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DISTRIBUTION OF THE CYANOGENIC PHENOTYPE OF *LOTUS CORNICULATUS* L. IN THE MURREE HILLS, THE JHELUM AND THE SWAT VALLEYS OF NORTHERN PAKISTAN

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Abstract

Sixteen *Lotus corniculatus* populations growing at altitudes ranging from 1036 to 2209 m were sampled for leaf-cyanogenesis. The areas chosen for this purpose were the Murree Hills, the Jhelum and the Swat valleys in the north of Pakistan. No evidence was obtained to suggest that there was any association between the distribution of the cyanogenic phenotype and altitude and hence cold temperature. All the populations sampled seemed to be monomorphic for the presence of both cyanoglucosides and linamarase.

Introduction

Most of the natural populations of both *Trifolium repens* L. and *Lotus corniculatus* L. in Europe are polymorphic for the character of leaf-cyanogenesis. Leaf-cyanogenesis in these two species is the release of free HCN that occurs when two cyanoglucosides, linamarin and lotaustralin are hydrolysed by the corresponding enzyme, linamarase (β-glucosidase) in the damaged leaves of the plants. Dawson (1941) revealed that leaf-cyanogenesis in Scandinavian material of *L. corniculatus* was determined by a dominant allele inherited tetrasomically although it is not clear whether he was studying the inheritance of the cyanoglucosides or of linamarase. Bansal (1966) had evidence that the production of linamarase is controlled by a dominant allele *Li* with some form of tetrasomic inheritance. Ramnani & Jones (1984) have studied the inheritance of both cyanoglucosides and linamarase production in the leaves of British material of *L. corniculatus* and found the situation to be much more complex than previously thought. The genetics of cyanogenesis in *T. repens* is, in essential respects, similar (Atwood & Sullivan, 1943) to that of *L. corniculatus.*

Both biotic and abiotic selective agents appear to be involved in the mechanisms that maintain the polymorphism of cyanogenesis in these species. Daday (1954a, b) suggested temperature as the most important factor, the cyanogenic form of *T. repens* being at disadvantage in winter or at the high altitudes. Evidence for the action of biotic factors comes from selective herbivory by snails, slugs and small mammals, the acyanogenic form of *T. repens* being preferred (Angseesing, 1974). Selective and differential eating has been reported for *L. corniculatus* (Jones, 1966; Ellis *et al.*, 1977; Compton *et al.*, 1983).

The present investigation reports the results of sampling populations of mature plants of *L. corniculatus* growing at various sites in the Murree Hills, the Jhelum and the Swat valleys and the pattern of distribution of the cyanogenic form of this species at these sites is compared with that obtained by Daday for *T. repens* in the European Alps.

Material and Methods

The areas chosen for this investigation involved the Murree Hills (Punjab Province), the Jhelum valley (Azad Jammu and Kashmir) and the Swat valley (North West Frontier Province) of North Pakistan. Samples ranging from 16 to 100 individuals were collected in 10 locations in the Murree Hills, 5 in the Jhelum valley and 1 in the Swat valley. The details of the locations are available from the main library of the Sind University, Khairpur, Sind. The altitudes of the locations surveyed ranged from 1036 to 2209 m. Populations were sampled on different dates as shown in the Table 1.

Table 1. Samples of *Lotus corniculatus* growing in the Murree Hills, the Jhelum and the Swat valleys which have been tested for cyanogenesis.

Location	Date	Phenotype ++	N	Approx. height (m) above sea level
THE MURREE HILLS				
Kenar Kas	8. 5.1985	26	26	1036
Upper Dewil Roadside	8. 5.1985	44	44	1283
Chitta mor	9. 5.1985	53	53	1585
Ghora Gali	17. 8.1984	60	60	1631
Ghora Gali	22.10.1984	47	47	1631
Forest Nursary Boor-Bun	8. 5.1985	47	47	1767
Golf Club, Boor-Bun	8. 5.1985	45	45	1810
Bansara Gali	23. 8.1984	63	63	1834
Bansara Gali	28.10.1984	48	48	1834
Bansara Gali	9. 5.1985	52	52	1834
Sunny Bank	9. 5.1985	40	40	1886
Governor's House Road	21. 8.1984	100	100	2005
Governor's House Road	29.10.1985	48	48	2005
Governor's House Road	8. 5.1985	49	49	2005
Barian	9. 5.1985	16	16	2045
THE JHELUM VALLEY				
Chakothi	10. 5.1985	65	65	1109
Chikar	12. 5.1985	53	53	1554
Loon Bagla Bazzar	12. 5.1985	61	61	1959
Loon Bagla Western Top	12. 5.1985	43	43	2133
Sudden Gali	12. 5.1985	83	83	2209
THE SWAT VALLEY				
Kalam	27. 8.1984	26	26	2073

++ = containing both Glucoside and Enzyme.

Since *L. corniculatus* propagates also vegetatively therefore the distance between two successive sampling points becomes important and there is likelihood of obtaining spurious effects if clone formation in this plant is ignored (Harberd, 1961). Based on the previous examination of cloning ability of *L. corniculatus*, Jones (1963) suggested a distance of 1 m between sampling points for most purposes. This method of sampling was followed because none of the populations sampled showed brown keel tip colour polymorphism (Hart & Wilsie, 1959) being monomorphic for yellow keel petals. This character can be used to distinguish between individual plants growing less than 1 m apart. Populations of *L. corniculatus* were sampled by removing leafy stems from plants growing at least 1 m apart.

