A COMPARATIVE STUDY OF CONVENTIONAL, LEAFLESS AND SEMI-LEAFLESS PHENOTYPES OF *Pisum sativum* L.

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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FOREWORD

This thesis was written in paper style according to regulations specified in the 1976 Plant Science Thesis Guide, sction 3. The tesis "A Comparative Study of Conventional,Leafless, and Semi-Leafless Phenotypes of Pisum sativum L." consists of three manuscript:

1- A Comparative Study of Conventional,Leafless and Semi-Leafless Phenotypes of Pisum sativum L.In vitro study of photosynthetic CO_2 fixation.

2- A Comparative Study of Conventional, Leafless and Semi-Leafless Phenotypes of Pisum sativum L. Effects on yield components.

3- A Comparative Study of Conventional, Leafless and Semi-Leafless Phenotypes of Pisum sativum L. Effects on root and shoot characters of young seedlings.

All manuscripts have been submitted for publication in the CANADIAN JOURNAL OF PLANT SCIENCE.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

Lafond, Joseph Guy Philippe. M.Sc., The University of Manitoba, October 1980.

A Comparative Study of Conventional, Leafless and Semi-Leafless Phenotypes of Pisum sativum L. Major Professor: Dr. L.E. Evans.

Two recessive gene mutations affecting foliar development in peas were studied. The mutation af converts leaflets into tendrils and st reduces the stipules to vestigial structures. Three phenotypes were examined afaf++(semi-leafless), afafstst(leafless) and ++stst(vestigal stipules) in four sets of isogenic lines of which Freezer and Canner had seven backcrosses and Century and Trapper four backcrosses.

We studied the effects of these particular conditions on the chlorophyll content, total protein, soluble protein, in vitro CO_2 fixation, water content and intercellular spaces. In all lines examined the chlorophyll content was reduced in the leafless and semi-leafless phenotypes. The leafless phenotypes had significantly higher levels of total protein but soluble protein levels were at best equal or lower than the leafed type. The water content is higher in the leafless phenotype with less free intercellular space in the tendrils as compared to the leaflet. In vitro measurements of CO_2 fixation revealed equal values between the different foliar phenotypes. We postulate that the lower in vivo CO_2 photo-assimilation values for tendrils would result from a higher CO_2 resistance into the tendril.

Two sets of isogenic lines Century and Trapper with their respective foliar phenotypes were also examined for effects on yield components. The genetic background greatly influenced the performance of these new plant models indicating the need for evaluation of as many genotypes as possible. The semi-leafless phenotype of Century yielded as well as the leafed type. Four sets of isogenic lines were examined for root and shoot characters of young seedlings. The leafless and semi-leafless phenotypes all exhibited equal or higher values for the characteristics studied. After 10 days of growth all characteristics of the leafless and semi-leafless phenotypes of Century exceeded those of the leafed type. The same was observed when they were grown in complete darkness indicating higher respiration rates by these foliar phenotypes. A hormonal imbalance caused by lack of leaflets is suggested to explain these results.

The results obtained indicate the feasibility of using these new crop models and justify the need for more research with these genotypes.

INTRODUCTION

The recording and maintenance of spontaneously occurring mutations in crop species is an obligation of all plant breeders and geneticists. Examples of the ultimate use of such mutations are the strains being developed from two recessive gene mutations in the species Pisum sativum. These mutations have improved agronomic traits which reduce the amount of haulm and the problem of lodging. They presently form the basis of a new crop model in peas.

The following study involves a continuation of the physiological and agronomic work initiated at the John Institute in England on this new model. It was regarded as necessary to examine as many characteristics of the new genotypes as possible in order to discover any possible and/or predictable effects these mutations have on the plant as a whole.

Extensive information exists on various aspects of the physiology of the garden pea (Sutcliff and Pate, 1977). Under normal atmospheric concentrations of carbon dioxide and saturating light intensity (17.6 KLux), the rate of net photosynthesis exhibits a broad temperature optimum spanning the range of 25 to 35°C. The carbon dioxide compensation point is relatively high (70 PPM at 27°C, 17.6 KLux), and the rate of net photosynthesis relatively low, from which Pate (1975) concludes that the pea operates on the Calvin pathway for photosynthesis.

A major concern is the reduced dry matter production of the leafless peas as compared to the conventional phenotype. When in vivo CO_2 photoassimilation is measured on an area basis, the tendril

is as efficient as the leaflet but when in vivo CO_2 photoassimilation is measured on a dry weight basis, the values are significantly lower for tendrils than leaflets (Harvey, 1972; Harvey and Goodwin, 1978). Answers to the cause(s) of this were sought by measuring the activity of the principal carboxylation enzyme ribulose biphosphate carboxylase. The phosphoenal pyruvate carboxylase enzyme activity was not measured since earlier results by Atkins et al (1977) showed the in vitro activity ratio of RuBP carboxylase to PEP carboxylase varied from 48:1 to 156:1 for blossom leaflets. Chlorophyll, soluble protein and Kjeldahl nitrogen were measured as were the fresh weight/dry weight ratio and intercellular space index in sets of isogenic lines.

The second major question deals with the mutant effects on yield components. Earlier results by Harvey (1978) and Snoad et al (1976) indicated a reduction in economic yield of the leafless peas. The reduction in yield resulted from fewer pods per plant and fewer seeds per pod. In this study thirteen characters affecting yield were measured in two different sets of isogenic lines, the purpose being to investigate how important the genotypic background was in affecting yield components of leafless and semi-leafless plants and whether or not the effects were similar.

A third objective was to examine root and shoot characteristics of 10 day old seedlings in four sets of isogenic lines to determine if any discernible effects could be attributed to changes in foliar morphology.

LITERATURE REVIEW

I. A New Crop Model for Peas

A. Genetic Basis for New Models

There is a great interest in the area of plant breeding in the creation of new plant models. It permits the reconstruction of plant types specific to certain agronomic needs. An example of a crop where drastic changes are being effected is in the vining and dried pea crop (Davies, 1977a).

There is a great deal of genetic variability in Pisum sativum L. (Yarnell, 1962; Marx, 1977). Three spontaneous mutations served as the basis for the new models; "af" which converts leaflets to tendrils; "tl" which converts tendrils into minute leaflets and "st" which reduces the stipules to vestigial structures.

The mutation "st" was first reported in 1915 and it appeared spontaneously in a single plant within a row of the variety Duke of Albany (Pellew and Sverdrup, 1923). Nilsson (1933) reported the spontaneous mutation "tl" and Kujala (1953) reported the "af" mutation.

B. Possible Models With These New Mutations

One of the proposed models assumed that an increase in photosynthetic area would be advantageous and envisaged the use of the genotype "afaftltl" which has a mass of small leaflets resulting in an increase in total leaf area (Harvey, 1972). This phenotype with its mass of small leaflets was soon eliminated from consideration because it exaggerated the top heavy condition of the normal form

(Davies, 1977b). The model also failed to improve lodging resistance and may have even aggravated the problem (Snoad, 1974).

Subsequently, it was suggested that the new model should decrease lodging, induce earlier drying and reduce the amount of haulm, thus reducing the bulk of foliage processed by the viners (Snoad and Davies, 1972).

C. Feature of the New Model

The proposed model has the leaflets converted to tendrils and the stipules left either in the normal condition (semi-leafless form) or reduced to vestigial structures (leafless form) (Snoad and Davies, 1972).

The leafless form attracted the most favourable comments from the agricultural industry for the dried pea crop. It remains upright until maturity, dries out well and permits direct harvesting with a combine harvester (Davies, 1977b).

The greater standing ability plus the lack of shading should improve the uniformity of maturity. The ability of light to penetrate the canopy should delay senescence of the lower parts of the plant. The lack of foliage may also lead to a reduction in herbicide scorching of the crop and more efficient control of weeds (Snoad, 1974).

II. Preliminary Assessment of the New Leafless and Semi-Leafless Models

A. Agronomic Considerations

1. Yield Potential. An important consideration in the development of the new model is the question of whether or not yields can be sustained. Snoad (1974) showed that the mean weight of dry seeds per plant in the semi-leafless forms from small experimental plots was comparable to that of some of the best available commercial varieties. Snoad and Ghent (1975) in yield trials, found no significant differences between the conventional and leafless type.

Gritton (1972) showed that both the semi-leafless and leafless forms had lower yields than the conventional one. His yield analyses, however, were made on trellises where the plants were not subjected to normal competitive field conditions.

Goldenberg (1973) reported no significant differences in yield between the "afila" mutants and the conventional types. Much of the discrepancy in yield trials in different areas could result from differences in genetic background and the lack of sufficient backcrossing before isogenic lines were tested.

B. Effects of Mutations on Yield

Snoad et al (1976) showed that any of the recessive genes singly had only a small effect upon yield but that two or three genes in combination reduced yield significantly in comparison with the conventional form. The biological yield (total dry weight of plant and seed) is influenced most by any combination of "af" and "st". Previous data from conventional peas showed that when economic yield is plotted

against biological yield, an r^2 of 96% is obtained (Snoad et al.1976) This indicates that the harvest index increases with plant size suggesting that large plants are more efficient in partitioning available photosynthate. In a breeding program, selection of large plants with high biological yields may ultimately increase yield.

C. Drying Rates

Snoad and Ghent (1974) showed no obvious differences in drying rates between conventional and semi-leafless peas but the fully leafless form appeared to dry faster. Semi-leafless and leafless peas showed improved standing ability with the leafless showing the highest resistance to lodging.

D. Effect of Varying Plant Densities

Snoad et al (1975) found that the dry seed yield per plant in semi-leafless and leafless types was influenced by planting densities due to the effects upon determinancy and upon the number of pods contributed by the basal branches. This response was similar in the conventional forms. The number of pods and therefore the number of dry seeds on the main stem was reduced as planting density increased.

The ease with which tendrils may link with other tendrils or stems is vital to the standing ability of the plants. The total inadequacy of the 40 cm row width was immediately apparent. The 20 cm row width except in the lowest plant densities was also not perfect. The plants have to be planted on the square to maximize opportunities for cross linking of tendrils. The development of two or three basal branches from each plant is useful in developing the standing ability and so relatively low densities may be suitable for dried seed production.

E. Nitrogen Fixation in Leafless and Semi-Leafless Peas

Snoad et al (1976) in examining N_2 fixation found the conventional and the semi-leafless plants were very similar in all the measured characters with the exception of root dry weight and the number of nodules which were somewhat lower in the semi-leafless plants. In contrast, the dry weights of the root and shoot, the number of nodules and the amount of nitrogen fixed in the leafless peas only amounted to one-third to one-half of that measured in the conventional forms. When the rates of acetylene reduction were expressed relative to plant dry weight, the values for all three genotypes were similar suggesting that the leafless peas were as efficient as the conventional peas in fixing nitrogen. It remains to be seen if the yields of leafless peas would respond favourably to added nitrogen.

F. Summary of Agronomic Potential

It is apparent that this new model has several advantages. The increased standing ability favours light penetration into the canopy, thereby favouring photosynthesis and possibly delaying senescence in the lower layers of the canopy. The microclimate surrounding leafless peas will be more illuminated and drier than in the conventional crop and should be less conducive to the establishment of pathogens.

