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FLEXIBILITY IN CYANOGENIC PHENOTYPE OF *LOTUS CORNICULATUS* L. IN RESPONSE TO LOW FLUCTUATING TEMPERATURES*

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Abstract

Synthesis of linamarase by approximately half the + – progeny of the cross A/13xC/27 was observed by exposing them to winter conditions in the field or in controlled environments suggesting that there was temperature-sensitive regulation of enzyme biosynthesis in these progeny. The appearance of linamarase-producing progeny in some of the crosses was justified because linamarase was detected in their parents under field conditions. The cyanoglucoside-producing progeny in some other crosses, however, could not be explained because none of the parents involved were observed to produce cyanoglucoside in the field, in the glasshouse or under any other conditions to which they were subjected. Genotype x environment interaction was shown to determine the phenotypic expression of individual plants of *L. corniculatus.* Low fluctuating temperature was believed to be one of the possible factors in the environment.

Introduction

Armstrong *et al.* (1912, 1913) were the first to investigate cyanogenesis in *Lotus corniculatus* L. (bird's foot trefoil). Cyanogenesis in this plant is the release of free HCN that occurs when two cyanoglucosides, linamarin and lotaustralin are hydrolysed by the corresponding enzyme, linamarase (a β-glucosidase) in the damaged leaves of the plant. They found that the plants of this species varied in their HCN concentration. They attempted to correlate the variability of cyanoglucosides and enzyme content with temperature and soil conditions. Neither were found to be an adequate explanation for the variation and they suggested subsequently (Armstrong *et al.* 1913) that there could be other factors concerned with the production of cyanoglucosides and enzyme. Dawson (1941) revealed that leaf cyanoglucoside production in *L. corniculatus* was determined by a dominant allele *Ac* inherited tetrasomically and Bansal (1966) showed that the production of linamarase is controlled by a dominant allele *Li* with some form of tetrasomic inheritance.

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Ellis *et al.* (1977) noted a considerable variation in the expression of cyanogenic phenotype in individual plants of *L. corniculatus* from an Anglesey (Wales) population grown under glasshouse conditions. They attributed this phenotypic variation to the temperature, because regression of the proportion of cyanoglucosidic plants against mean monthly minimum temperature and the percentage expression of the linamarase containing plants against mean monthly maximum temperature were both significant. In other words, at low temperatures some plants reduce cyanoglucoside content and at high temperature more plants gain enzyme activity. The present study was undertaken to measure the phenotypic stability in response to low temperature both of the parent plants used by the senior author in a breeding programme (Ramnani, 1979) and of their hybrid progenies.

Materials and Methods

The materials used for the breeding programme were plants A/6, A/8, A/13, A/16, A/36, A/38, C/23, C/27, C/32, D/8, and E/6, all obtained by Ellis *et al.* (1977) from Porthdafarch Anglesey. These were chosen because they were found to be consistantly stable *or* unstable in their phenotypic expression under all the conditions to which they had been subjected previously (Ellis *et al.* 1977). To examine them further for phenotypic stability or instability all the plants involved in the crosses accomplished in the summer of 1977 and 1978, together with those used for selfing, were regularly tested for cyanogenesis. These tests were carried out at one-monthly intervals, from the date of the establishment of the plants in the experimental field until December each year. The methods of testing leaves for cyanogenesis have been given in detail elsewhere (Jones, 1966).

A very useful characteristic of *L. corniculatus* is the ease with which the plant can be propagated from stem cuttings to give any number of ramets. Not only does this permit the same genotype to be subjected to an array of experimental treatments, but also enables replication of genotypes in each treatment.

The effect of temperature can be assessed by measuring seasonal variation in the expression of cyanogenic phenotypes of parent plants and their hybrid progenies growing in the field, in the glasshouse, and by growing the same in controlled environments.

Field testing

All the parent plants involved in the crosses made in 1977 and 1978 were allowed to remain in the experimental field until December each year and were tested monthly for cyanogenesis throughout that period.

