Studies on Lettuce Seed Quality: IV. Individually Measured Embryo and Seed Characteristics in Relation to Continuous Plant Growth (Vigor) Under Controlled Conditions[1]

Hillel Soffer[2] and O. E. Smith[3]
University of California, Riverside

Abstract. Physical characteristics measured individually for each lettuce (*Lactuca sativa* L.) seed and its embryo were significantly correlated with seedling and plant growth (vigor) up to a certain stage, possibly heading, after which the correlations diminished. Embryo physical measurements, although slightly better correlated with subsequent vigor than seed (achene) physical measurements, were highly correlated with those of the whole seed.

Of the 5 physical measurements studied, all but length were found to be associated with early vigor. Thorough statistical analyses place seed and embryo physical characteristics in a consistent and significant order in determining vigor: weight > thickness > density (as measured by an air column) > width > length.

For a given species and cultivar, the larger or heavier the seed, the greater will be the percent germination and the more vigorous will be the growing seedling (4). Lettuce seems to be included in this broad category (5, 6, 7). Sharples (6) found lettuce seed thickness to be related to percent germination and fresh wt of 4-day-old seedlings. He also showed that seed thickness was highly correlated ($r^2 = 0.758$) to seed wt. Scaife and Jones (5), in a research note, presented results of a single experiment with 21 lettuce plants that indicated a linear relationship between seed wt and the fresh wt of plant tops on the 49th day of the experiment. These results were substantiated by Smith et al. (7). Smith et al. (8) developed a vigor test for lettuce seed and used it to compare vigor of groups of seed which had been separated on screens and air columns. They showed a relationship of vigor to seed wt, width, and thickness. In another paper, Smith et al. (7) showed that the vigor test was indicative of emergence, seedling growth, and head yield in the field. Seed quality, however, was not examined on the merit of individually measured seed properties in either of these investigations.

For the studies presented in this paper, a new system of growing plants was developed. Initial measurements of root and shoot growth were made on seedlings from individual seeds whose physical properties had been measured. These same seedlings were retrieved and used for further growth measurements. This system provided a prolonged vigor test for successive performance measurements under favorable, uniform, and controlled conditions, and in the absence of interplant competition. The performance was then analyzed for relationships to the individual seed measurements.

The goals of the present studies were: 1) to evaluate relative importance of each of the physical characteristics of individual seeds in relation to subsequent growth performance; and 2) to determine how closely physical measurements of whole seeds parallel those of decoated embryos in relation to plant growth.

This information will provide the necessary background for future engineering of efficient seed separation equipment for use in agricultural practice.

Materials and Methods

'Calmar' lettuce seed from a commercial seed lot obtained in 1970 (Keystone Seed Co.) were used in 1971 for experiments 1-4. Seed used for experiments 5-7 were progenies of plants grown in a greenhouse in 1971 from seed of the 1970 seed lot. In order to ensure homogeneity, seed used for each experiment (expts 5-7) were from a single mother plant. Seeds varying in seed wt and size were obtained by separating them on an air column (General Seed Blower, Model ER) (8). The air column provided a seed separation which approximated a separation on the basis of seed wt, size, and shape that is referred to here as density. Arbitrary settings on the air blower of 19, 20, 21, 22, 23, 24, 25 corresponded to air velocities (MPH) of 5.7, 6.1, 6.6, 6.9, 7.1, 7.6, and 8.0. In experiments where only 3 densities were separated, the designations light, medium, and heavy (L, M, H) refer to > 6.1, 6.1-7.1, > 7.1 MPH, respectively. Density values represent groups of seeds, whereas length, width, thickness and wt values were specifically measured on individual seeds selected at random from the density groups. Seed wt was determined to ±0.001 mg. Measurements of seed length, width, and thickness to ±0.01 mm were made with a binocular microscope according to the method described by Brandenburg and Harmond (1). Since the mature lettuce seed (botanically an achene) retains both fruit and seed coat, it is difficult to get accurate measurements of seed size. However, by careful manipulation under a magnifying glass, the pericarp and seed coats (which are compressed together and their identity obscured) of mature achenes can be removed leaving the intact embryo surrounded by a thin layer of endosperm and an unbroken integumentary membrane. In experiments 5-7, physical measurements were made on both the whole seed and the embryo after it was decoated. For experiment 7 only, each individual embryo was placed in the air column and the blower setting (air velocity) required to carry the embryo over the column was determined.

