PLANT GENETIC RESOURCES

Birdsfoot Trefoil Flowering Response to Photoperiod Length

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ABSTRACT

Birdsfoot trefoil (*Lotus corniculatus* L.) is an indeterminate Old World perennial forage legume that is widely adapted to environments ranging from Scandinavia in the north to highlands near the equator in the south. Because of poor root and crown rot resistance, natural reseeding in pastures is desired. Therefore, birdsfoot trefoil genotypes that flower and set seeds at lower latitudes would be desirable to aid persistence and thus improve pasture forage quality. The purpose of this research was to determine the effects of photoperiod length and collecting site ecogeography on flowering of 68 birdsfoot trefoil accessions from the USDA-ARS National Plant Germplasm System (NPGS) collection. The flowering response index (FRI) described accession flowering in 10-, 13-, 16-, and 19-h photoperiod lengths. As photoperiod length increased from 13 to 19 h, the percentage of accession clones that flowered increased. Photoperiod lengths equal to 16 h were too long to differentiate germplasm differences, and thus are not suited as a selection criterion for flowering at low latitudes. Under 13-h photoperiod length, as collecting site latitude increased, the FRI and percentage of clones in an accession that flowered decreased. Only an induced mutant flowered in the 10-h photoperiod length treatment. Since wild accessions were collected from habitats as little as 7° N latitude, a new natural minimal critical photoperiod requirement at 12.5 h was inferred. When selecting genotypes for use in low latitude pastures, the flowering response at 13-h photoperiod length should be considered if reseeding is desired.

Birdsfoot trefoil is an indeterminate flowering Old World perennial forage that is adapted to a broad range of environments. It occurs naturally in habitats ranging from northern Scandinavian hemiboreal and boreal zones, to near-equatorial latitudes in the elevated plains and highlands of Ethiopia, Uganda, Kenya, and the Democratic Republic of Congo (Steiner, 1999). McKee (1963) reported that the flowering response to day length varied for birdsfoot trefoil germplasm from different geographic origins. Plant reproductive morphology is affected by latitude origin. Materials collected at lesser latitudes tend to display umbels more in the upper leaf axilla than those collected at higher latitudes that have umbels distributed at leaf axilla along the length of the stems (Steiner and Garcia de los Santos, 2001). Early research reports birdsfoot trefoil to have a minimum photoperiod requirement of approximately 14 h (Joffe, 1958; McKee, 1963) and a minimal flowering latitude from 26 to 30 that may be modified by native habitat altitude and ambient temperature (McKee, 1963). These reports conflict with later reports of naturally adapted materials from Ethiopia at lower latitudes (Steiner and Poklemba, 1994), as does an induced photoperiod insensitive mutant that flowers when grown at a 10-h photoperiod length (Steiner and Beuselinck, 2001).

Most North American birdsfoot trefoil cultivars require 16- to 18-h photoperiod lengths to flower (Beuselinck and Grant, 1995). When grown above 40 N latitude, Beuselinck and McGraw (1988) reported that intense flowering occurred in a contracted period of time. Li and Hill (1988) found that field grown plants may produce more than 70% of the total number of reproductive shoots in a 25-d period, even though reproductive shoots were produced over a protracted 7-mo period. Under short photoperiod lengths, birdsfoot trefoil plants produce fewer inflorescences and more sterile flowers when compared with plants grown under longer photoperiod lengths (Joffe, 1958). Photoperiod length can affect seed yields in that increased flowering occurs when plants grown for seeds are grown at higher latitudes. However, significant genetic × environment interactions are expected (McGraw et al., 1986).

Natural reseeding in pastures is desired, so identification of birdsfoot trefoil genotypes that flower and set seeds at lower latitudes would be desirable to aid persistence and thus improve pasture forage quality (McGraw et al., 1986). The purpose of this research was to determine the effects of photoperiod length and collecting site ecogeography on flowering of 68 birdsfoot trefoil accessions from the USDA-ARS National Plant Germplasm System collection.

MATERIALS AND METHODS

Sixty-eight birdsfoot trefoil accessions ($2n = 4x = 24$) from the USDA-ARS NPGS collection were concurrently examined for flowering response at 10-, 13-, 16-, and 19-h photoperiod lengths in CMP3244 growth chambers (Conviron, Pembina, ND) set at 23°C day temperature and a 15°C night temperature. Racks containing 21 40- × 300-mm containers filled with a commercial potting soil mix were planted with inoculated single seeds for each accession. One rack of each accession was placed in a growth chamber calibrated to the desired photoperiod and the containers watered as needed to prevent water stress. The racks were rotated through each growth chamber every 7 to 10 d to maintain relatively uniform lighting conditions for the racks of different accessions. The height of the lights in each growth chamber was adjusted as needed to maintain a photon flux density of 410 ± 15 μmol m^{-2} sec^{-1}. Temperatures were measured by the average reading from two HOBO H8 temperature loggers (Onset Computer Corporation, Pocasset, MA) positioned at plant level

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Abbreviations: FRI, flowering response index; FRI_{Comp}, composite flowering response index; HU, heat units; HU_{Ave}, average number of HU for an accession to flower; HU_{Max}, heat unit cut-off threshold; NPGS, USDA-ARS, National Plant Germplasm System; POP, percentage of population that flowered.

with measurements recorded at 10-min intervals. The growth chamber thermostats were adjusted as needed to maintain the desired day and night temperatures.

Plant emergence for each plant was scored the day that its cotyledons were fully expanded. To ensure that a minimum of 14 plants was evaluated for each accession, if no seedling emerged in a container after 14 d from planting, the containers without seedlings were reseeded. The average number of plants for each accession used in each photoperiod treatment was 18 plants.

The time to flowering for each plant was scored the day that the first flower on that plant was fully opened. Preliminary experiments determined that the time to flowering was generally as accurate as time to first bud formation (data not shown). The time to flowering was used because flowers could be directly observed, while measuring bud formation required dissecting buds within leaf axils.

The number of heat units to first flower for each plant was calculated from the date of emergence. Heat units were calculated as:

$$HU = [(L_{Light} \times 23 + L_{Dark} \times 15)/24] - 10 \quad [1]$$

where L_{Light} is the photo light period length (h) and L_{Dark} is the hours of the dark period. The 23 and 15 are the light and dark period temperatures (°C), respectively. The base temperature was 10°C. The average number of heat units for all clones that flowered (HU_{Ave}) and percentage of clones that flowered (POP) for each accession were determined for each photoperiod treatment.

To assess germplasm with diverse responses to photoperiod length, the flowering response index was devised and its limits tested by means of simulated sequential flower emergence patterns that vary about the normal distribution. The flowering response index (FRI) (Steiner et al., 2001) for each of the four photoperiod length treatments was calculated as:

$$FRI = \left(\frac{\sum_{i=1}^{t} \frac{n_i}{T_i}}{\sum_{i=1}^{t} \frac{n_i}{N}} \right) / HU_{Max} \times 10^6 \quad [2]$$

where n_i is the number of clones that flowered at time i; T_i is the number of heat units at time i that a clone required to flower; N is the number of clones for an accession that were used; HU_{Max} is the heat unit cut-off threshold for observations at all photoperiods; and t is the number of counts made until HU_{Max} was reached for an accession. For this experiment, HU_{Max} was 1550 when Eq. [1] was used. HU_{Max} was used so that plants grown in all photoperiod treatments received the same maximum number of heat units even though they received different photoperiod lengths. The coefficient 10^6 was used as a scaling constant. The FRI, HU, and POP are referred to as the individual flowering components.

