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Local Population Differentiation in *Trifolium alexandinum* L. in Response to Various Disturbance Regimes

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Abstract: Local population differentiation was studied in twenty populations of *Trifolium alexandrinum* L. Ten population were sampled from undisturbed sites while the other ten were collected from a variety of disturbed habitats. Each individual plant was regarded as genet and was analyzed for various biometric traits by taking consistent measurements for all characteristics. The biometric analyses revealed considerable phenotypic variability for a majority of morphological expressions. A significant differentiation between populations was found in response to local habitat conditions. This study suggested that phenotypic differentiation in *T. alxandrinum* was strongly influenced by environmental disturbance. Therefore, disturbance seems to play a major role in the generation of variation. The occurrence of phenotypic modification in response to environment was regarded as phenotypic plasticity and it appeared to be an important feature of adaptation to environmental fluctuation. This study concluded that the persistence of the species in spatially and temporally disturbed habitats presumably depends on the variability of certain morphological expressions.

Key words: Disturbance, biometric traits, plasticity, *Trifolium alexandrinum*

Introduction

Plant populations vary in space and time (Linhart *et al.*, 1981). Morphological differentiation is the reflection of the genetic organization of the individuals of a population and is strongly influenced by various environmental factors (Langlet, 1971). Population differentiation in response to disturbance is an important area of emphasis in ecological research for many years (Bazzaz, 1983; Levin and Paine, 1974).

Disturbance can be defined as an alteration in the environment and it can produce several detectable changes in the morphological expressions of plants (Bazzaz, 1996). Several plant species can maintain their populations despite their growth in disturbed habitats (Hedrick *et al.*, 1976). Although, various chemical, physiological and genetic changes have been reported in response to environmental instability (Brown, 1979; Hamrick, 1983) but biometric characteristics have not been emphasized although, they can readily be identified in plant phenotypes in relation to environmental disturbances (Thompson, 1990).

The species of the genus *Trifolium* belong to the family Fabaceae. The species can grow in a wide variety in habitats and can withstand different degrees of disturbance regimes (Turkington and Harper, 1979). ***Trifolium alexandrinum*** holds considerable botanical and economic importance because it is a main fodder crop of many sub-tropical countries of the world. The species is of great significance in maintaining soil fertility as it can add substantial amount of nitrogen to the soil. Moreover, it is used as green manure and for the reclamation of alkaline and saline soils.

The foremost objective of the study was to reveal stability and maintenance of the species in relation to disturbance so that it can be used as a substantial forage crop for unstable environments. ***T. alexandrinum*** L. Is used as a model species because it possesses variations for a wide array of characters (Burdon, 1980) an local occurring populations from diverse environments provided adequate material for the study. The present work repots biometric character analysis in several population of ***T. alexandrinum***, in relation to local environmental conditions.

Materials and Methods

Field study and collection

Ten populations of ***T. alexandrinum*** were collected from vast irrigated fields and were regarded as undisturbed populations whereas, the other ten were from disturbed habitats.
These sites and disturbance regimes are given below:

Sites	Disturbance
Eclectic power station	Heat and electric current
Brick furnace	Smoke and dust particles.
Sultan Ghee Mills	Effluent of mill containing nickel contents
Multan Tanneries, Multan.	Effluent of tanneries and highly salinized water
Pak Arab Fertilizer Factory, Multan.	SO_2, NH_3 & HNO_3 fumes
Busy road side	Smoke and smog
Beverage factory	Wastewater
Field irrigated with domestic waste water	Sewerage waste
Canal bank	High speed of water
General Bus Stand	Trampling and smoke

All provenances were form Southern Punjab, in a range of about 100 km from Multan, Pakistan (32° 20' N 71°'82 E). Ten plants, each regarded as genet, were collected 20-25 paces apart from all populations, The plants were uprooted carefully, excessive soil was removed by shaking, put in labeled paper bags and were analyzed for biometric traits in the laboratory.

