maintained separate in Fig. 4. Above about 110 tenderometer reading the percent yields separate distinctly. This separation of yields indicates a major influence of available soil water on the development of fresh peas in their later stages of growth. We suggest that this factor be carefully evaluated for experiments where irrigation or stored soil water is an experimental variable.

In passing, we note the failure of an appealing normalization procedure involving both yield and tenderometer reading. For each experiment, the maximum and minimum yield or tenderometer readings were noted and the normalized observation computed as $(u-u_{min})/(u_{max}-u_{min})$. The symbol u indicates the variable to be normalized. Nearly the whole range of normalized yield was noted for normalized tenderometer readings <0.5. Furthermore, there was much scatter providing little basis for a calibration.

Norton et al. (4) and Sayre (7) point out that 1 scale is not applicable to all pea cultivars. Norton et al. (4) add that the use of a well-developed scale for 1 cultivar to adjust another cultivar may introduce less error than using a scale developed from only a few points. Information presented in Fig. 4 is consistent with earlier results (1, 2, 4, 7) showing a similar relationship between percent yield and tenderometer readings in the range of 90 to 110. Percent yields changed between 1 and 2 percentage units with each unit change in tenderometer reading.

Experience by the authors indicates that fresh pea yield comparison at a common maturity is essential to good research. Harvesting each treatment at 2 or more times and interpolating the yield at 100 tenderometer is preferred. When only 1 harvest is possible, yields can be adjusted to 100 tenderometer by using a percent yield-tenderometer scale (Fig. 4) which provides more reliable data than merely using the unadjusted yields.

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Influence of the Multiflora-Grandiflora Genotypes of Petunia on Seed Germination, Seedling Growth, and Elemental Foliar Composition[1]

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Abstract. Three sets of *Petunia hybrida* Vilm. lines were used with each set comprised of the 3 genotypes, multiflora (*gg*), grandiflora (*GG*), and heterozygote (*Gg*). Seed germination was consistently high for the hybrid *Gg* (92%), intermediate for *gg* (77%) and low for *GG* (45%). The fresh and dry wt of 28-day-old seedlings was inconsistent but the *Gg* hybrid was the most vigorous at 49 days followed by the *gg* and *GG* genotypes. No differences were observed in N, P, K, Na, Mn, Fe, Cu, Zn, or Al in vegetative leaves of the 3 genotypes. Differences in Ca, Mg, and B occurred, but they were not uniform with respect to genotype or to genotypes within a set. Calcium and Mg were generally highest in *gg* and lowest in *GG*. Boron in 1 of 2 experiments showed the same pattern. The physiological roles of the observed differences in elemental composition with respect to chlorophyll composition, sugar metabolism, and vigor as indicated by an increase in fresh and dry wt, in the 3 genotypes are discussed.

Petunia cultivars are classified by plant and flower characteristics either as grandiflora or multiflora. Multiflora plants generally have dark green foliage, a large number of small flowers with small calyces and long, narrow sepals and slender filaments; in contrast, the typical grandiflora has light green foliage, fewer flowers, and calyces with short, broad sepals and short, thick anther filaments (6). It has been shown (1, 6, 12) that the grandiflora and multiflora types are determined by the *G* and *g* alleles, at a single locus respectively, and the homozygous *GG* showed degrees of sub-lethality due, perhaps, to low chlorophyll content. In addition, Bianchi (1) observed a certation effect which he concluded arose by linkage of self-sterility alleles with those determining flower size.

Reimann-Philipp (12) found no linkage between the self-sterility alleles and flower size and abscribed the reduced number of seeds to a zygotic lethal factor *l* (normal allele *L*) which often reduced fertilization in *l* pollen grains; so its function could also be explained as certation. He concluded that the low number of grandiflora homozygotes was due to sublethality of the genotype *GG* caused by a chlorophyll defect linked to the zygotic lethal factor. Ewart (6) also concluded that lethal and sub-lethal alleles may be closely linked with *G* resulting in a class of weak homozygous dominant petunias. He suggested also, that alleles of gene(s) controlling vigor may interact with the large flower-viability gene linkage.