The FRI was tested for its response to simulated sequential flower emergence patterns in hypothetical 100-plant populations and 1200 for HU_{Max} was used. These patterns for the flower emergence distributions included variation due to mode level (one to five equal sized classes) (Fig. 1); direction of skewness (γ_1) (skewed left, skewed right, and normally

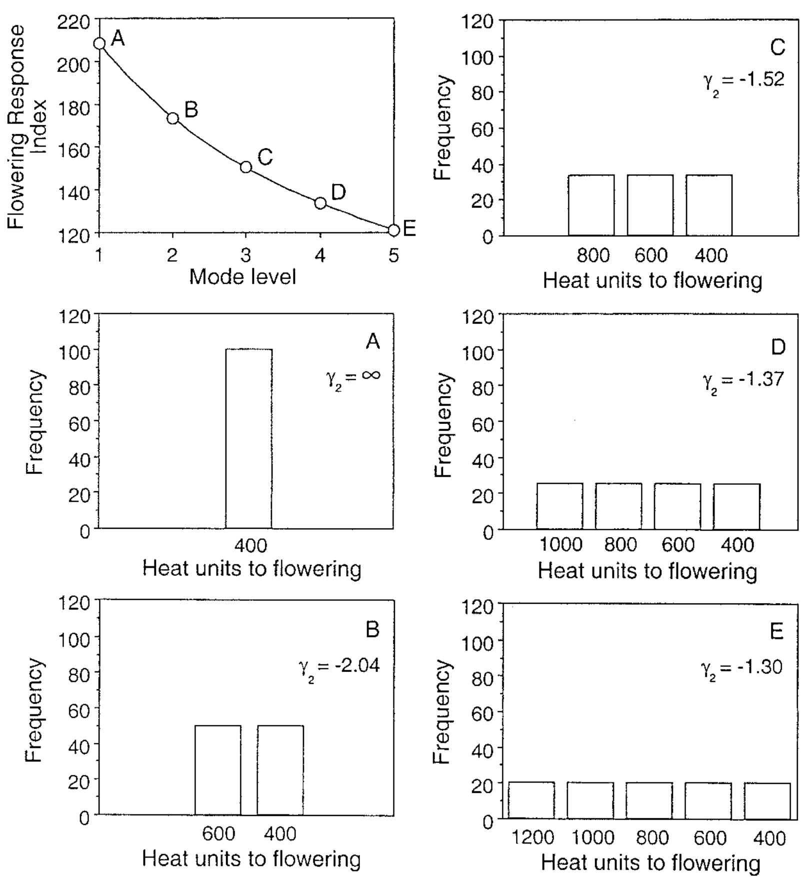

Fig. 1. Summary of the relationship between number of birdsfoot trefoil population mode levels of hypothetical clones flowering in a population and the flower response index (FRI). Also shown are the five modal-level distributions for each scenario (A, B, C, D, and E for 1, 2, 3, 4, and 5 equal frequency modes, respectively) used to test the FRI and the resulting amount of kurtosis (γ_2).

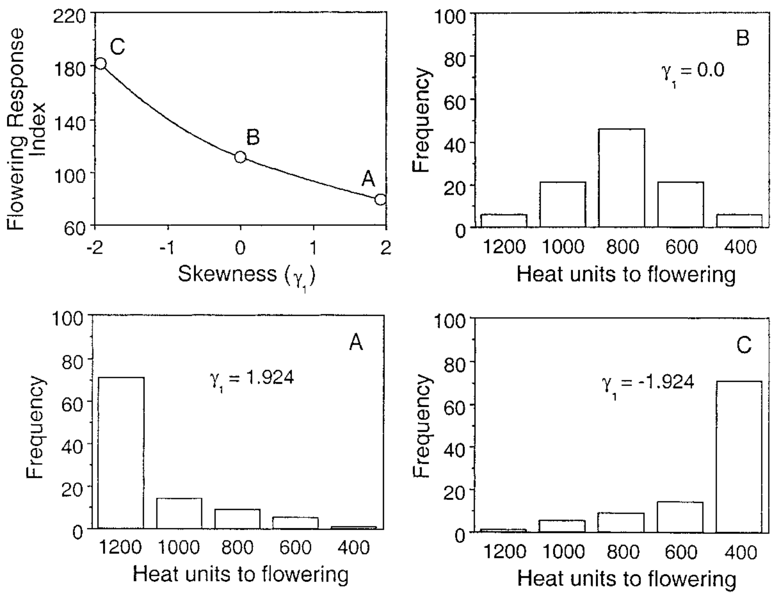

Fig. 2. The relationship between skewness (γ_1) of hypothetical clones flowering in a birdsfoot trefoil population and the flower response index (FRI). Also shown are the scenarios used for the range of skewed distributions about normal ($\gamma_1 = 0$) used to test the FRI.

distributed) (Fig. 2); and kurtosis (γ_2) (leptokurtosis and platykurtosis) (Fig. 3). The values of γ_1 and γ_2 were calculated from the hypothetical distributions by the formulas given in Sokal and Rholf (1981).

The distribution of accessions within 15 FRI classes between 0 and 2.8 were graphed and the class containing the average FRI (FRI_{Ave}) identified for the 13-, 16-, and 19-h photoperiod treatments. Only one accession flowered at 10 h, so that distribution is not presented. The aggregate photoperiod effects on an accession were measured as the FRI composite (FRI_{Comp}):

$$FRI_{Comp} = \sum_{i=1}^{pl} FRI_i \quad [3]$$

where FRI_i is one of each of the four photoperiod length (*pl*) treatments.

Pearson's correlation coefficient (*r*) was used to describe the associations among the FRI, HU_{Ave}, and POP variables at 13-, 16-, and 19-h photoperiod treatments. Correlation coefficients for FRI, HU_{Ave}, and POP variables at 13-, 16-, and 19-h photoperiod treatments with FRI_{Comp} were also determined. Probabilities for the significance of all correlation coefficients were determined by Bonferroni inequality adjustment (Snedecor and Cochran, 1980).

Multivariate factor analysis (Systat for the Macintosh, SPSS, Chicago, IL) using orthogonal factor rotation was done by the varimax method (Manly, 1986) to determine commonality among the FRI, HU, and POP variables at 13-, 16-, and 19-h photoperiod treatments with FRI_{Comp}. The number of factors used in the analyses was forced at two. Significant differences among loading factors were determined from the table of correlation coefficients and their probabilities.

A categorical class called an interpretive group (Steiner et al., 2001) was determined for FRI_{Comp} with the 48-accession

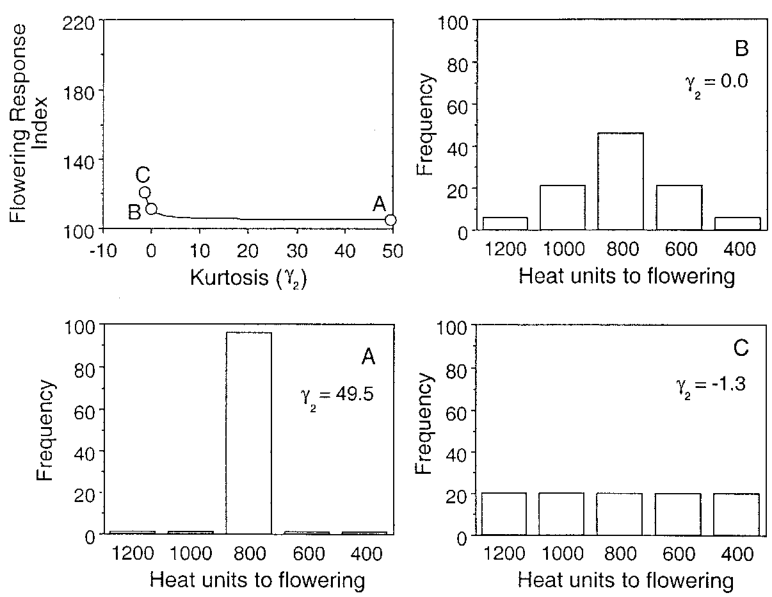

Fig. 3. The relationship between kurtosis (γ_2) of hypothetical clones flowering in a birdsfoot trefoil population and the flower response index (FRI). Also shown are the scenarios used for the range of kurtotic distributions about normal ($\gamma_2 = 0$) used to test the FRI.

