Effects of Photoperiod and Temperature on Rate of Node Development in Indeterminate Bean

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Abstract. The plastochron index was used to compare the effects by daylength, mean temperature, and diurnal temperature fluctuation, on the rate of node development of five indeterminate common bean (Phaseolus vulgaris L.) genotypes grown in eight growth chamber environments. Regression analysis described temporal trends in the plastochron index. Regression curves for the various genotypes—environment combinations were compared using canonical variates analysis. At a constant 17°C, extending daylength from 12 to 14 or 16 hr had no effect on rate of node development. The rate of node development increased at a constant 23°C when daylength was lengthened from 12 to 14 or 16 hr. The increase in rate of node development was more pronounced in genotypes with higher photoperiod sensitivity, as measured by delay of flowering. Temperature rise from 17 to 23 to 29°C also increased the rate of node development, with genotypes again exhibiting differential response. Diurnal fluctuation of 6°C about a mean of 23°C had the same node development rate as a constant 23°C.

We (Yourstone and Wallace, 1990) demonstrated that the plastochron index (PI) provides a suitable estimation of node development of indeterminate bean grown in a constant growth chamber environment. Use of PI was complicated by progressively increasing rates of leaf initiation over time, which produced a curvilinear rather than linear relationship. PI curves can be analyzed as a function of time in a similar fashion as the estimation and comparison of growth curves (Box, 1950; Grizzle and Allen, 1969). Growth analysis experiments are typically designed with repeated measurements of the experimental units that are analyzed as a split-plot in time. Eskridge and Stevens (1987) reviewed attendant statistical difficulties arising from analysis of repeated measures as a split-plot in time, which include lack of randomization of the time variable and increasing heterogeneity of variance with time. In addition, multiple measurements from the same experimental unit over time are correlated. This correlation is ignored in the traditional split-plot analysis, and tests may therefore be misleading.

Multivariate analysis of variance (MANOVA) was proposed as an alternative analysis (Cole and Grizzle, 1966; Potthoff and Roy, 1964), but is not feasible if the levels of time exceed the degrees of freedom for the whole-plot error. In addition, cases for which any of the repeated measures are missing must be excluded. An alternative procedure, which avoids these limitations, involves first fitting curvilinear regressions to the original data, followed by MANOVA of the regression coefficients (Eskridge and Stevens, 1987; Grizzle and Allen, 1969).

In this study, regression coefficients were calculated from PI data from five indeterminate bean genotypes grown in eight combinations of daylength and temperature in growth chambers. The coefficients were analyzed with MANOVA followed by canonical variates analysis. The objective was to determine the effects of mean temperature, diurnal temperature fluctuation, and daylength on the rate of node development for each of five indeterminate bean genotypes. The statistical methodology could also be applied to a wide range of studies involving the comparison of curvilinear functions or growth curves.

Materials and Methods

Five indeterminate bean genotypes were examined in this study (see Table 1, Yourstone and Wallace, 1990). ‘Jamapa’ and ‘P326’ had been classified as photoperiod-insensitive for delay to flowering, while flowering of ‘Magdalene’, ‘Porrillo Sintetico’, and ‘Rojo 70’ is delayed by long daylength (Yourstone, 1988). Plants were grown in walk-in growth chambers in 4.2-liter plastic pots filled with a mixture of 40% peatmoss, 60% vermiculite and dolomite lime, micronutrients, and 30 g of Osmocote (14N-14P-14K). Three seeds were sown in each pot. The seed coats were scarified before sowing to promote uniform germination. After emergence, the plants were thinned to one per pot. One pot was discarded to retain the four most-uniform plants of each genotype. Axillary branch buds were removed as they developed in nodes along the main stem, thus forcing all vegetative growth and flower development to occur on the main stem.