Prolonged vigor test for successive measurements. Ten measured lettuce seeds or embryos were placed on a platform made of 35 mesh Saran screen attached to an acrylic frame 27 x 2 cm. The platforms were floated on a pH 7.0 phosphate buffer solution in plastic boxes. The entire seed surface was covered by a single drop of solution placed on top of each seed. Due to surface tension, the seed remained completely wet throughout the imbibition and germination period. The boxes (Fig. 1) were placed in a dark, 25°C germination chamber. After various time periods (48-72 hr), the platforms containing the seedlings were transferred to a measuring device (Fig. 2) on which root and shoot length were determined while the seedlings remained untouched. Following vigor measurements, the seedlings were transplanted to polyurethane holders (3 plants/holder) floating on 1 liter of modified 1/2 strength Hoagland's solution (2) (Fig.

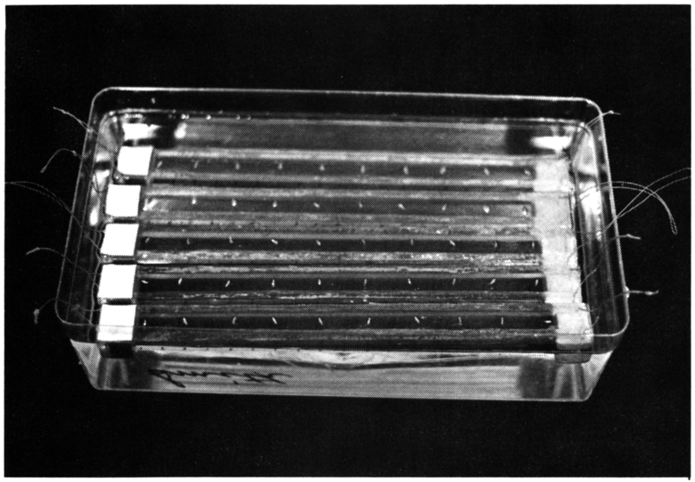
[1]Received for publication October 11, 1973. This research was supported in part by a grant-in-aid from the California/Arizona Lettuce Research Corporation. Technical assistance of J. Moore and statistical analysis by C. Adams are gratefully acknowledged. This report is to be submitted as partial requirement for the Ph.D. by the senior author.

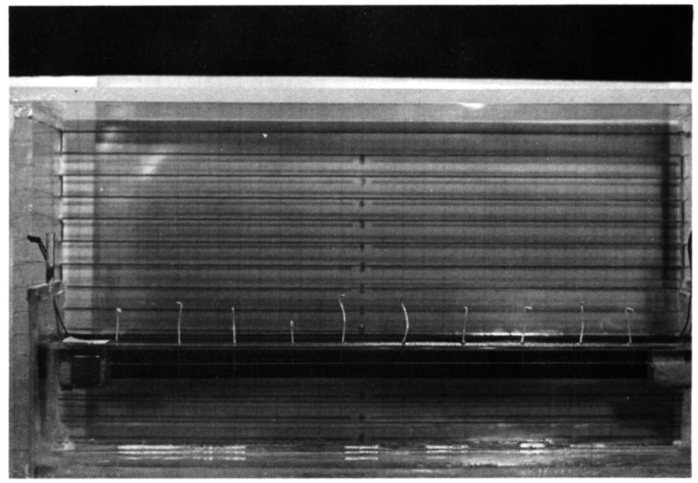
[2]Present address: Kibbutz Ein-Gedi, Israel.

