THE VASCULAR ANATOMY OF NORMAL AND VARIANT SEEDLINGS OF PHASEOLUS VULGARIS

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The investigations here summarized comprise a comparative and biometric study of the gross vascular anatomy of normal and variant seedlings of *Phaseolus vulgaris*.

Three morphological types have been considered, (*a*) the normal or *dimerous* seedling with two cotyledons and two primordial leaves, (*b*) the *trimerous* seedling with three cotyledons and three primordial leaves, and (*c*) the *hemitrimerous* seedling in which there are three cotyledons and two primordial leaves.

In normal seedlings, the vascular system of the root is typically tetrarch (with four protoxylem poles), and gives rise in the base of the hypocotyl to four pairs of double bundles which soon form a circle of eight bundles which continue to the cotyledonary node. At this point there is a complex vascular anastomosis. From it two strands are given off to each cotyledon. The remainder of the vascular tissue is reorganized into six strands, each of which typically soon divides into two, the twelve bundles thus formed comprising the vascular system of the epicotyl.

The trimerous seedlings typically possess six root poles instead of four, twelve bundles in the hypocotyl instead of eight, and nine primary epicotyledonary bundles instead of six. The nine primary epicotyledonary bundles do not all divide, however, so that the number of bundles in the central region of the epicotyl is variable ranging in general from fourteen to eighteen.

In both classes of seedlings, but more frequently in the normal type, additional or intercalary bundles appear in the hypocotyl, either *de novo* or as a result of division of the primary strands.

Four main groups of problems as to the vascular topography of these seedling types have been considered biometrically: First, the number of bundles at different levels in the seedling; second, the variability in bundle number; third, the differentiation in internal structure of seedlings which are externally dimerous, trimerous and hemitrimerous; and fourth, the interrelationship of bundle number in different regions of the seedling.

The following table of constants[1] summarizes the facts for number and variability of vascular bundles in various regions of the seedling and in the three types of seedlings.[2]

The constants in this table, and the frequency distributions from which the constants were computed, lead to the following conclusions.

	DIMEROUS SEEDLINGS			TRIMEROUS SEEDLINGS			HEMITRIMEROUS SEEDLINGS		
	Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.
Root poles									
Minimum	4.01	0.081	2.03	5.02	0.654	13.02			
Maximum	4.13	0.338	8.18	5.16	0.729	14.12			
Mean	4.05	0.171	4.19	5.09	0.707	13.87			
Primary double bundles									
Minimum	4.02	0.140	3.48	5.81	0.288	4.86	5.21	0.608	10.59
Maximum	4.52	0.666	14.74	5.98	0.581	10.01	5.74	0.750	14.07
Mean	4.19	0.411	9.66	5.91	0.405	6.87	5.49	0.676	12.37
Intercalary bundles									
Minimum	0.07	0.261	105.79	0.09	0.292	156.62	0.28	0.449	115.47
Maximum	0.83	1.024	355.48	0.29	0.686	381.67	0.53	1.148	214.68
Mean	0.49	0.687	182.70	0.19	0.491	274.92	0.44	0.737	163.82
Mid-region of hypocotyl									
Minimum	8.11	0.409	5.04	11.99	0.532	4.42	11.36	1.169	9.94
Maximum	10.62	1.645	17.34	12.29	1.283	10.44	12.32	1.524	12.87
Mean	9.23	1.193	12.67	12.16	0.883	7.24	11.94	1.307	10.96
Mid-region of epicotyl									
Minimum	12.11	0.406	3.35	14.89	1.152	7.74	12.93	1.245	9.07
Maximum	12.36	0.757	6.13	16.10	1.750	10.87	14.84	1.778	12.53
Mean	12.22	0.586	4.79	15.47	1.383	8.92	13.83	1.560	11.29

The modal number of primary double bundles in the region of transition from root to stem structure at the base of the hypocotyl is four in the dimerous and six in the trimerous and hemitrimerous seedling. In the normal seedlings more than four bundles may occur, but in no case have fewer than this number been observed. In the trimerous seedling variation both above and below the mode is found, the numbers ranging from four to eight. On the average the number is from 1.38 to 1.89 bundles higher (or from 30.5 to 47.0% higher) in the trimerous than in the dimerous seedlings.

Intercalary bundles, wh[illegible] rather uncommon in seedling anatomy in general, occur in fr[illegible]% of the normal seedlings, whereas they are found in only 9 to 29% of the trimerous and in 28 to 43% of hemitrimerous seedlings. The average number of intercalary bundles is also generally higher in the dimerous plantlets.

Considering the total bundle number at the base of the hypocotyl (primary bundles plus intercalary bundles) the trimerous and hemitrimerous seedlings have from 0.77 to 1.91 bundles, or from 14.4 to 46.7%, more than the dimerous seedlings. The differentiation of the dimerous and trimerous seedlings is conspicuously shown by the frequency distributions of two of the lines shown in diagram 1.