III. CO_2 Photoassimilation in Normal and Foliar Mutants of Unimproved Cultivars of Peas

Harvey (1972) was the first to do a comparative assessment of CO_2 photoassimilation in normal and foliar mutants of pea. The mutants were "afaf++" (leaflets converted to tendrils) and "afaftltl" (minute leaflets on a branched petiole). These mutants were derived from un-

improved cultivars. An interim study using infra-red gas analysis indicated that in terms of CO_2 photoassimilation per unit area of youngest expanded leaf of glasshouse grown plants, the mutants were similar to the normal. The phenotype with only tendrils was the least efficient of those assayed at utilizing light at an intensity below 100 J m^{-2} sec^{-1} (400-700 nm). The genotypes with only tendrils on a unit dry weight basis were only 18% as efficient as a normal leaved pea in photoassimilating CO_2, the other mutants being comparable to the normal in this respect. Comparison of dark respiration indicated that the foliar mutants were similar to the normal types, although no data was available for the mutant with tendrils only.

Harvey (1972) also showed that CO_2 photoassimilation by the youngest fully expanded leaf was a reproducible characteristic and not significantly affected by a transition from the vegetative to reproductive phase of growth.

IV. Comparison of Rates of Transpiration, Stomatal Frequency and Distribution; Chloroplast Distribution

In terms of rate of transpiration, all three foliar mutants were the same as the conventional types. The units for transpiration rate were ΔRH cm^{-2} leaf area (total surface) sec^{-1}. The value ΔRH (change in relative humidity) is the steady state difference between RH of air entering and leaving the assimilation chamber of the infra-red gas analyser. This would seem to indicate that CO_2 resistance into the plant is not the cause of low CO_2 photoassimilation on a unit dry weight basis (Harvey, 1972).

Harvey (1972) also examined the stomatal distribution and frequency. The relative number of stomata per unit area was on the average 25% less on upper than the lower surface of mature normal leaflets of conventional types. The epidermis of tendrils of

of normal leafed plants.

The chloroplast containing palisade and mesophyll tissues in normal pea leaflets were absent from the tendrils of "afafTLTL". Examinations of transverse sections of tendrils from the genotype "afafTLTL" indicated that the chloroplasts were confined to four or five consecutive sub-epidermal layers of cells which occupied the entire circumference of the tendril. The type of chloroplast distribution was characteristic of stems, petioles and tendrils of normal leaf plants (Harvey, 1972).

V. Effect of Mutation on the Physiology of the Pea Plants in Near Isogenic Lines

Harvey and Goodwin (1978) studied the effect of the leafless mutation in terms of foliage area, light interception, net CO_2 exchange and rate of dry matter production by comparing two near isogenic lines of the genotypes "afafstst" and "++++" (conventional).

A. Dry Matter Production

Harvey and Goodwin (1978) did a growth analysis study in the leafless and conventional phenotypes throughout the period of vegetative growth. Within each phenotype, in plants with one basal shoot, the main axis and basal shoot each had the same growth on a dry weight basis. Examinations of more genotypes with the leafless or semi-leafless condition might reveal otherwise.

B. Foliage Area Determination

Foliage area determination by Harvey and Goodwin (1978) showed the conventional phenotype attained a plateau at 220 cm^2, 56 days from seedling emergence. In the leafless phenotype, the aggregate tendril surface area was still increasing markedly at day 68 (from

seedling emergence) at which time this area was 168 cm^2. The fully expanded leaf area at individual nodes tended to increase with ascending nodal position in both phenotypes but the effect was more pronounced when tendrils replaced leaflets.

C. Seed Yield

Despite an apparently high photosynthetic potential observed for tendrils, there is consistently 50% less dry matter produced in the leafless phenotype (afafstst) during vegetative growth than in the conventional line (Harvey and Goodwin, 1978). An analysis of seed yield for the two phenotypes on a per plant basis showed that in the leafless mutant, there was a reduction of 50% in seed number despite commencing with a similar number of flowers and ovule initials per plant (Snoad et al, 1976; Harvey, 1978).

No reduction in yield was noticed for "afafSTST" or the "AFAFstst" phenotypes despite their respective reduction in leaf and stipule area (Harvey and Goodwin, 1978). Harvey (1974) showed that despite the changes in foliar morphology branches from leafless and semi-leafless plants when fed $^{14}CO_2$ are capable of exporting ^{14}C-photosynthate to the seed and pod wall in a manner comparable to normal leaves as previously determined (Harvey, 1973).

D. Photosynthetic Uptake of CO_2 by the Pod

There exists extensive reviews on seed and fruit development in P. sativum (Pate, 1975, Pate and Flinn, 1977). Between 6 and 30 days after anthesis, pod photosynthesis in conventional peas resulted in small gains of CO_2 from the external atmosphere, and the assimilation of most of the CO_2 respired by the fruit during the day. From then

until maturity (40 days) the fruit lost CO_2 during the day. Night losses of CO_2 increased with fruit age (Flinn et al, 1977). Measurements of photosynthesis in the pods of leafless phenotypes (afafstst) were done by Harvey (1978). The pods were more active photosynthetically in terms of CO_2 uptake from the atmosphere during the initial 18 days post-anthesis than were the corresponding fruits of the conventional phenotype. During the subsequent 16 day period of seed filling there was no marked difference between phenotypes and the fruit continuously lost CO_2 to the atmosphere, but significantly less CO_2 was lost in the light (40 KLux) than in the dark. Increased benefits from light available to the fruit within a sward canopy comprised of tendrils in place of leaflets is expected. Work by Atkins et al (1977) showed that the assimilation capacity of the inner epidermis is limited by radiant flux rather than carboxylation potential and any increases in incident radiation is likely to benefit CO_2 conservation. In the leafless plants, the more open canopy could reduce the amount of respiratory carbon loss from the fruit and act as an important yield determinant (Harvey, 1978).

E. Growth Analysis of the Fruit and Seed

A concurrent growth analysis of the fruit showed that the pod wall of each phenotype changed dry weight at a markedly different rate and attained a different maximum value (Harvey, 1978). The implication was that the gene "af" delayed maximal pod wall weight development by at least 6 days and also lowered the maximum wall weight attainable. The delay would result in competition for photosynthate on the part of the pod wall and the fruit. In the conventional types,

maximum elongation of the pod occurs before seed development starts (Pate, 1974). In the case of the leafless mutant, the delay in maximal wall development may result in an overlap with seed development such that competition for substrate causes ovule abortion. Nevertheless, the growth curves and mean dry weights per seed were not significantly different between the different phenotypes (Harvey, 1978).

However the dearth of information on the new leafless and semi-leafless plants in peas makes it difficult to do valid comparisons between tendrils and conventional leaflets. The tendrils although considered as modified leaves still possess a different structure and cell types making it difficult to extrapolate information from conventional leaflets to tendrils. Due to genotypic differences in conventional leaflets of peas for assimilate production and photosynthesis (Harvey, 1972; Pate, 1975), valid comparisons can only be done through the use of isogenic lines.

A Comparative Study of Conventional, leafless and Semi-Leafless Phenotypes of Pisum sativum L. In vitro study of CO_2 photosynthetic fixation.

ABSTRACT

The effect of the "leafless" mutations (in which tendrils replace leaflets, af and the stipules are reduced to a vestigal form, st) upon chlorophyll content, total protein, soluble protein, in vitro CO_2 fixation, water content, and intercellular space index were studied. Comparison of three isogenic lines of Century, Freezer and Trapper for the genotypes afafstst (leafless), afaf++ (semi-leafless) and ++stst (stipules only reduced) were examined.

Chlorophyll levels were reduced by as much as 50% in the leafless phenotypes, the semi-leafless being intermediate. The leafless phenotype showed significantly higher levels of total protein but measurement of soluble protein indicated the leafless plant was at best equal to the leafed type or lower.

The water content was significantly higher in the leafless phenotypes in the three cultivars examined. The high water content results from changes in morphology and possibly a lower transpiration rate. Intercellular spaces within the tendrils was significantly lower than in the leaflet. This would increase the diffusive resistances of CO_2 into the tendrils.

In vitro measurements of CO_2 photoassimulation per unit fresh weight showed no significant differences between the different foliar phenotypes. Lower dry matter accumulation by the leafless type can be accounted for by the higher CO_2 resistance in the tendrils. An additional feature of the leafless plant is an altered pattern of senescence. Under stress situations, the lower nodes of the leafed type senesced before the tendrils in the leafless plant, indicating greater resistance to adverse conditions by the leafless plant.

INTRODUCTION

A new plant model for peas has been created involving leafless (afafstst) and semi-leafless (afaf++) characters (Snoad and Davies 1972). The new model offers advantages with respect to reduced lodging, decreased disease susceptibility and a more open canopy which favors better air movement, CO_2 exchange and light penetration for photosynthesis. The presence of numerous tendrils also offers mutual plant support allowing greater ease for harvesting (Snoad 1974, Davies 1977).

Harvey (1972) examined carbon dioxide photoassimilation in normal and foliar mutants of Pisum sativum and found that CO_2 photoassimilation per unit area of the youngest expanded attached leaf in the mutants was comparable to the normal while on a dry weight basis, the leafless mutant was only 18% as efficient. Harvey and Goodwin (1978) reported that the leafless mutant consistently accumulated 50% less dry matter than the conventional plants. Yield of mature dry seed per plant showed a reduction both in seed number and total seed weight.

In the present study conventional and leafless strains of otherwise similar genotypes were compared relative to chlorophyll determination, fresh weight to dry weight ratio, percent total protein, soluble protein, intercellular space index, and in vitro incorporation of CO_2 by ribulose bi-phosphate carboxylase.

MATERIALS AND METHODS

A. Plant Material

Seed from the seventh backcross of near isogenic lines of two vining pea cultivars Freezer and Canner were obtained from DR. G. A. Marx, New York Agricultural Station, Geneva, New York 14456, U.S.A. (Marx 1974).

Seed from two field pea cultivars, Century and Trapper and a leafless and semi-leafless phenotype of each with four backcrosses were obtained from Dr. S. T. Ali-Khan of the Morden Agriculture Research Station, P.O. Box 3001, Morden, Manitoba, Canada ROG 1JO.

Single plants were grown in the clay pots 13 cm in diameter with a 1:1:1 mixture of peat, sand and soil. They were grown in a growth cabinet with 18hrs daylight; a day/night temperature of $15^{o}C/13^{o}C$ and a constant relative humidity of 50% and a light intensity of 21.5 KLux.

The plants were grown for approximately 36 days. The last expanded leaf was used for the $^{14}CO_2$ assay and soluble protein determination while the previous expanded node for chlorophyll determination. A node includes stipule, petiole, leaflets and/or tendrils. The percent total protein using the Kjeldahl digestion and fresh weight/dry weight ratio were determined from the remainder of the plant.

