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ARTICLE

Occurrence and Segregation of a Heterozygous Narrow-Leaf Mutant in Peanut

W.D. Branch*[1]; N. Brown[2]; J. P. Clevenger[3]; Z. Myers[4]

[1]Professor, Dept. of Crop and Soil Sciences, Univ. of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

[2]Assistant Professor, Dept. of Crop and Soil Sciences, Univ. of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

[3]Faculty Investigator, Hudson Alpha Institute for Biotechnology, Huntsville, AL 35806.

[4]Computational biologist, Hudson Alpha Institute for Biotechnology, Huntsville, AL 35806.

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Corresponding Author:
W.D. Branch
wdbranch@uga.edu

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ABSTRACT

The heterozygous Narrow-Leaf mutant phenotype found in the U.S. runner-type peanut (*Arachis hypogaea* L. subsp. *hypogaea* var. *hypogaea*) cultivar 'Tifguard' segregated into three phenotypes: Normal-leaf plant, smaller Narrow-Leaf plant, and a very small seed sterile Dwarf-leaf plant. Upon selfing individual plants of the Normal phenotype only Normal plants were produced. However, selfing individual plants of the Narrow-Leaf resulted in a 1 Normal: 2 Narrow-Leaf: 1 Dwarf genetic ratio. No other plant types were found within the segregating plant phenotypes. Following bulk segregant analysis the homozygous Normal and Dwarf phenotypes were found to be genetically more similar than the heterozygous Narrow-Leaf mutant type. Since these three phenotypes (genotypes) were also found to breed true-to-type for tan testa color, root-knot nematode resistance, normal-oleic, and leaflet length/width ratio, the results support a rare single gene mutation rather than natural outcrossing origin for the Narrow-Leaf mutants found in the Tifguard peanut cultivar.

INTRODUCTION

In the past, several different sterile dwarfs, small-plants, and micro-phenotypes have been reported in the cultivated allotetraploid peanut (*Arachis hypogaea* L.). Within the old 'Tennessee Red' valencia-type cultivar, Branch and Hammons (1983) found three distinct phenotypes resulted from selfing the heterozygous mini plants: micro, extremely reduced plants with very small leaves and stems; mini, plants with general overall small plant size; and typical normal size plants.

Sterile brachytic and sterile dwarf plants have also been found as homozygous recessive plants in F_2 populations (Branch, *et al.*, 2016). More recently, a novel 5-small leaflet mutant was found in the 'Georgia Green' cultivar to be heterozygous even after several self-generations and it segregated for 4-normal leaflets and 5-small leaflet plants (Branch, *et al.*, 2020).

Also, naturally and artificially drought-induced small-plants were found within the 'Georgia-10T' runner-type cultivar (Branch, *et al.,* 2019). Foliar treatment of these small plants with gibberellic acid (GA3) resulted in taller mainstems and longer internode lengths after one and two months in a greenhouse study. These results suggested the importance of early-season irrigation to avoid subsequent development of low-yielding, small-plants induced by drought stress.

In 2021, two smaller Narrow-Leaf plants were found within the 'Tifguard' cultivar (Holbrook, *et al.*, 2008). The objective of this study was to determine if these two Narrow-Leaf mutant plants segregated or bred true-to-type.

MATERIALS AND METHODS

In 2021, two "Narrow-Leaf" plants were isolated from 'Tifguard', a commercial cultivar of U.S. runner market type (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*) peanut. These two mutant plants had narrower leaves and more compact plant

appearance with reduced overall leaf size compared to the normal-type plants of Tifguard. However, the few pods and seed produced were only slightly smaller than the typical Tifguard cultivar (68 and 66 vs 72 and 70 g 100^{-1}, respectively). Subsequently, these two mutant plants were designated as Tifguard-1NL and Tifguard-2NL.

