# Inheritance of a Novel Heterozygous Peanut Mutant, 5-Small Leaflet

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## ABSTRACT

An unusual 5-Small Leaflet mutant plant was found within the 'Georgia Green' runner-type peanut (*Arachis hypogaea* L.) cultivar. Subsequent selfing has not established a true-breeding 5-Small Leaflet genotype. It continues to segregate normal and 5-Small Leaflet plants but with a reduced number of normal leaf plants upon selection for 5-Small Leaflet phenotypes after several self-generations. $F_1$, $F_2$, $F_3$, and $F_4$ data suggests that the 5-Small Leaflet trait is dominant or possibly pseudo-dominant. Likewise, the 5-Small Leaflet mutant can only be used as a pollen parent in crosses, and it has approximately a 1:1 ratio of elongated to normal stigmas, respectively, on individual plants. This is an example of a novel heterozygous peanut mutant plant found within the cultivated allotetraploid peanut.


Key Words: groundnut, *Arachis hypogaea* L., genetic ratios, cross combinations

Leaflets of normal (*Arachis hypogaea* L.) phenotypes have typically two-pairs of leaflets. However, the size of leaflets and plants may vary depending of genotypes (Ashri, 1970; Mouli and Kale, 1981; Branch and Hammons, 1983; Patil and Mouli, 1984; Essomba *et al*, 1993).

During 2001, an unusual individual mutant plant with 5-Small Leaflets (Fig. 1) was found within the 'Georgia Green' runner-type cultivar (Branch, 1996). Subsequently, continued selfing through the $S_9$ generation of the progeny from this unusual plant did not breed true-to-type for the 5-Small Leaflet characteristics. It has continued to segregate for four-normal leaflets and 5-Small Leaflet plant genotypes.

The objective of our study was to determine the inheritance of the 5-Small Leaflet mutant. Also, another underlying objective was to determine the cause for continuous segregation within this self-pollinated genotype.

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## Materials and Methods

During the fall and winter of 2003-04, reciprocal crosses were made in the greenhouse between the 5-Small Leaflet Mutant x Georgia Green, 'Georgia Valencia' (Branch, 2001), and 'Georgia-02C' (Branch, 2003). In 2005, $F_1$ seed were space-planted approximately 122-cm apart in one-row plots, 6.1 m long by 1.8 m wide at the UGA Coastal Plain Experiment Station, Gibbs research farms, Tifton, GA. $F_2$, $F_3$, and $F_4$ seed from each cross combination were space-planted each following year (2006, 2007, and 2008, respectively) approximately 30-cm apart in two-row plots with varying length by 1.8 m wide beds. These field genetic nursery plots were in a three-year rotation following corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). Each year, the soil-type was a Tifton loamy sand (fine-loamy, siliceous, thermic, Plinthic Kandidult). Recommended cultural practices with irrigation were used throughout each growing season.

In 2011, a $S_9$ generation 5-Small Leaflet Mutant yield test was conducted at the same location. Selfed seed from three individual plant selections were made and increased in 2010. The four entries included: Normal Leaf from normal leaf selection, Normal Leaf from 5-Small Leaflet selection, 5-Small Leaflet Mutant Selection, and the Georgia Green check cultivar. A randomized complete block field design was used with six replications. Each plot consisted of 2-rows, 6.1 m long by 1.8 m wide, and recommended cultural practices with irrigation were used for determining maximum yield potential among the four genotypes. Since segregation still occurred in some entries throughout the growing season, any off-type plants were rogued when apparent. The 5-Small Leaflet trait becomes noticeable about 3-4 weeks after emergence.

During the fall and winter of 2011-12, a full diallel crossing block was made between the three selections from the yield test in 2011 (Normal from normal leaf, Normal from 5-Small Leaflet, and 5-Small Leaflet Mutant). In 2012, $F_1$ seed were space-planted approximately 122-cm apart in one-row plots 6.1 m long by 1.8 m wide at the same location. $F_2$ and $F_3$ seed from each cross combination were also space-planted each year (2013 and 2014, respectively) approximately 30-cm apart in two-row plots with again varying length by 1.8 m wide beds.

**Fig. 1. The 5-Small Leaflet Mutant has an extra leaflet in top center of the four alternate leaflets.**

Phenotypic classifications were based upon individual plants for leaf characteristics. Segregation data was analyzed by chi-square analysis for goodness-of-fit ($P \leq 0.05$) to expected genetic ratios (Strickberger, 1968). Data from the 2011 yield test was subject to analyses of variance. Waller-Duncan's t-Test (k-ratio = 100) was used for mean separation of significant differences ($P \leq 0.05$).