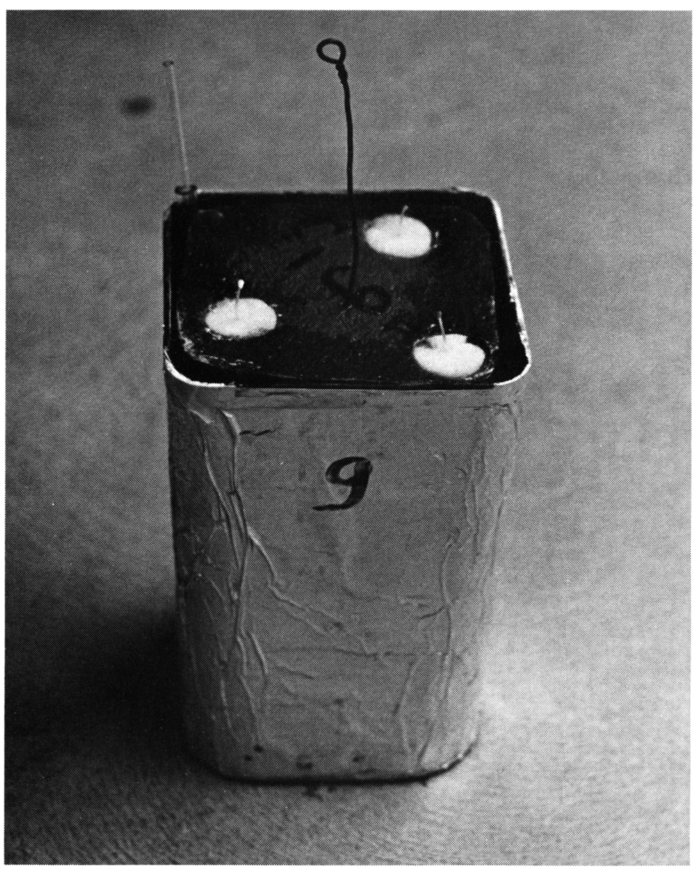
[3]Department of Plant Sciences.

3). The solution in each container was constantly aerated and mixed. In order to prevent considerable buildup or depletion of nutrient concentrations, the solutions were renewed weekly (expts 1-4) or daily (expts 5-7). Transplanted seedlings were grown in a growth chamber with the following environmental conditions: light 2000 ft-c (expts 1-4) and 3000 ft-c (expts 5-7), day-length 16 hr, day temp 23°C, night temp 20°C and an ambient RH of 40 to 50%.

In experiments 1-5 plants were harvested after 21 to 40 days of growth and fresh and dry wt of plants recorded. In experiments 6 and 7, plants were removed from the growth chamber 24 days after imbibition, weighed, and transplanted in hydroponics gravel culture beds in a greenhouse for further growth to head maturation. The culture solution remained unchanged and was pumped into the gravel medium 13 times a day.







Figs. 1-4. Prolonged vigor test sequence: Fig. 1. seeds imbibing on screen platform floating on buffer solution. Fig. 2. seedling vigor measuring device. Seedlings can be retrieved unharmed for further growth. Fig. 3. polyurethane float with 3 transplanted seedlings (3 days) on nutrient solution container used in growth chamber. Fig. 4. twenty-one day-old plants in the growth chamber. Roots were separated by plastic sheets to facilitate retrieval and measurements of single plant.

Results and Discussion

Seedling root length after 3 days in the dark at 25°C has been described as a measure of lettuce seed vigor (8). In this report, the term vigor will be used more broadly to reflect plant growth at various stages of development from seedling to head maturity. The results in the following tables are the product of statistical analyses of data in which correlations were examined between seed and embryo physical variables and growth variables.

Two sets of experiments were performed. The first set (expts 1-4) related physical characteristics of seeds to seedling and young plant performance. The second set (expts 5-7) compared physical characteristics of whole seeds and decoated embryos, measured separately, and correlated them to plant growth at various stages including head maturity (wt).

Seed physical properties in relation to plant growth (expts 1-4). The results of experiments 1 and 2, (Table 1) indicate that wt, thickness, and density (as determined by the air blower) were significantly correlated with seedling performance (expts 1 and 2) and plant performance (expt 1). Seed length and width were not correlated with subsequent growth in either experiment. Although seed wt was correlated to seedling (both expts) and plant performance (expt 1), better correlations were obtained between seed thickness and seedling vigor and between seed density and plant vigor (expt 1). Multiple regression analyses (not shown) using both stepwise and deletion methods, revealed simple results, i.e. there was only one dependent variable.

