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Single Marker Analysis Using Transposon Specific Markers (AhMITE1) for Yield, Foliar Disease Resistance and Oil Quality in a Mutant Population of Groundnut (*Arachis hypogaea* L.)

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A B S T R A C T

A population of 53 mutants, their parents and eight cultivated varieties belonging to two subspecies of groundnut were evaluated during *kharif* 2012 for productivity, oil quality traits and resistance to late leaf spot (LLS) and rust diseases to assess association between these traits and the *Arachis hypgaea* Miniature Inverted-Repeat Transposable Elements (*AhMITE1*) markers. Genotypes showed significant differences and high PCV and GCV for all the traits. Of the 41 transposable elements (TE) markers used for genotyping the population, 24 showed high to moderate genotype discriminating power in terms of polymorphism information content (PIC). Single marker analysis revealed significant association of AhTE205 with number of pods per plant, pod yield per plant and shelling percentage. While the other markers that should significant association are AhTE333 with test weight, AhTE343 with LLS (90 DAS), AhTE373 with oil content (%) and AhTE211 with oleate content and O:L ratio. These markers need to be validated for their trait association before they are employed for groundnut improvement.

Introduction

Cultivated groundnut (*Arachis hypogaea* L.) is a tetraploid with an AB-genome (2n=4x=40) of recent origin, arising from hybridization of two wild species followed by spontaneous chromosome duplication. Subsequent formation of two subspecies (ssp. *hypogaea* and ssp. *fastigiata*) and several botanical varieties of domesticated groundnut (Krapovickas and Gregory, 1994) probably took place when the primitive form of groundnut was used for cultivation where it was subjected to artificial selection pressures (Kochert *et al.*, 1996).

In addition to the artificial selection, several evidences have been presented in support of "Mutational theory" of evolution of groundnut. The possible role of spontaneous and induced mutations in the evolution of abundant morphological variation in groundnut was also evident from various subspecific changes brought about by

mutations in a few breeding programmes (Mouli *et al.,* 1979; Prasad, 1989; Gowda *et al.,* 1996).

Dharwad Early Runner (DER) a true breeding variant identified from a cross between two *fastigiata* cultivars *viz.*, Dh 3-20 and CGC-1, sharing the characters of both the subspecies of *A. hypogaea* upon mutagenesis with ethyl methane sulphonate (EMS) yielded a high frequency of mutants resembling all the four botanical varieties (Gowda *et al.,* 1996). Some of the mutants produced germinal reversions to DER in later generations, indicating genetic instability. In many cases, the breeding behaviour of mutants revealed several unusual features (such as homozygous mutations, mutation outbursts, segregation distortions, somatic mutations and multiple character mutations) that could not be explained through conventional mutation theory, indicating the activation of cryptic transposable elements (TEs) as the possible cause of mutations (Gowda *et al.,* 1996).

Further investigations on such mutants showed the activity of miniature inverted repeat transposable element (MITEs) in the mutational and evolutionary origin of botanical types of groundnut (Bhat *et al.,* 2008; Gowda *et al.,* 2010; Gowda *et al.,* 2011). MITEs are the non-autonomous class II type transposable elements (Osborne *et al.,* 2006) that make up the predominant TEs among plant genomes (Wessler *et al.,* 1995; Shan *et al.,* 2005; Naito *et al.,* 2006). Transposition preference for low copy genic regions signifies the role of MITEs in modulating gene expression (Wessler, 1998; Zhang *et al.,* 2000; Wessler, 2001; Lu *et al.,* 2012) and aiding crop evolution (Ma and Bennetzen, 2004; Shan *et al.,* 2005; Naito *et al.,* 2006).

Since the distribution pattern of MITE insertion varies across individuals and germplasm, TE-specific markers were developed (Bonin *et al.,* 2008; Grzebelus *et al.,* 2009; Monden *et al.,* 2009) and the distribution pattern and frequency of excision of a MITE were determined. A total of 504 PCR based markers were developed using primer pairs designed against both flanking sequences of each MITE element. These markers showed considerably high polymorphism (22.0%) (Shirasawa *et al.,* 2011) than that of the SSR markers (Koilkonda *et al.,* 2012; Pandey *et al.,* 2012). Later through *in silico* analysis, another 535 transposon markers were developed and validated (Shirasawa *et al.,* 2012b). Transposon markers, like SSR markers, represent potent, co-dominant, and PCR-based markers. They basically detect the transposition of *AhMITE1* at various sites, preferably in the genic region in the genome. A large population of independent mutants which differ for productivity, foliar disease resistance and quality traits was developed and characterized at UAS, Dharwad (Gowda *et al.,* 1989; Gowda and Nadaf, 1992; Gowda *et al.,* 1996). A detailed phenotypic and genotypic analysis with TE markers would help in identifying the markers that are significantly associated with productivity, foliar disease resistance and quality traits.