Seidel (13) showed that the *G* locus determining large flower in superbissima petunias (tetraploids) and in diploid grandifloras was the same. The genes determining flower size in *P. hybrida* grandiflora and in *P. axillaris* were found by Chlebowski (3) to be at the same locus. Petals with green margins in *P. hybrida* grandiflora and *P. hybrida* vulgaris (multiflora) also appeared to be linked with the grandiflora character (4). This linkage, like that involving lethality and fimbriate borders (4), is not universal to the species but is found only in certain genetic lines. Ewart (personal communication) indicated the linkage between *G* and the lethal gene(s) has been broken in breeding lines.

Hence, the grandiflora character is a monogenic inherited characteristic resulting from action of the genes *G* and *g* which control, by some as yet unknown physiological action, the flowering and growth type of petunia plants. We undertook to determine the influence of

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genes at this locus on physiological aspects of seed germination, seedling growth, and elemental content of vegetative leaves.

Materials and Methods

Three sets of lines of *P. hybrida* Vilm., homozygous multiflora (*gg*), homozygous grandiflora (*GG*), and their hybrid (*Gg*), obtained from Harris Seed Co. (Table 1) were used as the experimental genotypes. Seed germination was determined by placing 50 seeds on Whatmann No. 1 filter paper moistened with 5 ml of deionized-distilled water in a 10-cm petri dish. The dishes were placed in a chamber at 16 hr light-8 hr dark at 26°C and 22°C, respectively. The number of germinated seedlings was recorded daily beginning the second day and continued for 8 days, and a final germination count was made at 14 days. The standard deviation for each line was computed and the means of the 3 lines of each genotype were compared using the Studentized t-test (14).

In all other experiments, the 9 experimental accessions were considered as lines and were partitioned into 3 sources of variation: genotypes, sets (each comprised of the 3 genotypes), and the set by genotype interaction. When genotypes were significant in the AOV the mean for each genotype was compared by Tukey's hsd test (15) at the 1% level.

Plants for experimental purposes were obtained by sowing the seeds on dampened peat-lite mixed with terra-lite. After 2 to 3 true leaves appeared, 15 21 days from sowing, the seedlings were transplanted into 2.5 × 2.5-cm plastic bedding plant pots filled with dampened peat-lite mixed with terra-lite. The pots were watered on alternate days with 10 ml per pot of half-strength Hoagland's (7) nutrient solution and water. The plants were grown in a greenhouse at 22 to 27°C and with supplemental light when necessary to extend the daylength to 16 hr to induce flowering.

Growth data were obtained from seed sown April 12, 1972 and May 3, 1972. The fresh and dry wt and number of leaves were determined at 28 and 49 days after sowing and, in addition, plant ht was recorded at 49 days. In the first planting, 4 plants per line were sampled at 28 days and 2 at 49 days; whereas, in the second planting, 8 plants were sampled at 28 days and 5 at 49 days.

Elemental analyses were obtained for the vegetative foliage on plants in flower according to the procedure of Kenworthy (8). Petunia seedling growth can be divided into 2 stages: the vegetative leaves are alternate in the first stage. Opposite leaves are produced when flowering begins. Alternate leaves are usually larger and more abundant than opposite leaves; thus they were used for analysis. All of the alternate leaves were harvested from each plant and analyzed for 10 major and minor elements.

Results and Discussion

The results of the seed germination study (Fig. 1) are representative of 5 sets, each consisting of 3 genotypes, which were observed in a preliminary study. Germination of seeds of the 3 genotypes were alike 3 days after sowing. However, at maximum germination 8 days after sowing the seed germination differed among the 3 genotypes. The *Gg* genotype germinated best. Germination of the *gg* genotype was 78.4 percent of that of the hybrid *Gg* while *GG* was only 46.9 percent of the hybrid (Fig. 1). These data indicated that inbred multiflora and grandiflora breeding lines lacked the vigor in seed germination that was exhibited by their respective hybrids. High seed germination of the hybrid appeared to be primarily due to heterosis in contrast to the inherent weakness of both inbred homozygous multiflora and grandiflora genotypes.