Biometric characters analysis

Detail of characters and measurements are as follow:

Character	Measurement detail
Inflorescence length (cm)	Tip to the base of the top most inflorescence.

Leaflet length (cm)	Middle leaflet of a trifoliate leaf was measured from its point of attachment to the petiole to the tip.
Leaflet width (cm)	The maximum width of the leaflet
Petiole length. (cm)	From its point of attachment to the base of the leaflet.
Internodal distance (cm)	Distance between two nodes.
Stolon length (cm)	From one end to another end of a stolon from a fully-grown genet.
Oven dried biomass of shoot (g)	Shoot samples were dried at 90°C of 72 hours in an oven. Weight measurements were taken using an electronic balance (Chyo Balance Corporation).
Oven dried biomass of root (g)	Measurements were taken as described above.

Statistical analysis

Mean values for each parameter were calculated then the data for all parameters were subjected to statistical analysis. In order to elucidate differences between populations and habitats, a two way analysis of variance (ANOVA) was carried out using MS Excel, 2000. Duncan's Multiple Range Test (DMRT) was used to calculate least significant differences between character means.

Results

Inflorescence length

The results as mean values for inflorescence length (Fig. 1) depicted that populations from undisturbed habitats showed higher values for inflorescence length except populations No. 1,7 & 8. On the other hand, populations from disturbed sites exhibited lower means for this parameter (Table 1). However, it is evident from (Table 2) that population from varied habitats did not differ significantly, similarly, analysis of variance could not reveal significant differences between habitats as well as significant interaction is not evident (Table 2).

Leaflet length an width

It is obvious from Fig. 1 That populations from undisturbed habitats have longer leaflets except population No. 8 and 9 which had lower mean values for this trait. Reduction in leaflet length was observed in all populations from disturbed habitats (Table 1) but the maximum reduction was observed in the brick furnace population here, the lowest mean (1.48 cm) was recorded. Analysis of variance Table 2 revealed that leaflet length was significantly influenced by disturbance regimes. Marked differences between habitats were observed and a highly significant interaction is also evident (Table 2).

Leaflet width was consistently higher (3.56 - 6.18 mm) in all populations which were collected from undisturbed habitats while, populations from disturbed sites exhibited lower means. Thus, an overall decrease in leaflet width was observed under various disturbance regimes (Table 1). It became clear from Table 2 that disturbed environments had significantly reduced the leaflet

Table 1: Overall mean values for various biometric characters in populations of ***Triflium alexandrinum*** collected from diverse habitats during early summer, 2001

Characters	Undisturbed	Disturbed
Inflorescence length	1.50	1.43
Leaflet length	1.85	1.59
Leaflet width	4.29	3.20
Petiole length	1.56	1.14
Internodal length	6.71	5.75
Stolon length	2.98	2.15
Oven dried weight of shoot	1.87	3.20
Oven dried weight of root	0.31	0.29

Each mean value is across ten populations and n number of genet each replicated five times (n =10)

Table 2: Summary of analysis of variance for various biometric characters in populations of ***Trifoim alexandrinum*** collected from diverse habitats during early summer, 2001

Characters	MS_p	Significance F	Ms_h	Significance F	MS_I	Significance F
Inflorescence length	17.48	N.S	27.37	2.18 N.S	21.8	1.73 N.S
Leaflet length	19.0	2.67**	349.53	49.13***	26.19	3.68***
Leaflet width	2.22	3.65**	59.10	97.36***	3.28	6.29***
Petiole length	57.15	2.41**	119.97	5.07*	65.02	2.74**
Internode length	2681.21	14.03***	4086.9	25.16***	2564.7	13.42***
Stolon length	546.62	2.82**	2.2	0.01 N.S	439.38	2.54**
Oven dried (w) of shoot	53.42	1.38 N.S	89.94	2.32 N.S	31.22	0.8 N.S
Oven dried (w) of root	0.07	1.27 N.S	0.005	0.83 N.S	0.11	1.84 N.S

Ms_p, mean squares of differences between populations, Ms_h mean squares of differences between habitats, MSI, mean squares of interaction, **,P < 0.01, ***P< 0.001 and N.S, not significant

width. A significant contrast was present between two sites. Similarly, interaction between two factor was highly significant.

