was designated as *FAD2B*, and the mutant allele (*fad2B*) had a 1-bp insertion (A:T) at position 442 after the start codon, resulting in a frame-shift (Jung *et al.,* 2000). Chen *et al.* (2010) reported a PCR assay by designing allele-specific primers which can be used to detect mutant and wild-type alleles of *FAD2* on both the A and B genomes of groundnut. Details about the markers, its primer sequence are given in the table 1 which was used in the present investigation.

Table 1. Allele-Specific PCR Primers for characterization of *FAD2A* and *FAD2B* mutations (Chen *et al*., 2010)

Primers for *FAD* gene	Primer Sequence (5′ to 3′)	Expected size (bp)
F435-F	ATC CAA GGC TGC ATT CTC AC	-
F435IC-R	CTC CCT GGT GGA TTG TTC ATG T	250
F435WT-R	ACT TCG TCG CGG TCG	193
F435SUB-R	TGG GAC AAA CAC TTC GTT	203
F435INS-R	AAC ACT TCG TCG CGG TCT	195

RESULTS AND DISCUSSION

In our study with AS-PCR characterization of released varieties of India, we observed that out of 172 varieties studied 78 were found to have the *ahFAD2A* mutant allele. However none of the released varieties studied is found to have *ahFAD2B* mutant allele (Figure 1) in its background. Recently in a study by Mukri et al. (2012) on ICRISAT mini core collection, *ahFAD2A* mutant allele was found in 49.5% of the accessions and this mutation had a maximum contribution of 18.82, 12.98 and 10.52 towards the phenotypic variance of O, L and O:L ratio, respectively. However, genotypes with high oleic acid levels could not reveal 'A' insertion mutation in ahFAD2B. Further analysis of O/L ratio of these varieties and its association with SNP polymorphism in the ORF of *ahFAD* allele is underway.



(a) (b)

Figure 1. Mutant *FAD2A* (a) and *2B* (b) allele screening in the released varieties using AS-PCR markers.

CONCLUSION

Since, direct estimations of the kernel oil quality requires costly instrumentation therefore, identification of donor parents with mutations in *ahFAD* will help in breeding for high O/L. Genotyping and phenotyping data obtained for oil quality will be used for selecting parental genotypes with high O/L ratio which would be utilized in the marker assisted breeding programme for the development of cultivars with better O/L oil quality.

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