RESEARCH PAPER

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Morphological characterization of induced mutants in groundnut using RAPD Markers

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An experiment was conducted for morphological and molecular characterization of induced mutants. From both morphological and molecular analysis, it was revealed that TMV 2 and its mutant NLM were diverse and placed in different cluster, same holds good for DER and its mutants but for VL 1 and its mutants, there is lack of relationship between morphological and molecular diversity and RAPD failed to differentiate different botanical types of groundnut.

Key words : Groundnut**,** Mutants, Molecular Diversity, RAPD and TMV-2.

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INTRODUCTION

Groundnut is believed to have originated in the Bolivian region of South America where the greatest diversity is found (Krapovickas, 1969). Though *Arachis monticola* has been identified as the tetraploid progenitor, the A and B genome donors and mode of origin of two subspecies is still controversial (Smartt, 1990). Groundnut (*A. hypogaea*) is classified into two subspecies, *viz.,* ssp. *hypogaea* (Krap. and Rig.) and ssp. *fastigiata* (Wald.) based on variation in morphology. Further, the ssp. *hypogaea* is bifurcated into var. *hypogaea* (Virginia bunch/runner) and var. *hirsuta* (Peruvian runner), and likewise ssp. *fastigiata* into var. *fastigiata* (Valencia), Peruviana, aequatoriana and var. *vulgaris* (Spanish bunch) (Stalker and Simpson, 1995). Groundnut is one of the principle economic crops of the world, which has been exposed extensively to mutagenic treatments for induction of variability. Physical mutagens such as, X-ray, gamma rays, â-rays and fast neutrons (Shivaraj *et al.,* 1962) and chemical mutagens like, ethyl methane sulphonate (EMS), ethidium bromide, acryflavine (Levy, 1976), diethyl methane sulphonate (DES), N- nitroso-N- methyl – urea, N- ethyl–N-nitroso-urea, ethylene imine and sodium azide (Venkatachalam and Jayabalan, 1997) has been used to create genetic variability in groundnut. However, gamma rays and EMS are most widely used and most effective mutagens in groundnut. Mutants have been obtained in groundnut either spontaneously or induced by physical/ chemical mutagens. In cultivated groundnut very low or no polymorphism to abundant polymorphism in wild *Arachis* has been reported (Halward *et al*., 1991; Lanham *et al*., 1992; Paik-Ro *et al*., 1992). However, recent studies revealed polymorphism in cultivated groundnut using amplified length polymorphism (AFLP) (He and Prakash, 1997) randomly amplified polymorphic DNA (RAPD) (Bhagwat *et al*., 1997; Subramanian *et al*., 2000; Dwivedi *et al*., 2001) and simple sequence repeats (SSR) (Hopkins *et al*., 1999). Randomly amplified polymorphic DNA is a convenient, economic and rapid method as compared to other techniques as it requires no probes and prior sequence information. Further, relatively small number of primers can be used to generate a very large number of fragments from different regions of the genome and hence, multiple loci may be examined very quickly. This makes RAPD a powerful technique for screening the germplasm for assessing the genetic diversity. Therefore, this study was carried out to assess genetic diversity among 21 mutants representing four botanical types using RAPD.

RESEARCH METHODOLOGY

The experimental material comprised of 21 genotypes of groundnut representing Virginia bunch (3), Virginia runner (2), Valencia type (8), Spanish bunch (7), Dharwad early runner (1) was included (Table 1) for morphological and molecular characterization. Dharwad early runner (DER), a taxonomic freak was obtained from a cross between two *fastigiata* cultivars, *viz*., Dh 3-20 and CGC-1 DER on treatment with ethyl methane sulphonate (EMS) and gamma rays yielded very high frequency of mutants representing all the four botanical types (Spanish bunch, Valencia, Virginia bunch and Virginia runner) (Gowda *et al*., 1996) (Plate 1). DER on mutagenesis with EMS produced a Valencia type mutants, *viz*. VL 1 which showed high resistant to rust produced a DER revertant, VL 1 (R) in M_3 generation. The mutant and the revertant bred true in later generations. VL 1 yielded a high frequency of mutants with wide spectrum of variations for disease resistance reactions and morphological characters. The VL 1 derived mutants were evaluated for productivity and resistance parameters. A high level of resistance to late leafspot was evident in many mutants but these mutants, *viz*. VL 1-28-2, VL 1-110 combined early maturity, moderate to high productivity and desirable pod and kernel features (Motagi *et al*., 1996). TMV 2 is a Spanish bunch variety which on mutagenesis with EMS (0.2%) resulted in a 'narrow leaf mutant' (NLM) with a shift to Virginia type (Prasad *et al*., 1984). TMV 2 and its mutant differ for several morphological characters. NLM on treatment with azacytidine resulted in 'broad leaf variants', *viz*., 'NLM broad' (BLM). Treatment of DER with EMS and 5-