Petiole length

Data for petiole length (Fig. 1) revealed that undisturbed population exhibited longer petioles except population No. 10 that showed the lowest mean value (1.8 cm). By contrast, petioles were significantly shorter in populations of disturbed habitats (Table 1). Analysis of variance of the data (Table 2) revealed a profound effect of disturbance on petiole length. Likewise, a considerable disparity was found between habitats, Therefore, interaction being significant for this response.

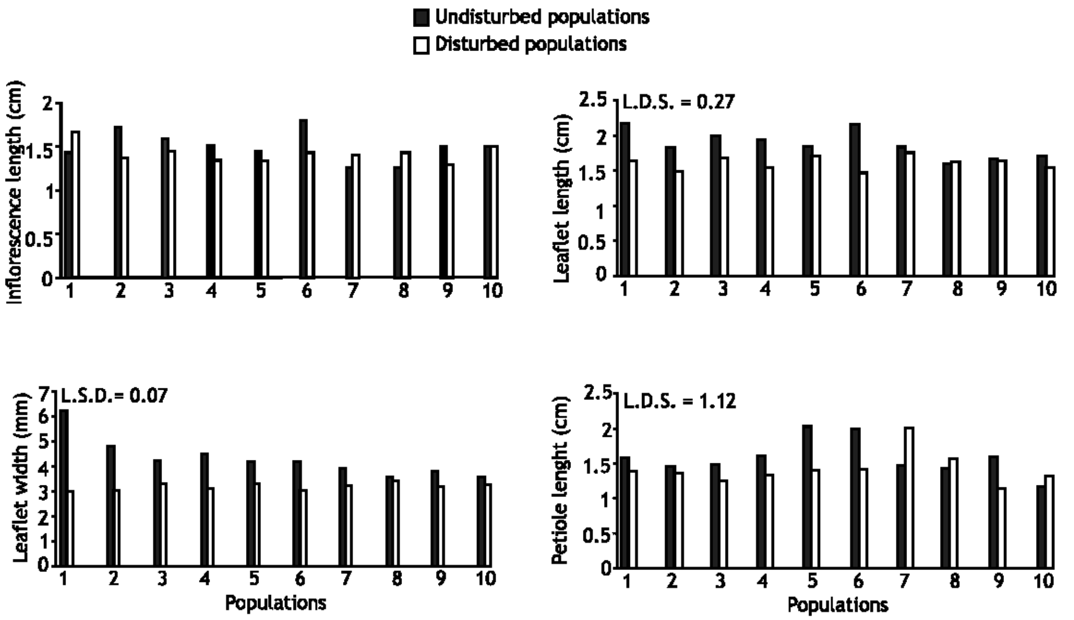

Fig. 1: Mean values of various biometric characters in populations of ***Trifolium alexandrium*** collected from diverse habitats during early summer, 2001

Internodal distance

The longest distance (11.0 cm) was recorded for population No. 1 of an undisturbed site (Fig. 2). Similarly, other disturbed populations also exhibited higher mean values for this attribute. However, population No. 7 showed the shortest distance (4.59 cm) between two nodes among these populations. It is also obvious from Fig. 2 that four populations (3,7,9,10) form undisturbed environments had higher mean values for this parameter. Although, an inconsistency was found in these two groups of populations for the trait but an overall reduction of internodes is evident from Table 1. Similarly, analysis of variance (Table 2) clearly indicated considerable effects of disturbance on internode length as well as a marked dissimilarity between populations and habitats.