In passing upward from the base of the hypocotyl, each primary bundle pair normally divides into two so that in the central region of the hypocotyl the bundle number is normally twice the number of primary double bundles at the base, plus the intercalary bundles. In many cases the number is somewhat in excess of this, however, showing either that new (intercalary) bundles have appeared or that some of the bundles have become subdivided.

The modal number of bundles in the mid-region of the hypocotyl is eight or ten in dimerous plantlets; in trimerous and hemitrimerous plantlets

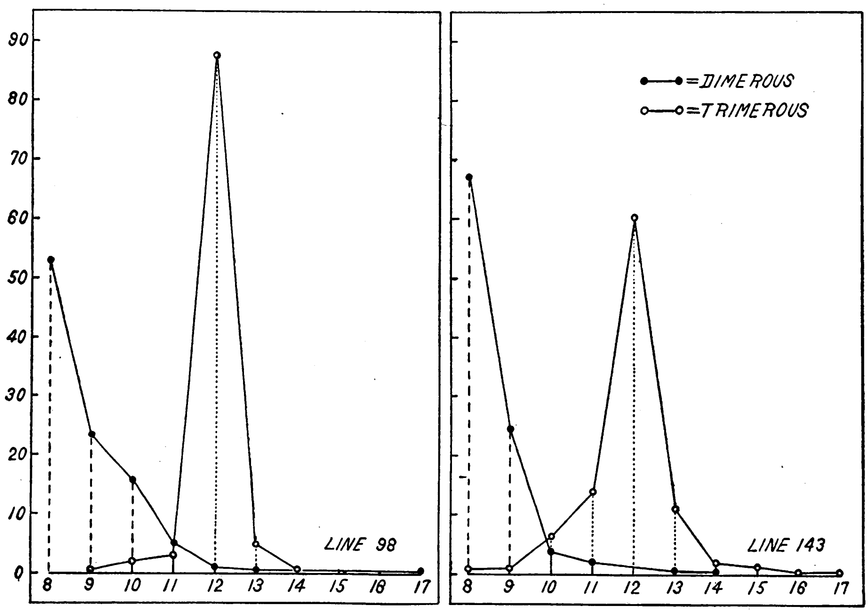

DIAGRAM 1

Percentage frequency distributions of total bundles (primary double bundles counted as two) at the base of the hypocotyl in dimerous and trimerous seedlings of two lines. Abscissae represent bundle numbers, ordinates represent percentage frequencies.

it is twelve. On the average the number is fr[illegible]3 to 3.8 bundles higher (or from 15.7 to 47.9% higher) in the trimerous than in the dimerous seedlings. The differentiation of the two classes of seedlings in their vascular anatomy at the level is clearly shown in diagram 2.

The bundles in the mid-region of the epicotyl show in dimerous plantlets a modal number of twelve, whereas in trimerous seedlings it is fifteen. On the average there are from 2.8 to 3.7, or from 23.0 to 30.2%, more bundles in the epicotyl of the trimerous than in the dimerous seedling.

The form of the frequency distributions for two of the lines is shown in diagram 3. The epicotyl of the hemitrimerous is in essentials of anatomy identical with that of the dimerous seedling.

Not only are there marked differences in the actual number of bundles, but the variability of bundle number changes from region to region of the seedling, and differs in the three seedling types. Whether judged by range, standard deviation or coefficient of variation, the variability of bundle number in the central region of hypocotyl tends to be distinctly higher in the dimerous than in the trimerous plantlets; but in the epicotyl just the reverse is true, the variability of the trimerous plantlets exceeding that of the dimerous. These differences are conspicuous in diagrams 2