As a result of experiments 1 and 2, seed length and width measurements, which were not correlated with plant performance, were not included in the analysis of experiments 3 and 4 (Table 2). Seed wt is the only parameter correlated to seedling and plant performance in experiment 3, while in experiment 4, density and thickness were also correlated to seedling and plant performance (Table 2). However, in all cases, seed wt provided the highest correlations. In experiment 3, seed wt was mainly correlated to seedling vigor, while in experiment 4, better correlations were obtained with performance up to 21 days for all three physical parameters.

Intercorrelations among seed measurements themselves (Table 3), showed that seed wt was consistently correlated with both seed density and thickness, while seed density and thickness were correlated one to the other only in experiments 1 and 2. In experiments 3 and 4, however, seed density and thickness were not correlated. These differences, in addition to the decline of correlations of seed thickness with vigor in experiments 3 and 4, from those of experiments 1 and 2, are due to the different method of separation used. Seed thickness was determined by screens for experiments 3 and 4. In all other experiments, thickness was measured under the microscope resulting in more accurate measurements.

The results of experiments 1-4 confirm, through measuring methods based on individual seeds and successive measurements of plant growth, the conclusion reached by Sharples (6), Scaife and Jones (5), and Smith et al. (7, 8): a positive correlation exists between seed physical characteristics, the seedling and young plant vigor.

Seed and embryo physical properties as related to plant growth (expts 5-7). Since none of the correlations between seed properties and vigor (expts 1-4) were consistently high, although they were significant, embryo physical characteristics in relation to subsequent growth were studied after stripping off fruit and seed coats. Table 4 presents correlations of seed and embryo physical parameters to growth variables for experiments 5-7.

Table 1. Correlations between growth variables and seed variables.

Growth measurements	wt	Seed measurement length (l)	width (w)	thickness (t)	density[z]
Experiment 1[y]					
seedling root length (2 days)	.353**	-.047	-.109	.447**	.346**
seedling shoot length (2 days)	.288*	-.033	-.069	.311*	.275*
seedling root length (3 days)	.565**	-.000	-.084	.641**	.554**
seedling shoot length (3 days)	.442**	-.073	-.199	.517**	.395**
final root length (40 days)	.219	.158	-.050	.198	.222
final shoot length (40 days)	.177	.014	.144	.215	.195
total root and shoot length (40 days)	.250*	.139	.010	.246*	.259*
final root fresh wt (40 days)	.280*	-.046	.047	.321**	.293*
final shoot fresh wt (40 days)	.364**	-.129	.198	.293*	.381**
total root and shoot fresh wt (40 days)	.367**	.116	.190	.305*	.384**
final root dry wt (40 days)	.205	-.095	.036	.243	.230
final shoot dry wt (40 days)	.334**	.113	.198	.296*	.361**
total root and shoot dry wt (40 days)	.332**	.103	.192	.298*	.360**
Experiment 2[x]					
seedling root length (2 days)	.412**	.084	-.007	.267*	.374**
seedling shoot length (2 days)	.459**	.019	-.129	.359**	.513**
seedling root length (3 days)	.187	.021	.042	.152	.155
seedling shoot length (3 days)	.479**	.155	-.013	.260	.404**
final root length (24 days)	.130	.090	-.075	.135	.034
final shoot length (24 days)	.338*	.000	-.187	.456**	.383**
total root and shoot length (24 days)	.204	.071	-.115	.243	.142
final root fresh wt (24 days)	.140	-.086	.036	.178	.188
final shoot fresh wt (24 days)	.153	.040	-.038	.197	.182
total root and shoot fresh wt (24 days)	.151	.009	-.020	.195	.186
final root dry wt (24 days)	.028	.010	-.042	.045	.119
final shoot dry wt (24 days)	.136	.007	-.054	.182	.191
total root and shoot dry wt (24 days)	.118	.007	-.053	.160	.181

[z]Density: arbitrary settings on the air blower (19, 20, 21, 22, 23, 24, 25) produced groups with average seed wt (mg) = 0.765, 0.804, 0.891, 0.938, 0.958, 0.999, 1.113.

[y]n = 66; terminated 40 days after imbibition.

[x]n = 55; terminated 21 days after imbibition.

*Significant at 5%.