Glasshouse testing

To determine whether the instability in phenotypic expression, observed in the parent plants, was due to the low temperature, or to boron deficient soil, replicate clones were removed from the experimental field, one replicate being potted in the soil in which it had been growing and the other in John Innes compost No. 2. These plants were placed in the warm glasshouse (minimum temperature 15°C). After 30 days they were tested for cyanogenesis.

Testing in controlled environments

Cuttings of plants A/6, A/8, A/13, A/36, A/38, C/23, C/27, C/32, D/8, and E/6 were propagated in the rooting medium of peat and sand (1:1 ratio) and were potted in (7.6 cm) pots after 6 weeks. They were then grown under MBFR/U lights (day length 16 hours) in a warm glasshouse for a further period of 4 weeks and were finally hardened off in a cooler glasshouse before being transferred to the controlled environment cabinet. The plants were arranged randomly Various day-air, night-air and soil temperatures and day length regimes were used. The details are shown in Table 3. Relative humidity was held constant at 80 per cent and the photon flux density from fluorescent lamps was 150-250μ moles $M^{-2}s^{-1}$. These plants were tested for cyanogenesis after different time periods as shown in Table 3.

Assessment of phenotypic stability of the hybrid progenies

Phenotypic stability of the progenies of the crosses, A/38 x A/8, A/38 x C/23 and C/27 x A/13 was assessed by testing the plants in the cooler half of the glasshouse (minimum temperature 7°C). The progenies of the crosses A/38 x E/6, C/27 x A/36, C/27 x A/6 and A/13 x C/27 were assessed by testing them in the cold frames and in addition by growing the progeny of the cross A/13 x C/27 in controlled environment.

Results

Almost all ramets belonging to the clones A/8, A/36, C/23 and C/32 were observed to be stable for the phenotypes expressed under warm glasshouse conditions throughout the period they remained in the experimental field, whereas the ramets belonging to the clones A/6, A/13, A/16, A/38, C/27, D/8 and E/6 synthesized linamarase and were hence regarded as unstable (Table 1).

One hundred per cent phenotypic instability was seen in the ramets belonging to the clones A/13, A/16 and E/6 while the instability percentage amongst the ramets belonging to the clones A/6, A/38, C/27 and D/8 was 41.66, 56.41, 19.0 and 68.75 respectively (Table 1).

Table 1. Effect of different environmental conditions on the phenotypic expression within the clones used as parent plants.

Clone No.	No. of ramets used	Phenotype in warm glass-house	Phenotype in in the field	Nos. of each phenotype	Percentage of unstable ramets
A/6	24	+–	+–	14	41.66
			–+	10	
A/8	17	++	++	17	00.00
A/13	17	+–	++	17	100.00
A/16	5	––	–+	5	100.00
A/36	17	++	++	17	00.00
A/38	39	––	––	17	56.41
			–+	22	
C/23	17	++	++	17	00.00
C/27	37	––	––	30	19.00
			–+	7	
C/32	17	–+	–+	17	00.00
D/8	16	+–	+–	5	68.75
			++	11	
E/6	16	+–	++	16	100.00

Because the instability was not detected until November in each year it appeared that a fall in temperature was probably one of the factors in the environment influencing this phenotypic expression. On the other hand Maevskaya, Troitskaya and Yakovleva (1976) have evidence that β-glucosidase synthesis can be stimulated in leguminous plants when they are grown in boron-deficient soils. There is independent evidence that boron deficiency can have serious deleterious effects on the physiology of *L. corniculatus* (MacQuarrie *et al.* 1983). To check on the possible effect of boron the following experiment was performed.

Two ramets per clone were removed from the experimental field, one replicate being potted in the soil in which it had been growing and the other in John Innes No. 2 compost. These ramets were placed in the warm glasshouse (minimum temperature 15°C). After 30 days linamarase was detected in none of the ramets. The plants were then returned to their former position in the field while still in pots. Daily testing for linamarase revealed that the enzyme was detectable after 3 days. It is clear therefore that the soil was having no observable effect on the synthesis of linamarase and so an

effect of boron deficiency was ruled out. There is from this experiment, however, further circumstantial evidence that temperature is influencing the synthesis of the linamarase, because the mean temperature in the glasshouse was 12°C higher than that of the experimental field. The results of this experiment are shown in Table 2.