Table 1. Response of 68 birdsfoot trefoil accessions from the USDA-ARS National Plant Germplasm System collection to four photoperiod lengths and descriptions of accession collecting site ecogeography.

			Flowering Response Index					Collecting site ecogeography								
												Temp.				
Entry	Origin	Cultivar	19	16	13	10	Comp	Latitude	Longitude	Snow	Precipitation	Low	High	Sunshine	Day length	FRI class†
			h					°		cm	mm	°C		%	min	
G 22102‡	USA	Fargo	1.64	0.67	0	0	2.31	46.9	−96.8	50	50	−14.5	21.5	56	950	1
G 22518‡	USA	Empire	1.58	1.42	0.1	0	3.10	42.7	−73.8	23	91	−4.6	22.1	52	917	1
G 31276§	Morocco		1.78	1.71	0.35	0	3.84	33.6	−4.9	0	770	3.6	22.0	72	863	2 DA
NSL 52607‡	USA	Dawn	1.70	1.35	0.09	0	3.14	38.5	−92.4	21	97	0.4	24.3	63	892	1
NSL 164710‡	Canada	Cree	1.41	1.30	0	0	2.71	52.1	−106.6	122	35	−17.6	19.2	52	1004	1
NSL 174057‡	USA	NC-83	1.96	1.80	0.14	0	3.90	44.8	−93.3	55	64	−11.2	22.5	59	932	2
NSL 174058‡	USA	Norcen	1.91	1.69	0	0	3.60	44.8	−93.3	55	64	−11.2	25.5	59	932	2
NSL 191436‡	USA	Fergus	1.83	1.50	0.15	0	3.48	38.1	−84.3	22	103	1.7	25.5	57	889	2
NSL 199053‡	USA	MU-81	2.16	1.84	0	0	4.00	44.8	−93.3	19	96	−1.0	25.8	63	932	2
PI 162456‡	Uruguay		2.15	1.73	0	0	3.88	34.0	−58.0	0	96	10.4	22.4	59	866	2
PI 180171‡§	Czech Republic		2.14	1.86	0	0	4.00	49.2	14.8	35	58	−2.8	17.1	37	973	2
PI 193725‡§	Sweden		2.06	1.63	0	0	3.70	55.9	13.1	2	62	−0.8	16.1	33	1052	2
PI 194228‡§	Croatia		2.47	1.93	0	0	4.39	46.0	16.0	31	79	−1.3	20.9	42	942	3
PI 226796‡§	Canada		2.20	1.45	0	0	3.65	55.0	4.5	0	80	1.7	17.2	33	1040	2
PI 226798‡§	Canada		2.13	1.65	0	0	3.78	52.0	4.5	0	80	1.7	17.2	33	1003	2
PI 227315‡§	France		2.32	1.79	0	0	4.12	48.8	2.1	7	58	3.2	18.4	39	968	2
PI 227512§	Iran		1.98	1.70	0.81	0	4.49	29.0	53.0	0	298	4.8	25.4	68	840	3 DA
PI 227849‡§	Iran		1.98	1.66	0.73	0	4.36	30.8	53.0	0	22	−0.6	22.8	72	849	3
PI 228286‡§	Iran		2.33	1.58	0.48	0	3.91	32.2	50.9	6	41	0.3	22.9	62	856	2
PI 232097‡§	Germany		2.19	1.66	0	0	3.86	50.8	9.5	36	74	−2.0	17.2	36	990	2
PI 232098‡§	Germany		2.03	1.45	0	0	3.48	50.8	9.5	36	74	−2.0	17.2	36	990	2
PI 233807‡§§	Italy		2.18	2.29	0	0	4.47	43.7	10.4	0	141	5.9	23.3	56	924	3
PI 234670§	France		1.56	1.21	0.09	0	1.55	48.1	−1.4	3	696	4.2	17.4	38	961	2 DA
PI 234670-S‡§	USA	Kalo	2.15	1.98	0	0	4.13	45.3	12.1	0	60	−0.1	17.5	34	930	2
PI 234692‡	Denmark		1.94	1.72	0	0	3.66	45.3	12.1	0	60	−0.1	17.5	34	936	2
PI 234811§	Switzerland		1.82	1.64	1.02	0	4.48	46.9	9.5	124	1075	−11.0	7.0	39	950	3 DA
PI 235525‡§	France		2.09	1.57	0	0	3.66	43.4	3.5	0	146	10.4	28.8	70	922	2
PI 237278‡§	Denmark	Late Roskilde II	2.23	1.60	0	0	3.83	55.6	12.6	0	60	0.3	16.5	37	1048	2
PI 246735‡§	Spain		1.75	1.34	0	0	3.09	40.4	−3.8	10	80	0.2	18.1	59	904	1
PI 249753‡§	Greece		1.72	1.24	0	0	2.96	40.5	23.8	0	51	5.5	24.4	59	904	1
PI 251143‡§	Macedonia		1.67	1.20	0	0	2.87	41.6	21.4	16	496	−5.6	16.5	46	910	1
PI 251146‡§	Serbia		2.17	1.71	0.15	0	4.04	42.1	20.4	18	98	−7.0	15.0	44	913	2
PI 255177‡§	Poland		2.34	1.80	0	0	4.14	52.4	16.9	19	53	−2.0	18.8	36	1008	2
PI 255302‡§	France		2.18	1.80	0	0	3.98	48.8	2.1	7	58	2.4	18.4	39	968	2
PI 255304‡§	France		2.16	1.75	0	0	3.91	48.8	2.1	7	58	2.4	18.4	39	968	2
PI 255305‡§	Italy		2.30	1.94	0	0	4.24	43.8	12.4	0	93	3.1	21.4	44	924	3
PI 258446‡§	Italy	Stripe 13	2.16	1.60	0	0	3.76	40.8	15.0	38	78	−1.9	18.5	51	906	2
PI 260268§	Ethiopia		1.97	1.59	0.98	0	4.54	9.4	41.5	0	685	12.9	18.8	67	761	3 DA
PI 260692‡§	Italy		1.28	1.09	0.14	0	2.51	42.5	12.5	9	949	4.1	23.2	53	916	1
PI 262529‡§	Czech Republic		2.20	1.75	0	0	3.95	49.0	15.5	95	57	−3.3	16.9	36	970	2
PI 262530‡§	Czech Republic		2.23	1.87	0	0	4.10	50.0	15.0	95	57	−3.3	16.9	36	981	2
PI 267060§	Poland		1.80	2.01	0	0	3.81	52.2	21.0	35	484	−3.5	19.2	34	1006	2 DA
PI 273937‡§	Ethiopia		1.89	1.46	0.93	0	4.27	7.0	37.0	0	102	21.4	24.1	51	752	3
PI 290717‡§	United Kingdom		2.16	2.48	0	0	4.64	51.7	−1.0	0	645	3.8	16.5	32	1000	3
PI 304067‡§	Uruguay		2.42	2.76	0.16	0	5.34	34.9	−56.0	0	102	10.4	22.4	64	971	3
PI 304523‡§	Turkey		2.63	2.51	0.63	0	5.77	39.0	39.0	16	43	−3.4	24.8	66	895	3
PI 306182‡§	USA	Leo	2.16	1.66	0	0	3.82	45.8	−71.2	34	101	−11.5	18.5	35	940	2
PI 310483‡§§	USA	Viking	2.14	1.69	0	0	3.83	42.3	−73.8	24	95	−3.8	21.9	55	914	2
PI 315082§	Kazakstan		2.58	2.60	0	0	5.18	43.2	76.6	99	480	−23.3	9.0	53	920	3 DA
PI 315454§	Russia		2.17	1.77	0	0	3.94	59.9	30.3	45	466	−8.3	17.2	30	1123	2 DA
PI 319021§	Spain		1.99	1.61	0	0	3.60	43.5	−5.3	0	909	2.5	14.6	43	922	2 DA
PI 319823§	Norway		1.63	1.85	0	0	3.48	59.6	6.0	17	2532	−7.0	10.4	26	1116	2 DA
PI 325369§	Russia		1.34	1.29	0.11	0	2.74	45.0	45.6	6	267	−6.2	25.5	42	933	1 DA
PI 325379‡§	Ukraine		2.00	1.20	0.14	0	3.34	44.3	34.1	1	452	−1.9	20.1	54	928	2
PI 369278§	Russia		2.41	2.56	0	0	4.97	55.0	82.6	195	388	−18.0	18.5	42	1040	3 DA
PI 380896‡§	Iran		1.44	1.08	0.24	0	2.76	36.4	48.8	63	132	−7.7	15.5	64	879	1
PI 383687‡§	Turkey		2.35	2.38	0.10	0	4.83	40.0	41.3	114	47	−9.6	18.8	57	901	3
PI 383689‡§	Turkey		2.34	2.23	0.75	0	5.32	36.2	29.5	12	109	−1.4	16.5	62	878	3
PI 384882‡§	Iran		1.96	1.46	0.13	0	3.55	35.3	53.3	0	842	3.6	24.0	69	873	2
PI 419228§	Greece		1.76	1.40	0.72	0	3.88	40.1	23.4	6	513	4.4	25.4	58	902	2 DA
PI 419233§	Greece		1.67	1.92	1.24	0	4.83	41.4	26.3	0	580	2.6	23.7	52	909	3 DA
PI 430546§	Russia		1.58	1.51	0	0	3.09	55.0	39.1	69	533	−13.0	17.9	36	1040	1 DA
PI 464682§	Turkey		1.77	1.41	0.10	0	3.27	39.5	36.1	39	333	−6.6	19.8	55	898	2 DA
PI 494653§	Romania		1.60	2.04	0.20	0	3.84	46.1	26.4	28	525	−5.2	16.4	42	943	2 DA
PI 547080‡§	USA	AU Dewey	1.73	1.89	0	0	3.62	32.6	−85.5	0	124	6.7	26.2	65	858	2
PI 566818§	USA	AG-S4	1.71	2.12	1.51	0	5.33	–¶	–	–	–	–	–	–		3 DA
PI 592503§	USA	ARS-2620	2.28	1.74	0	0	4.02	44.3	−123.2	15	1338	5.3	16.9	47	928	2 DA
PI 613539§	USA	RG-BFT	2.82	2.82	2.77	1.55	9.96	–¶	–	–	–	–	–	–		3 DA