**Significant at 1%.

Table 2. Correlations between growth variables and seed variables.

Growth measurements	Experiment 3[z] Seed measurements			Experiment 4[y] Seed measurements		
	density	thickness[x]	weight	density	thickness[x]	weight
seedling shoot length (3 days)	-.030	.162	.346**	-.137	.074	-.066
seedling root length (3 days)	-.035	.227	.384**	.099	.308*	.389*
final shoot fresh wt	.111	.071	.128	.535**	.361**	.614**
final shoot dry wt	.245*	.184	.257*	.349**	.258*	.474**
final root dry wt	.153	.053	.125	.372**	.166	.444**
final root and shoot dry wt	.237	.170	.244*	.359**	.249	.478**

[z]n = 66; terminated 32 days after imbibition.
[y]n = 60; terminated 21 days after imbibition.
[x]Seeds were separated for thickness by 3 different size screens in contrast to actual measuring seed thickness as in experiments 1 and 2.
*Significant at 5%.
**Significant at 1%.

Table 3. Intercorrelations of seed measurements.

	length	width	thickness	density
Experiment 1				
wt	.187	.230	.703**	.947**
length		-.013	-.144	.144
width			.039	.218
thickness				.681**
Experiment 2				
wt	.352**	.300*	.554**	.710**
length		.128	-.020	.026
width			-.175	-.172
thickness				.638**
Experiment 3				
wt			.719**	.434**
thickness				.006
Experiment 4				
wt			.718**	.564**
thickness				.106

*Significant at 5%.
**Significant at 1%.

Compared to seed, embryo physical measurements were slightly better correlated with growth because the embryo is the seedling itself before germination. Seed coats and pericarp may contribute nothing to the growth of the seedling, but they detract little from correlations between seed and subsequent performance. This might be explained by the intercorrelations between physical parameters of seed and embryo which were as follows: wt 0.987**, length 0.391*, width 0.904** and thickness 0.964**. In other words, lettuce seed physical characteristics are, with the exception of length, very closely related to those of the embryo. Seed and embryo dimensions, especially length and width, are visibly different (Fig. 5). The seed were illuminated from underneath on a microscope in such a way that the embryo was clearly outlined.

The relative importance of each physical parameter of seed and embryo is obtained by combining results of experiments 5, 6, and 7 (Table 5). A priority list of the physical characteristics is similar for both seed and embryo: wt > thickness > density > width > length. All 5 parameters were correlated with seedling growth in varying degrees. This information is necessary for future engineering of efficient seed separation equipment to improve the uniformity and quality of seed lots.

Prolonged vigor test. Significant correlations between seed physical characteristics and seedling vigor were shown in all 7 experiments. In all but one experiment significant correlations between seed parameters and intermediate growth stages (21-40 days) were found. In Experiments 6 and 7, wt of marketable heads was recorded. The results (Table 4) indicate no correlation between any seed or embryo physical characteristic and plant measurements (vigor) at head stage. Since seed wt was consistently the best correlation parameter, it was plotted in relation to growth at 3 stages in experiment 7 (Fig. 6). Similar results were obtained in experiment 6 (not shown).

The information in Table 4 and Fig. 6 clearly suggests a diminishing correlation between physical parameters of seed and embryo and plant growth. The early stages of growth were correlated, but at an unknown stage during vegetative growth,