List of quantitative traits	List of qualitative traits
Plant height	Growth habit
Number of primary branches	Branching pattern
Number of secondary branches	Stem pigmentation
Leaf length	Stem hairiness
Leaf width	Leaf colour
Leaf length/width ratio	Leaflet shape
One line pod weight	Leaflet margin
Test weight	Peg colour
Seed length	Flower colour
Seed width	Leaflet surface
Shelling per cent	Pod beak
Pod length	Pod constriction
Pod width	Pod reticulation
Number of pods/plant	Seed size
Number of seeds/pod	Seed shape
LLS (90 days)	Testa colour
RUST (90 days)	
LLS (at harvest)	
19. Pod yield (g)/plant	

Table 1: List of mutants and their origin

Sr. No.	Mutants	Botanical types	Pedigree
1.	DER	-	Dh 3-20 x CGC -1
2.	SB 3	Spanish bunch	Gamma rays mutant of DER
3.	SB 6	Spanish bunch	Gamma rays mutant of DER
4.	VL 1	Valencia type	EMS mutant of DER
5.	VL 3	Valencia type	EMS mutant of DER
6.	VL 1 (dwarf)	Valencia type	Mutant of VL 1
7.	VL 1 (R)	Valencia type	Revertant of VL 1 resembling DER
8.	M 28-2	Spanish Bunch	EMS mutant of VL 1
9.	M 28-2 (S)	Valencia type	Mutant of M 28-2 susceptible to LLS
10.	M 110	Spanish Bunch	EMS mutant of VL 1
11.	M 110 (S)	Valencia type	Mutant of M 28-2 susceptible to LLS
12.	VB 1	Virginia Bunch	Virginia Bunch type mutant from DER
13.	VB 3	Virginia Bunch	Virginia Bunch type mutant from DER
14.	VR 8	Virginia Runner	EMS mutant of VL 1
15.	SB necrotic	Spanish Bunch	Disease mimic mutant from DER
16.	SB normal	Spanish Bunch	Non- necrotic mutant from SB necrotic
17.	VL necrotic	Valencia type	Disease mimic mutant from DER
18.	VL normal	Valencia type	Non- necrotic mutant from VL necrotic
19.	TMV 2	Spanish Bunch	Mass selection from Gudhiatham bunch
20.	NLM	Virginia Bunch	EMS mutant of TMV2
21.	NLM broad	Virginia Runner	Azacytidine induced mutant of NLM

azacytidine has resulted in necrotic disease mimic mutants namely 'Spanish bunch necrotic' (SB necrotic) and 'Valencia necrotic' (VL necrotic), respectively. In *Kharif* season, the experimental materials were sown on 26th September 2007. Regular cultivation practices were followed to raise a good crop and observations were recorded for the following characters according to 'Groundnut descriptors'.

The 19 quantitative and 16 qualitative traits and were given were subjected for Principal component analysis (PCA) Sneath and Sokal (1973). For molecular characterization DNA was extracted by (CTAB) method (Saghai -Maroof *et al.*, 1984) and subjected to polymerase chain reactions (PCR) using 27 random primers. The PCR products were separated in 1.2 per cent agarose gel of 1x TAE buffer containing ethidium bromide (5 µl/100ml). The amplification profiles of all the 21 genotypes for all the primers were scored depending on the presence (1) ore absence (0) of amplified DNA fragments and 0, 1 matrix was constructed. The genetic similarity was completed by DICE coefficient using NT –Sys-PC 2.0 Software programmes (Dice, 1945 and Nei and Li, 1979). The clustering was done using SAHN and dendrogram was constructed by using unweighted pair group arithmetic mean (UPGMA).