In 2022 and 2023, selfed seed from Tifguard-1NL and Tifguard-2NL were spaced-planted 30.5 cm apart in field nursery plots at the Gibbs research farm near the University of Georgia, Coastal Plain Experiment Station, Tifton Campus. Each year, plots consisted of two rows with variable length depending upon number of seed x 1.8m wide beds. The soil type was a Tifton loamy sand (fine-loamy, siliceous, thermic, Plinthic Kandiudult). Recommended cultural practices with irrigation were followed throughout both growing seasons. Individual plants were harvested near optimum maturity based upon days after planting and above-ground plant appearance. After harvest, pods were dried with forced warm air to approximately 6% seed moisture content before weighing and shelling.

Phenotypic classification was based upon individual plants prior to harvest. Segregation data was analyzed by chi-square analysis for goodness-of-fit ($P \leq 0.05$) to expected genetic ratios (Strickberger, 1968).

RESULTS

During 2022, the selfed progeny rows from each of the two mutant plants, Tifguard-1NL and Tifguard-2NL, segregated into three distinct phenotypes: Normal, Narrow-Leaf, and Dwarf both above-ground and below-ground (Figure 1). Segregation from both mutant plants showed an acceptable fit to a 1 Normal: 2 Narrow-Leaf: 1 Dwarf expected genetic ratio for monogenic inheritance (Table 1). Total, summed, and homogeneity chi-square values were also found acceptable to a 1:2:1 ratio for each of the two mutant selfed plants. It was interesting to note that no other plant types were detected as might be expected with natural outcrossing. Likewise, the testa color was tan for all seed from Normal and Narrow-Leaf plants; whereas, the Dwarf plants were all seed sterile.

Figure 1. Normal, Narrow-Leaf, and Dwarf plants separated and together above-ground (top left to right) and inverted below-ground plants (below left to right), respectively.

Table 1. Segregation of individual plant selections for number of Normal, Narrow-Leaf, and Dwarf plants from two Narrow-Leaf 'Tifguard' mutant plants, 2022.

Narrow-Leaf Selections	No. Plant Segregation			χ^2 (1:2:1)	ρ
	Normal	Narrow-Leaf	Dwarf		
Tifguard-1NL	5	14	4	1.174	0.50-0.75
Tifguard-2NL	2	2	4	3.000	0.25-0.10
Total				4.174	0.25-0.50
Summed	7	16	8	0.097	0.950-0.975
Homogeneity				4.077	0.10-0.25

Selfed seed from five 2022 individual Narrow-Leaf and five 2022 Normal-Leaf plants were replanted in 2023. Again, as in 2022, only three distinct phenotypes (Normal, Narrow-Leaf, and Dwarf) were observed. Segregation from both mutant plants likewise showed an acceptable fit for a 1 Normal: 2 Narrow-Leaf: 1 Dwarf expected genetic ratio for monogenic inheritance (Table 2), except for one progeny rows, Tifguard-1NL-3. Total, summed, and homogeneity chi-square values were also found acceptable for several selfed individual Narrow-Leaf plant progeny rows. Also, all the Normal-Leaf plant progeny rows bred true-to-type in 2023.

Table 2. Segregation of individual plant selections for number of Normal, Narrow-Leaf, and Dwarf plants from Narrow-Leaf 'Tifguard' mutant plants, 2023.

Narrow-Leaf Selections	No. Plant Segregation			χ^2 (1:2:1)	ρ
	Normal	Narrow-Leaf	Dwarf		
Tifguard-1NL-3	13	35	28	6.395	<0.05*
Tifguard-1NL-4	19	36	13	1.294	0.50-0.75
Tifguard-1NL-5	23	40	15	1.692	0.25-0.50
Tifguard-1NL-6	19	36	19	0.054	0.950-0.975
Tifguard-2NL-11	25	38	14	3.156	0.10-0.25
Total				12.591	0.10-0.25
Summed	99	185	89	0.560	0.75-0.90
Homogeneity				12.031	0.10-0.25

*Significantly different from the 1:2:1 expected ratio.