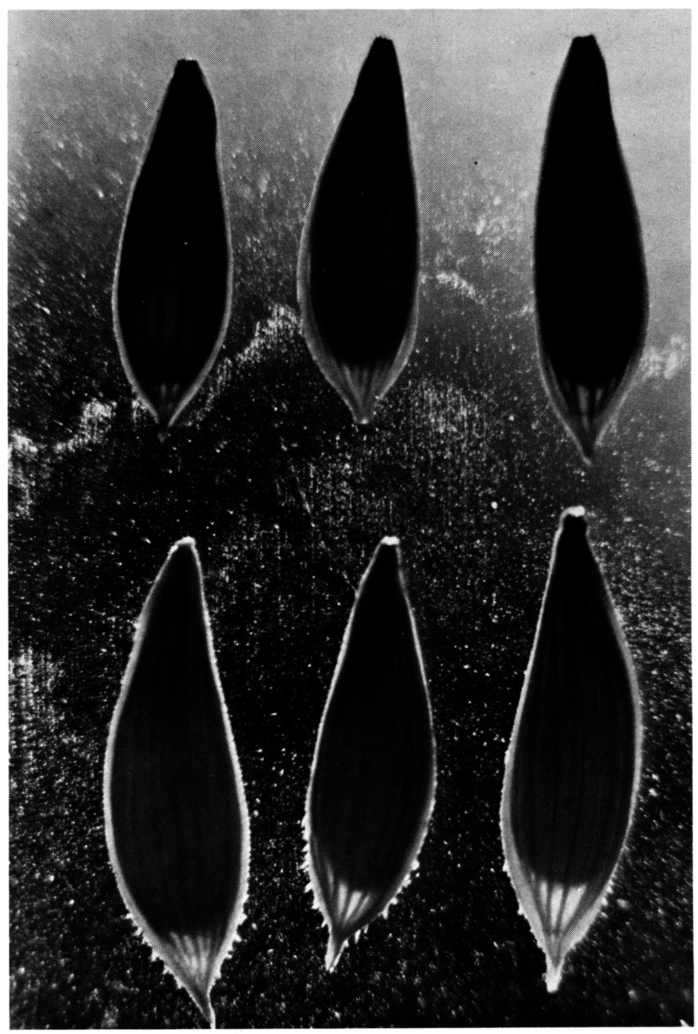


Fig. 5. Lettuce seed illuminated from beneath the microscope (X12.5). Embryos are clearly outlined inside the seeds.

Table 4. Correlations between growth variables and physical variables of seed and embryos.

Growth measurements	Physical measurements									
	Seed					Embryo				
	density[z]	wt	length	width	thickness	density[y]	wt	length	width	thickness
Experiment 5[x]										
seedling root length (3 days)	.671**	.557**	.230	.424**	.583**		.580**	.274	.383*	.600**
seedling shoot length (3 days)	.336*	.247	.009	.214	.241		.290	.057	.190	.223
fresh wt root (25 days)	.461**	.656**	.245	.441**	.618**		.687**	.406*	.423**	.573**
fresh wt shoot (25 days)	.344*	.668**	.330*	.532**	.576**		.697**	.431**	.466**	.487**
total fresh wt (25 days)	.391*	.686**	.315	.522**	.608**		.717**	.438**	.469**	.529**
Experiment 6[w]										
seedling root length (3 days)	.487**	.632**	-.034	.325*	.609**	.679**	.632**	.052	.252	.652**
seedling shoot length (3 days)	.410**	.553**	.074	.153	.626**	.573**	.544**	.050	.058	.630**
total seedling length (3 days)	.505**	.670**	.034	.256	.708**	.705**	.663**	.058	.158	.731**
plant fresh wt (24 days)	.154	.559**	.464**	.260	.335*	.212	.566**	.439**	.281*	.260
head fresh wt (53 days)	.103	.186	.190	-.004	.069	.137	.207	.239	.066	.088
root fresh wt (53 days)	-.039	.218	.233	.077	-.002	.109	.229	.307*	.078	.033
total plant fresh wt (53 days)	.097	.192	.196	.001	.066	.138	.212	.247	.068	.086
Experiment 7[v]										
seedling root length (3 days)	.582**	.628**	.207	.237	.561**		.600**	.126	.259	.640**
seedling shoot length (3 days)	.674**	.579**	.267	.277	.595**		.608**	.132	.375*	.575**
total shoot and root length (3 days)	.745**	.697**	.285	.306	.679**		.706**	.151	.386*	.700**
plant fresh wt (24 days)	.753**	.765**	.022	.501**	.708**		.746**	-.148	.606**	.765**
head fresh wt (73 days)	-.064	-.018	.100	.096	.066		-.032	.071	.062	-.020
root fresh wt (73 days)	.095	.005	.093	-.007	-.006		-.025	-.032	.006	.001
marketable head wt (73 days)	-.010	.039	.162	.087	.106		.018	.196	.028	.014

