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Research Note

Analysis of a mutant population in groundnut

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Abstract
Fifty three mutants derived from Dharwad Early Runner (DER), a true breeding variant from a cross between two Valencia varieties of groundnut were evaluated for taxonomic, productivity and quality traits for assessing its suitability to ascertain marker-trait association. Mutants were confirmed for subspecific changes. Sixteen independent mutants shared common taxonomic shift from DER type to that of ssp. *hypogaea* var. *hypogaea*. Seventeen and nine mutants showed taxonomic shift to ssp. *fastigiata* var. *fastigiata* and ssp. *fastigiata* var. *vulgaris*, respectively. Four mutants had a shift from var. *fastigiata* to var. *vulgaris*. Significant shifts both in positive and negative direction were observed for most of the productivity and quality traits along with resistance to late leaf spot and rust. Since these mutants are derived from a common source (Dharwad Early Runner), those contrasting for any trait are expected to differ for a small genomic region. Role of transposons being significant in groundnut mutations, genotyping such mutants with transposon-specific markers might reveal marker-trait associations useful for groundnut improvement.

Key words: Groundnut, induced mutants, taxonomic and productivity traits, marker-trait association

Groundnut (*Arachis hypogaea* L.) is a major oilseed and legume crop grown throughout the world. Improving groundnut for its productivity, quality and resistance to biotic and abiotic stresses is the major objective in breeding. Though conventional methods of breeding have been successful, the rate is limited by various factors that demand the use of markers for efficient and rapid development of varieties. Therefore, identification of markers associated with the traits is a prerequisite for their use in molecular breeding.

Groundnut, an allotetraploid (2n=4x=40) carrying A and B genomes contributed by *A. duranensis* and *A. ipaensis*, respectively, has evolved into two subspecies (ssp. *hypogaea* and ssp. *fastigiata*) and botanical varieties (Krapovickas and Gregory, 1994) due to artificial selection during domestication (Kochert *et al.*, 1996) and spontaneous mutations (Mouli *et al.*, 1979; Prasad, 1989; Gowda *et al.*, 1996). The role of mutations, possibly involving transposons, in intraspecific differentiation of groundnut was demonstrated using induced mutations (Gowda *et al.*, 1996; Gowda *et al.*, 2011).

A population consisting of a large number of mutants derived from a common source but sharing common shifts in important traits provides a resource for identifying marker-trait association when subjected to genotyping with a marker system like transposon-specific markers. Such a population of independent mutants differing for major taxonomic traits was developed and characterized at UAS, Dharwad (Gowda *et al.*, 1989; Gowda and Nadaf, 1992; Gowda *et al.*, 1996). An effort was made to analyze this mutant population for the kind of shifts in taxonomic, productivity and quality traits in addition to resistance to late leaf spot and rust for ascertaining its use in marker-trait association studies.

The study used a mutant population consisting of 42 primary mutants, 7 secondary mutants, 4 tertiary mutants and their parents representing the two subspecies and four botanical varieties of groundnut. All the primary mutants originated upon mutagenesis of Dharwad Early Runner (DER) with ethyl methane sulphonate (EMS) (0.5%). DER was recovered from a cross involving two *fastigiata* cultivars, *viz.* Dh 3-20 and CGC-1 (Gowda *et al.*, 1989).

These genotypes were evaluated for taxonomic, productivity and quality traits apart from resistance to late leaf spot and rust traits in a randomized complete block design with two replications during *kharif* 2012 at the IABT Garden, Main Agricultural Research station, Dharwad. Each replication consisted of two rows of 2.5 mt length with 45 cm space between them. The seeds were sown every 10 cm within each row. Five randomly selected plants from each mutant in each replication were studied for the taxonomic traits like main stem flowering, growth habit and type of inflorescence. Productivity traits (number of pods/plant, pod yield/plant, shelling percentage and test weight) and quality traits (protein content, oil content, and oleic acid and linoleic acid content) were recorded and the mean was calculated. Quality parameters were estimated by near infrared spectroscopy

(NIRS) at Seed Quality Testing and Research Laboratory, Seed Unit, UAS, Dharwad.

The genotypes were subjected for field screening for rust and LLS reaction using spreader row technique (Subrahmanyam *et al.*, 1995) in which the disease spreader plants (TMV 2 and mutant 28-2) were planted at regular interval of 10 rows. Disease scoring for both rust and LLS was done at 90 days after sowing (DAS) according to modified 9-point scale (Subbarao *et al.*, 1990).