Comparison of the three plant phenotypes found upon selfing the heterozygous Narrow-Leaf individual plants showed that Normal and Narrow-Leaf had similar and significantly taller mainstem heights than the Dwarf phenotype (Table 3). Significant differences were likewise found among the three phenotypes for leaflet length and width, but surprisingly no differences were noted between length/width ratios (Figure 2). After harvest and shelling, average pod weight per normal plant were found to be significantly greater than Narrow-Leaf plants; whereas, 100-seed weight and oleic to linoleic fatty acid ratios were not significantly different (Table 3).

Figure 2. Leaflets from Normal, Narrow-Leaf, and Dwarf plant phenotypes segregating from the two Tifguard-1NL and -2NL mutant plants (left to right, respectively).

Table 3. Ten plant average comparison among three segregating plant phenotypes found in the 'Tifguard' mutant plants, 2023.

Plant Phenotype	Mainstem Height	Leaflet Characteristics			Pod Weight	100 Seed Weight	Oleic to Linoleic
		length (L)	width (W)	L/W			
	(cm)	(mm)	(mm)	(ratio)	(g)	(g)	(ratio)
Normal	21.6 a*	54.8 a	21.2 a	2.58 a	91.2 a	56.2 a	1.8 a
Narrow-Leaf	21.1 a	31.5 b	12.2 b	2.60 a	76.7 b	58.5 a	2.2 a
Dwarf	5.8 b	6.7 c	2.8 c	2.44 a	-	-	-

*Means within columns followed by the same letter are not significantly different at P≤0.05.

Individual leaf samples were also collected during the 2023 growing season for marker assisted selection (MAS) to determine root knot nematode (RKN) resistance. All three plant phenotypes were found to have RKN resistance like Tifguard by four different molecular markers. These findings strongly suggest that the Narrow-Leaf mutant plants did not originate from natural outcrossing. Instead, both mutant plants found in two different test plots during 2021 possibly originated from one 2-seeded pod as a single point mutation.

Bulked segregant analysis (Michelmore *et al.*, 1991) was then utilized to determine relative genetic relationships between these three genotypes vs phenotypes (Normal, Narrow-Leaf, and Dwarf). Figure 3 shows the relative genetic distant relationship (cladogram) between these three genotypic bulks based on numerous SNP's. Despite no clear genetic signature explaining the phenotype, the majority of the Narrow-Leaf bulks are grouped together in the top half, but the lower half predominantly contained the Normal and Dwarf bulks. These results suggest that the homozygous Normal and Dwarf bulks are genetically closer together than the heterozygous Narrow-Leaf bulks. This coincides with the inheritance data of 1 Normal and 1 Dwarf vs 2 Narrow-Leaf genetic ratio.