[z]Density - seed were separated by the air blower into 3 groups; light, medium and heavy.
[y]Density - individual embryo density was determined by the air blower (experiment 7 only).
[x]n = 37; terminated 25 days after imbibition.
[w]n = 49; terminated 53 days after imbibition.
[v]n = 32; terminated 73 days after imbibition.
*Significant at 5%.
**Significant at 1%.

rate of growth of plants from seeds of different size and wt changed. Either plants from small light seeds accelerated their growth rate and finally closed the initial vigor gap brought about by the differences in seed properties or plants from large seeds failed to maintain their initial growth advantage. Partial explanation might be derived from work of Zink et al. (11) which indicated a rapid acceleration of lettuce growth rate during the last 3 weeks before head harvest. Plants in their study produced in excess of 70% of their fresh wt in the 21 days immediately preceding first harvest. During this period of rapid growth, the slightest difference in growth rate in favor of the small and light seed could result in equal yield regardless of initial vigor. Phenotypic variability, according to Went (10), is least under the most nearly optimal growing condition, in which the growth rate is the fastest. Went observed that plants from larger pea seeds grew more slowly than those from smaller ones and that after some time plants from larger seed caught up with the plants from the smaller seed and reached the same overall size.

Our results indicated a growth advantage of plants from large seed over small seed during early growth. However, Went's observation that differences between individuals do not become magnified but decrease relatively, may apply to lettuce as well. With lettuce, plants from small light seeds were small initially, but later, possibly at the heading stage, growth rate accelerated rapidly, and they reached yield equality with plants from large

Table 5. Correlations between seed and embryo physical variables and vigor variables at day 3 and 24, and intercorrelations among vigor variables.[z]

Seed, embryo variables	Vigor variables			
	root length day 3	shoot length day 3	root & shoot day 3	plant fresh wt day 24
seed density	.490**	.430**	.529**	.316**
seed wt	.659**	.382**	.581**	.501**
embryo wt	.608**	.429**	.587**	.566**
seed length	.305**	.065	.198*	.183*
embryo length	.299**	.067	.191*	.276**
seed width	.388**	.194*	.296**	.286**
embryo width	.291**	.177	.262**	.302**
seed thickness	.568**	.410**	.554**	.348**
embryo thickness	.572**	.422**	.565**	.333**
Vigor intercorrelations				
root length day 3		.467**	.806**	.238**
shoot length day 3			.900**	.196*
root & shoot day 3				.249**

[z]Experiments 5, 6 and 7 pooled together n = 118. (experiment 5 was terminated on the 25th day.)
*Significant at 5%.
**Significant at 1%.