Dharwad Early Runner (DER) showed the characteristics of both the subspecies as it was observed earlier (Gowda *et al.*, 1989). However, 42 primary mutants derived from DER, 7 secondary mutants and 4 tertiary mutants could be clearly classified into ssp. *hypogaea* or ssp. *fastigiata* (Fig. 1). DER was therefore considered to resemble *A. monticola*, a primitive progenitor of groundnut (Gowda *et al.*, 1996).

Based on the presence or absence of main stem flowering as observed during *kharif* 2012, the primary mutants from DER were classified into two subspecies (Table 1) (Fig. 1). Sixteen genotypes belonged to *A. hypogaea* ssp. *hypogaea* (VB: Virginia bunch and VR: Virginia runner) and 26 belonged to *A. hypogaea* ssp. *fastigiata*. However, there were a few exceptions. VB 2, VB 8b, VR 2, and VR 8 mutants classified as ssp. *hypogaea* had main stem flowering, while DER VL (a mutant classified as ssp. *fastigiata*) did not have main stem flowering. In the past, studies have indicated the possibility of either *A. hypogaea* ssp. *hypogaea* (Krapovickas, 1969) or *A. hypogaea* ssp. *fastigiata* (Singh, 1988) being more primitive. Since the mutants were randomly selected in this study, nothing could be concluded about the primitive subspecies.

The mutants were evaluated for growth habit and the type of inflorescence to classify them further into botanical varieties. But all genotypes within the population including *A. hypogaea* ssp. *fastigiata* var. *fastigiata* (VL: Valencia types) showed compound inflorescence; hence was not used for classification. Based on the growth habit the primary mutants were classified into 9 VB, 7 V), 17 VL, and 9 SB (Spanish bunch, ssp. *fastigiata* var. *vulgaris*) types. But VR 3, VR 5, VR 7 and VR 8 though classified as ssp. *hypogaea*, showed erect growth habit.

Secondary mutants like VB 8b, VR 1b and VL 4b did not involve any shift in the taxonomic traits as compared to their respective parents. But the secondary mutants 28-2, 45, 98 and 110 originating from VL 1 involved taxonomic shift from VL to SB. The tertiary mutants 28-2 (S), 45 (S), 98 (S), 110 (S) did not involve any taxonomic shift.

Field evaluation of these mutants also revealed significant shifts in various productivity and quality traits, in addition to resistance to late leaf spot (LLS) and rust (Table 2). Significant shifts in both the directions were noticed for number of pods/plant (NPP), pod yield/plant (PYP) and shelling percentage (SP). But test weight (TW) showed significant shifts only in positive direction. The shifts took place in both the directions; though in negative direction were more frequent for protein content and oleic acid content. But equally frequent shifts in both the directions were noticed for oil content. Shifts towards higher linoleate was more common compared to those with shift in negative direction, which resulted in frequent shifts towards reduced O:L among the mutants.

For disease resistance, the shifts were mostly towards resistance. VL 1 was susceptible to late leaf spot (LLS) disease, but its four SB mutants were resistant to LLS (Table 2). However, the spontaneous revertants (tertiary mutants) from all these mutants were susceptible to LLS.

These mutants having common origin (DER) are expected to differ for a limited region of the genome. Yet they showed significant shifts representing contrasting phenotypes for taxonomic, productivity and quality traits in addition to resistance to late leaf spot and rust. Therefore, these mutants make up an ideal genetic resource to study the association of specific genetic changes with the important traits. Earlier, Cavanagh *et al.* (2008) observed the appropriateness of using mutant population for gene-trait association studies in crop plants. Since, most of the mutations in groundnut involve the activity of transposable elements (TE), transposon-specific marker system (Bhat *et al.*, 2008; Shirasawa *et al.*, 2012) might serve as a robust tool in detecting the specific genetic changes (involving transposition) among these mutants. An investigation to check the strength of co-segregation between a specific genetic change and the phenotype among several independent mutants sharing similar shifts for each trait would identify marker-trait association. Since these transposons have transpositional preference to genic regions (Wessler *et al.*, 1995), the genetic changes detected by TE markers may correspond to genes thereby enabling trait-specific gene tagging for future groundnut improvement.