Leaflet measurements were recorded from each plant three times per week (Monday, Wednesday, Friday) at similar times during the day. For each measurement, the number of trifoliate leaves (n) with central leaflet length exceeding 20 mm was counted. Then, lengths of the central leaflet of leaf n and leaf n + 1 were measured to the nearest 0.1 mm using a vernier caliper. Measurements were terminated at anthesis, at which time the node of first flower (cotyledonary node was considered node one) and days from planting to flowering were also re-

| Daylength (hr) | 24-hr mean temp |
|---------------|-----------------|
| 12            | 17/17 23/23, 29/17 29/29 |
| 14            | 17/17 23/23 |
| 16            | 17/17 23/23 |

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corded. PI was calculated from the leaf length data using the equation of Erickson and Michelini (1957):

\[
PI = n + \frac{l_n(L_n) - ln(R)}{ln(L_n) - ln(L_{n+1})}
\]  

[1]

where \( n \) is the number of leaves longer than the reference length (\( R = 20 \) mm), and \( L \) is the length of middle trifoliate leaflet of leaf \( n \) or leaf \( n + 1 \).

The growth chamber environments compared the effects of mean temperature, diurnal temperature difference, and daylength (Table 1). Each environment received 12 hr of light at 180 µmol·s⁻¹·m⁻² (measured at pot level) provided from a mix of incandescent and fluorescent lighting. Daylength was extended to 14 and 16 hr with 25% of full light. When day/night temperatures fluctuated, day temperature corresponded to the 12-hr period of full light. Relative humidity was maintained at 40% under all conditions; thus, the vapor pressure deficit varied among chambers and with time. Temperature levels were monitored daily using thermographs.

Analysis of the plastochron index data proceeded as follows. First, for each experimental unit (individual plant), a polynomial regression of PI on time was fitted according to the model:

\[
PI = \beta_1 + \beta_2 (DFP) + \beta_3 [(DFP - 30)^2]
\]

[2]

where \( PI \) is the plastochron index, DFP is the days from planting, and \( \beta_{1,3} \) are regression coefficients?

Subtracting 30 from the polynomial time term reduced the simple correlation coefficient \((r)\) between the linear and squared terms from 0.98 to 0.44 (Snecorde and Cochran, 1980). Thirty was near the overall mean DFP.

Second, the regression coefficients \( \beta_1, \beta_2, \) and \( \beta_3 \) were analyzed as dependent variables in a MANOVA (see appendix A). The analysis was performed using the MANOVA statement within the PROC, GLM procedure of the Statistical Analysis System (SAS). The model partitioned the model total sums of squares and cross products (SSCP) into SSCP for environments, genotypes, and genotype x environment interaction. These SSCP matrices were assigned as the H matrix for significance testing of each partitioned component.

Third, discrepancies between the null hypothesis and the data were evaluated using canonical variates analysis (CVA) (see appendix B). Chatfield and Collins (1980) present a complete description and derivation of CVA. They argue that CVA provides better protection against a Type I error than confidence intervals based upon Wilks’ criterion. In addition, CVA provides visual identification of the significant differences between treatment means by a plot of the first two canonical variates. A microcomputer spreadsheet model was constructed to calculate canonical variates. When dimensionality (appendix C) is 2, the treatment means can be plotted using the first two canonical variates as coordinates.

Coordinates calculated from the first canonical variate were plotted to show the effects of daylength and temperature for each genotype. Circles centered on the coordinates depicted statistical significance between treatments. Nonoverlapping circles were significantly different at the 0.05 level. The circles were drawn with a radius equal to the least significant interval (LSI) (Andrews et al., 1980). Because the canonical variates are normalized, the LSI can be calculated as \( LSI = \frac{t\sqrt{2/m}}{2} = \sqrt{1.96} \sqrt{m} \), where \( t = 1.96 \) and \( m \) is the number of replications.