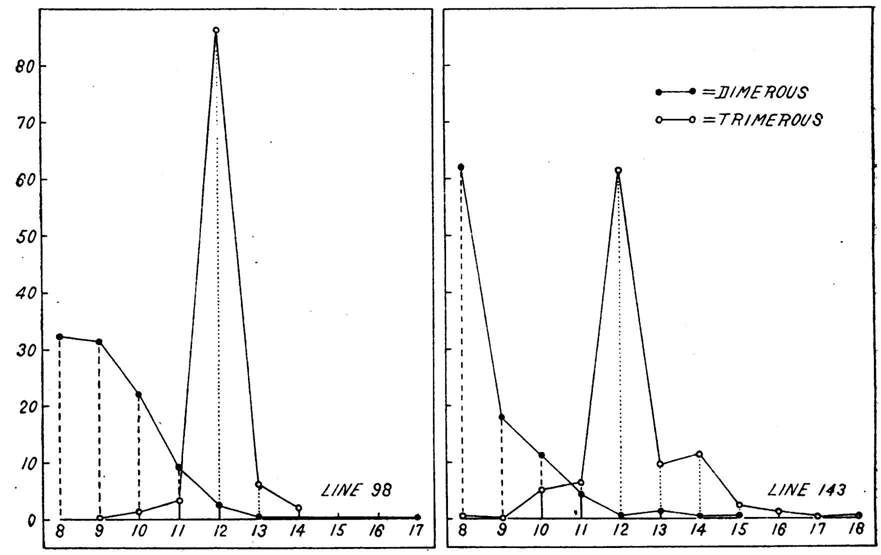

DIAGRAM 2

Percentage frequency distribution of number of bundles in central region of hypocotyl in dimerous and trimerous seedlings. Abscissae represent bundle numbers, ordinates represent percentage frequencies.

and 3. In the first case it is the dimerous plantlets, in the second case it is the trimerous ones which show the greater variability. Apparently this is due to differences in the number of intercalary bundles in the hypocotyl, and to the extent of division of the bundles in the epicotyl, of the two types of seedlings.

The coefficients of correlation between various bundle systems also differ widely. In both trimerous and dimerous seedlings there is a negative correlation between the number of primary double bundles and the number of intercalary bundles at the base of the hypocotyl. Thus the number of intercalary bundles is smaller in seedlings with larger numbers of primary double bundles and vice versa. This result for seedlings of the same (external) morphological type is in agreement with those obtained by a comparison of seedlings which are externally dimerous and trimerous, since the latter frequently have a larger number of primary double bundles but a smaller number of intercalary bundles than the former. In both types of seedlings variation in the number of intercalary bundles is the primary factor in determining variation in the total number of bundles at the base of the hypocotyl.

Turning to the problem of the interrelationship of bundle number at different levels in the seedling we find that there is a substantial correla-

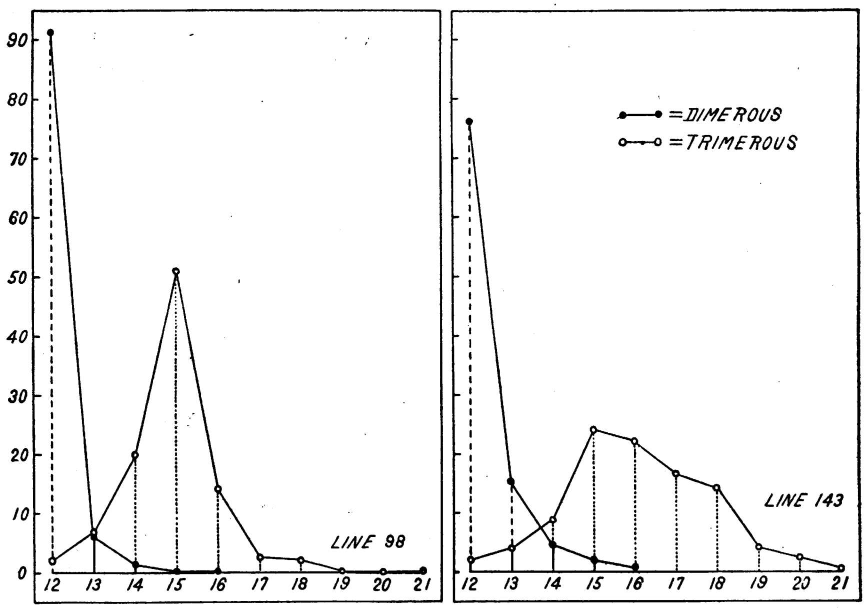

DIAGRAM 3

Percentage frequency distributions of total bundle number in the central region of the epicotyl of dimerous and trimerous seedlings of two lines. Abscissae represent bundle numbers, ordinates represent percentage frequencies.

tion between the numbers of the three classes of bundles—primary double bundles, intercalary bundles, and total bundles—at the base of the hypocotyl and the number of bundles in the central region of the hypocotyl. In the normal seedlings the coefficients average +0.509 for number of primary double bundles and number of hypocotyledonary bundles, +0.629 for intercalary bundles and hypocotyledonary bundles, and +0.813 for total bundles and hypocotyledonary bundles. In the trimerous plants these correlations average +0.381, +0.238 and +0.598, respectively. The correlations for normal plantlets are practically without exception higher than those for abnormal seedlings.

The correlations between the number of bundles in the hypocotyl (both basal region and central region) on the one hand and the number of bundles in the central region of the epicotyl on the other differ conspicuously from those found between the numbers of bundles at the two levels in the hypocotyl itself, in that the coefficients are extremely slight. They are positive in some series, but negative in others.