Table 2. Effect of soil on the phenotypic expression of the clones used as parent plants.

Clone No.	Phenotype in warm glass-house	Phenotype in the field	Phenotype in warm glasshouse	
			Field soil	John Innes No. 2 compost
A/6	+–	+–⇆–+	+–	+–
A/13	+–	+–⇆++	+–	+–
A/16	––	–+	––	––
A/38	––	–+	––	––
C/27	––	–+	––	––
D/8	+–	++	+–	+–
E/6	+–	++	+–	+–

Controlled environment experiments

Experiments were devised to determine whether changes in temperature would affect the synthesis of linamarase. Plants were propagated from the clones of the parent plants and were grown in the controlled environment cabinet. The ramets were tested for cyanogenesis after prescribed time periods. The various day-air, night-air and soil-temperatures used, together with different day-lengths and the duration of the experiments are shown in Table 3. The various day lengths and temperatures used in these experiments correspond to those prevailing during November to February 1977-78 and 1978-79 at the Botanic Gardens at Hull (England). Because within clone variation in stability had been detected amongst the parent plants in the experimental field all the ramets involved in these experiments were given serial numbers so that a particular ramet showing phenotypic stability or instability could be identified.

Ellis (personal communication, 1976) found that her plants showed the symptoms of physiological drought rather than of frosting when subjected to a temperature lower than –3°C and she concluded that the water availability to the plants is reduced at that temperature. For this reason under-soil heating was used for some of the experiments described below, although it was considered unnecessary when temperatures higher than 0°C were used.

Ramets of clones D/8 and E/6 were not found to show phenotypic instability at temperatures between 0°C and 5°C (Table 3), whereas 2 out of 7 ramets of clone D/8 and 4 out of 7 ramets of clone E/6 respectively were observed to show instability in phenotypic expression at temperatures –5°C to 5°C. Temperatures of –5°C to 5°C had no effect on the phenotypic expression of the ramets of the clones C/27 and A/38 whereas at temperatures as high as 2.5°C to 10.5°C and 2.5°C to 13°C ramets of clones A/6, A/38, and C/27 were observed to be unstable. Clearly not all the unstable clones nor all the ramets of the same clone express their phenotypic instability at the same temperature. In other words different unstable clones express their phenotypic instability at different temperatures. However, when the temperature is lowered there is general tendency for these plants to synthesize linamarase. On the other hand, clones A/8, A/36, C/23 and C/32 and A/13 were observed to be stable in their phenotypic

Table 3. Effect of temperature on phenotypic expression within clones in the controlled environment experiment

				Temperature						
Clone No.	No. of ramets used	Serial No. of ramets	Phenotype in warm glasshouse	Day air	Night air	Soil	Day length	Duration of experiment	Phenotype after treatment	Nos. of each phenotype
D/8	7	1,2,3,6,13, 14, 17.	+–	5°C	0°C	5°C	8 hrs.	5 days	+–	all
E/6	7	1,3,5,7,9, 10,11	+–						+–	all
D/8	7	5,8,11,15, 18,19,20	+–	5°C	–5°C	5°C	8 hrs.	5 days	+–	5
									++	2 (5,8)
E/6	7	2,4,6,8,12, 13,14	+–						+–	3
									++	4 (4,6,8,12)
D/8	5	3,6,8,19,23	+–	5°C	–5°C	2°C	9 hrs.	8 days	+–	all
A/38	2	13,19	––						––	
C/27	2	4,7	––						––	
D/8	5	7,9,10,12,16	+–	5°C	–5°C	5°C	9 hrs.	6 days	+–	all
C/27	4	17,19,20,22	––						––	all
A/38	4	13,18,20,21	––						––	all
A/8	4	11,12,17,20	+–						++	all
C/23	4	2,10,14,18	+–						++	all
A/36	4	12,14,16,20	++						++	all
C/27	4	14,15,17,18	––	7.5°C	2.5°C	-	9 hrs.	8 days	––	all
A/13	5	1,2,3,5,6,	+–						+–	all
C/27	2	17,20	––	10.5°C	2.5°C	-	7 hrs	7 days	––	!
									–+	1 (17)
A/13	1	3	+–						+–	
A/6	1	5	+–						––	
C/32	1	1	–+						–+	
A/6	1	8	+–	13.0°C	2.5°C	-	7 hrs.	7 days	–+	
C/32	1	1	–+						–+	
D/8	1	14	+–						++	
C/27	1	20	––						–+	
A/38	1	13	––						–+	