## Results

It became apparent that none of the crosses using 5-Small Leaflet mutant as a female (♀) parent were successful. Only when 5-Small Leaflet mutant was used as a male (♂) parent were hybrid seed produced. Subsequently, stained pollen from the flowers of the 5-Small Leaflet mutant were indeed found to be normal with plump sound looking pollen grains.

A striking elongated stigma was also noticeable which ranged from 1-15 mm above the keel and was common among about half of the flowers from individual 5-Small Leaflet Mutant plants (Fig. 2). This would reduce self-fertilization and allow for potential cross-pollination; however, after numerous attempts to hand-pollinate the elongated stigma, none were successful.

**Fig. 2. Elongated stigma above the keel from flowers of 5-Small Leaflet Mutant with the wing petals removed.**

$F_1$ plants had the 5-Small Leaflet trait among the following crosses: Georgia Green (♀) x 5-Small Leaflet (♂), Georgia Valencia (♀) x 5-Small Leaflet (♂), and Georgia-02C (♀) x 5-Small Leaflet (♂). This suggests that the 5-Small Leaflet trait is dominant to the normal 4 leaf.

The $F_2$ individual plant segregation from these three cross combinations showed an acceptable fit for both a 9 (5-Small Leaflet) to 7 (normal) and 1 (5-Small Leaflet) to 1 (normal) genetic ratios, respectively (Table 1). Total, summed, and homogeneity chi-square values were found acceptable for each of these two expected ratios, except for the summed $\chi^2$ value for the 1:1 ratio.

$F_3$ and $F_4$ progeny row segregation among $F_{2:3}$ normal leaf and $F_{2:4}$ 5-Small Leaflet both showed an acceptable fit for a 1 segregating to 1 non-segregating ratio (Table 2). However, the $F_{2:4}$ normal leaf showed an acceptable fit to a 1 segregating to 2 non-segregating ratio, and all of the progeny rows from the $F_{2:3}$ 5-Small Leaflet segregated.

The 2011 yield test resulted in significant differences ($P \leq 0.05$) in pod yield among four entries (Table 3). The normal leaf from normal

**Table 1. $F_2$ individual plant segregation for 5-small leaflet mutant and normal leaf genotypes from three cross combinations.**

| Cross (♀ x ♂) | No. $F_2$ Plants: 5-Small | No. $F_2$ Plants: Normal | $\chi^2$ (9:7) | $\rho$ | $\chi^2$ (1:1) | $\rho$ |
|---|---|---|---|---|---|---|
| Georgia Green x 5-Small Leaflet | 52 | 41 | 0.004 | 0.90 – 0.95 | 1.301 | 0.25 – 0.50 |
| Georgia Valencia x 5-Small Leaflet | 45 | 30 | 0.429 | 0.50 – 0.75 | 3.000 | 0.05 – 0.10 |
| Georgia-02C x 5-Small Leaflet | 155 | 130 | 0.402 | 0.50 – 0.75 | 2.193 | 0.25 – 0.50 |
| Total | | | 0.835 | 0.75 – 0.90 | 6.494 | 0.10 – 0.05 |
| Summed | 252 | 201 | 0.071 | 0.75 – 0.90 | 5.745* | 0.02 – 0.01 |
| Homogeneity | | | 0.749 | 0.50 – 0.75 | 0.752 | 0.50 – 0.75 |

*Significantly different at $P \leq 0.05$.

**Table 2. $F_3$ and $F_4$ progeny row segregation among $F_2$ and $F_3$ genotypes from the 5-small leaflet and normal leaf plants from the Georgia-02C x 5-small leaflet mutant cross combination.**

| Genotype | No. $F_3$ and $F_4$ Progeny Rows | | $\chi^2$ | $\rho$ |
|---|---|---|---|---|
| | Segregating | Non-Segregating | | |
| $F_3$ (from $F_2$ 5-Small Leaflet) | 30 | 0 | - | - |
| | | | (1:1) | - |
| $F_3$ (from $F_2$ Normal Leaflet) | 8 | 7 | 0.067 | 0.75 – 0.90 |
| | | | (1:1) | |
| $F_4$ (from $F_3$ Normal from $F_2$ 5-Small Leaflet) | 8 | 7 | 0.067 | 0.75 – 0.90 |
| | | | (1:2) | |
| $F_4$ (from $F_3$ Normal from $F_2$ Normal Leaflet) | 5 | 10 | 0.000 | 1.00 |