† FRI class was based on a discriminant analysis of the 48-accession birdsfoot trefoil core subset collection using the 13-, 16-, and 19-h FRI as the discriminant variables. The DA next to the FRI class number indicates the accession was placed by the discriminant analysis.

‡ Indicates accession is a member of the USDA-ARN National Plant Germplasm System birdsfoot trefoil core subset collection.

§ Indicates the accession is from a wild Old World population.

¶ Indicates accession was developed in the greenhouse and not subjected to selection in the field, so no ecogeographic descriptors are estimated.

core subset accessions (Table 1) that were a part of the 68 accessions tested. The three interpretive group classes were determined by cluster analysis based on Euclidean distance and Ward's (1963) clustering technique (Systat for the Macintosh, SPSS, Chicago, IL) and the optimal number of classes (c_{opt}) method for germplasm collection analyses (Steiner et al., 2001):

$$c_{opt} = \lim [D_n = 0.5 \times D_g]; \text{ whenever } n > 2 \quad [4]$$

where D_g was the greatest amalgamation distance between two clusters and D_n was the least successive amalgamation distance between two clusters that was greater than or equal to one-half D_g. The 48-accession birdsfoot trefoil core subset was used as a reference collection (Beuselinck and Steiner, 1992) to predict the placement of the remaining 20 accessions into one of the three FRI classes by step-wise discriminant analysis (SPSS Inc., Chicago, IL) and with the FRI_{Comp} class number as the grouping variable (Steiner et al., 2001). The percentage of accessions from the core subset that were correctly classified was also determined.

The origins of the accessions were estimated from passport data reported in the USDA-ARS Germplasm Resources Information Network (GRIN). Forty-four of the 68 accessions were collected from wild populations in the Old World. When actual collecting site coordinates were not recorded, the latitude and longitude were determined by the retroclassification method (Steiner and Greene, 1996). For the remaining cultivars, germplasm, or New World naturalized accessions, the location of breeding selection or seed production recorded in the cultivar–germplasm registrations was used to estimate the geographic origin and determine the ecogeographic variables. Ecogeographic estimates were not made for PI 566818 and PI 613539 because these were not subjected to any selection pressures in the field.

The ecogeographic data describing the collecting sites or breeding selection environments were acquired from the U.S. Environmental Protection Agency and National Oceanic and Atmospheric Administration (EPA/NOAA) Global Ecosystems Databases (Kineman and Ohrenschall, 1992, 1994). The ecogeographic variables described were lowest (low) and highest (high) monthly temperature, monthly accumulated precipitation (precipitation), and monthly percentage of sunshine hours (sunshine) (Leemans and Cramer, 1992), and monthly accumulated snow depth (snow) (Chang et al., 1990). From the Smithsonian Meteorological Table no. 171 (Smithsonian Institution, 1963), the annual longest day (day length on 21 June) for 24 latitudes between 0 and 65 were plotted (Fig. 4) and the latitude for 10-, 13-, 16-, and 19-h photoperiod lengths determined by the equation

$$L = -789.05 + 2.2101 \times p - 2.1172 \times 10^{-3} \times p^2 + 9.7595 \times 10^{-7} \times p^3 - 1.2809 \times 10^{-10} \times p^4 \quad [5]$$

where L is the estimated latitude and p is the photoperiod length in minutes.

Correlations were determined among POP, HU, and FRI at 13-, 16-, and 19-h photoperiods, and FRI_{Comp}, for all 68 accessions (Table 2). From the 44 wild or naturalized Old World accessions, correlations were determined for POP, HU, and FRI at the three photoperiod lengths with the eight ecogeographic variables (Table 3). Six 10° latitude categorical classes were made between 0 and 60 N latitude and the wild accessions sorted within each class. The FRI_{Comp}, POP, and HU_{Ave} for the genotypes within the accessions that flowered were determined for the accessions placed in each latitude class. All differences are significant at $P \leq 0.05$ unless otherwise indicated.

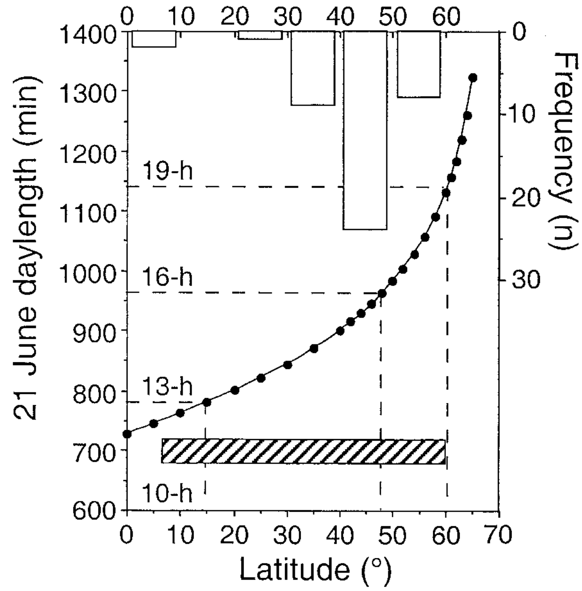

Fig. 4. The relationship between latitude and 21 June day length (-) and their correspondence to the 10-, 13-, 16-, and 19-h photoperiod treatments. The hatched horizontal bar indicates the latitude range for the 44 wild or naturalized USDA National Plant Germplasm Systems birdsfoot trefoil accessions used in the experiment. The downward vertical bars at the top of the figure indicate the frequency accessions within each of the six latitude classes.