RESULTS AND ANALYSIS

Out of sixteen qualitative traits; first seven PCs contributed significantly and explained 81.675% variation and the hierarchical cluster analysis revealed seven clusters at Sij of 10.0

(Table 2a, b and Fig. 1). Out of nineteen quantitative traits, first six principal components (PCs) contributed significantly and explained 83.031% of variation in mutants and the hierarchical cluster analysis revealed six clusters at Sij 7.50 (Table 3 a, b and Fig. 2). The combined hierarchical cluster analysis generated eight clusters at Sij 10.0. TMV 2 and its mutant NLM were distinct and grouped in different clusters; VL 1 and its mutants 110 and M 28-2 were also distinct and were placed in different clusters; DER and its mutants grouped in distinct clusters; SB 3 and SB 6, SB necrotic and SB normal clustered together; DER and NLM broad clustered together (Table 4a, b and Fig. 3).

Table 2a: Frequency of mutants in each cluster based on qualitative traits

Cluster	Total	Frequency (%)
I	1	4.76
II	1	4.76
III	1	4.76
IV	1	4.76
V	2	9.52
VI	2	9.52
VII	13	61.90
Total	21	100

Table 2b: Distribution of mutants in different clusters based on qualitative traits

Cluster	Total	Mutants
I	1	DER
II	1	VL 1(R)
III	1	M 110
IV	1	NLM broad
V	2	VL necrotic ,VL normal
VI	2	SB necrotic, NLM
VII	13	SB 3,SB 6,VL 1,VL 3,VL 1dwarf, M 28-2, M 28-2 (S), M 110 (S), VB 1,VB 3,VR 8, SB normal, TMV 2

For the molecular diversity 21 mutants were subjected to RAPD assay using 27 primers. Twenty one mutants were subjected to RAPD assay using 27 primers. Twenty seven primers generated a total of 253 amplified fragments out of which 77 (2.85) showed polymorphic bands. The polymorphism ranged from 9.09 (OPK 14 and OPM 12) to 71.42% (OPV 16) with over all mean of 30.16 %. Number of amplified fragments ranged from 6