expression at all the temperatures used in these experiments (Table 3). It will be noted that only a small number of ramets were used for the last two experiments. The reason for this is that these experiments were primarily designed to study the effect of temperature on the plants of hybrid progenies and the parent plants were included only for comparison. Anticipating the results of the experiments on the progenies, the conclusion is that the phenotypic instability is not related to absolute temperature as such, but rather to a fluctuating low temperature, in this case between 2.5°C to 10.5°C and 2.5°C to 13°C.

To estimate the effects of physiological drought on the phenotypic expression, the ramets of the clones A/38, C/27 and D/8 were divided into two groups, each consisting of 6 ramets (one ramet of clone A/38, one ramet of C/27 and 4 ramets of clone D/8). Each ramet was in a separate pot. One group of these ramets was placed on the surface of the soil with no under-soil heat whereas the other was put into the soil (with the soil surface level with the top of the pot) with under-soil heating applied. Testing the ramets of both groups after a period of 7 days in controlled environments, where day-air, night-air, and soil-temperatures, day length and relative humidity were set at 5°C, –5°C, 2°C, 9 hours and 80% respectively, showed that the phenotypes were the same as those expressed under warm glasshouse conditions. These ramets were subjected to 15 hours a day frosting for a continuous period of 7 days and showed no change in their phenotypic expression. It clearly suggests that physiological drought has no influence on the phenotypic expression of the plants used in this experiment.

Assessment of phenotypic stability of the hybrid progenies

The variation in phenotypic expression observed within the clones of the parent plants A/6, A/38, C/27, D/8 and E/6 under experimental field conditions and in controlled environments seems to suggest that the phenotypic expression is determined by genotype x environment interaction, probably stimulated by low fluctuating temperature.

To test this hypothesis 133 plants from the progenies of the crosses A/38 x A/8, A/38 x C/23 and C/27 x A/13 were moved at the beginning of February 1978 from the warmer half (minimum temperature 15°C) into the cooler half of the glasshouse in which the mean minimum temperature during February, March and April 1978 was 7.5°C, 8°C and 9.5°C respectively. These plants consisted of 28 containing the cyanoglucosides and linamarase (++), 40 plants containing only the cyanoglucosides (+–), 30 plants with linamarase (–+) and 35 plants negative for both cyanoglucoside and linamarase (––). Testing these plants after 3 months (at the end of April 1978) showed that all the plants belonging to the ++ and –– phenotypic classes remained stable for the phenotypes expressed under warm glasshouse conditions (Table 4) whereas an average of 49.7 per cent of the plants of the +– and –+ phenotypic classes showed instability

Table 4. Effect of temperature on the phenotypic expression in the hybrid progenies in the cooler half of the glasshouse

Cross	No. of progeny	Phenotype in warm half of glasshouse	Nos. of each phenotype	Phenotype in cooler half of glasshouse	Nos. of each phenotype	Percentage of unstable progeny
A/38 x A/8	45	++	16	++	16	46.6
		+–	10	+–	1	
				++	2	
				––	7	
		–+	12	––	12	
		––	7	––	7	
A/38xC/23	59	++	12	++	12	54.23
		+–	16	++	5	
				+–	1	
				–+	2	
				––	8	
		––	18	–+	1	
				––	17	
		––	13	––	13	
C/27 x A/13	29	––	14	––	14	48.27
		––	15	––	15	

of their phenotypic expression, probably in response to low temperature. In general, there was loss of ability to produce either the cyanoglucosides or linamarase in the cooler glasshouse.