B. Ribulose bi-Phosphate Carboxylase Enzyme Assay

The RuBP caboxylase enzyme assay for measuring the in vitro incorporation of $^{14}CO_2$ was done according to the procedure of Quebedeaux and Chollet (1975). The plant material was homogenized with a VirTis homogenizer in a tris-HCl buffer of pH 8 under a flow of dinitrogen gas. The homogenate was then transferred to a volumetric flask filled with dinitrogen gas. The reactions were initiated using ribulose bi-phosphoric acid. The assays lasting 3 minutes were conducted at $30^{o}C$ under a flow of dinitrogen gas to reduce any oxygen interference and were terminated using 6N glacial acetic acid. The assays were carried out in scintillation vials. The material was then dried at $90^{o}C$, liquid scintillation fluid added to the vials and read in a Mark III 6880 liquid scintillation system. Three plants were assayed for each phenotype and three assays conducted per plant.

C. Soluble Protein Assay and Total Protein Assay

The dye binding method of Bradford (1976) was used. The dye CoumassieBrilliant Blue G250 binds to proteins which causes a shift in the absorption maximum of the dye from 465 to 595 nm. Increase in absorption at 595 nm was monitored on a Zeiss spectrophotometer. Bovine serum albumin served as a standard protein. The dye concentrate was obtained from Bio-Rad Laboratories, catalog #500-0006.

The nitrogen assay was done using the Kjeldahl digestion method (AACC 1969). Percent protein was calculated using a factor 6.25 x nitrogen. The values for both assays were determined on a dry weight basis.

D. Chlorophyll Determination

Chlorophyll content was determined according to the procedure outlined by Harbone (1973). Three measurements were made per plant.

E. Determination of Intercellular Spaces

Conventional and leafless plants from the Century and Freezer cultivar were used for this experiment. Petioles were cut at the stem, and the cuttings were placed in water for two hours to attain maximum turgor. After the two hour period, branches were removed from the water, the cut petiole ends sealed with wax and the weights recorded. The branches were then infiltrated under vacuum in distilled water. When the water started to bubble, the vacuum was released causing the water to infiltrate the intercellular spaces. This was repeated five times followed by weighing the branch. The ratio of the two weights after/before served as the index relating to intercellular spaces (Salisbury and Ross 1978).

F. Fresh Weight/Dry Weight Ratio Determination

The ratio was determined by taking the fresh weights of the plants then oven drying them to constant weight and recording their weight.

G. CO_2 Compensation Experiment

This experiment consisted of placing a leafed and a leafless plant in a closed container in order to determine which plant would senesce first. After watering, the pots were covered with plastic bags (excluding the plant) to minimize the effects of carbon dioxide from soil respiration. The jars were sealed and put in a growth cabinet. The temperature inside the jars was monitored and found to be approximately $25^{o}C$.

RESULTS

The measurements for the incorporation of radiolablelled CO_2 by the enzyme ribulose di-phosphate carboxylase are presented in Table 1. Measurements based on fresh weight (Table 1) showed no significant differences among the different foliar phenotypes. Measurements based on a mg of chlorophyll (Table 1), showed significant differences between the leafless and leafed phenotype and in the case of the cultivar Freezer, there was also a significant difference between the leafed and semi-leafless type. This suggests that the leafless phenotype is more efficient in incoporating carbon dioxide on a per unit chlorophyll basis. Although differing in magnitude similar results were obtained with both Century and Freezer.

The chlorophyll content of the different phenotypes for the three cultivars are presented in Table 2. All showed similar significant

Table 1 The amount of $^{14}CO_2$ incorporated per mg fresh weight and per mg of chlorophyll basis by foliar mutants and conventional plants for two sets of isogenic lines.

Variety		Genotypes ++++ (conventional)	afafstst (leafless)	afaf++ (semi-leafless)	++stst (reduced stipule)	F-test
	umoles $^{14}CO_2$ hr^{-1} mg. f.wt.$^{-1}$ ($x10^{+4}$)	1.80 ± .29a+	1.63 ± .49a	1.40 ± .11a	----	1.98
Century						
	umoles $^{14}CO_2$ hr^{-1} mg chlorophyll^{-1} ($x10^{+2}$)	2.54 ± .14a	3.70 ± .60	2.62 ± .03a	----	16.78**
	umoles $^{14}CO_2$ hr^{-1} mg f.wt.$^{-1}$ ($x10^{+4}$)	1.10 ± .16a	1.26 ± .19a	0.88 ± .20a	1.02 ± .25a	2.92
Freezer						
	umoles $^{14}CO_2$ hr^{-1} mg chlorophyll^{-1} ($x10^{+2}$)	4.73 ± .87	9.31 ± 1.59	2.34 ± .53a	1.81 ± .53a	67.99**

+ Values represent mean ± s.e. for 3 plants and 3 samples per plant

Values with same letters are not significantly different with L.S.D. P = 0.05 within each row.

** Significant at p = .01.

Table 2 Total chlorophyll content (mg/g f.wt.) for three sets of isogenic lines and their respective foliar phenotypes.

Variety	++++ (conventional)	afafstst (leafless)	afaf++ (semi-leafless)	++stst (reduced stipule)	F-test
Century	+2.33 ± .01	1.39 ± .01	3.78 ± .23	-----	62.73**
Freezer	6.49 ± .51	3.65 ± .34	4.26 ± .43	7.62 ± .15	32.87**
Trapper	7.59 ± .79	3.43 ± .71	5.83 ± .47	-----	29.04**

+ Values represent mean ± s.e. for 3 plants and 3 samples per plant.

All values within each variety are significantly different with L.S.D. P = .01 within each row.

** Significant at p = .01.

differences among the different phenotypes. The leafless mutant had significantly less chlorophyll in all three cases. High levels of chlorophyll were noticed for the ++stst (stipules reduced only) phenotypes in Freezer.

Protein content was determined according to the Bradford method (soluble) and the Kjeldahl digestion total methods. Results of both determination are included in Table 3. The leafless phenotypes of all three cultivars showed a higher protein percentage compared to the other phenotypes using the Kjeldahl method. However, using the Bradford method, the protein content of the leafless phenotype was either equal or lower than the other phenotypes. The additional mutant (++stst) in the Freezer cultivar showed high amounts of protein which correlates well with the high levels of chlorophyll (Table 2). The cultivar Trapper on the other hand, showed no significant differences in soluble protein (Bradford method) between the three phenotypes. The cultivar Century showed the highest content and extreme phenotype differences in protein content. The high levels of protein in the tissue maybe an indicator of its genotypic superiority and breeding value.

The data for the fresh weight/dry weight ratio are summarized in Table 4. In all cultivars studies, the leafless phenotype had a significantly higher ratio than the leafed types. The semi-leafless types were similar to the leafed type except in the cultivar Trapper where it was lower.

Measurements of the intercellular spaces in leafed and leafless plants of Century and Freezer (Table 5) showed significantly greater intercellular spaces in the leafed type. The differences were in the same direction and magnitude for both phenotypes. Differences between the two cultivars are an indication of genetic variability.

Table 3 Protein determination using two different methods in three sets of isogenic lines with their respective foliar phenotypes (mg / g d.wt.).

		Genotypes				
Variety	Method	++++ (conventional)	afafstst (leafless)	afaf++ (semi-leafless)	++stst (reduced stipule)	F-test
†Century	Kjeldahl	261.70 ± 14.00	293. ± 14.10	224.70 ± 3.50	---------	24.06**
	Bradford	232.20 ± 27.20	111.89 ± 15.78	168.97 ± 28.93	---------	96.99**
Freezer	Kjeldahl	190.50 ± 20.35a	230.00 ± 21.40b	206.50 ± 7.80ab	192.30 ± 20.50a	5.64*
	Bradford	61.99 ± 6.31a	79.38 ± 3.44ab	91.89 ± 11.51bc	114.05 ± 17.68c	6.28*
Trapper	Kjeldahl	236.00 ± 26.50a	273.70 ± 5.90	243.70 ± 16.20a	--------	6.56*
	Bradford	83.35 ± 19.10a	58.53 ± 14.68a	69.90 ± 9.45a	--------	2.67

\+ All values within this cultivar are significantly different with L.S.D. P = .01.

++ Values represent mean ± s.e. for 3 plants and 3 samples per plant.

Values with same letters are not significantly different with L.S.D. P = .05.

* Significant at p = .05.

** Significant at p = .10.

Table 4 Fresh weight/dry weight ratio for three sets of isogenic lines and their respective foliar phenotypes.

Cultivar	++++ (conventional)	afafstst (leafless)	afaf++ (semi-leafless)	++stst (reduced stipule)	F-test
Century	+7.58 ± .45a	10.05 ± 1.34	7.34 ± .12a	----	9.08*
Freezer	5.95 ± .83a	7.49 ± .27b	6.65 ± .28ab	5.76 ± .79a	5.04*
Trapper	7.82 ± .49	9.17 ± .26	7.27 ± .14	----	17.41**

\+ Values represents mean ± s.e. for 3 plants

Values with same letters within cultivars not significantly different with L.S.D. P = .05 within each row.

* Significant at p = .05.

** Significant at p = .01.

Table 5 Intercellular space index (weight after infiltration/weight before infiltration) for two sets of isogenic lines and their respective foliar phenotypes.

Cultivar	++++ (conventional)	afafstst (leafless)	F-test
Century	+1.41 ± .06	1.23 ± .13	11.43**
	(8)	(5)	
Freezer	1.29 ± .09	1.14 ± .01	7.34*
	(4)	(4)	

\+ Values represent mean ± s.e., ( ) represent number of plants.

* Significant at p = .05.

** Significant at p - .01.

In an attempt to determine if senescence could be induced in the leafless type as a result of an assumed higher CO_2 compensation point than in the leafed type, it was found that the leafless plant showed and altered pattern of senescence. Senescence could not be induced in the leafless plant while the leafed type showed extreme senescence in the lower nodes.

In another experiment, a leafless and leafed type were placed in a jar with a strong basic solution of solium hydroxide to maintain low atmospheric CO_2 levels for 13 days. The temperature inside the jars was monitored at 25^{o}C, with a day/night of 16 and 8 hours. Senescence of the lower parts of the leafed plant took place while the leafless plant no apparent yellowing or senescence occurred. It was also observed that when the pots were taken from the jars, that the soil mixture was wetter with the leafless type than with the leafed type indicating a lower transpiration rate by the leafed type.

From these preliminary observations it appears that the presence of tendrils alters the pattern of senescence and has important implications with respect to the potential use of this new model.

DISCUSSION

The results from the in vitro $^{14}CO_2$ fixation assay indicates that on a fresh weight basis (Table 1), there were no significant differences between the different phenotypes within each cultivar. Since the water content varies significantly between phenotypes (Table 4) it can be argued that had the measurements been made on a dry weight basis, appreciable differences would exist. Examining the data for protein content (Table 3) in the cultivar Century, there is a significant difference between the leafed and semi-leafless phenotypes but no

differences in water content (Table 4) or ability to fix $^{14}CO_2$ (Table 1) were observed (the leaf type also had significantly more tissue proteins than the leafless type). Similar results were obtained for the cultivar Freezer. This then suggests that in the case of the leafless phenotype (Table 1), if the results had been put on a dry weight basis, they would appear to be able to fix significantly more CO_2. This means that the photosynthetic efficiency is higher in the leafless types than the leafed type and that limitations to dry weight production would result from the high diffusive resistances imposed by the tendrils. These results agree with those of Harvey (1972) and Harvey and Goodwin (1978) who found significant differences on a dry weight basis in an in vivo assay of CO_2 photoassimilation using an infrared gas analyser, the leafless in this case being less effective.