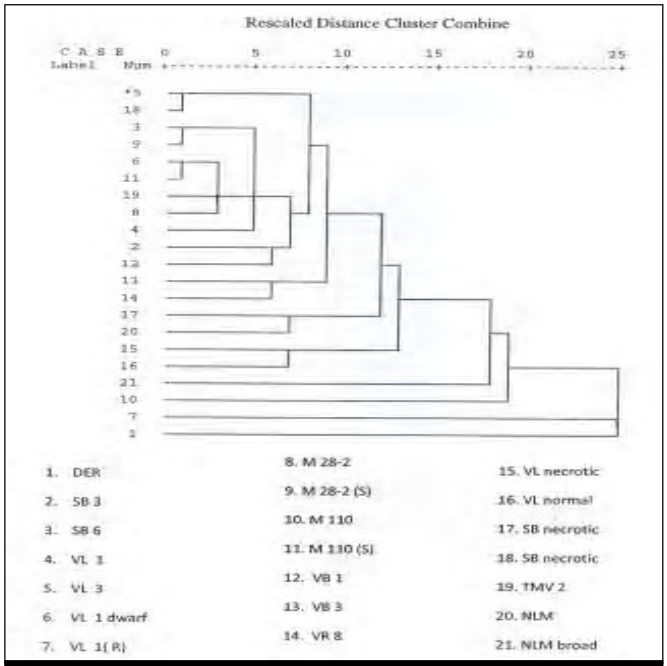


Fig 1: Dendrogram depicting genetic diversity in mutants for qualitative traits

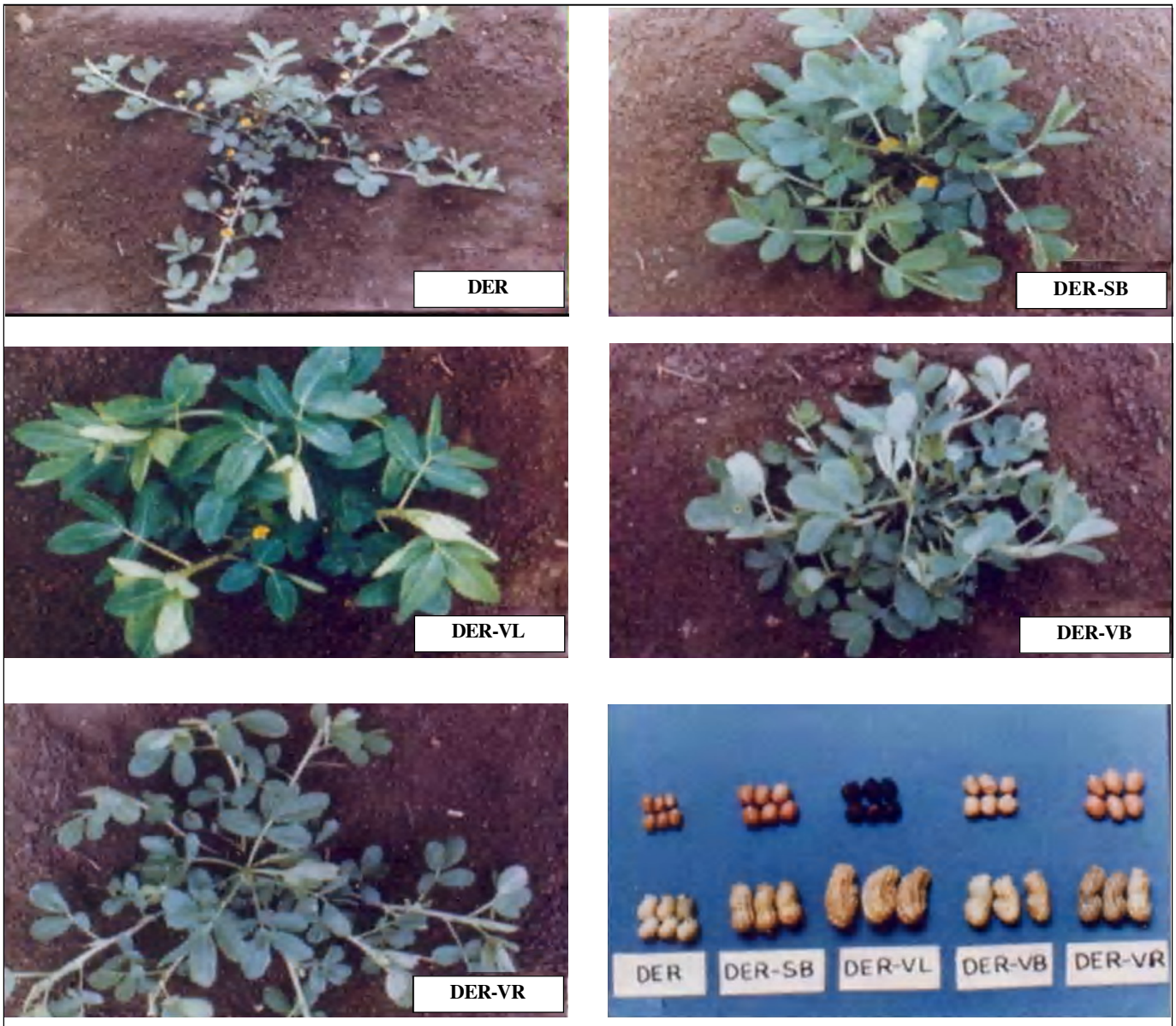


Plate 1: DER and its taxonomic metants

Table 3a: Frequency of mutants in each cluster based on quantitative characters

Cluster	Total	Frequency%
I	1	4.76
II	1	4.76
III	1	4.76
IV	1	4.76
V	14	66.66
VI	3	14.28
Total	21	100

Table 3b: Distribution of mutants in different clusters based on quantitative characters

Cluster	Total	Mutants
I	1	NLM
II	1	110 (S)
III	1	VL1 dwarf
IV	1	VL 1
V	14	SB 3, SB 6, VL 3, VL 1 (R), M 28-2, M 28-2 (S),M 110, VB 1,VB 3,TMV 2, VL necrotic, VL normal, SB necrotic, SB normal.
VI	3	DER, NLM broad, VR 8

Table 4a: Frequency of mutants in each cluster based on combined qualitative and quantitative traits

Cluster	Total	Frequency %
I	1	4.76
II	1	4.76
III	2	9.52
IV	2	9.52
V	1	4.76
VI	1	4.76
VII	1	4.76
VIII	12	57.14
Total	21	100

Table 4b: Distribution of mutants in different clusters based on combined qualitative and quantitative characters