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Table 1. Mutants and their parents along with the taxonomic shift

Parent	Mutant	Taxonomic shift
	Primary	
DER	VB 1, VB 2, VB 3, VB 4, VB 5, VB 6, VB 7, VB 9, VB 8a	DER to VB
DER	VR 2, VR 3, VR 5, VR 6, VR 7, VR 8, VR 1a	DER to VR
DER	SB 1, SB 2, SB 3, SB 4, SB 5, SB 6, SB 7, SB 8, SB 9	DER to SB
DER	VL 1, VL 2, VL 3, VL 4a, VL 6, VL 7, VL 8, VL 9, VL 10, VL 11, VL 12, VL 13, VL 14, VL 16, VL 17, DER VL, DER VL purple	DER to VL
	Secondary	
VB 8a	VB 8b	VB to VB
VR 1a	VR 1b	VR to VR
VL 1	28-2, 45, 98, 110	VL to SB
VL 4a	VL 4b	VL to VL
	Tertiary	
28-2	28-2 (S)	SB to SB
45	45 (S)	SB to SB
98	98 (S)	SB to SB
110	110 (S)	SB to SB

Table 2. Performance of mutants and their parents for productivity and quality traits along with reaction to late leaf spot and rust

Sl. No.	Genotype	NPP	PYP	SP	TW	Protein	Oil	O	L	O:L	LLS (90 DAS)	Rust (90 DAS)
1	VB 1	9.7	4.1	40.0	35.0	32.6	46.5	49.9	33.6	1.5	8.0	8.0
2	VB 2	19.8	15.0	62.3	34.5	32.4	49.6	41.2	38.8	1.1	7.0	7.5
3	VB 3	16.3	7.1	48.5	35.3	31.6	44.6	55.2	28.3	2.0	8.0	7.0
4	VB 4	14.2	7.0	43.8	34.5	31.7	49.0	47.2	33.9	1.4	8.0	5.0
5	VB 5	11.9	6.5	45.0	34.3	31.2	46.5	47.3	35.1	1.4	7.5	7.0
6	VB 6	12.8	4.1	38.3	27.3	25.6	44.2	60.9	23.4	2.6	7.5	7.5
7	VB 7	15.8	10.5	42.5	34.5	30.8	46.3	47.0	33.9	1.4	8.0	7.0
8	VB 8a	22.8	7.8	51.5	29.3	31.8	47.6	47.1	33.2	1.4	6.5	8.5
9	VB 8b	13.5	10.4	65.0	37.5	28.1	45.2	47.4	34.2	1.4	5.5	9.0
10	VB 9	4.1	1.4	22.8	20.5	28.1	45.2	47.8	33.0	1.5	5.5	7.0
11	VR 1a	5.8	6.0	57.5	30.5	33.1	46.7	49.9	32.0	1.6	7.5	7.5
12	VR 1b	16.4	8.4	49.3	26.5	33.8	44.5	48.7	35.0	1.4	8.0	6.5
13	VR 2	10.0	7.6	22.0	20.0	31.9	46.8	58.9	24.2	2.4	7.0	8.0
14	VR 3	38.0	21.9	49.3	27.5	31.6	50.4	48.1	34.0	1.4	6.5	5.0
15	VR 6	13.1	12.9	49.8	24.0	22.4	46.8	50.4	33.7	1.5	6.5	8.0
16	VR 5	14.5	15.5	60.0	53.5	31.4	50.7	47.4	33.7	1.4	7.0	8.0
17	VR 7	12.8	9.0	46.0	41.0	32.6	47.1	47.9	34.4	1.4	7.0	8.0
18	VR 8	20.6	13.3	63.5	31.5	23.9	50.0	50.1	31.7	1.6	6.5	5.0
19	SB 1	26.9	11.3	72.0	35.0	30.3	48.3	40.5	43.6	0.9	7.0	8.0
20	SB 2	12.0	12.6	65.0	35.5	30.7	47.8	40.9	42.5	1.0	9.0	8.0
21	SB 3	27.5	9.0	63.0	24.5	31.1	46.7	44.4	38.6	1.2	7.5	5.0
22	SB 4	16.8	14.3	62.8	43.3	34.3	52.7	38.6	38.1	1.0	8.5	5.5
23	SB 5	16.3	9.0	62.3	30.8	30.0	48.1	40.5	41.4	1.0	8.0	8.0
24	SB 6	13.0	7.1	71.5	20.3	29.2	50.1	37.9	41.1	0.9	6.5	8.0