Another 30 plants positive for both the cyanoglucosides and linamarase (++), 85 plants positive for only the cyanoglucoside (+–), 18 plants positive for only the linamarase (–+) and 99 plants negative for both the cyanoglucoside and the linamarase (––) from the progenies of the crosses A/38 x E/6, C/27 x A/36, C/27 x A/6 and A/13 x C/27, were moved from a warmer glasshouse minimum temperature 15°C) into cold frames at different times during January, February, March and April 1979. The recorded mean temperature outside the frames during January, February, March and April 1979 was –2°C, 0.5^{o}C and 1.5^{o}C respectively. Testing these plants after different periods of time (Table 5) showed that again almost all plants of the ++ phenotypic class and 96 per cent of the –– phenotypic class remained stable (Table 5) for the phenotypes expressed under the warm glasshouse conditions, whereas instability of phenotypic expression was observed in the plants of the +– and –+ phenotypes. The percentage of the plants showing unstable phenotypes in each progeny is shown in Table 5. As high a proportion as 39.72 per cent of unstable plants was recorded in the progeny of the cross A/13 x C/27; the majority of these unstable plants belonged to the +– phenotypic class and they synthesized linamarase and lost the cyanoglucoside.

Table 5. Effect of temperature on the phenotypic expression in the hybrid progenies in cold frames

Cross	No of progeny	Pheenotype in warm glasshouse	Nos. of each phenotype	Phenotype in cold frames	Nos. of Each phenotype	Period in cold frames	Percentage of unstable progeny
A/38 x E/6	79	++	20	++	20	14 days (21 Feb - 7 Mar 1978)	11.25
		+–	11	+–	7		
				++	2		
				––	2		
		–+	3	–+	2		
				++	1		
		––	45	––	42		
				–+	3		
C/27 x A/36	40	++	8	++	8	27 days (23 Mar - 27 Apr 1978)	17.5
		+–	12	+–	9		
				++	1		
				–+	1		
				––	1		
		–+	13	–+	9		
				––	4		
		––	7	––	7		
C/27 x A/6	40	++	2	++	2	23 days (28 Feb - 23 Mar 1978)	7.5
		+–	22	+–	21		
				++	1		
		–+	2	–+	2		
		––	14	––	14		
A/13 x C/27	73	+–	40	+–	12	23 days (1–24 Jan 1979)	39.72
				++	25		
				––	3		
		––	33	––	32		
				+–	1		

N.B. Most of the 33 –– progeny of A/13 x C/27 were very small, and after the original test for cyanogenesis in the glasshouse there was insufficient material left with which to carry out satisfactory further tests for cyanogenesis. These data are included in the table, however because there was one plant in which a phenotypic change was detected.

In addition, 32 plants positive for only the cyanoglucoside (+–) including those which had been observed to synthesize linamarase under cold frame conditions, and 30 plants negative for both the cyanoglucoside and the β-glucosidase (––) from the progeny of the cross A/13 x C/27 were grown at different times in controlled environments (Table 6). Not one of the 25 –– plants showed instability in their phenotypic expression when subjected to an environment in which day-air, night-air and soil-temperature, day length and the duration of the experiment were 5°C, –5°C, 2°C, 9 hours and 7 days respectively (Table 6); but 2 of these were found to be unstable when day-air, night-air temperature, day-length and duration of the experiment were set at 10^{C}, 2.5^{C}, 9

Table 6. Effect of temperature on the phenotypic expression of the progeny of the cross of A/13 x C/27 under controlled environmental conditions