RESULTS AND DISCUSSION

The FRI was tested by means of hypothetical 100-plant populations used to simulate different flower emergence patterns for clones in an accession population. The range for γ_1 was -1.924 (skewed left) to 1.924 (skewed right). The γ_1 values used were symmetrical for left and right skewness as well as $\gamma_1 = 0$ (no skewness and normally distributed). The range for γ_2 was -1.3 (platykurtosis) to 49.5 (leptokurtosis) for five frequency classes and with $\gamma_1 = 0$.

As the number of mode classes increased, the FRI decreased, indicating that the more rapid and uniformly a population of clones in an accession flowered, the greater the FRI (Fig. 1). Similarly, the more skewed right the population of clones flowered (an initial flowering flush followed by fewer later flowering clones), the greater the FRI compared with normally distributed and skewed left populations (Fig. 2). Among the scenarios for population mode number, skewness, and kurtosis, the FRI was least affected by differences between the kurtosis population distributions, with the FRI range for near absolute leptokurtosis (peaked deviation from normal) and platykurtosis (flattened deviation from normal) ($\gamma_2 = 49.5$ and -1.3, respectively) being only 105 and 121, respectively (Fig. 3). On the basis of these findings, it was concluded that the greater the rate of flowering and the greater the percentage of clones in a population that flowered, the larger the FRI value at a specific photoperiod length. The FRI values were greater than 0 when at least one clone in an accession flowered.

The Old World wild accessions used in this research were collected from habitats between 7 and 60° N lati-

Table 2. Pearson correlation coefficients (*r*) for relationships among flowering response measurements for 68 birdsfoot trefoil accessions from the USDA-ARS NPGS collection.

	Percentage of population flowering (POP)			Heat units for flowering (HU)			Flowering response index (FRI)			
Descriptor	13-h	16-h	19-h	13-h	16-h	19-h	13-h	16-h	19-h	FRI_{Comp}
	r									
POP-13	1.00									
POP-16	0.12	1.00								
POP-19	0.11	0.31	1.00							
HU-13	0.58***	−0.07	−0.08	1.00						
HU-16	−0.22	−0.42*	−0.03	0.06	1.00					
HU-19	0.03	−0.54***	−0.20	0.28	0.64***	1.00				
FRI-13	0.95***	0.12	0.12	0.45**	−0.28	−0.03	1.00			
FRI-16	0.23	0.62***	0.13	−0.08	−0.94***	−0.66***	0.29	1.00		
FRI-19	0.03	0.48**	0.43*	−0.28	−0.61***	−0.90***	0.10	0.69***	1.00	
FRI_{Comp}	0.63***	0.47**	0.25	0.13	−0.74***	−0.60***	0.73***	0.81***	0.69***	1.00

* Indicates *r* significance at $P = 0.05$, as determined by the Bonferroni inequality adjustment.
** Indicates *r* significance at $P = 0.01$, as determined by the Bonferroni inequality adjustment.
*** Indicates *r* significance at $P = 0.001$, as determined by the Bonferroni inequality adjustment.

tude (Table 1). These collecting sites corresponded with maximum 21 June day lengths ranging from 752 to 1123 min, respectively. The natural collecting site maximum day lengths were bracketed by 10- to 19-h photoperiod length treatments, with a majority of the accessions collected from European habitats between 40 and 50 N latitude (Fig. 4). The percentage of accessions in the NPGS Old World birdsfoot trefoil collection originated between 50 to 60, 40 to 50, 30 to 40 and <3°0 N latitude were approximately 39, 38, 21, and 2%, respectively. Few accessions with limited diversity were available in the NPGS collection from extreme latitudes less than 30° or greater than 55°. The only near-equatorial accessions in the NPGS collection are those from the highlands of Ethiopia (eight accessions). Also, no accessions were in the NPGS collection that originated from between 10 and 20° N, though the most likely areas where populations could be found would be in the mountainous regions of northern Ethiopia, Eritrea, or Yemen above the Gulf of Aden and the Red Sea. Specimens collected from North Africa (Egypt, Algeria, and Libya; 20–35° N) are reported (McKee, 1963), but examination of other NPGS accessions from the northern Africa region has proven the accessions to be diploids ($2n = 2x = 12$) and therefore not birdsfoot trefoil (Steiner and Beuselinck, 1992, unpublished data). The general lack of birdsfoot trefoil germplasm from this region and at these latitudes and examination of accessions from regions peripheral to typical European materials (Steiner et al., 2001) suggests that future expeditions may yield unique materials different from those already found in the NPGS collection.

The previously reported minimum latitude range for birdsfoot trefoil flowering was from 26 to 30° N (McKee, 1963). The plant materials used by McKee (1963) were domestic cultivars and wild or naturalized materials that originated from between 42 and 58° N latitude, so no source materials from lower latitudes were used. The relatively long 14-h photoperiod minimum reported by Joffe (1958) was probably also due to plant materials used that were from greater latitude origins than those used in this study.

The FRI captured multiple sequential flowering attributes of birdsfoot trefoil populations in response to four photoperiod length treatments. All 68 accessions flowered at the 16- and 19-h photoperiod length treatments, but only 59% flowered in the 13-h photoperiod treatment. Of all the accessions, only the RG-BFT rapid flowering induced mutant (PI 613539) flowered in the 10-h photoperiod treatment. As photoperiod length increased (13–16 and 16–19 h), the distribution of FRI values became more normal (less skewed to the right and less leptokurtotic; γ_1 and $\gamma_2 \rightarrow 0$) (Fig. 5). These findings support the general observation that birdsfoot trefoil flowering intensity can be enhanced as photoperiod length increases (Joffe, 1958).

Since the two Ethiopian accessions examined were collected from habitats as low as 7° N (Table 1), the minimal

Table 3. Pearson correlation coefficients (*r*) for seven collecting site ecogeographic variables with flowering response measurements for 44 wild USDA-ARS NPGS Old World birdsfoot trefoil accessions.

	Population percentage flowering			Heat units to flowering			Flowering response index (FRI)			
Descriptor	13 h	16 h	19 h	13 h	16 h	19 h	13 h	16 h	19 h	FRI_{Comp}
	r									
Snow	−0.22	0.19	0.15	−0.15	−0.39	−0.24	−0.12	0.41	0.25	0.27
Precipitation	0.03	−0.22	−0.20	0.13	0.01	0.52*	0.09	−0.07	−0.43	−0.18
Latitude	−0.73***	0.01	−0.06	−0.57**	−0.25	−0.04	−0.62**	0.22	0.08	−0.15
Longitude	0.30	−0.05	0.12	0.29	−0.23	−0.17	0.22	0.21	0.16	0.29
Low temp.	0.38	−0.05	0.03	0.17	0.42	0.17	0.32	−0.37	−0.15	−0.11
High temp.	0.30	−0.19	−0.02	0.24	0.26	0.12	0.19	−0.25	−0.09	−0.08
Sunshine	0.58**	0.04	0.14	0.59*	0.24	0.03	0.46	−0.17	−0.02	0.12
Daylength	−0.66***	−0.01	−0.04	0.60**	−0.29	−0.05	−0.56**	0.24	0.06	−0.12

* Indicates *r* significance at $P = 0.05$, as determined by the Bonferroni inequality adjustment.
** Indicates *r* significance at $P = 0.01$, as determined by the Bonferroni inequality adjustment.
*** Indicates *r* significance at $P = 0.001$, as determined by the Bonferroni inequality adjustment.