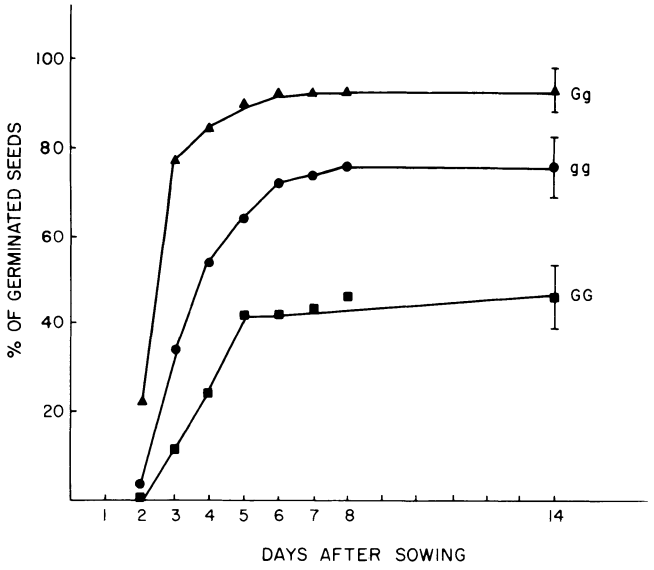

Fig. 1. Relationship between grandiflora *GG*, multiflora *gg* and hybrid *Gg* genotypes of petunia and seed germination. Vertical line at 14 days is the standard deviation.

In both experiments on seedling growth (Tables 2 and 3), lines were significantly different for fresh and dry wt at 28 days but differences between genotypes within lines were not significant. This variance was due to set and/or set by genotype interaction, independent of the multiflora-grandiflora genotype influence. At 49 days, the mean fresh and dry wt for each genotype were significantly different. The *GG* genotype was lowest in both parameters followed by the *gg* genotype, which was intermediate, and the hybrid (*Gg*), which had the highest fresh and dry wt (Table 2). Lines were significantly different for plant ht but not genotypes.

In the second planting some lines were significantly different from one another, but this was not due to differences in genotype. Thus, the means of the 3 lines of the same genotype were not combined as in the first planting (Table 3). However, in considering each set independently, *gg* and *Gg* genotypes had a higher fresh and dry wt than the *GG* lines at 49 days. Plant ht followed the same trend as in planting 1, *Gg* was the tallest followed by *gg* and lastly *GG* lines (Table 3).

The differences in vigor as indicated by these growth data are related to genotype with F_1 hybrids accumulating up to twice the quantity of fresh and dry matter as either parent genotype at 49 days. Whether this increase is the result of a more efficient genotype-related metabolism or other factors is unknown since there was no interaction between genotype and set. The grandiflora inbreds, genotype *GG*, were the slowest growing as indicated by their slower increase in mean fresh and dry wt. This may have been due to inherent weakness of the genotype by lowered chlorophyll and/or elemental content.

Although significant differences in some elements were observed between various lines in the foliar analysis study, only these elements which were significantly different at the 1% level for genotypes as well as lines were investigated in detail (Table 4). Significant differences in percent Mg and Ca in vegetative leaves of mature multiflora and grandiflora genotypes were observed. In all sets, *GG* was lower in Ca than *gg* and in all but the second set from *Gg* lines. The percent Mg in

Table 1. *Petunia hybrida* Vilm. breeding and hybrid lines employed in this study.