Results

Regressions of PI on time (days) from planting had coefficients of determination (\( r^2 \)) that ranged from 0.94 to 0.99 among experimental units (plants). Residual plots indicated that the model (Eq. [2]) was appropriate. MANOVA partitioning of environmental, genotypic, and genotype x environment interaction effects were all significant at the 0.0001 level (Table 2). Correlation coefficients indicated a high linear relationship between the intercept (\( \beta_0 \)) and linear (\( \beta_1 \)) regression coefficients (\( r = 0.94 \)) and between the intercept (\( \beta_0 \)) and polynomial (\( \beta_2 \)) coefficients (\( r = 0.82 \)), but smaller linear relationship between the linear (\( \beta_1 \)) and polynomial (\( \beta_2 \)) coefficients (\( r = 0.63 \)). The test of dimensionality follows (appendix C):

\[
\text{HO: Dimensionality} = 1: x = 130.5 \ln(4.2605 * 1.5807) = 248.89 < \chi^2_{\text{LSI}}(76) \approx 95.
\]

\[
\text{HO: Dimensionality} = 2: x = 130.5 \ln(1.5807) = 59.75 > \chi^2_{\text{LSI}}(37) \approx 51.
\]

The test of dimensionality equals two was significant. The canonical variates are (appendix B): \( Z_1 = 3.6388 + 86.8598 + 419.931 \beta_r + 1.8228 + 269.6988 + 2.733(\beta_r) \).

Figure 1 plots the first (\( Z_1 \)) and second (\( Z_2 \)) canonical variates for the 40 possible genotype-environment combinations. A very different response by 'Rojo 70' when grown at 29°C contributes much of the variability of the second canonical variate. Rate of leaf initiation for 'Rojo 70' in this warm environment decreased over time. For all other genotypes and environments, the rate increased over time. Removal of the 'Rojo 70' data of this environment reduces the dimensionality to one: \( H_0: \text{Dimensionality} = 2: x = 126.0 \ln(1.1570) = 18.37 < \chi^2_{\text{LSI}}(36) \approx 51 \).

The first canonical variate is a reduced representation of the original regression coefficients. Because the first canonical variate captures most of the variation of the regression coefficients, it provides a single measure estimate of rate of node development. Further evidence that the first canonical variate represents the rate of development is its high correlation with the calculated PI (\( r = 0.95 \) after 20 DFP and \( r = 0.99 \) after 30 DFP). The

| Source | df | SSCP matrix (omitting lower triangle) | Wilks' criterion |
|--------|----|--------------------------------------|-----------------|
| Model  | 39 | 1141.9286 -58.9387 3.4052 1.5303 0.0020*** | 0.0020*** |
| Environment | 7 | 485.7890 -32.6568 2.2698 0.4643 0.0190*** | 0.0290 |
| Genotype | 4 | 142.6819 -6.7421 0.3422 0.1893*** | 0.0064 |
| G x E  | 28 | 513.4577 -19.5400 -0.8813 0.0419*** | 0.0299 |
| Residual | 113 | 142.4040 -5.3318 0.2239 0.2351 | 0.0073 |
| Total   | 152 | 1284.3326 -64.2705 1.7654 3.6291 0.0722 0.0036 |

***P < 0.0001.
second canonical variate had low correlations with calculated PI ($r = -0.29$ and $r = -0.028$).

The first canonical variate contains the information of interest to this study, so it will be considered in detail. The canonical variate analysis reduced the data to a single number representing the rate of node development, and provides LSD regions for comparisons among genotypes and among environments.

Coordinates of the first canonical variates are depicted on one-dimensional plots for each of the five genotypes. The effects by 12-, 14-, and 16-hr daylength at means of 17 and 23C are compared in Fig. 2. The effects by 17, 23, and 29C at 12 hr of daylength are compared in Fig. 3. Each circle encompasses the LSD region. Therefore, overlapping circles represent nonsignificant differences in the magnitude of the first canonical variate (rate of node development) within that genotype. Circles lying within the same horizontal plane indicate nonsignificant differences between genotypes.