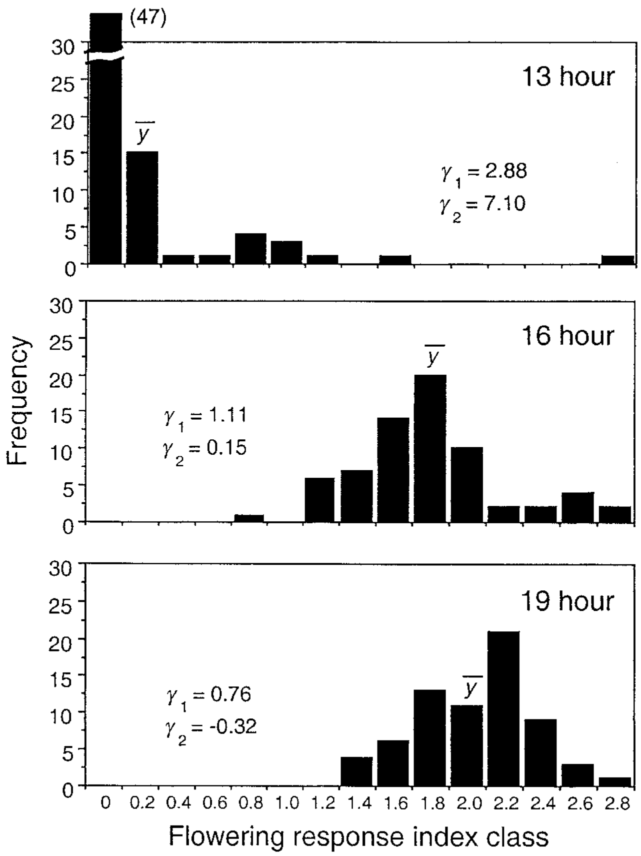

Fig. 5. The frequency of 68 USDA National Plant Germplasm System birdsfoot trefoil accessions by flowering response index (FRI) classes when grown at 13-, 16-, and 19-h photoperiod lengths. The class that contains the mean FRI is marked by y-bar. γ_1 and γ_2 indicate the amount of skewness and kurtosis about the normal distribution (γ_1 and γ_2 for the normal distribution = 0).

critical photoperiod requirement for naturally occurring birdsfoot trefoil may be as few as 752 min on the basis of the Ethiopian PI 273937 collected at 7° N latitude (Fig. 4). The maximum daylengths at these collecting sites were less than the 13-h photoperiod treatment, but greater than the 10-h treatment. The Ethiopian accessions flowered at a 13-h photoperiod, but not at a 10-h photoperiod. These findings extended the minimum 14-h photoperiod requirement for birdsfoot trefoil from earlier reports (Joffe, 1958; McKee, 1963) and the minimal 26 to 30° flowering latitude (McKee, 1963). The materials used by the Joffe (1958) and McKee (1963) experiments did not include low latitude origin germplasm, so those reported conclusions would be expected.

The FRI, number of heat units to flowering, and POP components of flowering for accessions grown in 13-h photoperiod conditions were generally correlated with collecting site latitude, day length, and sunshine percentage (Table 3). These three ecogeographic variables were highly collinear with one another (absolute *r* range among the three was 0.72–0.95). Other ecogeographic descriptors generally did not affect the photoperiod responses. In contrast, the individual flowering components at 16- and 19-h photoperiod lengths and the FRI_{Comp} were not correlated with any ecogeographic variables (Table 3).

Table 4. Interrelationships among flowering responses to three photoperiod lengths and the composite flowering response index from 68 birdsfoot trefoil accessions from the USDA-ARS NPGS collection.

	Photoperiod length condition	
Descriptor†	**Unlimited**	**Limited**
	Rotated factor loadings	
FRI-19	0.893a‡	−0.125
FRI-16	0.890a	0.150
HU-19	−0.865a	0.179
FRI_{Comp}	0.825a	0.541ab
HU-16	−0.794a	−0.151
POP-16	0.593a	0.023
POP-13	0.140	0.968bc
FRI-13	0.214	0.936bc
HU-13	−0.166	0.577c
POP-19	0.274	0.035
Variance explained (%)	41.7	25.3

† FRI, flowering response index, HU, average population heat units required for flower initiation; and POP, percentage of population that flowers; FRI_{Comp}, composite flowering response index.

‡ Rotated factor loadings with the matrix followed by the same letter are significantly correlated using Pearson's coefficients and the Bonferroni inequality adjustment. The 13, 16, and 19 indicate photoperiod length. Descriptor factor loadings without a letter are not significantly associated with any other descriptor according to Pearson's correlation and Bonferroni inequality adjustment.

Also, the individual components of flowering under 13-h photoperiod lengths were not correlated with those at 16 and 19 h, thus two distinct photoperiod response classes (limited and unlimited, respectively) were identified (Table 4). Within the limited and unlimited photoperiod response classes, the individual flowering components were generally associated with one another. Because FRI_{Comp} was correlated with the flowering components from both limited and unlimited photoperiod response classes, its use as a general descriptor of flowering response over the range of photoperiod length conditions was supported. These findings also confirmed that accession pools representing different ecogeographic regions could be distinguished by their different responses to 13-h photoperiods, but not at the longer 16- and 19-h photoperiod (Steiner et al., 2001).

Wild or naturalized accessions collected from environments <30° N latitude (two accessions from Ethiopia and one from Iran, PI 227512) and grown under the limited 13-h photoperiod conditions exhibited greater FRI and POP responses than accessions collected at latitudes >30° (Fig. 6A and 6B, respectively). Accessions collected between 30 and 50° N latitude were highly variable for FRI and POP responses, with none to 67% of their clones flowering. Among these are accessions that are from ecogeographic regions known to hold uniquely diverse materials (e.g., PI 234811 from Switzerland and PI 419233 from Greece) different from those of European origins (Steiner et al., 2001). No clones flowered from any accession collected above 50° N latitude and grown under the 13-h photoperiod length. Interestingly, for those clones of accessions that flowered, collecting site latitude did not effect the average number of heat units to flowering (Fig. 6C). These findings support earlier field research that showed genetic × photoperiod length (environment) interactions occur in birdsfoot trefoil (McGraw et al., 1986).