Identification of marker(s) significantly associated with any trait of economical and agronomical importance is always useful in crop improvement. Trait-marker mapping can be done through linkage and/or association mapping (Pasupuleti *et al.,* 2013). Identification of DNA markers strongly associated with productivity, foliar disease resistance and quality traits may prove useful for groundnut improvement through marker assisted selection. The present investigation was undertaken to evaluate and characterize mutants for associating productivity, disease resistant and quality traits using transposon specific (*AhMITE1*) markers.

Materials and Methods

Field experiment

The study employed a mutant population of 53 mutants their parent DER and eight check varieties. The field experiment was conducted during *kharif* 2012 at IABT Garden of Main Agricultural Research Station, UAS, Dharwad belonging to transitional tract of Karnataka. The experimental block consisted of vertisol soil with pH of 7.0-7.5. The mutants and parents of the mutant population were sown with two rows of 2.50 m in a Randomized Complete Block Design (RCBD) with a spacing of 45 cm between rows and 10 cm between plants in two replications. Standard package of practice was followed to raise the crop. Mutants and their parents were evaluated for growth, morphological, productivity and nutritional traits, and response to late leaf spot and rust disease. Most of the field observations were recorded on five randomly selected plants as per the groundnut descriptor (IBPGR\ICRISAT, 1992) and the mean value was computed for each genotype.

Genotyping of mutants with markers transposon specific (*AhMITE1*) markers

DNA was isolated from the young leaves of mutants and parents and check varieties grown in the field by following the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Cuc *et al.,* 2008). DNA yield was quantified by using Nano Drop (UV technologies, USA). DNA concentration and purity was also checked by running the samples on 0.8% agarose gel with known concentration of uncut lambda DNA of 50 ng/µl, 100 ng/µl and 200 ng/µl. The DNA stocks of the samples were diluted to a working concentration of 5 ng/µl. Mutants and parents were genotyped with transposon marker (AhTE markers) (Shirasawa *et al.,* 2012a; Shirasawa *et al.,* 2012b). DNA amplification was performed in a 20 µl reaction mixture with appropriate PCR profile using Eppendorf Mastercycler® pro and BIO-RAD T100^{TM} Thermal cyclers. Touch-down PCR was carried out in a final volume of 20 ll containing 50 ng genomic DNA, 10X PCR buffer, 2 mM dNTPs, 10 pmol of each primer and 1 U of Taq DNA Polymerase (New England Biolabs, Ipswich, MA, USA) for the rust and LLS resistance-linked markers. Amplification was carried out in a mastercycler (Eppendorf, Hamburg, Germany) by setting the conditions for one cycle of pre-denaturation (94°C for 5 min), 38 cycles of denaturation (94 °C for 2 min), annealing (starting from 52 °C for 1 min with a decrease of 1 °C/cycle for the first five cycles) and extension (72 °C for 2 min). One cycle of final elongation (72 °C for 10 min) was included before the product was held at 4 °C for 30 min. The PCR products were mixed with 2 µl of loading dye (Bromophenol blue) and loaded on 1.5% agarose gel prepared in 1X TAE buffer containing ethidium bromide (5 µl/100 ml). Products were separated at 80 volts till they clearly resolved. The gel was observed and documented with DNA Bio-Imaging system.

Scoring the alleles

Specific PCR product was identified for each marker (Shirasawa *et al.,* 2012a; Shirasawa *et al.,* 2012b) and scored as "2" (with *AhMITE1* insertion), "1" (without *AhMITE1* insertion) or "0" (absence of specific PCR product).