Cluster	Total	Mutants
I	1	NLM
II	1	M 110 (S)
III	2	DER, NLM broad
IV	2	VL 1 ®, VL necrotic
V	1	VL1 dwarf
VI	1	VL 1
VII	1	M 110
VIII	12	SB 3, SB 6, VL 3, M 28-2, M 28-2(S), VB 1, VB 3, VR 8, VL normal, SB necrotic, SB normal , TMV 2

to 13 in a given primer. On an average 9.4 bands per primer were amplified and 3.00 bands per primer were polymorphic. All 27 primers showed polymorphism but OPB 19 (50%), OPB 11 (44.44%) OPV 16 (71.42%), OPA 12 (44.44%), OPJ 17 (53.84%), OPK 18 (46.15%) OPA 07 (45.45%), OPB 9 (50%) and OPB 13 (66.66%) were highly polymorphic. The band profile obtained from 27 primers is summarized in (Table 2a and b). The Dendrogram revealed five distinct clusters at Sij 0.95 and TMV2 and its mutant NLM were distributed in different clusters indicating diversity at molecular level. DER and its mutants, *viz.* (SB 3, SB 6, VL 1, VL 3, VB 1, VB 3 and VR 8) also showed high genetic divergence. But VL 1 and its mutants, *viz.* (M 110 and M 28-2) clustered together. Based on the Dice - coefficients the mean similarity indices for 21 genotypes ranged from 0.88 to 0.98 (mean 0.93) indicating that accessions had 93 per cent of their RAPD fragments in common.

Among the qualitative traits; growth habit, stem pigmentation, leaflet surface, pod constriction, pod reticulation, pod beak, stem hairiness, flower colour and testa colour had high loading value indicating their importance in differentiating the mutants. Shelling per cent, pod yield per plant, test weight and one line pod weight among quantitative traits had high loading value indicating their importance for diversity. Among the mutants VL 1,

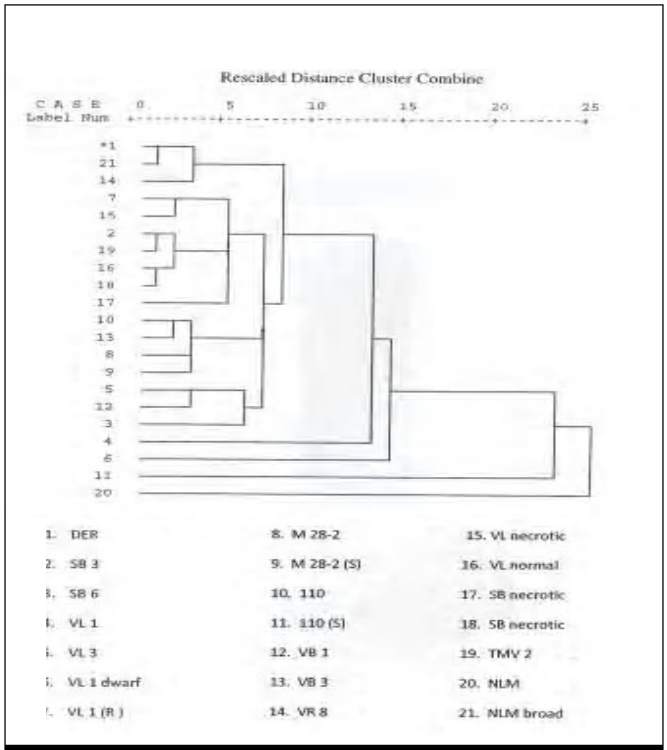


Fig. 2: Dendrogram depicting genetic diversity in mutants for quantitative characters