No. of progeny	Serial nos. of progeny	Phenotype in warm glasshouse	Temperature: Day air	Temperature: Night air	Temperature: Soil	Day length	Duration of experiment	Phenotype after treatment	Nos. of each phenotype
25	2,3,7,10,11,12,13, 16,18,27,30,41,42, 43,46,48,58,60,66, 67,69,71,72,73,75.	––	5°C	–5°C	2°C	9 hrs	7 days	––	all
			5°C	0°C	-	9 hrs.	8 days		
12.	44,45,49,53,54,56, 61,62,63,64,65,68	+–						+–	all
			7.5°C	2.5°C	-	9 hrs.	8 days		
7	44,45,54,62,63,64, 68	+–						+–	all
2	41,73	––						––	
			10°C	2.5°C	-	9 hrs.	8 days		
12	42,43,46,48,58,60, 66,67,69,71,72,75	––						–– –+	10 2 (43, 60)
23	8,17,20,21,25,26, 28,29,31,33,36,44, 45,47,49,52,53,62, 63,64,68,70,77	+–						+– ++	22 1 (49)
			13°C	2.5°	-	9 hrs	8 days		
16	15,41,42,43,48, 58,65,67,69,71, 72,73,75,T-3(2) T-3(4), T-3 (7)	––						–– –+ +–	9 6 (43,48,67, 69,T-3 (2), T-3 (7). 1 (15)
28	8,17,20,21,25,26, 28,29,31,33,36,37, 44,49,53,54,56,61, 62,63,64,68,70,77, T-3 (1), T-3 (3), T-3 (9), T-3 (10)	+–						+– ++	11 17 (8,17,20, 25,26,33,63, 44,49,53,56, 62,64,T-3 (1) T-3 (3), T–3 (9), T-3 (10).

hours and 8 days respectively. Furthermore, 6 out of 16 plants of –– phenotypic class were observed to gain linamarase and one gained the cyanoglucoside when day-air temperature was raised as high as 13°C, while the rest of the conditions were not changed. Under these latter environmental conditions 17 out of 28 plants of +– phenotypic class were observed to synthesize linamarase and these were the same plants which had been observed to synthesize the linamarase under cold frame conditions during the month of January 1979. It was seen that different individuals of the same phenotypic class reveal their instability under different environmental conditions and it therefore suggests that the phenotypic expression is determined by genotype x environment interaction, the environmental effect probably being the fluctuating low temperature.

Discussion

The clones used in the breeding programme were chosen because they had stable phenotypes under all the conditions to which they had been subjected previously or showed a pattern of instability that was repeatable. Nevertheless it was considered prudent to test all the replicates of the clones used in the breeding programme throughout the period they remained in the field beginning from April. As a result of this regular testing for cyanogenesis it was discovered that some of the original stable clones remained stable whereas others did not. That these unstable clones gained the ability to synthesize linamarase was first discovered in November 1977. Because a change in phenotypic expression in these clones was not detected until late autumn it is believed that a fall in temperature was one of the principal factors in the environment influencing the phenotypic expression. It appears that a new type of phenotypic flexibility in *L. corniculatus* has now been described where plants can gain the ability to synthesize linamarase and/or cyanoglucosides when the temperature is lowered rather than losing this ability. Adjustment in the rate of synthesis of cyanoglucosides at different stages of the life-cycle has also been reported in some other cyanogenic plants, e.g. *Sorghum sudanese* (Piper) Stapf (Hogg and Ahlgren, 1943), *Zea mays* L. (Nass, 1972), *Trifolium repens* L. (de Waal, 1942, Till , 1983), *Linum usitatissimum* L. (Trione, 1960), *Tinantia erecta* Schlecht, *Bambusa bambos* (L.) Voss., *Dendrocalamus latiflorus* Munro (Tjon Sie Fat, 1978a, b), *Trochodendron aralioides* Sieb. and Zucc. (van Valen, 1978), *Tetragonolobus purpureus* Moench, *T. requienii* (Mauri ex Sanguinetti) Sanguinetti, *Ornithopus compressus* L. *O. roseus* Dufour (van Valen, 1979), *Juncus articulatus* L., *J. acutiflorus* Ehrk, *J. alpino-articulatus* Chaix ex Vill, s.l., *J. subnodulous* Schrank (Zandee, 1976), *Molinia caerulea* (L.) Moench (Tjon Sie Fat and van Valen, 1978), *Dactylotenium radulans* P. Beauv., *Campanula rotundifolia* L., *Glyceria fluitans* (L.) R.Dr. (Tjon Sie Fat, 1977a,b), *Ranunculus repens* L. (Tjon Sie Fat, 1979, *Sorghastrum nutans* (L.) Nash and *S. pellitum* Parodi (Haskins *et al.* 1979). The HCN concentration in *S. nutans* and *S. pellitum* was shown to vary when the same plant was tested for cyanogenesis at different times of the same day. HCN-content in cyanogenic species of *Lotus* has also been reported to vary with developmental stage and season (Borsos, 1977). Ellis *et al.* (1977) began a similar kind of study on *L. corniculatus.*