At 17C, daylength did not significantly affect the rate of node development of the five genotypes (Fig. 2), except that node development occurred slightly faster at 12 hr than 14 or 16 hr for ‘P326’. Differences between genotypes were also minimal at the low temperatures. Nodes of ‘Rojo 70’ tended to develop slightly faster than those of the other four genotypes. At 23C, daylength extension from 12 to 14 hr resulted in faster node development for all genotypes. The significant genotype x environment interaction (Table 2) is graphically illustrated by long daylength accelerating the rates of ‘P326’ and ‘Jamapa’ less than those of ‘Porrillo Sintetico’, ‘Magdalena’, and ‘Rojo 70’ (Fig. 2). Extending the daylength from 14 to 16 hr did not further enhance the rate of node development. ‘Porrillo Sintetico’ had a significantly lower rate of node development at 16 than 14 hr of daylength. Longer daylength tended to enhance rate of node development for genotypes with sensitivity to long daylength for delay of flowering (‘Magdalena’ and ‘Rojo 70’) more so than insensitive genotypes. Enhanced rate of node development corresponds to promotion of vegetative growth that attends daylength-modulated reduction of reproductive growth. ‘Porrillo Sintetico’ is moderately photoperiod-sensitive for delay of flowering. At a 14-hr daylength in the growth chamber, its rate of development was similar to the more-sensitive genotypes ‘Mag-
dalena’ and ‘Rojo 70’, but it responded similarly to the insensitive genotypes ‘Jamapa’ and ‘P326’ at a 16-hr daylength (Fig. 2). This results indicates that other genetic and/or environmental factors exist that modify rate of development.

Compared to 17C, 23C increased the rate of node development for ‘P326’, ‘Jamapa’, ‘Porrillo Sintetico’, and ‘Magdalena’ (Fig. 3). It did not further increase the relatively high rate of ‘Rojo 70’. The rate of node development for all five genotypes was greater at 29C than at 23C. The five genotypes responded to increasing temperature from 17 to 23 to 29C with an exponential increase in rate of development. Response of the photoperiod-insensitive genotype ‘P326’ to this range of temperatures, was less than that of the other four genotypes. For all genotypes, the effect of alternating day (29C) and night (17C) temperatures around a mean of 23C was similar to the constant 23C environment.

**Discussion**

At the low temperature (17C), increasing daylength minimally affected rate of node development. This parallels observations in bean (Gniffke, 1985; Yourstone, 1988) and pea (Pisum sativum L.) (Berry and Aitken, 1979) of reduced photoperiod gene activity and hence less daylength-caused delay of flowering at low temperatures. At the moderate temperature (23C), extending daylength increased rate of node development for these five bean genotypes. Similar results have also been reported for soybean (Glycine max L.) (Snyder and Bunce, 1983). The effect of daylength on rate of node development was genotype-specific, creating a large genotype × daylength interaction. Both minimal photoperiod gene activity at low temperature and genotype × daylength interaction correspond to observed photoperiod gene activity as measured by delay in time to flower in bean. Furthermore, there appears to be a relationship between genotypes in this study in that those that showed the largest response to lengthening daylength for rate of node development have also been previously classified as photoperiod-sensitive for flowering (Yourstone, 1988). While this is certainly too small a sample of bean genotypes to determine conclusively a relationship between these two characteristics, it does suggest the possibility of common or linked genetic control. Rising temperature increased the rate of node development differentially for the five genotypes, with insensitive ‘P326’ responding the least and sensitive ‘Magdalena’ the most. That all genotypes were similarly affected by both long daylength and high temperature (29C) suggests that both environmental factors affect the same physiological process, e.g., photoperiod gene activity.

We hypothesize that photoperiod gene activity regulates the partitioning of photosynthates between vegetative and reproductive structures in bean. If so, high rates of vegetative or node production should be associated with delayed node to flowering. This was the generalized observation among this set of five genotypes (Yourstone, 1988).

Wallace (1985) proposed a time-to-flowering model in which photoperiod acted to modify the node of first flower and temperature acted to control both the rate of node development and the photoperiod gene activity. The results of this study implicate photoperiodic control over the rate of node development. The same photoperiod–temperature complex that modulates flowering may also influence the rate of node development, perhaps through control of partitioning of assimilates. Thus, a bean

**Table 3. Mean days to first flower (DFF), first canonical variate (FCV), and node to first flower (NFF, cotyledonary node equals node one) for bean cultivar Magdalena grown in eight daylength/temperature regimes.**

| Temp (°C) | 12 | 14 | 16 |
|----------|----|----|----|
|          | DFF | FCV | NFF | DFF | FCV | NFF | DFF | FCV | NFF |
| 17       | 59.5 | 13.6 | 13.8 | 58.5 | 12.6 | 13.8 | 65.3 | 13.6 | 16.8 |
| 23       | 41.8 | 18.0 | 12.3 | 47.0 | 26.2 | 17.0 | 42.0 | 25.9 | 16.3 |
| 29       | 41.3 | 28.6 | 19.3 | 41.3 | 28.6 | 19.3 |
| 29/17    | 51.8 | 17.3 | 19.5 |