Stolon length

Fig. 2 indicated that ll disturbed population had higher mean values for stolon length except population No. 10 which showed the lowest mean for this parameter. Conversely, populations of undisturbed environments had shown lower mean values for stolon length. A significant elongation of stolon was observed in response to various type of disturbances (Table 2). The analysis of variance of the data (Table 2) revealed significant differences between two groups (undisturbed and disturbed) of populations for this phenotypic expression.

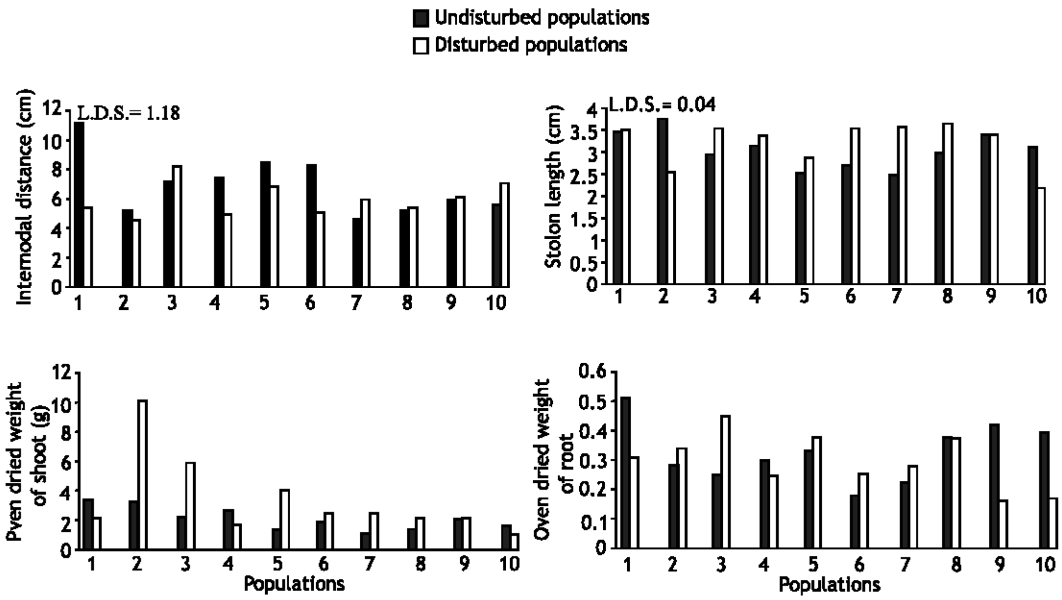

Fig. 2: Mean values for biometric and biomass characters in populations of ***Trifolium alexandrinum*** collected from diverse habitats during early summer, 2002

Oven dried biomass of shoot and root

Table 1 showed that undisturbed populations had produced lower oven dried biomass of shoot. On the contrary, populations from disturbed environments showed an overall higher mean for dry biomass of shoot. Fig. 2 showed that the maximum dry biomass was produced by population No. 2 (10.05 g) while, population No. 10 exhibited the lower biomass under a disturbance regime. However, populations No. 1 & 2 from normal habitats had shown comparatively higher mean values for this parameter (Fig. 2). Although, an overall increase in shoot biomass was recorded in disturbed populations but analysis of variance revealed that this increase was insignificant (Table 2). Similarly, statistical analysis did not reveal any significant distinction between population from contrasting habitats.

Fig. 2 depicted that populations of undisturbed sited had shown relatively lower mean values for dry biomass of root that their corresponding populations from disturbed habitats. However, population No. 1 from a normal habitat showed the higher mean (0.51 g) for this response. Analysis of variance (Table 2) revealed that various types of disturbances had not imposed any significant effect on root dry biomass and differences between populations and habitats were found to statistically non-significant.

Discussion

This study addresses morphological differentiation in several populations of ***Trifolium alexandrinum*** sampled from contrasting environments. Disturbed habitats have imposed a wide range of phenotypic variation for variation for various biometric attributes. Similarly, habitats differed distinctly and significant variability due to the interaction of populations with environments is evident.