Increased enzyme activity has also been found in other leguminous plants. Krasnuk *et al.* (1978) demonstrated that activities of amylases and leucine-amino peptidases were greater under simulated winter conditions than under summer conditions in both the cold-tolerant variety 'Vernal' and the cold sensitive variety 'Sonora' of alfalfa (*Medicago sativa*). They also detected new esterase forms and greater quantities of a heat-stable amylase in both varieties under winter conditions. Nicholas *et al.* (1976) found an increased activity of nitrate reductase in the leaves of field grown soybeans (*Glycine max* (L.) Merr) under cooler night temperatures ($16^{o}C$). Rose (1967) believes

that in an inducible enzyme system temperature can influence enzyme synthesis by changing the effective concentration of a specific repressor. Synthesis of linamarase in the progeny of the cross A/13 x C/27 at low temperature suggests that it may be possible that the product of the regulatory gene synthesized at low temperature may act as an inducer of linamarase. As far as we know an increase in the activity of linamarase at low temperature has not been reported prior to this study.

The variation in phenotypic expression observed between the clones of the parent plants in the field and in the controlled environments suggested that the phenotypic expression of *L. corniculatus* from Porthdafarch is determined by genotype x environment interaction, probably stimulated by low fluctuating temperature. In our experiments the phenotypic instability was detected only when temperature fluctuated between 2.5°C and 10°C and between 2.5°C and 13°C.

As a consequence of this variation in the synthesis of linamarase and cyanoglucoside cyanogenesis in *L. corniculatus* is influenced by fluctuation in temperature, but also Hogg and Ahlgren (1943) reported a highly significant positive correlation between temperature and the hydrogen-cyanide content of *Sorghum sudanase*. In contrast, Gorashi (1977) reported higher hydrogen-cyanide concentration in this species at lower temperatures. The work with *Lotus corniculatus* shows that temperature also influences cyanogenesis in both directions.

There is also another possibility that linamarase in *L. corniculatus* may have multiple molecular forms with different temperature co-efficients. It may be that at low temperature, some metabolites essential for synthesis of sub-units are formed which may account for an increase in the concentration of a linamarase isoenzyme with low temperature co-efficient. Gerloff *et al.* (1967) associated an increase in the activity of soluble oxidative enzymes and the formation of two new isoenzymes with an increase in the content of soluble proteins in alfalfa roots during hardening.

The ability of some *L. corniculatus* plants to change their cyanogenic phenotypes under certain temperature conditions must be remembered when estimating the frequency of the cyanogenic phenotypes in natural populations of *L. corniculatus*. Because of this phenotypic plasticity comparison between population of *L. corniculatus* sampled at different times of the year could be very misleading. Moreover the variability in the expression of cyanogenesis shows that this character cannot be used as genetic marker, contrary to the suggestions of Bansel (1966) and of Miri and Bubar (1966). Furthermore any correlation between cyanogenesis and other (morphological, physiological and genetical) characteristics in *L. corniculatus* should be interpreted with more than usual caution.

The work of Ellis *et al.* 1977 showed that some *L. corniculatus* plants from Anglesey tended to lose glucoside and enzyme activity at low temperature. In contrast, in the present study some *other* plants from the same population were found to gain glucoside and/or linamarase activity under these conditions.

Another possibility worth exploring is that the linamarase produced at low temperature could be structurally different from the one present in those *L. corniculatus* plants which do not seem to be influenced by low temperature. It would be interesting to purify the enzyme produced at low temperature and elucidate its structure.

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