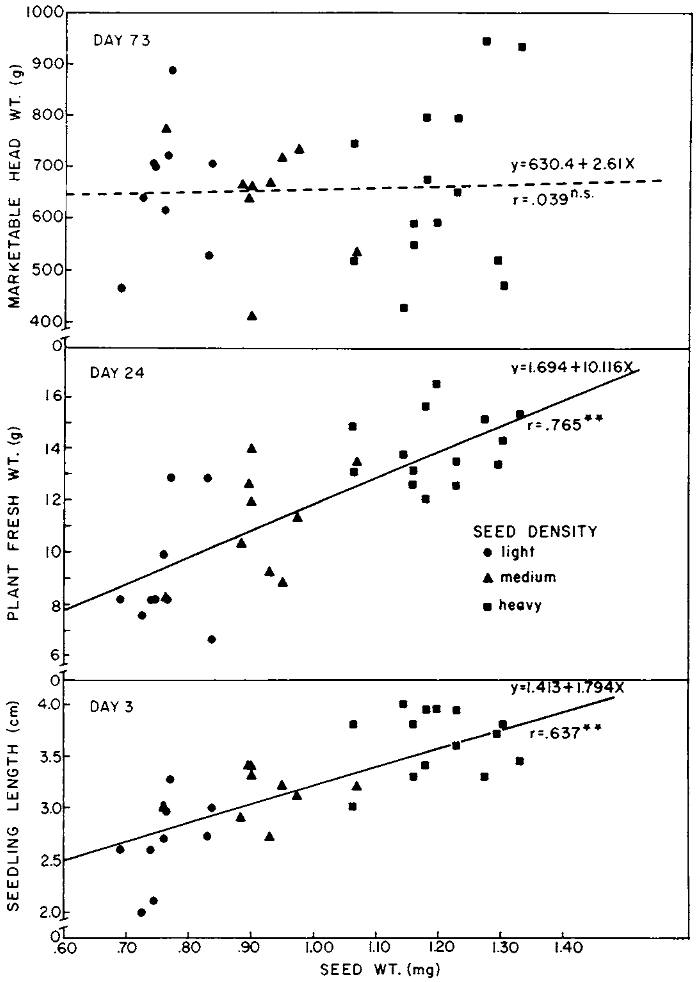


Fig. 6. Three consecutive growth measurements (vigor) of the same plants (expt 7) grown in growth chamber and hydroponics gravel culture in relation to initial seed wt. At the early growth stage (day 3 and day 24) a direct correlation was found between vigor and seed wt. However, this correlation diminished at head harvest time.

heavy seed.

Lettuce plants are almost entirely self-pollinated (3). All seed used for each experiment (5 to 7) were obtained from a single mother plant. Although found to vary widely in size and wt (9), they should be considered genetically homogeneous. Consequently, the genetic difference may not be responsible for the non-uniform late performance of genetically homogeneous plants grown under uniform conditions. Present lettuce production clearly demands uniformity at harvest time as a prerequisite for the forthcoming once-over mechanical harvester. Our findings show that seed and seedling vigor were not associated with either head wt or uniformity. The lack of uniformity was equally distributed for all seed sizes. Lettuce seed properties and seedling vigor were previously to be correlated with marketable head wt under field conditions (7). Further studies correlating seedling vigor with uniformity of both head wt and maturity are clearly needed.

It should be emphasized that the growing and measuring methods used in this study were considerably different from those used in previous reports. The plants in this study were not exposed to any known measurable stress, diseases, or insects. The aim was to make the growing conditions as uniform as possible. The seedlings were recovered unharmed from the vigor test. The nutrient culture in the growth chamber and in the greenhouse hydroponics beds provided uniform and favorable growth conditions throughout the growth period, and eliminated plant competition.

The results and assessments of this work could not and should not be extrapolated to lettuce field production. On the contrary, one can readily visualize situations in the field in which initial growth advantages would in fact be magnified.

Literature Cited

1. Bradenburg, N. R., and J. E. Harmond. 1966. Separating seed by length with special indent cylinders. *Tech. Bul.* 88, Agr. Exp. Station, Oregon State Univ., Corvallis.
2. Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soils. *Calif. Agr. Expt. Sta. Cir.* 347.
3. Jones, H. A. 1927. Pollination and life history studies of lettuce (*Lactuca sativa* L.). *Hilgardia* 2:424-480.
4. Pollock, B. M., and E. E. Roos. 1972. Seed and seedling vigor. p. 313-387. *In* Seed Biology, Vol. I. (T. T. Kozlowski, Ed.) Academic Press, NY.
5. Scaife, M. A., and D. Jones. 1970. Effect of seed weight on lettuce growth. *J. Hort. Sci.* 45:299-302.
6. Sharples, G. C. 1970. The effect of seed size on lettuce germination and growth. *Prog. Agr. AZ* 2:10-11.
7. Smith, O. E., N. C. Welch, and O. D. McCoy. 1973. Studies on lettuce seed quality: II. Relationship of seed vigor to emergence, seedling weight, and yield. *J. Amer. Soc. Hort. Sci.* 98:552-556.
8. ———, ———, and T. M. Little. 1973. Studies on lettuce seed quality: I. Effect of seed size and weight on vigor. *J. Amer. Soc. Hort. Sci.* 98:529-533.
9. Soffer, Hillel, and O. E. Smith. 1974. Studies on lettuce seed quality: III. Relationship between flowering pattern, seed yield, and seed quality. *J. Amer. Soc. Hort. Sci.* 99:114-117.
10. Went, F. W. 1953. Gene action in relation to growth and development. I. Phenotypic variability. *Proc. Nat. Acad. Sci.* 39:839-848.
11. Zink, F. W., and M. Yamaguchi. 1962. Studies on the growth rate and nutrient absorption of head lettuce. *Hilgardia* 32:470-500.