leaf and Georgia Green (Fig. 3) resulted in the two highest yielding entries at 6227 and 5800 kg/ha, respectively. Normal Leaf from 5-Small Leaflet was next highest followed by the lowest yielding entry, the 5-Small Leaflet Mutant (Fig. 4) at 2357 kg/ha which equals approximately 40% yield reduction compared to the Georgia Green cultivar. Likewise, total sound mature kernels (TSMK) percentage, 100 pod and 100 sound mature kernels (SMK) weights were lower for the 5-Small Leaflet compared to Georgia Green.

A full diallel crossing block of these same three selections used in the yield test (Normal from normal, Normal from 5-Small Leaflet, and 5-Small Leaflet) resulted in only $F_1$ seed from (Normal from normal) x (Normal from 5-Small Leaflet) and (Normal from normal) x (5-Small Leaflet) crosses. The other four possible diallel cross combinations did not produce any viable seed, only an occasional immature pod. $F_1$ hybrid plants from these two crosses exhibited the 5-Small Leaflet trait which again suggests that it is dominant to normal leaf.

The $F_2$ individual plant segregation from both cross combinations showed an acceptable fit for a 9 (normal) to 7 (5-Small Leaflet) and a 1 (normal) to 1 (5-Small Leaflet) genetic ratios, respectively (Table 4). The 9 (normal) to 7 (5-Small Leaflet) is the reverse compared to the earlier cross combinations (Table 1), with a 9 (5-Small Leaflet) to 7 (normal). Total, summed, and homogeneity chi-square values were also found acceptable for both of these two expected genetic ratios 9:7 and 1:1 (Table 4).

$F_3$ progeny row segregation among $F_{2:3}$ normal leaf from both normal from normal and normal from 5-Small Leaflet showed an acceptable fit for a 2 segregating to 1 non-segregating ratio (Table 5). Whereas, the $F_{2:3}$ 5-Small Leaflet progeny rows were all segregating from both normal from normal leaf and normal from 5-Small Leaflet cross combinations.

## Discussion

These findings suggest that the 5-Small Leaflet mutant is heterozygous since it continues to segregate upon selfing for numerous generations. The fact that the 5-Small Leaflet mutant can only be used as a male and not a female parent, could be related to its elongated stigma abnormality which was only noticeable on about 50% of the flowers from individual plants during crossing. Many attempts to make crosses with 5-Small Leaflet as a female on both normal and elongated stigma were not successful. It also exhibits pleiotropic effects for several other plant characteristics: smaller canopy size (Figure 4), lower pod yield, reduced pod and seed weight (Table 1), as well as elongated stigmas (Figure 2).

The inheritance of the 5-Small Leaflet mutant trait appears to be quite novel. In certain cross combinations it appears dominant (Table 1) while in other crosses it appears to be recessive (Table 4).

**Table 3. Selfed normal and 5-small-leaflet genotypes in preliminary yield test at the University of Georgia, Coastal Plain Experiment Station, Tifton, GA 2011.**

| Selfed Genotype | Pod Yield | TSMK | Pod wt. | SMK wt. |
|---|---|---|---|---|
| | (kg/ha) | (%) | (g/100) | (g/100) |
| Normal Leaf (from Normal Leaf) | 6227 a* | 79 | 140 | 60 |
| Georgia Green (ck) | 5800 a | 79 | 140 | 59 |
| Normal Leaf (from 5-Small Leaflet) | 3648 b | 76 | 115 | 61 |
| 5-Small Leaflet Mutant | 2357 c | 77 | 109 | 57 |

*Pod yields within the column followed by the same letter are not significantly different at $P \leq 0.05$.