Data for the intercellular space index indicates lower free space within the tendrils than the leaflets. A decrease in intercellular space would result in higher CO_2 resistances making carbon dioxide more limiting. Since carbon dioxide is a major factor in dry matter accumulation, the end result would be lower dry matter accumulation which is consistent with the lower dry matter production in leafless peas.

The lower levels of chlorophyll in the leafless phenotype would not present any limitations. The fact that on a chlorophyll basis (Table 1) the leafless phenotype was more effective in fixing CO_2 indicates that enough chlorophyll is present. Work by Highkin et al. (1969) showed that a pea mutant deficient in chlorophyll was at least double that of the normal in terms of efficiency of CO_2 fixation. The chlorophyll A to chlorophyll B ratio deviated greatly (appendix I) from the earlier reported value of 3:1 (Smillie and Krotkov 1961)

In all three cultivars, the leafless phenotype showed the

highest percent protein per gram dry weight based on a Kjeldahl digestion. When soluble protein was determined according to Bradford's method, the leafless phenotypes showed the lowest amount of the protein (Table 3). The differences may be due to the higher efficiency of the leafless phenotype to convert assimilates into insoluble protein. The drastic changes in cell types going from leaflets to tendrils could have serious implications on the levels and activity of the nitrate reductase in the roots.

The significantly higher fresh weight/dry weight ratio in the leafless phenotype in all three cultivars also has important implications (Table 4). This suggests greater water holding capacity by the leafless plant. This could explain the observation of similar rates of transpiration between the leafless and leafed type due to a higher proportion of water in the tissue of the leafless plant. From our observations in closed containers, the soil of the leafless type did not dry as quickly suggesting a lower transpiration rate by the plant. It is important to note that on a whole plant basis, the total leaf area in the leafless phenotype is less than a leafed one, making the evaporative surface lower in the leafless type.

In the CO_2 compensation experiments, the difference in senescence pattern under varying conditions indicates that the leafless phenotype can possibly withstand more adverse conditions. A probable cause is the difference in the cell type of a leaflet compared to a tendril. A major consequence of change in morphology would be changes in the action of certain hormones. Since the morphology of the tendril is similar in many respects to a petiole, it is likely that in a leaf type, the leaflets will senesce before the petiole.

The choice of a suitable parent is an important factor in a

breeding program. Based on our results, major differences exist between cultivars in terms of protein content, CO_2 fixation and chlorophyll content. There is great potential for this new model and research is required to more fully understand these phenotypes.

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A Comparative Study of Conventional, Leafless and Semi-Leafless phenotypes of Pisum sativum L. Effects on yield components.

ABSTRACT

Isogenic lines of two field pea cultivars, Century and Trapper differing in the leafless (afafstst), semi-leafless (afaf++) and reduced stipules only (++stst) loci were studied for effects on yield components.

There were no significant differences between yield components of the normal and semi-leafless phenotypes of Century. The leafless phenotype of Century had a significantly lower number of reproductive nodes, fewer seeds per plant, fewer pods per plant but a significantly longer pod and higher number of seeds per pod than the leafed counterpart. The phenotype of Century with only reduced stipules had a significantly higher number of pods per node. The semi-leafless phenotype of Trapper had a significantly lower number of reproductive nodes, fewer pods per node, fewer seeds per plant, fewer pods per plant, a reduced total seed weight per plant and reduced branching. The length of the last petiole was significantly longer in the leafless and semi-leafless types of Century and the semi-leafless type of Trapper. Significant positive correlation coefficients occurred between the length of the past petiole and certain yield components.

INTRODUCTION

Snoad et al. (1976) in describing the fruiting characteristics of one normal and three foliar recessive mutants of P. sativum showed that individually the recessive genes had little effect upon yield but that in combination the two genes reduced yield significantly. Similar results were obtained by Harvey (1978) and Harvey and Goodwin (1978).

Hedley and Ambrose (1979) studied the effects of shading on the yield components of six leafless genotypes. The similarity between

growth-curves and yield characteristics of the unshaded and 30 per cent shade treatment strongly indicated that there was no source limitation to the yield of the "leafless" pea phenotypes at normal spring and early summer light intensities.

In this study, the yield components of isogenic lines of field peas possessing different foliar genotypes, normal leafed (++++), semi-leafless (afaf++), leafless (afafstst) and one where the stipules are reduced (++stst) were compared.

MATERIALS AND METHODS

The study was conducted at the Agriculture Canada Research Station in Morden, Manitoba, Canada during the summer of 1979. Isogenic lines of the cultivars Century and Trapper were measured for thirteen parameters affecting yield. The leafed type of Tara, a high yielding cultivar was also studied.

The Century lines studied consisted of one normal cultivar, fifteen semi-leafless, one leafless and two where the stipules only are reduced. The Trapper line included a normal type and fifteen semi-leafless,

The lines were grown in single row plots five meters long. All lines were replicated twice in a randomized complete block design. Ten random plants (five in each of two replicates) were examined in each line. Measurements were made approximately three weeks prior to harvest. The following variables were studied: number of reproductive nodes, number of pods per node, number of seeds per pod, number of seeds per plant, total seed weight per plant, individual seed weight, number of basal shoots, number of pods per basal shoot, length of pods, diameter of seed, length of last petiole, number of pods per plant and number of branches resulting from the development of axillary buds.

RESULTS

The statistical analysis was performed using a computer program developed by Susan R. Beal and S.G. Carmer of the Department of Agronomy, University of Illinois. The program, Analysis of Variance of Multi-Factor Experiments handles analysis of variance for a randomized complete block design with subsampling and single degree of freedom comparisons for treatment main effects.

The means of each character for each foliar phenotype within each cultivar are included in table I along with their respective level of significance.

The semi-leafless (afaf++) condition in the cultivar Century did not affect any of the yield components. The dry weight of seed produced was similar in the leafed and semi-leafed types. The leafless (afafstst) phenotype produced significantly less reproductive nodes and fewer seeds per plant but significantly larger pods and more seeds per pod. The seed weight per plant was reduced by 56%. The overall number of pods per plant was significantly reduced. We noted however that the uniformity in seed size and maturity was very obvious and the pods themselves were more resistant to shattering. In the phenotype with only reduced stipule (++stst) the average number of pods were significantly higher at each node but there was no significant difference for final seed weight, although the seed weight per plant tended to be slightly lower. In the case of the cultivar Trapper, the semi-leafless (afat++) condition was characterized by a significantly lower number of reproductive nodes, fewer pods per node, fewer seeds per plant, lower seed weight and less branching.

The length of the last petiole was also recorded for each plant examined. We earlier observed that certain leafless and semi-leafless plants were able to produce a long petiole resulting in larger tendrils.

TABLE 1. Mean of characters affecting yield for three cultivars and their respective phenotypes.

	CUTLIVAR						
	CENTURY				TRAPPER		TARA
	PHENOTYPES						
Character	++++ (conv.)	afaf++ (s-L)	afafstst (LL)	++stst (red. stip.)	++++ (con.)	afaf++ (s-L)	++++ (conv.)
Reproductive Nodes	5.00	6.10	2.30	4.40	9.60	6.55	9.20
Pods/node	1.73	1.63	1.55	2.24**	2.30	1.67**	1.49
Seeds/pod	4.93	4.34	5.87**	4.67	4.77	4.88	5.16
Seeds/plant	43.20	42.00	26.30*	34.65	80.00	56.42**	80.40
Seed weight/plant (g)	9.85	9.26	4.34**	8.25	12.16	8.69*	16.18
Individual seed weight (mg)	0.226	0.221	0.225	0.249	0.146	0.155	0.198
Basal shoots	0.10	0.03	0.20	0.10	0.00	0.03	0.20
Pods/basal shoot	0.00	0.10	0.00	0.60	0.00	0.144	0.50
Length of pods (mm)	51.96	51.12	58.60	53.70	50.01	51.01	53.17
Diameter of seed (mm)	6.71	6.53	6.97	6.81	5.85	5.96	6.36
Length of last petiole (cm)	3.51	5.19**	6.90**	4.18	3.31	4.98**	3.86
Pods/plant	9.00	9.81	4.90*	8.55	18.30	11.16**	14.80
Branches/plant	0.60	0.61	0.00	1.00	1.90	0.54**	0.60

** Significant at p = .01

* Significant at p = .05

Significant or non significance were determined from the F ratio of single degree of freedom comparison between the normal phenotype and the other phenotypes taken singly within each cultivar. All characters in the foliar phenotype other than the leafed one with no asterisk are considered non significant.

An increase in length causes an increase in girth resulting in a higher leaf area. Correlation coefficients relating length of last petiole to yield components were calculated for each leafless and semi-leafless phenotype within each cultivar. In the cultivar Century, there were significant positive correlation coefficients in the semi-leafless phenotype between length of last petiole and number of reproductive nodes ($r = .21$), seeds per plant ($r = .24$), individual seed weight ($r = .19$), length of pod ($r = .26$), pods per plant ($r = .20$), axillary development ($r = .28$), and seed weight per plant ($r = .29$). There were no significant correlation coefficients in the leafless phenotype of Century. In the cultivar Trapper, the semi-leafless phenotype showed significant positive correlations for seeds per pod ($r = .19$), individual seed weight ($r = .23$), length of pod ($r = .37$) and length of last petiole.

We assume in the course of this study that any change in environment would affect all phenotypes within a cultivar similarly as a result of its isogenic nature.

DISCUSSION

The data clearly show that lines can be developed with reduced leaf area (afat++) which maintain comparable yields with the leafed types. An extensive reduction in the leaf area (afafstst) however is not compensated for by the tendrils only and a 56% reduction in yield occurred. The tendrils offer many advantages, e.g., better light penetration, smaller boundary layer, better air movement within the canopy but the resistance to CO_2 within the tendrils imposes limits on the potential of the leafless plant (Appendix I).

The genotypic background is also of considerable importance. The semi-leafless phenotype in the cultivar Century shows no differences for yield and yield components when compared to the leafed type. However

in the cultivar Trapper, the semi-leafless phenotype showed significant differences for some of the yield components (Table 1). Previous data indicate a reduction in the number of seeds per pod for the leafless phenotype (Harvey 1978; Harvey and Goodwin 1978) while our data showed the opposite. An efficient breeding program involving production of the leafless types requires the incorporation of the foliar mutations into as many backgrounds as possible.

The significant correlation coefficient relating length of last petiole to yield components indicates genetic variability for selecting highly productive plants. It can be argued however that the volume of the tendril and petiole will increase faster than the surface area. This indicates that a larger volume may be increasing intercellular spaces favoring better CO_2 diffusion within the tendril and/or more photosynthetic machinery.

The two most important characters contributing to total seed yield are the number of reproductive nodes produced and/or the number of branches that develop on the plant. The two cultivars Tara and Trapper which show high yields both produce a high number of reprductive nodes and/or high number of branches. Paudey and Gritton (1975) found that plant height was often positively correlated with pods per plant, seeds per plant and yield per plant. Plant height is the result of the number of nodes x length of internode. A plant that can produce many fruiting nodes is exhibiting potential for greater seed yield. The yield depression in the semi-leafless type of the cultivar Trapper is due to a lower number of reproductive nodes and smaller number of branches produced.