The regression straight lines and the empirical means for number of bundles in the hypocotyl (lower line) and epicotyl (upper line) associated with various numbers of primary double bundles at the base of the hypocotyl in two of the lines is shown in diagram 4. The irregularity of the means is at least in part due to the fact that the frequencies are largely concentrated in the two classes of 4 and 5 primary double bundles. The

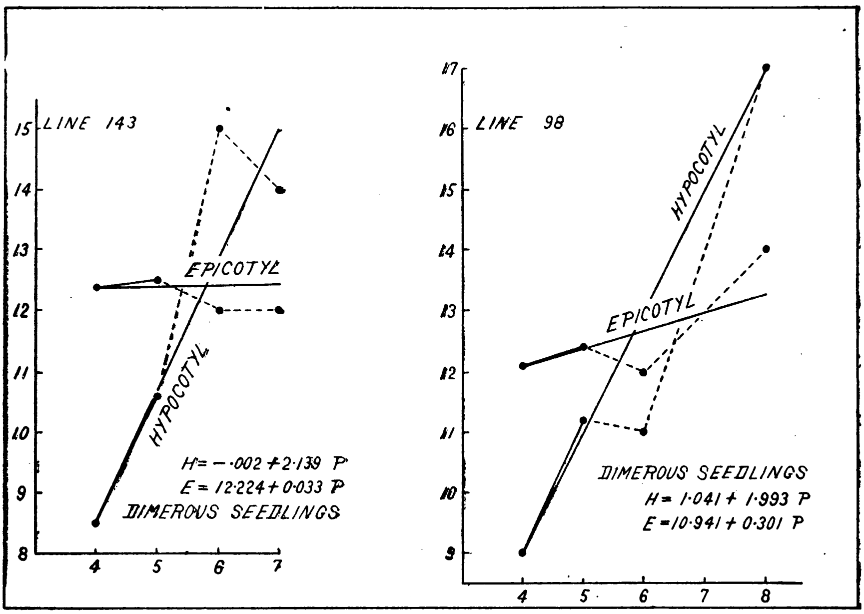

DIAGRAM 4

Regression of number of bundles in central regions of hypocotyl and of epicotyl on number of primary double bundles at base of hypocotyl. Abscissae represent primary double bundles, ordinates represent mean number of bundles in hypocotyl and epicotyl.

slope of the line representing change in hypocotyledonary bundles associaated with variation in number of basal bundles is steep, showing a rather close dependence; but the line showing the change in epicotyledonary bundles associated with the same variations in basal bundles is very slight, showing the laxness of the interdependence of bundle number in these two regions.

On the basis of the data available it is impossible to assert that there is any relationship at all between bundle number above the cotyledons and bundle number below them. These results indicate that there is a *complete reorganization of the vascular system at the cotyledonary node.*

It will be evident from the foregoing outline that the vascular-structures of the seedling are not constant but are highly variable, even within genetically very homogenous material. It is quite possible, therefore, that anatomical investigations based on but a few sections for each species might lead to erroneous results. Seedlings differing in external form are differentiated in their internal anatomy. Such differentiation is evident not only in mean number of bundles but in the degree of variability in bundle number. Thus, in normal seedlings the variability is higher in the hypocotyl than in the epicotyl, whereas in seedlings with three cotyledons and three primordial leaves just the reverse is true. The external form and the internal structure of the seedling are highly but not perfectly correlated. Finally in both normal and variant seedlings, the number of vascular elements of the several regions of the seedling are correlated in very different degrees; the correlation between some is high; between others it is practically wanting.

Papers to appear in the *American Journal of Botany* may be consulted for details.

[1] The values given in each case are the maximum and minimum constants for the lines investigated. For the trimerous and dimerous seedlings all the averages are based on five lines. For the hemitrimerous seedlings the averages for primary double bundles and intercalary bundles are based on five lines while for the mid-region of hypocotyl and the mid-region of epicotyl they are based on six lines. Data for number of root poles are available for only three lines.

[2] Because of the extreme rarity of hemitrimerous seedlings it is not feasible to discuss variability of bundle number in this group.

THE PRESENT STATUS OF THE LONG-CONTINUED PEDIGREE CULTURE OF PARAMECIUM AURELIA AT YALE UNIVERSITY

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As a matter of record it seems advisable to bring up to date and summarize the chief results derived from the writer's main pedigree culture of Paramecium aurelia.

This culture, designated Culture I, was started on May 1, 1907, by the isolation of a "wild" individual which was found in a laboratory aquarium.[1] The original specimen was placed in about five drops of culture fluid on a glass slide having a central ground concavity, and when this animal had produced four individuals, each of these was isolated on a separate slide to form the four lines of the culture. The four lines have not been kept distinct from one another throughout the work, but have been replenished by cells from one of the sister lines when, through accident or otherwise, one or another of the lines has become extinct.