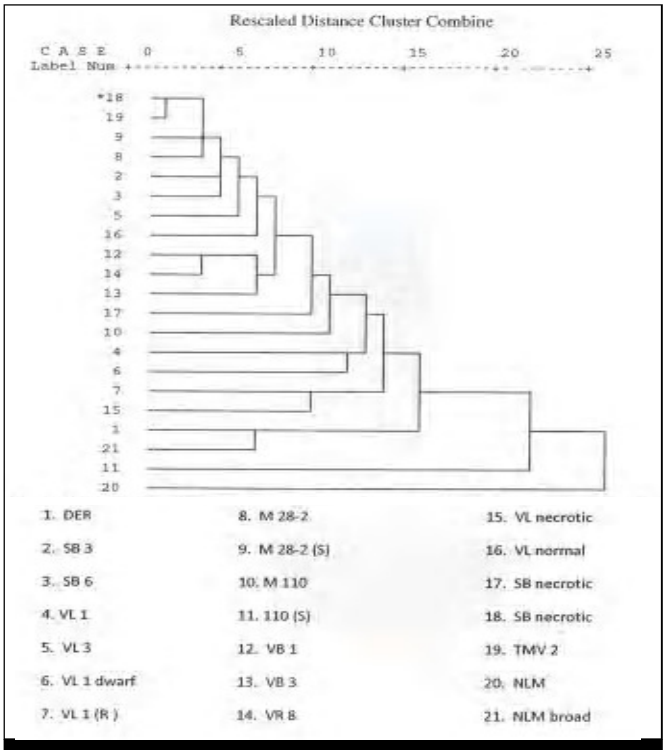
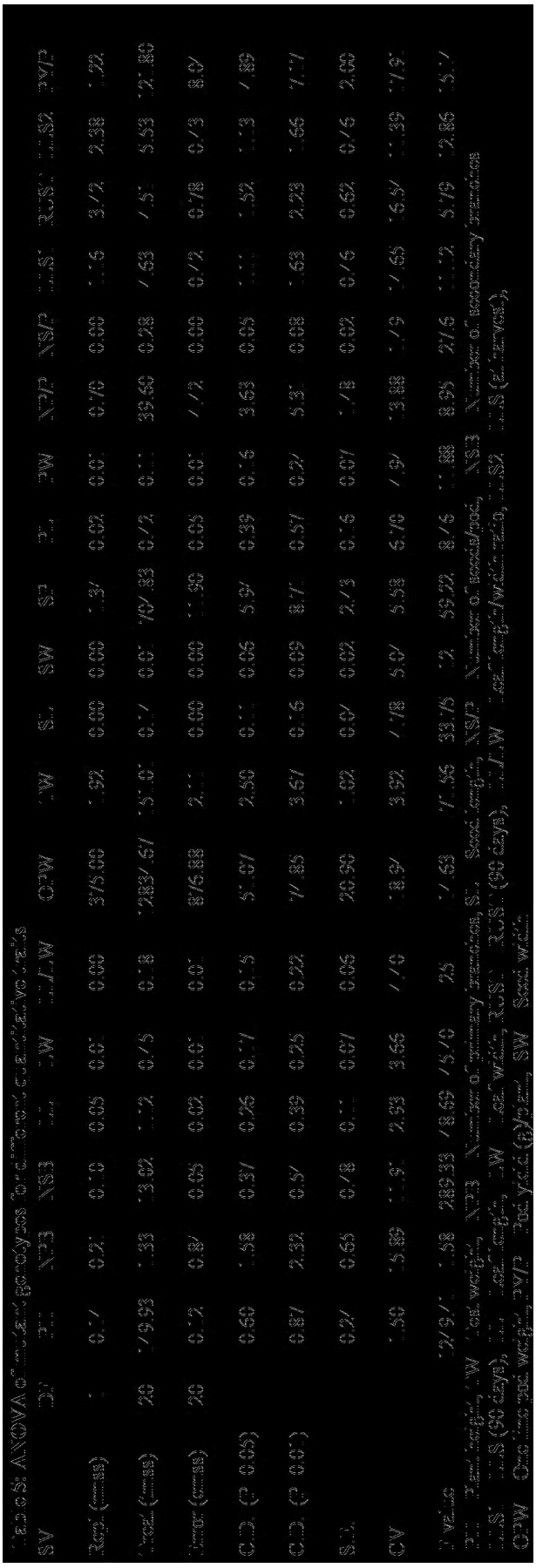


Fig. 3: Dendrogram depicting genetic diversity in mutants for combined qualitative and quantitative charcters



VL 3, M 28-2 and M 110 combined resistance to either rust or LLS with productivity and they could be utilized in future breeding programs. By combined investigation on both morphological and molecular diversity, it is found that TMV 2 and its mutants NLM were diverse, same holds good for DER and its mutants, but VL 1 and its mutants M 110 did not much differ at molecular level. Although, morphological characterization show different clusters for different botanical types, but not much differentiation was evident at molecular level, so lack of relation between morphological and molecular diversity arises because mutation in certain loci leading to phenotypic effects and the little polymorphism at the DNA level, as evident by molecular analysis among various botanical varieties of two subspecies indicated that *A. hypogaea* might have arises as a result of a single polyploidization event which occurred relatively recently on an evolutionary time scale (Halward *et al.*,1991and Kochert *et al.*,1991). In contrast, AFLPs and SSRs (He and Prakash, 1997) were found efficient in grouping different botanical types based on molecular diversity. Hence, there is a need to apply these tools to gain more insight into the mutants at molecular level in future investigation.

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