Genotype and Set	Description
Set 1	
gg[z]	Salmon Perfection
GG	Salmon Perfection, very weak.
Gg	Hybrid, Salmon Perfection, fringed, glowing salmon.
Set 2	
gg	Ace of Hearts inbred.
GG	Ace of Hearts inbred, weak.
Gg	Hybrid, Ace of Hearts, bright red.
Set 3	
GG	Roulette inbred, weak.
gg	Roulette inbred.
Gg	Hybrid, Roulette, red and white bicolor.

[z] gg—multiflora; G—grandiflora.

Table 2. Seedling fresh and dry wt at 28 and 49 days and plant ht at 49 days for grandiflora and multiflora genotypes in Experiment 1.

Genotype and set number	g/seedling				Plant ht 49 days (cm)
	Fresh wt		Dry wt		
	28 days	49 days	28 days	49 days	
GG-1	0.034[z]	2.734	0.003a	0.170	4.6a
GG-2	0.105ab	7.232	0.008ab	0.395	3.2a
GG-3	0.027a	3.428	0.001a	0.125	5.1ab
		4.465a		0.230a	
gg-1	0.281c	9.945	0.017c	0.634	11.0d
gg-2	0.187bc	9.213	0.014bc	0.634	5.6ab
gg-3	0.126ab	7.989	0.009abc	0.524	7.5c
		8.989b		0.620b	
Gg-1	0.217bc	19.848	0.015bc	1.373	11.8d
Gg-2	0.137ab	15.879	0.013bc	1.213	11.3d
Gg-3	0.188bc	17.684	0.011bc	1.298	8.4c
		17.804c		1.290c	
	$HSD_{0.01}$ 0.160	4.45	0.009	0.329	1.8

[z] Means within a column having the same letter are not significantly different.

Table 3. Seedling fresh and dry wt at 28 and 49 days and plant ht at 49 days for grandiflora and multiflora genotypes in Experiment 2.

Genotype and set number	g/seedling				Plant ht 49 days (cm)
	Fresh wt		Dry wt		
	28 days	49 days	28 days	49 days	
GG-1	0.148a[z]	6.093a	0.012a	0.415a	3.58a
GG-2	0.210ab	8.494ab	0.012a	0.554bcd	3.22a
GG-3	0.208ab	5.787a	0.011a	0.360abc	5.92b
gg-1	0.457bc	13.936c	0.030a	0.952e	12.44d
gg-2	0.240abc	12.195bc	0.016a	0.692bcde	5.62b
gg-3	0.212ab	8.179ab	0.013ab	0.502a	9.44c
Gg-1	0.405abc	11.894bc	0.030ab	0.764cde	13.94d
Gg-2	0.488bc	12.165bc	0.033b	0.777de	10.54c
Gg-3	0.563c	12.156bc	0.038b	0.811de	13.34d
	$HSD_{0.01}$ 0.295	4.230	0.022	0.244	1.56

[z] Means within a column having the same letter are not significantly different.

Table 4. Elemental analysis of 49 day old seedlings of grandiflora and multiflora genotypes in experiments 1 and 2.

Genotype and set number	Exp. 1		Exp. 2		
	% Dry wt		% Dry wt		ppm
	Ca	Mg	Ca	Mg	B
GG-1	2.06a[z]	1.10ab	2.00a	1.12ab	44.2ab
GG-2	2.54bcd	1.20abcd	2.83b	1.32bc	47.3abc
GG-3	2.33ab	1.23bcd	2.52ab	1.46c	37.3a
gg-1	3.17	1.20abcd	3.54cd	1.24abc	68.8d
gg-2	2.94	1.30cd	3.58cd	1.47c	57.9bcd
gg-3	3.46	1.41d	3.89d	1.78d	52.3bc
Gg-1	2.80cde	0.98a	3.00bc	1.01a	58.9cd
Gg-2	2.47bc	1.04ab	2.77l	1.15ab	46.6abc
Gg-3	2.87de	1.10abc	3.62cd	1.44c	44.8ab
	$HSD_{0.01}$ 0.37	0.23	0.68	0.27	13.7

[z] Means within a column having the same letter are not significantly different.

sets 2 and 3 for the *GG* genotypes was numerically intermediate between the *Gg* and *gg* lines. The *Gg* and *gg* lines in sets 2 and 3 differed in their Mg content with *gg* having the highest Mg content. In set 1, the 3 genotypes did not differ in percent Mg (Table 4). No differences were observed in N, P, K, Na, Mn, Fe, Cu, Zn, or Al among genotypes in either planting.