Further research with this new crop model is justifiable. The semi-leafless phenotypes (afaf++) have yielding potential. The choice of

a suitable parent is very difficult because of the varied effects of the foliar mutants in different genotypic background. With regard to the leafless (afafststst) type, reduction in the leaf area is too drastic and greatly depresses yield. We noticed however that the uniformity of maturity and seed size in the leafless type may have merit for the vining pea industry.

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A Comparative Study of Conventional, Leafless and Semi-Leafless Phenotypes of Pisum sativum L. Effects on root and shoot characteristics of young seedlings.

ABSTRACT

Two recessives mutations affecting leaf morphology in Pisum sativum were studied for effects on early root and shoot characteristics in ten day old seedlings. The "af" mutation converts leaflets to tendrils and "st" changes the stipules to a vestigial structure. Four isogenic lines Freezer, Canner, Century and Trapper were examined. The root and shoot characters in the semi-leafless (afaf++) and leafless (afafstst) phenotypes in all four isogenic lines were either similar or greater in magnitude than the leafed types except in the Canner line where shoot weight in the semi-leafless (afaf++) and reduced stipule (++stst) phenotypes were significantly lower than the leafed (++++) type. The latter phenotype in Canner also exhibited a significantly shorter root length to last lateral and shorter main shoot than the leafed one. The leafless phenotype (afafstst) in the Freezer line was greater in all characters except for the length of the longest lateral root. The Century line exhibited significantly higher values for all characters in the semi-leafless (afaf++) and leafless (afafstst) phenotypes. Etiolated plants of the semi-leafless (afaf++) and leafless (afafstst) phenotypes of Century were greater in values of all characters except for length of main root. This suggests higher growth rates in the leafless and semi-leafless phenotypes resulting from changes in hormonal balance. Therefore,

the leafless and semi-leafless plants would benefit greatly at the onset of crop establishment.

INTRODUCTION

The early establishment of a crop is an important consideration in the development of new cultivars. Snoad and Arthur (1974) studied the genetics of seed size and the initial stages of plant development in peas. They found polygenic control for all characters examined with additive gene action for seed weight and dominance being important for seedling characters. Contin and Marx (1974) studied the resistance to uprooting in peas. They found that it was a heritable characteristic influenced by root thickness and branching. The use of mutagens in peas resulted in the production of many mutants of variable root type (Zobel, 1974).

Ali-Khan and Snoad (1977) studied the variability and heritability of seven root and shoot characteristics of seedlings in a large number of genotypes. Heritability values exceeding 50% were estimated for lateral root number, shoot length and root and shoot weight. Ali-Khan et al (1977) studied the effect of temperature at 13°C, 16°C and 19°C on shoot and root characters in seedling peas. Growth responses determined 9 days after sowing were found to be linear in relation to temperature. Such responses, however, did not bear a linear relation to accumulated heat units.

The purpose of this study was to examine the effects of foliar mutations on root and shoot characteristics in four isogenic lines.

MATERIALS AND METHODS

Seed from the seventh backcross of near isogenic lines of two vining pea cultivars Freezer and Canner were obtained from Dr. G.A. Marx, New York Agricultural Station, Geneva, New York 14456, U.S.A. (Marx, 1974). Seed from two field pea isogenic lines, Century and Trapper, each with four backcrosses were obtained from Dr. S.T. Ali-Khan of the Morden Agriculture Research Station, P.O. Box 3001, Morden, Canada R0G 1J0.

Single plants were grown in clay pots 13 cm in diameter with a 1:1:1 mixture of peat, sand and soil. They were cultured in a growth room with 18 hours daylight, a day/night temperature of 22°/17°C and a constant relative humidity of 50%. Soil temperatures varied from 19°C to 21°C and a light intensity of 21.5 KLux.

In each of the isogenic lines Freezer and Canner, four foliar phenotypes were examined; leafed (++++), semi-leafless (afaf++), leafless (afafstst) and one with reduced stipules (++stst). In the isogenic field pea lines Century and Trapper, three foliar phenotypes were examined; leafed (++++), semi-leafless (afaf++), leafless (afaf stst). Seven seeds for each foliar phenotype within each isogenic line were weighed, sown and grown for 10 days after which the plants were removed from the pots and the following characters measured (Figure 1); length of main root, length to last lateral root, number of primary roots, length of longest lateral, length of main shoot, fresh weight of root, fresh weight of shoot. This was repeated for three consecutive times and served as three replicates. The plants were divided in a completely randomized arrangement in the growth room.

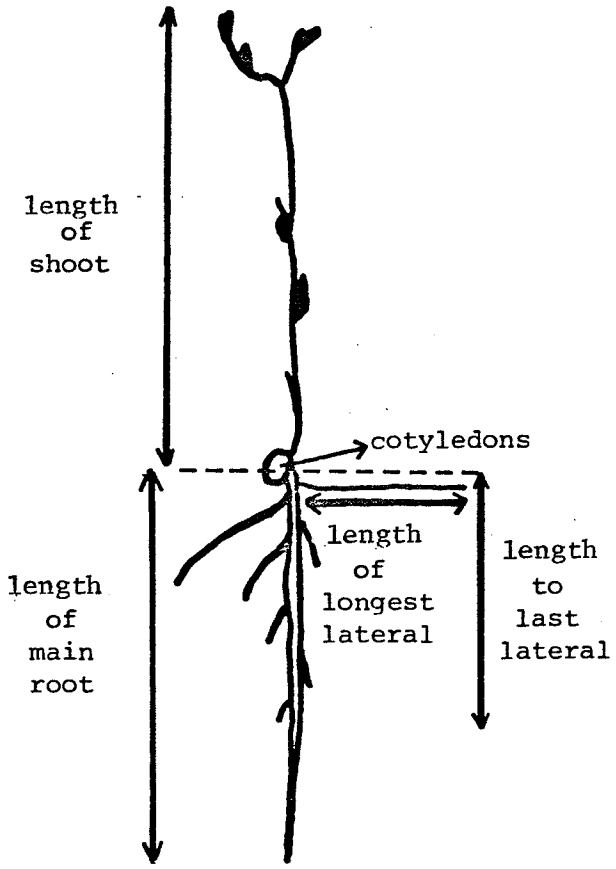

Fig.1 Characters of pea seedlings measured 10 days after sowing.

The same characters were measured for etiolated plants of the isogenic line Century. They were grown at a constant temperature of 20°C in the dark for a period of 10 days. The dry weights of the roots and shoots were also recorded. This was repeated twice and served as our two replicates.

The statistical analysis was carried out using a two way analysis of variance for a randomized complete block design with single degree of freedom comparisons for treatment main effects. Each replication was treated as a block. The single degree of freedom comparison involved pair wise comparison between the leafed type and the other foliar phenotypes within each isogenic line.

RESULTS

The mean value of eight seed, root and shoot characters for different foliar phenotypes within the four isogenic lines Freezer, Canner, Century and Trapper are included in Tables 1 and 2 along with their level of significance.

The data for the isogenic line Freezer (Table 1) indicate that the semi-leafless phenotype (afaf++) had a significantly higher root length to last lateral, a longer main shoot and a larger shoot weight. The leafless (afafstst) phenotype showed a significantly greater length of main root, length to last lateral, number of primary roots, longer main shoot and root weights. The phenotype with only reduced stipules (++stst) showed no significant differences with the leaf type for the characters studied.

In the isogenic line Canner, the semi-leafless (afaf++) phenotype had only a significantly reduced shoot weight. The leafless (afafstst) phenotype had a significantly longer main root and the phenotype with

Table 1. Mean of eight seed, root and shoot characters in four foliar phenotypes of two isogenic lines, Freezer and Canner grown for 10 days at 20°C †.

	CULTIVAR							
	FREEZER				CANNER			
	Genotype							
Character	++++	afaf++ §	afafstst	++stst	++++	afaf++	afafstst	++stst
Weight of 7 seeds(g)	1.758	1.688	1.676	1.612	1.729	1.736	1.684	1.551
Length of Main Root (mm)	129	142	148*	117	111	117	146*	101
Length to Last Lateral (mm)	62	69*	69*	60	58	56	61	46*
Number of Primary Roots	28	30	31*	28	29	28	30	24
Length of Longest Lateral (mm)	65	66	69	64	52	49	52	44
Length of Main Shoot (mm)	62	75**	72**	60	61	59	61	55*
Weight of Root (mg)	462	528	573*	451	506	444	539	430
Weight of Shoot (mg)	339	427**	391*	332	421	355*	369	327**

**Significant at p=.01.
* Significant at p=.05.
† Each figure represents mean of fifteen plants
§ Any character of the foliar phenotypes within an isogenic line marked with an asterisk is significantly different with the leafed type, otherwise it is not significant.

Table 2. Mean of eight seed, root and shoot characters in three foliar phenotypes of two isogenic lines, Century and Trapper grown for 10 days at 20°C †

	CULTIVAR					
	CENTURY			TRAPPER		
	GENOTYPE					
CHARACTER	++++	afaf++§	afafstst	++++	afaf++	afafstst
Weight 7 seeds (g)	1.745	1.664	1.731	1.035	1.202	0.586
Length of Main Root (mm)	106	183**	151**	88	115*	98
Length to Last Lateral (mm)	49	83**	79**	50	51	50
Number of Primary Roots	22	41**	44**	25	24	21
Length of Longest Lateral (mm)	47	64**	67**	53	47	49
Length of Main Shoot (mm)	77	119**	115**	70	85	66
Weight of Root (mg)	471	626**	680**	300	338	219
Weight of Shoot (mg)	317	546**	640**	242	315	212

**Significant at p=.01
*Significant at p=.05
†Each figure represents mean of 15 plants
§See footnote in Table 1

reduced stipules (++stst) was significantly lower in length to last lateral, had a shorter main shoot and lower shoot weight.

In the isogenic line Century (Table 2), the semi-leafless (afaf ++) and leafless (afafstst) phenotypes were significantly higher in values for all characters examined except seed weight.

In the case of Trapper (Table 2), the semi-leafless (afaf++) phenotype had only a longer main root. The leafless phenotype showed a significantly smaller seed size with all other characters not significantly different.

When the Century foliar phenotypes were grown in complete darkness (Table 3), the semi-leafless phenotype showed higher significant values for all characters except for length of first internode, length of main root and length of shoot. The leafless (afafstst) phenotype showed significantly greater differences for all characteristics examined except for length of first internode and length of main root.

DISCUSSION

The effects of the semi-leafless (afaf++), leafless (afafstst) and reduced stipule (++stst) condition on early root and shoot characteristics were studied in four isogenic lines of peas. Since the photo-assimilation potential is higher in the "leafless" mutants when measured on an area basis (Harvey and Goodwin, 1978) but lower on a dry weight basis (Harvey, 1972), we wanted to see whether or not we could detect differences in early root and shoot characteristics 10 days after sowing.