Statistical analysis

Statistical analysis for the phenotypic data was carried out using statistical package Windostat Version 8.1 available at Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. Analysis of variance (ANOVA) for

different characters was computed by using the mean phenotypic data on each genotype in order to partition the variation due to different sources following the method given by Panse and Sukhatm (1954). Molecular marker data was analyzed for polymorphic information content (PIC), and the association of the markers with productivity, disease resistance and quality traits was tested by Single marker analysis (SMA) using WinQTL Cartographer version 2.5 (Wang *et al.,* 2007).

Results and Discussion

Analysis of variance for the mutant population consisting of a mutant population consisting of 53 mutants their parent DER and eight check varieties showed significant genotypic differences for all the productivity and quality traits studied. Genotypes also differed significantly for rust and LLS resistance except for LLS at 70 DAS. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) revealed high variability for number of pods/plant and pod yield/plant, and moderate variability for other traits (Table 1). LLS and rust resistance at three stages exhibited moderate variability (Table 2). Similarly, nutritional traits showed moderate or low variability (Table 3). Number of pods/plant and pod yield/plant also showed very high heritability and genetic advance over mean, indicating the scope for selection among the genotypes. SMK, test weight and pod length showed high heritability though they had moderate level of variability (Table 1). Moderately high heritability was observed for LLS and rust resistance at 80 and 90 DAS compared to 70 DAS (Table 2). In general, all nutritional traits recorded very high heritability with moderate GAM. However, O/L recorded highest genetic advance over mean (56.01%), indicating the scope for selecting superior genotypes for this trait (Table 3).

Polymorphism information content (PIC) analysis

Power of each marker to discriminate the genotypes was calculated by estimating polymorphism information content (PIC) value. PIC values ranged from 0 (AhTE347, AhTE442, AhTE445, AhTE376 and AhTE300) to 0.50 (AhTE121, AhTE489, AhTE426, AhTE205, AhTE113 AhTE148 and AhTE319).

Markers were classified as high (≥ 0.5), medium (0.26 to 0.49) and low (≤0.25) based on the PIC value. In total, 7, 17 and 17 markers were identified as high, medium and low for their genotype-discriminating power, respectively. Twenty four (58%) out of 41 AhTE markers used for molecular assay reported moderate to high PIC value, this indicates that most of the *AhMITE1* specific markers posses better genotype-discriminating power over commonly used SSR markers in groundnut (Shirasawa *et al.,* 2012a; Koilkonda *et al.,* 2012; Pandey *et al.,* 2012).

Single marker analysis (SMA)

The mutant population such as the one used in this study allows analysing marker-trait association. Single marker analysis was performed using the phenotypic data on all 62 genotypes to determine the strength of association between 41 AhTE markers and a given trait by calculating *F* statistic and simple linear regression coefficient (Haley and Knott, 1992) using WinQTL Cartographer version 2.5 (Wang *et al.,* 2007). Statistically significant association of at least one marker was observed with the traits studied. AhTE205 had a significant association and high R^2 for number of pods per plant, pod yield per plant and shelling percentage.

Table.1 Estimates of genetic parameters for agronomic traits among mutant population and check varieties of groundnut

Traits	Mean	Range		PCV (%)	GCV (%)	h^2 (broad sense) (%)	GA	GAM
		Min	Max					
Plant height (cm)	28.39	15.06	42.30	18.04	13.11	53	0.25	19.61
Primary branch length (cm)	33.95	21.56	64.57	13.86	4.46	10	0.10	2.96
No. of primary branches	7.11	3.40	14.20	15.38	13.43	76	1.42	24.17
No. of secondary branches	2.92	0.00	26.40	17.15	14.62	73	1.83	25.68
Leaf length (cm)	5.50	3.57	7.74	13.32	8.44	40	0.40	11.01
Leaf width (cm)	2.66	2.08	4.41	17.69	15.27	75	1.57	27.15
Shelling percentage (%)	51.36	22.00	72.00	18.95	16.36	75	2.02	29.09
Sound mature kernel (%)	81.77	19.00	96.50	9.31	9.15	97	1.96	18.52
Test weight (g)	31.88	20.00	53.50	14.93	14.26	91	0.81	28.07
Pod length (cm)	2.80	1.76	3.99	12.06	11.90	97	11.63	24.17
Pod width (cm)	1.29	0.92	2.43	18.04	13.11	53	0.25	19.61
No. of pods per plant	15.12	4.05	37.95	39.09	37.95	94	11.48	75.89
Pod yield per plant (g)	9.98	1.40	30.63	50.33	49.18	96	9.88	99.02