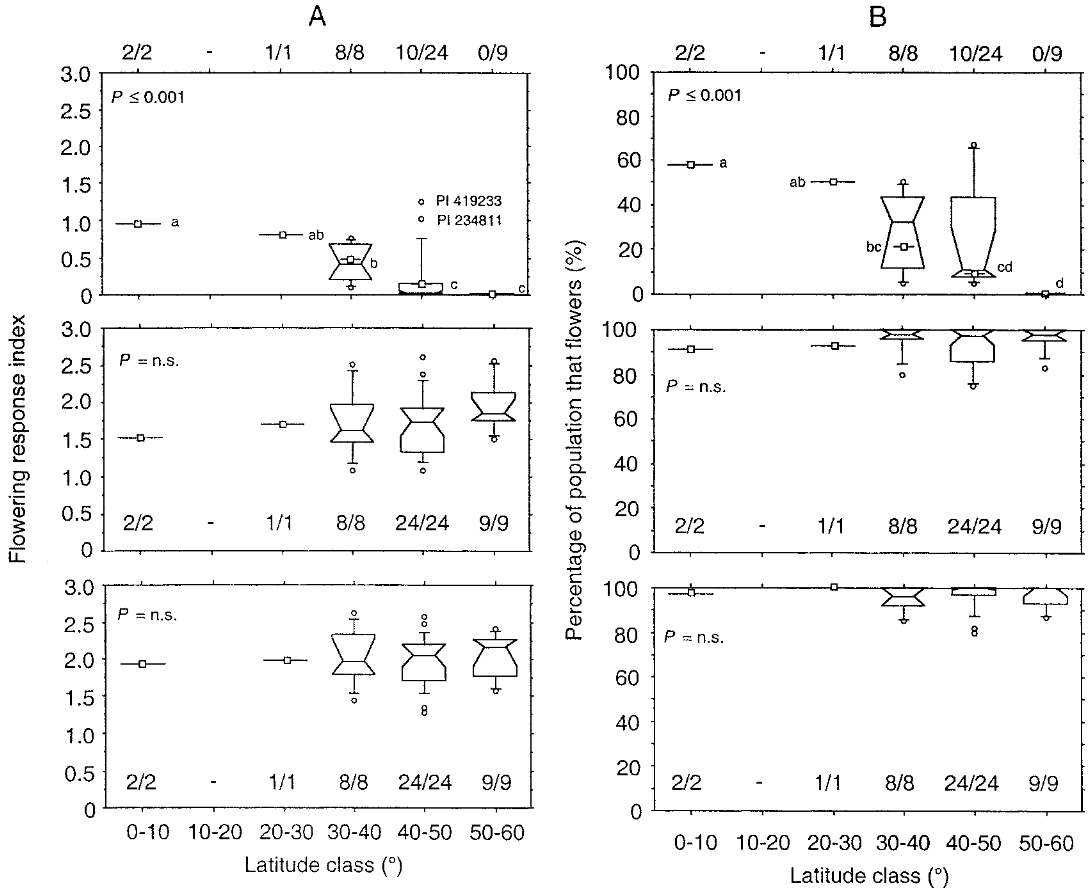

Fig. 6. Continued on next page.

When the wild or naturalized accessions were grown at 16- and 19-h photoperiod lengths, no impact was found for collecting site latitude on the components of flowering, although there were individual differences among the accessions. In the 16-h treatment, the percentage of clones in an accession that flowered ranged from 75 to 100%. In the 19-h treatment, the number of clones in an accession that flowered ranged from 82 to 100%. Previous research showed birdsfoot trefoil collected from greater latitudes had longer photoperiod requirements and tended to bear their inflorescences at leaf axilla along the entire stem, compared to those from lower latitudes (Steiner and Garcia de los Santos, 2001). These findings suggested birdsfoot trefoil populations naturally selected under climate-limited reproductive conditions have morphology and flowering time optimized to the time of the year with longest photoperiod length and for maximum floral sites available to increase the chances of seed production success. Practically, photoperiod lengths equal to 16 h were too long to differentiate among birdsfoot trefoil germplasm and thus should not be used as a selection criterion for determining photoperiod response differences or reproductive adaptation to specific ecogeographic regions.

The induced mutant RG-BFT was the only accession that flowered in the 10-h photoperiod treatment (Table 1). All RG-BFT clones flowered in all four photoperiod treatments, which differs from the other 67 accessions examined. Not only did RG-BFT flower under all photoperiod conditions, but it also appeared to be photoperiod insensitive because the FRI was similar in the 13-, 16-, and 19-h treatments (Fig. 7). All other accessions, including AG-S4 the progenitor of RG-BFT (Steiner, 1993), had optimal FRI at either 16- or 19-h photoperiod lengths, with a FRI that decreased at 13 h, if flowering even occurred. The FRI values varied because of the number of clones in an accession that flowered at each photoperiod length. RG-BFT did not have an optimal photoperiod length and no differences were observed in the average number of heat units needed or the number of clones that flowered. The number of heat units for RG-BFT plants to flower in the 10-h treatment was greater than the three other treatments (average HU needed were 1003, 560, 553, and 554 for the 10-, 13-, 16-, and 19-h treatment, respectively). This was possibly the result of limited available daily photoenergy in the 10-h treatment that restricted the flower development rate. Even though RG-BFT flowered at the 10-h trestment, it did not produce any pods, even when left to grow beyond the HU_{Max} 1550 heat unit cutoff period.

A 10-h daylength is shorter than the daylength time at the equator (600 and 727 min, respectively) (Fig. 4). Therefore, the 10-h light condition is not found in nature at a time of the year when seed production could occur. The inability of RG-BFT to produce pods in limited

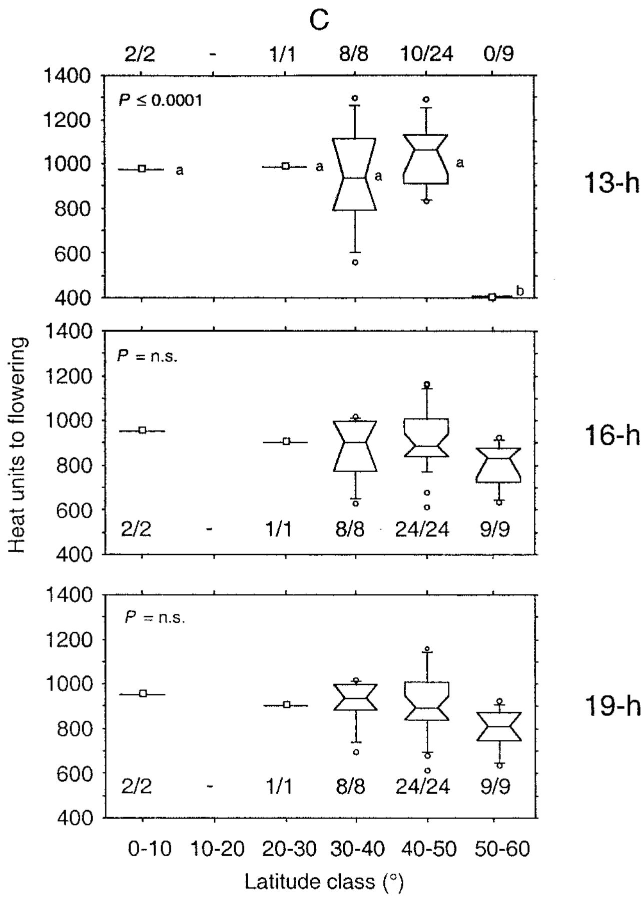

Fig. 6. (A) The flowering response index (FRI); (B) percentage of clones in an accession that flower (POP). (C) Number of heat units to flowering time of accessions that flower (HU) at 13-, 16-, and 19-h photoperiod lengths for 44 wild or naturalized Old World birdsfoot trefoil accessions sorted by six collecting site latitude classes. Notched-box plots labeled with different letters indicate comparisons between latitude classes are different at $P = 0.01$ according to Fisher's least significant difference test. The notches represent the 95% confidence bands. The small square with a horizontal line indicates the mean response. Latitude classes with no box plot have too few accessions within the class for calculation. Also shown are the number of accessions within a latitude class that flowered.

light conditions supported earlier field research that demonstrated birdsfoot trefoil reproduction in materials from higher latitudes was restricted by short photoperiod lengths and produced fewer and more sterile inflorescences than when grown under longer daylength conditions (Joffe, 1958). It appeared that the AG-S4 photoinduction mechanism was inactivated by the mutating irradiation that imparted photoperiod insensitivity in RG-BFT. Mutating radiation has been shown to cause simple genetic changes in other species such as rapid flowering, photoperiod insensitivity, and short plant stature (Sigurbjornsson, 1983). The photoinduction mechanism among accessions may be sensitive to different photoperiod lengths, but appears to be unaffected by collecting site latitude as shown by the absence of any differences among accessions when classified by latitude classes (Fig. 6C).