In all four isogenic lines studied, the characteristics were similar or greater in magnitude for the semi-leafless and leafless

Table 3. Mean of ten root and shoot characters of etiolated plants for three foliar phenotypes of the isogenic line, Century grown for 10 days at 20°C†

	CULTIVAR		
	CENTURY		
	Genotype		
Character	++++	afaf++§	afafstst
Length first internode (mm)	58	61	55
Length of Main Root (mm)	147	179	173
Length to last lateral (mm)	48	76**	78**
Number of Primary Roots	17	32**	37**
Length of Longest Lateral (mm)	29	66**	62**
Length of Shoot (mm)	175	219	239*
Weight of Root (mg)	199	433**	416**
Weight of Shoot (mg)	547	854*	994**
Dry Weight of Root (mg)	79	175**	170**
Dry Weight of Shoot (mg)	33	50**	62**

**,* Significant at p=.01 and p=.03
†Each figure represents mean of 10 plants
§See footnote of Table 1.

phenotypes except for the Canner isogenic line where the weight of the shoot was lower for the semi-leafless (afaf++) and reduced stipule (++stst) phenotypes. The latter phenotype (++stst) also showed a significantly shorter length to last lateral and a shorter main shoot (Table 1). The isogenic line Freezer with its leafless phenotype (Table 1) demonstrated higher values for all characters studied except for length of longest lateral. The isogenic line Century exhibited higher significant values for all characters in the semi-leafless (afaf++) and leafless (afafstst) phenotype. On the other hand, the semi-leafless phenotype in Trapper demonstrated only one significantly different characteristic, a longer main root, We can then argue for the Century foliar phenotypes that a growth analysis study might reveal differences either for growth rate or final dry matter accumulated differing with the earlier results of Harvey and Goodwin (1978) who showed that the growth rates were similar between the leafed and leafless phenotype.

As a result of vigorous seedlings produced by the leafless and semi-leafless forms of Century, an experiment was conducted in complete darkness. The same trends (Table 3) were obtained as when the plants were grown in the light (Table 2) except for the length of main root which was similar for all phenotypes. This suggests higher growth rates in the leafless and semi-leafless plants increasing overall growth. This, however, is different than the earlier observations by Harvey and Goodwin (1978) who had found similar dark respiration rates between the leafless and leafed phenotypes. The leaves also represent important sites for hormone synthesis and the replacement of leaflets by tendrils may be causing a hormonal imbalance

resulting in increased growth and respiration as indicated by similar root lengths in the etiolated plants as opposed to longer main roots in the leafless and semi-leafless phenotypes grown in the light. Auxins inhibit growth in pea roots and etiolated plants have higher levels of auxins in the shoots (McComb, 1977).

These data clearly indicate that the background genotype greatly influences the performance of the leafless and semi-leafless phenotypes stressing the need for evaluation into more genotypic backgrounds for a successful breeding program. These present findings indicate the advantages some semi-leafless and leafless plants would gain at the onset of crop establishment in terms of root penetration, water availability and competition.

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GENERAL DISCUSSION

In the hope of distinguishing distinct/predictable effects of the foliar mutations on the physiology of the pea plant, it was found that the effects depended on the genotypic background into which the foliar mutations were incorporated. This was clearly indicated in three sets of experiments carried out during this study.

The first question investigated dealt with the lower yield and lower dry matter accumulation of the leafless plants. Although in vivo CO_2 photo-assimilation measurements per unit leaflet/tendril area were similar (Harvey 1972), the rates were significantly lower for tendrils than leaflets per unit dry weight(Harvey 1972). It was postulated that the foliar mutations might be affecting some aspect of the photosynthetic machinery. Measurements by Harvey (1972) on transpiration rates (change RH cm^{-2} sec^{-1}) indicated that the tendrils transpired at the same rate as the leaflets which rules out higher CO_2 stomatal resistance.

Measurement of the activity of the principal carboxylation enzyme, ribulose bi-phosphate carboxylase along with other physiological parameters revealed no significant difference between the tendrils and leaflets(Appendix I) for the in vitro assay of RuBP carboxylase. This indicates that the photo-synthetic machinery is not affected and that the limiting factor might be the inability of CO_2 to diffuse within the tendrils. Diffusion of CO_2 decreases substantially when it goes from a gaseous to liquid state. A measure of the intercellular space index (Appendix I) revealed that intercellular space in the tendrils was less than in the leaflets in two sets of isogenic lines. A close examination of the anatomy of the tendril shows an epidermis of collenchyma type cells with thickened outer and inner tangential walls and thinner radial walls. Under the epidermis the normal ground tissue has very little intercellular space typical of pith, cortex and parenchyma and

characteristic of mid-rib ground tissue. The chloroplasts are concentrated sub-epidermally in three or four cell layers encompassing the entire circumference of the tendril. There is a marked reduction in chloroplasts in the more central ground tissue. There are three distinct vascular bundles, protoxylem elements with spiral thickenings and phloem elements. In terms of inert tissue, leaflets would appear to have just as much or more than tendrils.

The cylindrical structure of the tendril probably plays an important role. The boundary layer is smaller and heat dissipation through evaporation is probably more efficient. Heat loss through convection although not measured is possibly significant. The surface to volume ratio in tendrils is lower than in leaflets and the higher water content in the tendril allows more heat to be absorbed. The energy balances would than be different in tendrils than leaflets. More data is required before it can be stated that similar transpiration rates occur in leaflets and tendrils.

Harvey and Goodwin (1978) measuring CO_2 photoassimilation in tendrils and leaflets of near isogenic lines found on a unit area that tendrils were more efficient than leaflets. They discussed the problem of measuring the photosynthetically active surface area of tendrils. Since tendrils approximate a cylinder, they corrected their leaf area measurements with $\pi/2$. Therefor the higher values obtained by Harvey and Goodwin (1978) could result from:

1- the tendril capturing incident light on more than ½ of the surface area as compared to the leaflet.

2- the lower surface to volume ratio of the tendril makes more photosynthetic machinery available per unit area.

The lower CO_2 photoassimilation values per gram dry weight obtained by Harvey (1972) would result from a higher CO_2 "mesophyll" resistance in the tendrils and the lower yield of peas (Harvey,1978; Snoad et al, 1976) from

less available photosynthetic area in the leafless plants.

Given this information, the plant breeder could select plants with increased tendril area. We have obtained significant corelation coefficients between lenght of last petiole and some yield components. This was discussed in the section dealing with effects on yield components. Another means of increasing yield would be to increase leaf area index through increase in number of plants per unit area. Very little information exists on the effects of varying plant densities in the leafless peas.

The study of yield and characteristics affecting yield revealed that the background genotype greatly influenced the yielding performance of the foliar phenotypes. The semi-leafless phenotype showed the greatest promise since the presence of normal stipules would maintain a higher leaf area than in the leafless phenotypes thereby compansating for the reduction in leaflet area. With the semi-leafless phenotype you still have all the advantages of the tendrils. Our data showed that some semi-leafless lines of the cultivar Century (Appendix II) could outyield the leafed lines, while the semi-leafless lines of Trapper were on the average inferior to the leafed type. We recommend extensive testing of these foliar mutations into as many background genotypes as possible.

Interesting results were obtained for root and shoot characteristics of young seedlings (Appendix IV). In all four isogenic lines, most characteristics of the foliar mutants examined were similar or greater than their leafed counterpart. The differences in root weight and root lenght for the leafless and semi-leafless phenotypes of Century were very high compared to the leafed type. One possible reason discussed earlier was a higher growth rate accounting for the greater root weight. Measurements on etiolated plants of Century leafless and semi-leafless plants also showed higher shoot and root

weights. The fact that root lenghts were similar in the Century lines when grown in the dark as compared to longer roots when grown in the presence of light would possibly involve a plant hormone explaining the differences in root lenghts. The reason for a higher respiration rate remains to be elucidated. Although Harvey and Goodwin (1978) reported similar dark respiration rates between leaflets and tendrils, they were examining different pea genotypes. It is also possible that the energy requirements for producing leaflets and tendrils differ. It may be that less energy is spent in producing tendrils as compared to leaflets. No unfolding is required and as soon as the tendrils are exposed to light they may become operative, being able to export assimilates and grow at the same time.

In order to conclude this study, Iwould like to discuss a few future research topics with this new model:

1- Growth analysis study

The influence of the genotypic background warrants the need to study leafless and semi-leafless phenotypes into promising genotypes like Century and examine their growth characteristics more closely.

2- Respiration study

The higher respiration rate expressed by etiolated leafless and semi-leafless plants for higher root and shoot weights warrants the need for a closer examination of the factors responsible for the higher respiration rate if such is the case.

3- Foliar applications of Auxins on Leafless and Semi-Leafless plants

This idea comes from an experiment we did to see whether or not we could reverse the tendrils back to a leaflet through foliar application alpha-naphtalene acetic acid and gibberelic acid.These hormones may be involved in the differentiation pathway. We sprayed leafed, leafless and semi-leafless plants with no apparent change in morphology. We did

notice however that there seemed to be a stimulatory effect observed in the leafless plants when sprayed with alpha-NAA. The plants were larger and seemed to be able to produce more reproductive nodes. Whether or not beneficial effects could be obtained through foliar applications of alpha-NAA remains to be investigated.

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APPENDIX TABLE I. Data for all physiological variables studied in four isogenic lines with their respective foliar phenotypes.

Freezer	AFST			afST			af st			AFst		
Plant	1	2	3	1	2	3	1	2	3	1	2	3
Chlorophyll A (mg/g f. wt)	2.06	2.09	2.08	1.28	1.26	1.28	3.32	3.71	3.71	7.14	7.57	7.20
Chlorophyll B (mg/g f. wt)	0.25	0.25	0.25	0.11	0.11	0.16	0.20	0.21	0.19	0.34	0.23	0.35
Total Chlorophyll (mg/g f. wt)	2.31	2.34	2.33	1.39	1.38	1.39	3.52	3.91	3.91	7.51	7.79	7.55
Chlorophyll A/B Ratio	8.29	8.27	8.28	11.30	11.37	11.05	16.955	18.78	19.08	21.15	33.02	20.55
Fresh Weight (g)	6.28	3.54	4.85	4.26	4.31	4.40	8.60	7.92	7.67	4.96	3.68	4.89
Dry Weight (g)	0.97	0.55	0.97	0.59	0.58	0.56	1.29	1.14	1.21	0.77	0.75	00.83
Fresh Wt/Dry Wt Ratio	6.44	6.43	4.99	7.24	7.44	7.78	6.67	6.92	6.36	6.45	4.90	5.92
Total Protein (mg/g Dry wt)	230.70	170.50	180.70	254.00	245.00	249.00	193.00	212.00	202.00	216.00	180.00	181.00
Soluble Protein (mg/g dry wt)	74.09	66.62	46.91	73.23	82.04	83.02	82.97	91.72	100.33	141.85	103.62	96.54
Moles CO_2/hr/ mg fr. wt ($X10^{-4}$)	1.22	1.11	0.97	1.01	1.31	1.41	0.74	0.76	1.14	0.90	1.07	1.41
Moles CO_2/hr/ mg Chlorophyll ($X10^{-2}$)	5.78	4.75	3.65	7.29	9.45	10.14	1.95	2.05	2.99	1.18	1.41	1.90