Cyanogenic phenotypes were distinguished by using the modified Sodium Picrate Test described by Jones (1977). Tests of plants left for 2 h at ambient room temperature gave satisfactory results. As cyanogenesis results from the interaction between β-glucosidase and cyanoglucosides, linamarin and lotaustralin (Conn, 1979) the presence of both enzyme and substrate is essential for cyanogenesis to occur. As will be seen in the results there was no need to test for the presence of the glucosides in the absence of linamarase, nor for linamarase in the absence of the glucosides.

Results

The frequency of the cyanogenic plants, details of the sample sizes and location altitudes are given in the Table 1. All populations examined were monomorphic for the presence of both cyanoglucosides and β-glucosidase. Re-testing for leaf-cyanogenesis the plants growing at Ghora Gali, Bansara Gali and Governor's House Road in 1984 and 1985 showed that the plants retained a stable phenotype over a period of 11 months.

Discussion

The results of the survey of *L. corniculatus* populations for leaf-cyanogenesis are not consistent with those obtained by Daday (1954b) for *T. repens* who found a decline in the frequency of both cyanoglucoside and β-glucosidase forms in seed samples collected at successively higher locations in the European Alps. His hypothesis that is correlation between the distribution of the cyanogenic forms of *T. repens* and the mean winter temperature was essentially based on his alpine data. Some evidence has been presented by Brighton & Horne (1977) supporting a relationship between the frequency of the cyanogenic phenotype of *L. corniculatus* and altitude, presumably cold temperature, in the Scottish Highlands. On the other hand Jones (1977) finds no evidence of such a relationship in the Scottish Highlands and the French Alps since the locations at which, Brighton & Horne (1977) tested plants are included in his data. Recent results obtained by Compton *et al.*, (1983) from a survey of *L. corniculatus* populations in the Jostedal valley in Norway showed a high frequency of the cyanogenic plants in the

north of the valley where weather conditions are more extreme and the proportion of cyanogenic plants in these populations was positively correlated with altitude. Similar evidence has been reported by Urbanska & Wildi (1975) for both *L. corniculatus* and tetraploid *Lotus alpinus* in the Swiss Alps where the frequency of the cyanogenic diploid *L. alpinus* increased with increasing altitude. Certainly these results are contrary to those found with *T. repens* (Daday, 1954b). Under certain conditions some plants of this species are shown to change their cyanogenic phenotype (de Waal, 1942; Till, 1983), hence, in addition to the possible contamination by cultivars of this species (Jones, 1977) instability of cyanogenic phenotype presents a problem when studying wild populations of *T. repens*. The conclusion drawn by Daday (1954b) is based on only seed samples collected from all over the world and no mention is made of the temperature conditions under which the seeds were grown and the seedlings tested for cyanogenesis. As the plants were grown at the Welsh Plant Breeding Station Aberystwyth there can be little doubt that the seedlings were raised under different environmental conditions from those that the parent plants had been experiencing in their natural habitats. It cannot be assumed, therefore, that the frequency of the cyanogenic form based purely on seed samples, would really represent the true frequency of the cyanogenic form in natural populations where the adult plants have experienced the rigours of establishment and survival.

An explanation for the observation by Daday (1954b) that the acyanogenic forms of *T. repens* are commoner at higher altitudes has been suggested by Jones (1966); that is the distribution of the selective herbivores which preferentially eat acyanogenic *L. corniculatus* and *T. repens* may be controlled by the January mean temperature. If the numbers of these herbivores are high in environments which have higher temperatures then the correlation between glucoside and enzyme frequencies and January mean temperature obtained by Daday (1954a, b) appear to be secondary. Daday (1965) believes that the cyanogenic plants of *T. repens* are at a selective disadvantage in colder areas because low temperature appears to activate the enzyme, there is production of HCN which irreversibly inhibits the respiratory system of the leaves and eventually leads to death. Daday's argument cannot be applied to the *L. corniculatus* populations surveyed in the present study. On the contrary, all the *L. corniculatus* plants tested for cyanogenesis in different populations growing in the Murree Hills, the Jhelum and the Swat valleys were found to be monomorphic for the presence of both leaf-cyanoglucosides and β-glucosidase production irrespective of altitudes. These results can be compared neither with those of Daday (1954b) for *T. repens* and nor with those of Jones (1977) for *L. corniculatus*. Our results suggest that cold temperature does not act as a selective force on the polymorphism of cyanogenesis in *L. corniculatus* in Pakistan.

Another example of essentially the same genetic polymorphism in two closely related organisms responding to the same selection in different ways, is seen in the exceedingly similar snails, *Cepaea nemolaris* and *C. hortensis* (Clarke, 1960). It can

therefore be concluded that although the genetic polymorphism of cyanogenesis appears to be the same in *T. repens* and *L. corniculatus* there is no reason to suppose that distribution of the cyanogenic forms will also be the same in these two species.

Urbanska (1984) has shown higher frequencies of the cyanogenic phenotype of *L. alpinus* growing upon the carbonate soils as compared to those growing upon the acidic silicious soils in the Swiss Alps. Thus other climatic, edaphic and biotic factors need to be examined in the areas involved in the present study to determine what effects they may have on the pattern of distribution of the cyanogenic form.

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