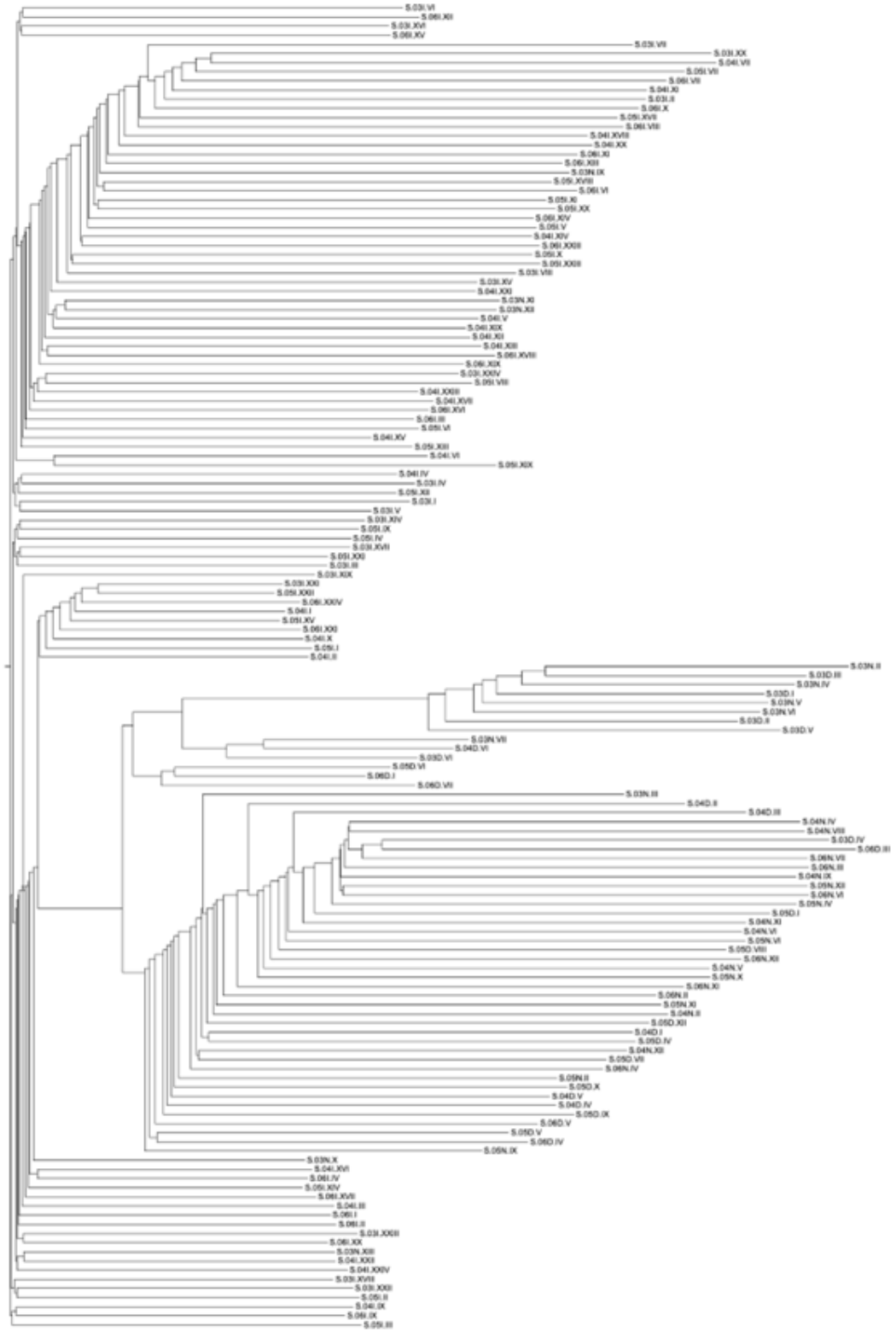

Figure 3. Cladogram results from bulk segregate analysis showing the relative genetic relationship among three genotypes: Normal, Narrow-Leaf, and Dwarf phenotypes.

DISCUSSION

Both segregation and bulk segregant analyses support a single locus mutation rather than natural outcrossing origin for the Narrow-Leaf mutants found in the Tifguard cultivar. Tifguard originated from a cross of 'C-99R' (Gorbet and Shokes, 2002) and 'COAN' (Simpson and Starr, 2001). COAN was developed from five-backcrosses to the recurrent female parent 'Florunner' (Norden *et al.*, 1969) x a 3-way

interspecific hybrid, TxAG-6 (Simpson *et al*., 1993). Tifguard has the long-segment introgressed region with the very-high RKN resistance on chromosome 9 similar to COAN. It is possible that somewhere along this long-introgressed region resulted in a single gene mutation or deletion without effecting the RKN resistance. Unfortunately, QTL variant analysis with deletions included did not find any signal between Normal and Dwarf genotypes, but it is still possible that the mutation is a small cryptic variant that has no buildup of SNP's in linkage around it that would allow for genomic mapping. In summary, this study documents the occurrence and segregation of a rare point mutation in the cultivated peanut, possibly a deletion based upon the 1:2:1 ratio observed for ++, +-, --, respectively.

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