Canner	AFST			afST			af st			AFst		
Plant	1	2	3	1	2	3	1	2	3	1	2	3
Chlorophyll A (mg/g f. wt)	2.20	3.28	3.34	1.62	1.58	1.09	1.77	2.73	2.36	3.51	3.32	2.48
Chlorophyll B (mg/g f. wt)	0.20	0.31	0.44	0.07	0.11	0.10	0.17	0.12	0.20	0.39	0.31	0.32
Total Chlorophyll (mg/g f. wt)	2.40	3.59	3.34	1.69	1.69	1.19	1.94	2.86	2.56	3.90	3.62	2.79
Chlorophyll A/B Ratio	11.97	10.77	7.80	22.49	19.17	12.63	11.11	24.74	11.89	8.90	16.55	7.98
Fresh Weight (g)	2.93	2.72	1.90	4.10	6.22	4.86	3.14	1.570	2.060	4.33	5.19	5.41
Dry Weight (g)	0.40	0.34	0.29	0.40	0.61	0.48	0.43	0.21	0.30	0.62	0.78	0.82
Fresh Wt/Dry Wt Ratio	7.41	8.13	6.65	10.33	10.24	10.06	7.28	7.60	6.87	6.99	6.66	6.60
Total Protein (mg/g dry wt)	190.00	200.00	165.00	207.00	213.00	212.00	168.00	184.00	150.00	139.00	126.00	134.00
Soluble Protein (mg/g f. wt)	47.05	113.41	27.58	103.30	68.54	54.32	122.30	139.84	35.02	50.33	50.28	57.88
Moles CO_2/hr/ mg fr. wt ($X10^{-4}$)		-			-			-			-	
Moles CO_2/hr mg Chlorophyll ($X10^{-2}$)		-			-			-			-	

Century	AFST			afST			af st			AFst		
Plant	1	2	3	1	2	3	1	2	3	1	2	3
Chlorophyll A (mg/g f. wt)	5.71	6.26	6.67	3.23	3.40	3.87	4.59	5.14	5.41		-	
Chlorophyll B (mg/g f. wt)	0.25	0.29	0.30	0.14	0.14	0.16	0.20	0.21	0.22		-	
Total Chlorophyll (mg/g f.wt)	5.96	6.55	6.97	3.37	3.54	4.03	4.79	5.35	5.63		-	
Chlorophyll A/B Ratio	22.84	21.58	22.23	23.07	24.29	24.19	22.95	24.48	24.59		-	
Fresh Weight (g)	6.68	6.12	6.49	3.97	3.31	4.00	8.88	7.83	7.55		-	
Dry Weight (g)	0.940	0.803	0.811	0.450	0.288	0.406	1.226	1.071	1.010		-	
Fresh wt/Dry wt Ratio	7.11	7.62	8.00	8.82	11.49	9.85	7.24	7.31	7.48		-	
Total Protein (mg/g dry wt)	246.00	273.00	266.00	276.00	298.00	305.00	225.00	221.00	228.00		-	
Soluble Protein (mg/g f. wt)	247.48	218.67	227.84	99.49	109.04	124.31	99.04	71.37	78.03		-	
Moles CO_2/hr/ mg f. wt. ($X10^{-4}$)	1.91	1.81	1.69	1.48	2.33	1.35	1.21	1.40	1.49		-	
Moles CO_2/hr/ mg Chlorophyll ($X10^{-2}$)	2.56	2.76	2.42	4.38	3.70	3.36	2.60	2.59	2.65			

Trapper	AFST			afST			af st			AFst		
Plant	1	2	3	1	2	3	1	2	3	1	2	3
Chlorophyll A (mg/g f. wt)	7.01	6.69	8.09	3.21	4.04	2.66	5.25	5.40	6.14		-	
Chlorophyll B (mg/g f. wt)	0.31	0.28	0.39	0.13	0.14	0.11	0.24	0.23	0.23		-	
Total Chlorophyll (mg/g f. wt)	7.32	6.97	8.48	3.34	4.18	2.77	5.49	5.63	6.37		-	
Chlorophyll A/B Ratio	22.61	23.89	20.74	24.69	28.85	24.18	21.87	23.48	26.70		-	
Fresh Weight (g)	6.82	5.71	6.53	4.96	6.04	4.05	7.64	6.90	9.40		-	
Dry Weight (g)	0.87	0.69	0.89	0.53	0.68	0.43	1.06	0.93	1.31		-	
Fresh Weight/ Dry Weight Ratio	7.81	8.33	7.34	9.29	8.88	9.35	7.20	7.43	7.18		-	
Total Protein (mg/g dry wt)	235.00	263.00	210.00	276.00	278.00	267.00	262.00	238.00	231.00		-	
Soluble Protein (mg/g dry wt)	80.05	70.30	97.40	58.16	42.98	75.17	73.84	61.45	56.15		-	
Moles CO_2/hr/ mg f. wt		-			-			-			-	
Moles CO_2/hr/ mg Chlorophyll		-			-			-			-	

APPENDIX TABLE II. Data for 13 variables related to yield components of foliar phenotypes within three cultivars.

Cultivar Century		RN	PPN	SPP	SPPL	SWPPL (g)	ISWT (g)	BS	PPBS	LP (mm)	SDIA (mm)	PL (cm)	PPPL	AXDEV
Gen.	Line													
AFST	1	5.00*	1.73	4.93	42.20	9.85	0.226	0.10	0.00	51.96	6.71	3.51	9.0	0.6
afSt	2	5.40	1.32	4.45	31.00	6.94	0.220	0.20	1.10	49.87	6.63	5.60	7.0	0.5
	3	5.10	1.68	4.60	35.80	8.21	0.232	0.00	0.00	50.34	6.63	5.22	7.9	0.9
	4	5.80	1.61	4.76	56.60	13.34	0.234	0.00	0.00	51.42	6.55	5.83	12.2	0.4
	5	6.40	1.44	4.80	49.90	12.47	0.244	0.10	0.40	51.22	6.80	5.28	10.5	0.7
	6	7.30	1.83	3.51	57.40	11.77	0.210	0.00	0.00	53.97	6.69	5.85	14.3	0.9
	7	7.70	1.58	5.01	50.30	10.24	0.201	0.10	0.00	54.83	6.60	5.41	10.3	1.3
	8	5.60	1.68	4.58	47.20	11.25	0.245	0.00	0.00	51.65	6.80	6.03	9.8	0.8
	9	4.20	1.71	4.21	26.60	4.66	0.176	0.00	0.00	46.66	6.27	4.68	6.4	0.1
	10	7.70	1.24	3.91	32.20	6.78	0.210	0.00	0.00	45.67	6.54	4.52	8.3	0.0
	11	6.30	1.82	3.76	47.30	9.93	0.210	0.00	0.00	49.33	6.36	4.12	13.1	0.9
	12	6.60	1.83	3.73	41.50	9.06	0.217	0.00	0.00	51.93	6.42	5.23	11.2	1.1
	13	7.10	1.48	4.89	47.40	10.54	0.247	0.00	0.00	56.78	6.40	5.59	10.8	0.6
	14	5.10	1.72	3.91	28.40	6.15	0.218	0.00	0.00	48.24	6.28	5.46	7.6	0.5
	15	6.60	1.51	4.62	36.80	8.92	0.252	0.00	0.00	55.22	6.66	4.92	8.1	0.3
	16	4.60	1.90	4.35	41.70	8.69	0.202	0.00	0.00	49.59	6.28	4.15	9.7	0.1
afst	17	2.30	1.55	5.87	26.30	4.34	0.225	0.20	0.00	58.60	6.97	6.90	4.9	0.0
AFst	18	5.00	1.70	4.91	33.40	7.68	0.243	0.20	1.20	53.40	6.58	4.49	8.9	0.4
	19	3.80	2.78	4.44	35.90	8.83	0.504	0.00	0.00	54.00	7.05	3.86	8.2	1.6

Cultivar / Trapper		RN	PPN	SPP	SPPL	SWPPL (g)	ISWT (g)	BS	PPBS	LP (mm)	SDIA (mm)	PL (cm)	PPPL	AXDEV
Gen.	Line													
AFST	1	9.60	2.30	4.77	80.00	12.16	0.146	0.0	0.0	50.01	5.85	3.31	18.3	1.9
afST	2	7.70	1.80	4.73	69.40	8.41	0.139	0.0	0.0	48.69	5.83	5.40	14.7	0.9
	3	6.80	1.31	4.72	40.40	6.17	0.151	0.0	0.0	51.58	5.93	4.74	8.6	0.4
	4	6.80	1.56	5.20	66.60	9.83	0.150	0.0	0.0	56.12	6.07	5.00	13.2	0.6
	5	4.90	1.45	4.63	30.80	5.27	0.173	0.0	0.0	54.93	5.96	6.14	6.7	0.0
	6	7.40	1.82	4.80	59.40	9.56	0.159	0.0	0.0	51.15	5.54	4.76	12.5	1.2
	7	7.20	1.58	4.65	67.90	12.04	0.178	0.0	0.0	50.23	6.08	4.09	15.0	0.4
	8	5.50	1.59	4.62	41.20	6.57	0.160	0.2	1.0	49.39	6.20	5.01	8.5	0.9
	9	7.70	1.96	5.22	81.40	12.85	0.154	0.0	0.0	51.57	5.94	5.09	15.5	0.6
	10	5.10	1.59	4.88	39.20	5.95	0.149	0.0	0.0	50.53	5.91	5.29	8.0	0.0
	11	8.20	2.36	4.87	104.80	16.27	0.160	0.2	0.9	50.73	6.05	4.86	22.0	1.5
	12	4.50	1.50	4.44	34.80	4.82	0.148	0.0	0.0	42.65	6.16	4.11	7.6	0.1
	13	6.40	1.56	5.85	48.00	7.94	0.163	0.0	0.0	52.82	6.02	5.26	9.9	0.3
	14	7.60	1.52	4.61	64.60	10.86	0.169	0.0	0.0	51.53	6.05	5.57	14.5	0.4
	15	6.60	1.71	4.99	55.80	8.86	0.157	0.0	0.0	53.24	5.89	4.98	11.4	0.3
	16	7.00	1.63	5.56	59.70	9.09	0.151	0.0	0.0	54.86	6.09	5.02	10.7	0.2
	17	6.90	1.47	4.87	46.50	7.31	0.152	0.0	0.0	52.37	5.91	5.22	9.7	0.3
	18	4.50	1.89	4.86	44.50	5.38	0.132	0.1	0.4	45.90	5.73	4.24	10.0	0.6
	19	7.10	1.67	5.30	60.60	9.15	0.149	0.1	0.3	49.92	5.87	4.93	11.4	1.0

Cultivar Tara		RN	PPN	SPP	SPPL	SWPPL (g)	ISWT (g)	BS	PPBS	LP (mm)	SDIA (mm)	PL (cm)	PPPL	AXDEV
Gen. Line														
AFST	1	8.20	1.49	5.16	80.40	16.18	0.198	0.2	0.5	53.17	6.36	3.86	14.80	0.60

*Each value represents the mean of 10 observations.

APPENDIX TABLE III. Data for % seed protein content for plots within each isogenic line and their respective foliar phenotypes.