Table.2 Estimates of genetic parameters for LLS and rust resistance traits among mutant population and check varieties of groundnut

Traits	Mean	Range		PCV (%)	GCV (%)	h^2 (broad sense) (%)	GA	GAM
		Min	Max					
Late leaf spot at 70 DAS	3.52	3.00	5.50	13.86	4.46	10	0.10	2.96
Late leaf spot at 80 DAS	5.88	4.00	8.00	15.38	13.43	76	1.42	24.17
Late leaf spot at 90 DAS	7.10	4.00	9.00	17.15	14.62	73	1.83	25.68
Rust at 70 DAS	3.60	3.00	5.00	13.32	8.44	40	0.40	11.01
Rust at 80 DAS	5.78	4.00	7.50	17.69	15.27	75	1.57	27.15
Rust at 90 DAS	6.95	4.00	9.00	18.95	16.36	75	2.02	29.09

Table.3 Estimates of genetic parameters for nutritional quality traits among mutant population and check varieties of groundnut

Traits	Mean	Range		PCV (%)	GCV (%)	h^2 (Broad sense) (%)	GA	GAM
		Min	Max					
Palmitic acid	10.58	7.20	12.12	9.31	9.15	97	1.96	18.52
Stearic acid	2.87	1.44	4.07	14.93	14.26	91	0.81	28.07
Oleic acid	48.14	33.21	60.91	12.06	11.90	97	11.63	24.17
Linoleic acid	33.73	22.00	47.33	15.11	14.95	98	10.27	30.45
Oleic: Lenoleic acid	1.49	0.70	2.68	27.97	27.58	97	0.83	56.01
Arachidic acid	1.09	0.76	1.59	19.66	19.50	98	0.43	39.83
Eicosenoic acid	0.97	0.76	1.18	9.00	8.85	97	0.18	17.94
Behenic acid	3.75	3.07	4.86	10.80	10.63	97	0.81	21.55
Lignoseric acid	1.23	1.02	1.47	8.70	8.43	94	0.21	16.81
Protein content (%)	29.85	19.60	36.88	10.61	10.57	99	6.48	21.70
Oil content (%)	47.8	42.5	54.4	4.86	4.89	98	4.70	9.95

Table.4 Potential markers associated with important productivity, nutritional traits and disease resistance

Sl. No.	Marker	Trait	R^2 (%)
1	AhTE205	Pods per plant	21
2	AhTE205	Pod yield per plant	14
3	AhTE205	Shelling percentage	29
4	AhTE333	Test weight	23
5	AhTE343	LLS (90 DAS)	14
6	AhTE373	Oil content	20
7	AhTE211	Oleate	19
8	AhTE211	O:L ratio	15

Similarly, AhTE333 for test weight, AhTE343 for LLS (90 DAS), AhTE373 for oil content (%) and AhTE211 for oleate content and O:L ratio had significant association and high R^2 (Table 4). AhTE205 showing significant association with most of the productivity traits like number of pods per plant, pod yield per plant and shelling percentage is of significance in groundnut breeding. However, the markers identified in this study as associated with productivity, disease resistance and quality traits need to be validated across genotypes. Similar marker trait association studies have been attempted to identify markers linked to taxonomic, agronomic, productivity, foliar disease resistance and nutritional quality traits in groundnut for their utilization in groundnut improvement through marker assisted selection (Anita *et al.*, 2015; Hake *et al.*, 2017a; Hake *et al.*, 2017b; Zongo *et al.*, 2017).If phenotypic variation explained (PVE) by a marker is more than 10%, it is considered to be a major marker and this method has been used by several researchers to signify the marker and trait association (Collard *et al.*, 2005; Zongo *et al.*, 2017).

In conclusion out of 41 markers used for marker trait association, five markers (AhTE205, AhTE333, AhTE343, AhTE373 and AhTE211) reported more than 10% PVE for different traits in the study, indicating their importance in trait selection through MAS. More importantly, since the transposon specific (*AhMITE1*) markers used in this study indicate the site of transposition, they would prove useful in unraveling the gene(s) involved in such transpositions leading to trait variation.

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