25	SB 7	7.5	8.9	52.8	27.3	31.3	49.8	44.9	35.4	1.3	7.0	8.5
26	SB 8	10.3	9.5	50.0	36.3	29.5	48.9	46.8	35.0	1.3	8.0	8.0
27	SB 9	17.8	10.9	53.0	38.0	29.2	48.0	46.4	36.9	1.3	8.0	8.0
28	VL 1	12.3	9.0	55.0	38.0	28.3	47.5	45.6	36.5	1.3	8.0	5.5
29	28-2	14.5	10.6	51.8	36.0	32.8	54.4	49.6	31.5	1.6	5.0	8.5
30	28-2 (S)	12.0	13.4	49.8	33.8	30.3	47.7	49.3	31.3	1.6	9.0	7.5
31	45	22.0	16.9	61.0	40.3	30.9	49.1	46.8	35.3	1.3	5.5	6.5
32	45 (S)	17.0	13.5	56.5	34.5	28.5	48.4	44.3	37.1	1.2	8.0	8.0
33	98	13.0	8.7	57.8	29.3	31.4	46.4	48.9	32.2	1.5	5.0	8.0
34	98 (S)	21.0	12.0	55.8	45.5	28.6	46.4	56.9	25.3	2.3	6.0	6.0
35	110	15.8	11.6	57.5	36.8	31.0	46.9	52.8	31.4	1.7	5.5	6.5
36	110 (S)	18.0	22.7	56.0	46.0	32.5	50.3	52.8	29.9	1.8	8.0	7.5
37	VL 2	16.4	8.1	53.0	23.0	24.6	49.2	49.7	31.4	1.6	5.5	5.5
38	VL 3	10.8	10.2	57.8	31.8	24.2	48.7	33.2	47.3	0.7	5.5	8.0
39	VL 4a	11.3	4.2	30.0	26.0	30.9	47.8	50.1	33.0	1.5	8.0	6.5
40	VL 4b	7.4	3.2	24.5	28.0	29.2	42.5	48.9	35.0	1.4	8.0	8.0
41	VL 6	12.0	3.9	54.0	27.0	31.7	48.6	50.9	34.5	1.5	8.0	8.0
42	VL 7	9.3	7.9	35.0	29.3	29.3	46.6	51.6	32.4	1.6	8.0	5.5
43	VL 8	16.5	4.9	56.5	30.0	30.6	50.6	38.7	41.7	0.9	8.0	7.0
44	VL 9	15.5	12.8	57.5	37.5	29.7	51.7	39.7	40.9	1.0	6.5	6.5
45	VL 10	15.3	9.3	47.0	35.5	30.5	48.6	47.6	33.6	1.4	5.0	6.0
46	VL 11	10.8	11.9	51.8	39.3	28.5	47.2	47.8	35.8	1.3	8.0	5.0
47	VL 12	10.5	3.3	36.5	24.8	29.8	42.9	48.2	32.3	1.5	7.5	8.0
48	VL 13	13.8	7.8	54.3	36.5	22.4	52.0	49.0	34.1	1.4	8.0	5.0
49	VL 14	11.5	12.2	62.0	31.0	25.7	47.5	53.7	27.3	2.0	7.5	6.0
50	VL 16	11.1	10.1	43.5	28.0	27.8	46.8	53.4	29.3	1.8	8.0	5.0
51	VL 17	19.2	13.8	56.8	27.5	31.5	49.4	35.7	44.0	0.8	5.5	8.5
52	DER VL	29.5	10.4	64.3	32.3	32.9	46.7	56.0	28.8	2.0	7.0	6.0
53	DER VL purple	15.8	8.0	47.8	23.0	27.6	47.4	59.0	22.0	2.7	8.0	6.5
54	DER	11.3	4.5	39.8	20.8	33.5	47.9	58.4	24.5	2.4	8.0	8.0
	CV (%)	13.3	15.1	17.3	17.6	1.3	0.8	2.8	3.2	6.6	12.7	13.5
	C.D. (5%)	4.0	3.0	17.8	11.2	0.8	0.7	2.7	2.1	0.2	1.8	1.9

NPP: Number of pods/plant, PYP: Pod yield/plant (gm), SP: Shelling percentage, TW: 100 seed weight in gm, Protein: Protein content (%), Oil: Oil content (%), O: oleic acid content (%), L: Linoleic acid content (%), O:L: ratio of O to L, LLS (90 DAS): LLS score at 90 DAS and Rust (90 DAS): Rust score at 90 DAS.

Fig. 1. DER and its mutants representing ssp. *hypogaea* and ssp. *fastigiata*

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