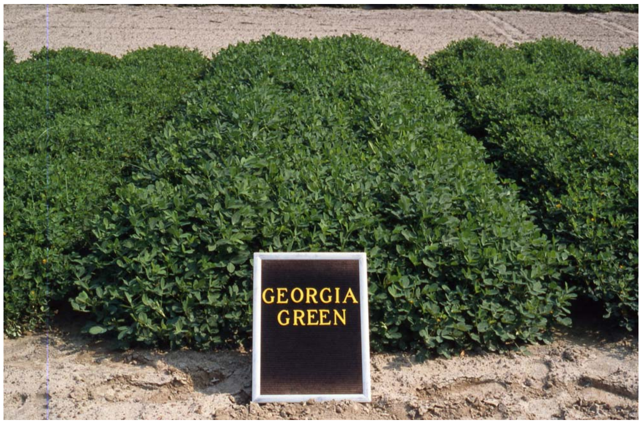


**Fig. 3. Two-row plot of the 'Georgia Green' cultivar from which the 5-Small Leaflet Mutant was found.**

**Fig. 4. Two-row plot of the 5-Small Leaflet Mutant showing the small decumbent growth habit.**

Whereas in all $F_3$ progeny rows, 5-Small Leaflet continues to segregate without any non-segregating progeny rows (Tables 2 and 5). However in the $F_{2:4}$, 5-Small Leaflet progeny rows did show an acceptable fit to a 1:1 ratio of segregating to non-segregating progeny rows, respectively (Table 2). The reason or cause for continuous segregating is not clear, but it could be related to the fact that the 5-Small Leaflet plants cannot be used as a female parent in crosses.

Another unique aspect of the 5-Small Leaflet inheritance and subsequent segregation appears to be related to an acceptable fit for a (1:1) genetic ratio. Modified genetic ratios have previously been reported for another small leaf character in peanut (Patil and Mouli, 1984). This imparipinnate mutant appeared to be recessive compared to normal leaf, and "preferential" segregation in favor of normal leaf was suggested for the modified monohybrid ratio of 6:1 found to fit imparipinnate inheritance.

Branch and Hammons (1983) likewise reported upon a partial dominant micro phenotype found in peanut. The $F_1$ and $F_2$ data suggest monogenic inheritance for the micro phenotype.

The 5-Small Leaflet mutant appears dominant at least in the three wider genetic cross combinations (Table 1), but it has continuous segregation which suggest it is heterozygous and does not breed true-to-type upon selfing. These characteristics might suggest a chromosome deletion, thus pseudo-dominance inheritance.

Genomic mapping of the cultivated allotetraploid peanut was recently completed through an international effort (Bertioli *et al.*, 2019). These

**Table 4. $F_2$ individual plant segregation for 5-small leaflet and normal leaf genotypes from two cross combinations.**

| Cross (♀ x ♂) | No. $F_2$ Plants | | | | | |
|---|---|---|---|---|---|---|
| | Normal | 5-Small | $\chi^2$ (9:7) | $\rho$ | $\chi^2$ (1:1) | $\rho$ |
| Normal (from Normal Leaf) X 5-Small Leaflet | 146 | 128 | 0.979 | 0.25 – 0.50 | 1.182 | 0.25 – 0.50 |
| Normal (from 5-Small Leaflet) X 5-Small Leaflet | 92 | 77 | 0.226 | 0.50 – 0.75 | 1.331 | 0.10 – 0.25 |
| Total | | | 1.205 | 0.50 – 0.75 | 2.513 | 0.25 – 0.50 |
| Summed | 238 | 205 | 1.148 | 0.25 – 0.50 | 2.458 | 0.10 – 0.25 |
| Homogeneity | | | 0.057 | 0.75 – 0.90 | 0.055 | 0.75 – 0.90 |

**Table 5. $F_3$ progeny row segregation among $F_{2:3}$ genotypes from the normal from normal leaf and normal from 5-small leaflet x 5-small leaflet mutant cross combination.**

| $F_2$ Genotype | No. $F_3$ Progeny Rows | | | |
|---|---|---|---|---|
| | Segregating | Non-Segregating | $\chi^2$ (2:1) | $\rho$ |
| (Normal from Normal) | | | | |
| $F_{2:3}$ Normal Leaf | 20 | 10 | 0.000 | 1.00 |
| $F_{2:3}$ 5-Small Leaflet | 30 | 0 | - | - |
| (Normal from 5-Small Leaflet) | | | | |
| $F_{2:3}$ Normal Leaf | 21 | 9 | 0.150 | 0.90 – 0.95 |
| $F_{2:3}$ 5-Small Leaflet | 28 | 0 | - | - |

authors found that variable deletions were common (i.e. chromosome Arahy .04/Arahy .14) among different *A. hypogaea* genotypes representing both subspecies, *hypogaea* and *fastigiata*. It could be possible that the maternal-sterile outcrossing trait is linked and found in a similar deletion. This could also explain the lack of obtaining a true-breeding 5-Small Leaflet genotype.

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