When the elemental analysis was repeated with plants grown at a later date, significant differences were observed for the elements Ca, Mg, and B. In sets 1 and 3, *Gg* lines had a higher percent Ca than the *GG* lines; the *gg* lines had the highest percentage of Ca and was greater than *Gg* lines for sets 1 and 2. The *gg* lines had a higher quality of Mg than *Gg* lines in sets 2 and 3 and the *GG* line in set 3. In set 1, there were no differences in Mg content. In sets 1 and 3, B was higher in leaves of *gg* than in those of *GG*, and *Gg* of set 1 was higher in B than any *GG* genotype. There were no differences in set 2 in B content (Table 4).

Calcium was generally highest in lines with the *gg* genotype, lowest in *GG* lines, while Mg also followed this pattern. Boron, in the second planting also followed this pattern. Calcium is involved in the maintenance of cell membranes and functions in ion transport, especially in chloroplasts and mitochondria. In fact, these organelles are sites of Ca accumulation along with Mg (9, 10, 11, 16). Magnesium is a primary constituent of chlorophyll as well as being active as a co-factor in numerous biochemical pathways, especially those involving phosphate transfer. Unlike Ca, Mg is a mobile ion, readily translocated from older tissues to young meristematic regions (5). Boron is involved in the translocation of sugars from leaves and especially in switching the degradation of glucose, to either glycolysis or the pentose shunt (5). Whether or not any of these differences in ion concn is of physiological importance in the petunia genotypes studied herein cannot be determined from the above data alone, since these elements were usually found present in super-optimal concn in the plant.

Differences in Ca, Mg, and B may be related to chlorophyll content and/or metabolic differences. Since in most cases, Mg is especially high in the *gg* lines, this may indicate that these plants do in fact have a higher concn of functional chlorophyll than do the *GG* lines. Whether these differences are the result of the grandiflora-multiflora gene locus and a direct effect of these alleles, cannot be definitely determined by this study. Future studies to determine the actual chlorophyll content of these genotypes may partially answer this question.

It must be recognized that these differences may be the result of other genes common to the 3 sets and which may mask or act synergistically with this particular gene locus. This possibility is indicated by the difference in elemental content in the 3 sets. The elemental values obtained for the hybrids do closely resemble those reported (2) for the grandiflora hybrid 'Pink Magic' as optimum.

In conclusion, the breeding lines we employed were not isogenic with respect to the *G-g* alleles; therefore, the differences we observed cannot be attributed solely to variation in gene action at this locus. Certainly, the genetic background is not similar in the homozygous *gg* multiflora and *GG* grandiflora inbreds; and the hybrid vigor of the *Gg* genotype accounted in part for its superior seed germination and seedling growth. Similarly, in the lines we used, the lethal gene *L* was not linked with *G*; had it been, perhaps the *GG* genotype would have responded in a much less vigorous manner. We observed consistent lower vigor in the inbred *GG* genotypes, but no abnormally weak or malformed seedlings or flowering plants.

The use of 3 sets each, of the 3 genotypes of diverse origins allowed us to gain a broader understanding of the influence of the *G* gene on growth and flowering of petunia seedlings. In all sets, the multiflora petunias appeared to have greater vigor than the grandiflora inbreds as shown in the growth studies, the hybrids, as expected, surpassed these in terms of seed germination and early seedling growth. Except for these obvious differences in viability and vigor, however, all 3 genotypes appeared to have generally the same physiological makeup in levels of major and minor elements.

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