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of nodes to flowering and the rate of node development were the same for both. Increase in the number of nodes to flowering is the classical response of photoperiod-sensitive genotypes to delaying photoperiod. "Magdalena" exhibited this response to 14-hr daylength at 23°C, but not 17°C (Table 3). That photoperiod effects are reduced or nonapparent at low temperatures was observed by Wallace (1985), Gnifike (1985), and Yourstone (1988).

In this study, diurnal temperature fluctuation did not affect the rate of node development. Mean temperature determined the rates of node development. The temperature range of this study, however, was relatively small and extrapolating this result to other temperature regimes would not be appropriate. High day temperature (29°C) alone was sufficient to increase the number of nodes to flowering as much as a constant 29°C, producing a significant delay in time to flowering of "Magdalena" bean. Thus, diurnal temperature fluctuation must be considered as another factor that influences time to flowering.

**Appendix A**

Univariate analysis of variance partitions variation as represented by sums of squares (SS) into sources. In a k-dimensional MANOVA, there are k SSs plus measures of covariance between pairs of the k components. In MANOVA, therefore, partitioning of variation consists of k × k symmetric matrices of sums of squares and cross products (SSCP) such that:

| Source | df | SSCP matrix | Matrix notation |
|--------|----|-------------|-----------------|
| Total  | Y'Y |             |                 |
| Model  | h B'X'Y | H |                 |
| Error  | e Y'Y − B'X'Y | E |                 |

Since matrices cannot be divided, the matrix E'H is the basis for test statistics. Several test statistics are available for multivariate hypothesis, with considerable discussion but no definitive conclusion on which is the best. (For example, see Lee, 1971; Pillai and Jayachandran, 1967; Schatzoff, 1966.) Wilks’ criterion is commonly used and is the test statistic used by Chatfield and Collins (4). Wilks’ A, like the other test statistics, is a function of the characteristic roots or eigenvalues (λ) of the matrix E'H. Wilks’ criterion (A) is transformed to an approximate F distribution that allows for the rejection or acceptance of the null hypothesis at a prescribed level of significance.

**Appendix B**

Canonical variates (Z) are linear compounds of the vector of coefficient means (x) for a given treatment: Z = a’x.

The number of possible canonical variates is equal to the number of coefficients means (k). The vector a is chosen such that the first canonical variate (Z1) maximizes the variance ratio (VR): VR = [(a’Ha)/h]/[(a’E a)/e], where a is a vector of k elements, H is the model SSCP matrix, E is the residual SSCP matrix, h is the degrees of freedom of the H SSCP matrix, and e is the degrees of freedom of the E SSCP matrix.

The second canonical variate (Z2) maximizes VR with the constraint Cov(Z1, Z2) = 0, and so forth. The vector a is determined by solving the equation (H − λE) a = 0, obtaining a unique solution by adding the constraint a’Ea = e.

Multiplication of the a vector by the coefficient means vector (x) consisting of β1, β2, and βk for each treatment gives the canonical variate of that treatment corresponding to the characteristic root (λ).

**Appendix C**

The test statistic to determine dimensionality is given by:

\[ x^2 = \left( e - \left( p - h + 1 \right) \right) \ln \left( \frac{\hat{p}}{\sum_{\lambda_i} \left( 1 + \lambda_i \right)} \right) \]

where e is degrees of freedom of the E SSCP matrix, p is the number of variates measured per experimental unit, h is degrees of freedom of the H SSCP matrix, and t is the dimensionality of the null hypothesis with t ≤ k.

This test statistic follows a χ² distribution with (p − t)(h − t) degrees of freedom (Bartlett, 1947; Chatfield and Collins, 1980). The test of dimensionality equal to zero is the Wilks’ A test.

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