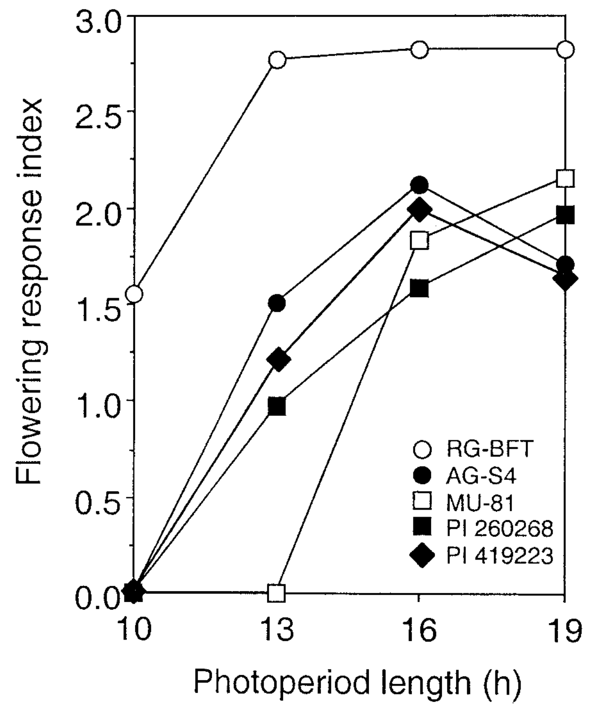

Fig. 7. The flowering response index for RG-BFT, AG-S4, MU-81, and PI 260268 birdsfoot trefoil accessions grown at 10-, 13-, 16-, and 19-h photoperiod lengths.

CONCLUSIONS

The birdsfoot trefoil accessions that originated from habitats ranging from 7 to 60° N latitude exhibited a wide range of flowering responses to 10-, 13-, 16-, and 19-h photoperiod lengths. As photoperiod length increased (13 to 16 to 19 h), the distribution of FRI values became more normally distributed and thus flowering intensity was enhanced as photoperiod length increased. Photoperiod lengths equal to 16 h were too long to differentiate differences among accessions, and thus should not be used as a selection criterion for enhancing flowering in germplasm that is to be utilized at low latitudes. Under 13-h photoperiod length conditions and increasing collecting site latitude, the FRI and percentage of clones in an accession that flowered decreased. These results demonstrated that when selecting genotypes to flower and reseed naturally in pastures at low latitudes, knowledge about the flowering response of the source materials should be considered. If source materials originate from natural high latitude populations, then flowering will be reduced in shorter day length environments as found at low latitudes. Accessions were identified (e.g., PI 304067, PI 304523, and PI 315082) that had high rates of flowering at shorter photoperiod lengths that should be suitable for use in breeding programs to enhance cultivar reseeding capability. Also, autogamous accessions with high flowering percentages in short photoperiod lengths were identified that have potential as germplasm sources to develop breeding materials that may not need pollinators for reseeding when grown under grazed conditions (i.e., PI 260268 and PI 273937). The FRI was also shown to be a useful tool for describing the flowering response of birdsfoot trefoil, and should be suitable for use with other photoperiod sensitive species.

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REFERENCES

Beuselinck, P.R., and W.F. Grant. 1995. Birdsfoot trefoil. p. 237–248. *In* R.F Barnes et al. (ed.) Forage, Vol. 1. An introduction to grassland agriculture, Iowa State Univ. Press, Ames.

Beuselinck, P.R., and R.L. McGraw. 1988. Indeterminate flowering and reproductive success in birdsfoot trefoil. Crop Sci. 28:842–844.

Beuselinck, P.R., and J.J. Steiner. 1992. A proposed framework for identifying, quantifying, and utilizing plant germplasm resources. Field Crops Res. 29:261–272.

Chang, A.T.C., J.L. Foster, D.K. Hall, H.W. Powell, and Y.L. Chien. 1990. Nibus-7 SMMR derived global monthly snow cover and snow depth data set for October 1978 through August 1987. *In* Global ecosystems database, Version 1.0: Disc B, documentation manual. Key to geophysical records documentation no. 27. USDOC/NOAA National Geophysical Data Center, Boulder, CO.

Joffe, A. 1958. The effect of photoperiod and temperature on the growth and flowering of birdsfoot trefoil, *Lotus corniculatus* L. S. Africa J. Agric. Sci. 1:435–450.

Kinemann, J.J., and M.A. Ohrenschall. 1992. Global ecosystems data base, Version 1.0: disc A, documentation manual. Key to geophysical records documentation no. 27. USDOC/NOAA National Geophysical Data Center, Boulder, CO.

Kinemann, J.J., and M.A. Ohrenschall. 1994. Global ecosystems database, Version 1.0: disc B, beta test. USDOC/NOAA National Geophysical Data Center, Boulder, CO.

Leemans, R., and W.P. Cramer. 1992. The IIASA database for mean monthly values of temperature, precipitation, and cloudiness on a global terrestrial grid. *In* Global ecosystems database, Version 1.0: disc A, documentation manual. Key to geophysical records documentation no. 27. USDOC/NOAA National Geophysical Data Center, Boulder, CO.

Li, Q., and M.J. Hill. 1988. An examination of different shoot age groups and their contribution to the protracted flowering pattern in birdsfoot trefoil (*Lotus corniculatus* L.). J. Appl. Seed Prod. 6: 54–62.

McGraw, R.L., P.R. Beuselinck, and R.R. Smith. 1986. Effect of latitude on genotype × environment interactions for seed yield in birdsfoot trefoil. Crop Sci. 26:603–605.

McKee, G.W. 1963. Influence of daylength on flowering and plant distribution in birdsfoot trefoil. Crop Sci. 3:205–208.

Manly, B.F.J. 1986. Multivariate statistical methods, a primer. Chapman and Hall, London.

Sigurbjornsson, B. 1983. Induced mutations. p. 153–176. *In* D.R. Wood (ed.) Crop breeding. ASA and CSSA, Madison, WI.

Smithsonian Institution. 1963. Smithsonian meteorological tables. 6th ed. Duration of sunlight, Table 171. Smithsonian miscellaneous collections, 114. Washington, DC.

Snedecor, G.W., and W.G. Cochran. 1980. Statistical methods. Iowa State Univ. Press, Ames, IA.

Sokal, R.R., and R.J. Rohlf. 1981. Biometry, 2nd ed. W.H. Freeman and Co., San Fransisco.

Steiner, J.J. 1993. Registration of AG-S4 autogamous broad-leaf birdsfoot trefoil germplasm. Crop Sci. 33:1424–1425.

Steiner, J.J. 1999. Birdsfoot trefoil origins and germplasm diversity. p. 81–96. *In* P.R. Beuselinck (ed.) Trefoil: The science and technology of *Lotus*. ASA-CSSA Spec. Publ. 28. Madison, WI.

Steiner, J.J., and P.R. Beuselinck. 2001. Registration of RG-BFT photoperiod insensitive and rapid-flowering autogamous birdsfoot trefoil genetic stock. Crop Sci. 41:607–608.

Steiner, J.J., P.R. Beuselinck, S.L. Greene, J.A. Kamm, J.H. Kirkbride, and C.A. Roberts. 2001. A description and interpretation of the NPGS birdsfoot trefoil core subset. Crop Sci. 41:1968–1980.

Steiner, J.J., and G. Garcia de los Santos. 2001. Adaptive ecology of *Lotus corniculatus* L. genotypes: I. Plant morphology and RAPD marker characterization. Crop Sci. 41:552–563.

Steiner, J.J., and S.L. Greene. 1996. Proposed ecological descriptors and their utility for plant germplasm collections. Crop Sci. 36: 439–451.

Steiner, J.J., and C.J. Poklemba. 1994. *Lotus corniculatus* classification by seed globulin polypeptides and relationship to accession pedigrees and geographic origin. Crop Sci. 34:255–264.

Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58:236–244.