Cult. & Gen.	Plot #	Wt (g)	mg/seed	Seed size (mm)	% Prot.
QENTURY					
AFST	663	4.35	.218	6.73	25.4
	685	4.68	.234	6.68	27.0
afST	713	4.33	.217	6.70	25.8
	666	4.19	.210	6.56	24.4
	629	4.42	.221	6.81	23.6
	701	3.95	.198	6.45	21.4
	637	4.73	.237	6.62	23.2
	673	4.44	.222	6.47	21.8
	658	4.62	.231	6.75	24.2
	677	4.85	.243	6.85	25.8
	707	4.41	.221	6.62	23.8
	716	4.51	.226	6.76	24.8
	644	4.10	.205	6.47	23.4
	682	4.77	.239	6.72	24.8
	649	5.01	.251	6.73	26.0
	699	5.11	.256	6.84	24.0
	662	3.24	.162	5.99	26.0
	695	4.04	.202	6.55	24.8
	634	3.86	.193	6.42	28.6
	748	4.34	.217	6.66	29.2
	703	4.48	.224	6.28	25.8
	561	4.65	.233	6.45	27.4
	631	3.94	.197	6.29	23.6
	674	4.62	.231	6.54	24.6
	636	4.00	.200	6.32	25.6
	675	4.41	.221	6.47	23.0
	667	4.10	.205	6.29	23.6
	686	4.09	.205	6.27	24.8
	635	4.65	.233	6.70	29.4
	693	4.58	.230	6.63	25.2
	660	3.54	.177	6.14	25.6
	683	4.38	.219	6.63	28.0

Cult. & Gen.	Plot #	Wt (g)	mg/seed	Seed size (mm)	% Prot.
afst	653	4.98	.249	6.87	27.6
	672	4.67	.234	7.06	24.0
AFst	656	3.99	.200	6.38	22.6
	692	4.54	.227	6.77	25.4
	639	5.13	.257	7.01	26.0
	688	5.18	.259	7.09	26.4
TRAPPER					
AFST	638	2.96	.148	5.89	25.8
	708	2.62	.131	5.82	27.0
afST	648	3.12	.156	5.79	25.8
	676	2.83	.142	5.88	24.2
	655	3.19	.160	5.87	25.6
	671	2.78	.140	5.99	23.8
	641	2.73	.137	5.86	25.2
	678	3.36	.168	6.27	27.6
	652	3.42	.171	6.18	27.2
	669	2.78	.140	5.73	23.8
	647	3.47	.174	4.91	28.2
	696	3.29	.165	6.16	25.2
	630	3.34	.167	5.96	22.6
	704	3.55	.178	6.20	22.4
	668	3.25	.163	6.01	25.8
	680	3.64	.182	6.39	27.6
	640	2.97	.149	5.81	26.0
	684	3.32	.156	6.06	26.2
	661	2.87	.144	5.78	24.6
	670	2.99	.149	6.03	24.0
	657	3.11	.156	6.29	24.4
	689	3.10	.155	5.81	25.0
	665	3.34	.167	6.16	27.0
	702	3.60	.180	6.16	26.2

Cult. & Gen.	Plot #	Wt (g)	mg/seed	Seed size (mm)	% Prot.
	659	3.20	.160	5.90	26.6
	681	2.92	.146	6.14	27.0
	632	3.20	.160	6.28	27.4
	700	3.49	.175	5.83	24.4
	642	2.90	.145	5.75	23.2
	706	3.13	.157	6.03	22.4
	645	2.69	.135	5.72	26.2
	698	3.56	.178	6.47	25.2
	654	2.82	.141	5.89	25.6
	705	3.29	.165	5.92	25.2
	664	2.74	.137	5.77	25.2
	691	2.87	.144	5.70	25.4
	650	3.20	.160	5.83	25.2
	679	2.80	.140	5.92	25.8
TARA					
AFST	646	3.70	.185	6.37	25.6
	687	4.16	.208	6.37	24.2

APPENDIX TABLE IV. Data for early root and shoot characteristics of four isogenic lines and their respective foliar phenotypes. (All values represent the mean of five observations.)

Gen. Freezer / Rep	Shoot Root Ratio	Weight of 7 seeds (g)	Length of main root (mm)	Length to last lateral (mm)	Number of primary roots	Length of longest lateral (mm)	Length of shoot (mm)	Fresh weight root (mg)	Fresh weight shoot (mg)
AF	0.879	1.810	120.80	67.80	32.40	81.60	69.20	482.00	400.00
ST	0.737	1.765	160.80	66.40	31.40	73.60	65.00	580.00	360.00
	0.840	1.710	106.20	50.80	21.20	39.00	51.60	324.00	258.00
$\bar{X}$	0.819	1.758	129.27	61.66	28.33	64.73	61.93	462.00	339.33
af	0.820	1.748	140.60	69.40	30.60	80.00	79.80	590.00	482.00
ST	0.801	1.655	155.40	76.20	33.60	74.00	78.40	692.00	494.00
	1.109	1.660	129.20	60.00	24.40	43.40	68.00	304.00	306.00
$\bar{X}$	0.910	1.687	141.73	68.53	29.53	65.80	75.40	528.66	427.33
af	0.714	1.664	143.60	77.00	31.80	90.20	78.20	676.00	472.00
st	0.702	1.615	179.60	72.40	35.20	70.40	73.60	642.00	426.00
	0.699	1.750	120.20	57.40	25.80	46.20	63.80	402.00	276.00
$\bar{X}$	0.705	1.676	147.80	68.90	30.93	68.93	71.86	573.33	391.33
AF	0.770	1.631	119.40	58.40	32.00	74.00	65.40	554.00	408.00
st	0.728	1.630	125.40	67.80	32.00	78.60	61.80	564.00	366.00
	1.009	1.575	107.60	54.00	21.40	39.40	53.20	234.00	222.00
$\bar{X}$	0.836	1.612	117.47	60.07	28.46	64.00	60.13	450.66	332.00

Gen. / Canner / Rep	Shoot Root Ratio	Weight of 7 seeds (g)	Length of main root (mm)	Length to last lateral (mm)	Number of primary roots	Length of longest lateral (mm)	Length of shoot (mm)	Fresh weight root (mg)	Fresh weight shoot (mg)
AF ST TL	0.834	1.718	78.60	54.00	32.60	70.80	72.20	654.00	546.00
	0.955	1.760	157.40	73.60	34.20	46.40	62.00	484.00	434.00
	0.870	1.710	97.80	47.40	20.80	39.80	47.40	380.00	284.00
$\bar{X}$	0.886	1.729	111.26	58.33	29.20	52.33	60.53	506.00	421.33
af ST TL	0.866	1.718	109.60	65.60	32.20	77.60	68.80	474.00	394.00
	0.979	1.770	149.00	62.80	27.80	35.20	54.20	390.00	350.00
	1.123	1.720	93.40	40.40	24.60	33.80	54.60	448.00	320.00
$\bar{X}$	0.989	1.736	117.33	56.26	28.20	48.86	59.20	444.00	354.66
af st TL	0.681	1.743	147.40	65.40	36.40	80.80	78.80	762.00	514.00
	0.729	1.670	170.40	73.00	32.20	48.60	63.00	544.00	392.00
	0.924	1.640	121.00	43.60	20.20	25.40	41.40	310.00	200.00
$\bar{X}$	0.778	1.684	146.26	60.66	29.60	51.60	61.06	538.66	368.60
AF st TL	0.845	1.342	111.60	61.80	32.60	68.40	61.40	686.00	440.00
	1.288	1.610	112.80	47.60	22.40	38.40	55.40	294.00	318.00
	0.785	1.700	79.20	28.00	16.20	25.80	47.00	310.00	222.00
$\bar{X}$	0.973	1.551	101.20	45.80	23.73	44.20	54.60	430.00	326.66

Gen. Trapper / Rep	Shoot / Root Ratio	Weight of 7 seeds (g)	Length of main root (mm)	Length to last lateral (mm)	Number of primary roots	Length of longest lateral (mm)	Length of shoot (mm)	Fresh weight root (mg)	Fresh weight shoot (mg)
AF ST TL	0.489	1.024	82.20	49.20	25.00	63.20	74.00	520.00	245.00
	1.384	1.121	96.80	44.80	20.20	46.00	67.20	190.00	218.00
	1.457	0.960	86.00	54.60	30.20	49.80	67.40	190.00	262.00
$\bar{X}$	1.109	1.035	88.33	49.53	25.13	53.00	69.53	300.00	241.66
af ST TL	0.772	1.256	165.80	70.00	28.80	65.20	88.80	448.00	334.00
	1.681	1.110	101.60	53.80	26.00	54.80	109.00	292.00	420.00
	1.764	1.240	77.80	30.40	15.80	20.40	57.20	273.00	190.00
$\bar{X}$	1.405	1.202	115.06	51.40	23.13	46.80	85.00	337.93	314.86
af st TL	0.946	0.632	103.00	56.80	25.80	60.80	71.40	300.00	242.00
	1.100	0.546	114.20	56.00	21.60	51.80	63.20	197.00	197.00
	2.386	0.580	75.40	37.60	15.60	33.20	62.80	159.60	196.00
$\bar{X}$	1.477	0.586	97.53	50.13	21.00	48.60	65.80	219.06	212.07

Gen. Century / Rep	Fresh weight shoot / root ratio	Weight of 7 seeds (g)	Length of main root (mm)	Length to last lateral (mm)	Number of primary roots	Length of longest lateral (mm)	Length of shoot (mm)	Fresh weight root (mg)	Fresh weight shoot (mg)	Dry weight shoot (mg)	Dry weight shoot (mg)	Dry wt ratio shoot / root
	0.481	1.766	95.80	48.20	19.40	61.20	77.20	638.00	274.00			
AF	1.248	1.724			21.20	31.80	68.60		240.00			
ST	1.526	1.792	100.00	45.00	15.00	27.50	64.00	193.20	207.50		239.50	0.166
TL		1.773	90.50	44.50	24.71	44.14	79.71	136.00				
	0.806		115.00	50.42				471.42	380.00	39.83		
$\bar{X}$	1.02	1.764	100.32	47.03	20.08	41.16	72.37	359.50	275.38			
	1.022	1.666	189.00	86.40	43.00	82.20	129.40	656.00	632.00			
af	0.768		143.40	74.80	38.20	59.40	99.20	766.00	520.00			
ST	1.035	1.663	144.25	65.00	30.00	44.25	78.25	362.50	375.00	59.92	277.00	0.216
TL	0.937	1.370	197.42	85.41	49.92	58.33	122.83	555.83	520.83			
		1.628										
$\bar{X}$	0.926	1.582	168.52	77.90	40.28	61.04	107.42	585.08	511.75			
	0.856	1.718	124.80	66.60	40.80	66.60	102.40	626.00	528.00			
af	0.882	1.744	107.80	78.40	49.00	70.60	117.20	808.00	714.00			
st	0.638	1.465	120.00	64.50	33.00	40.00	78.00	635.00	405.00	59.20	319.00	0.186
TL	1.04	1.873	175.20	82.20	42.70	63.50	116.80	612.00	634.50			
$\bar{X}$	0.854	1.700	131.95	72.93	41.37	60.18	103.60	670.25	570.25			