The phenotypic expressions are pertinent to habitats and differentiation is the reflection variable environmental conditions. Consequently, populations from dissimilar habitats might show variable morphological expressions. Furthermore, these expressions may likely to be environmentally controlled, phenotypically plastic (gross inflorescence size and vegetative characters) while, floral and fruiting characters are supposed to be genetically controlled thus phenotypically rigid (Stebbins, 1950; Schmid, 1992). Using a phenotypically plastic character that is gross inflorescence that disturbed populations but a significant contrast was not present between two groups of populations from different habitats. Although, inflorescence length is assumed to be a plastic character but it did not vary in populations. These results are no surprising because they confirm the early finding of Bradshaw (1959) where similar pattern of variation for inflorescence was observed in populations of a grass species collected from diverse habitats.

It is obvious that leaflet traits were significantly influenced by disturbance regimes. Populations from disturbed environments have distinctly shorter leaflets (length and width) than undisturbed populations. The overall compactness of leaflet in disturbed populations indicated that leaf could be considered as a plastic organ. Therefore, these results supported Bradshaw (1974) who reported changes in leaf morphology in two clover species; ***Trifolium repens*** and ***T. subterranean***. Furthermore, consistent decline of leaflet width is in lines with Turkington and Burdon (1983) who demonstrated reduction of leaflet width in response to local environment. However, these alteration did not indicate any adaptive change.

Morphological differentiation for petiole length is evident in response to local environmental conditions. The extreme alteration of petiole in ***Trifolium*** species is well reported by many workers (Hill, 1977; Brougham ***et al.***, 1978). In fact, Bradshaw (1974) regarded the petiole of ***Trifolium*** as one of the most plastic plant organ. Moreover, the existence of variability for petiole morphology confirms the findings of Evans and Turkington, (1988) who examined considerable flexibility of petioles in ***T. repens*** in relation to environmental heterogeneity.

Populations from varied habitats displayed significant variation for internode length. Five populations from undisturbed environment exhibited higher levels of morphological variation and at the same time, disturbed population were also found to be responsive to their habitats. However, the pattern of differentiation was inconsistent. Since, vegetative characters are more liable to change and may show incoherent variability (Marshall and Jain, 1968).

The observed increase in stolon length under varying disturbance regimes is presumably an adaptive change because an expanded genet allows a plant to do well in variable environment (Newton, 1986). Moreover, increased stolon length seems to be a familiar response of ;plants growing in unstable environments. Aston and Bradshaw (1966) also documented stolon elongation in ***Agrostis stolonifera*** from disturbed maritime habitat.

The results presented for shoot and root biomass demonstrated that populations from disturbed and undisturbed habitats do not differ significantly for biomass production. However, populations from disturbed environments produced grater biomass for shoot. The greater shoot biomass production can easily be related to stolon length. Elongated stolon have produced more ramets and thus greater biomass. Turkington (1983) regarded it is a survival strategy in ***T. repens*** where it facilitated the persistence of the species in spatial and temporal disturbed environments.

This study clearly demonstrated that populations of ***T. alexandrinum*** have responded morphologically to various environments. The ability of plant to alter its morphology in response to changes in environments can be regarded as phenotypic plasticity (Bradshaw, 1965; Schlichting, 1986). Population resistance to a changing environment is usually achieved by plastic phenotypic reponed (Levins, 1968) because it has been agreed that plastic response are necessary for species for the occupancy of disturbed habitats (Thompson, 1991). This study suggested that populations of ***T. alexandrinum*** are phenotypically differentiated in response to environmental conditions. Therefore, phenotypic plasticity appeared be an important feature of species adaptation (Watson ***et al.***, 1997).

This work indicated that ***T. alexandrinum*** seems to be a flexible species and its populations are well adapted to varied habitats. Therefore, it is likely that the species can withstand environmental disturbances by sustaining variability of certain biometric traits.

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