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Response Flexibility in *Trifolium alexandrinum* L. : A Phenomenon of Adaptation to Spatial and Temporal Disturbed Habitat

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Abstract: Two populations of *Trifolium alexandrinum* were studied from a normal and a disturbed site for phenotypic differentiation. Morphological variation was assessed from twenty individual plants from each of two field provenances. Similarly, phenotypic and genetic components of variability were revealed after their transplantation to common garden conditions. Consistent measurements for various biometric and biosynthetic attributes revealed that both populations were morphologically differentiated but alteration of certain character expressions was more profound for the disturbed provenance. Likewise, common garden cultivation revealed that much of this variation has environmental component rather than genetic variability. Therefore, environmental fluctuations appear to generate flexibility of morphological responses. It is much likely that response flexibility has allowed the species to withstand spatial and temporal disturbances and seems to be an underlying phenomenon of adaptation to environmental constraints.

Key words: Environmental fluctuations, character expressions, response flexibility, *Trifolium alexandrinum*

INTRODUCTION

We are becoming increasingly aware that an individual cannot be considered out of the context of its environment. Therefore, at the present time there is a great deal of interest in the way in which an individual maintains its stability in the face of varying environmental situations[1]. Habitat conditions reflect the characteristics of an individual because they are pertinent to environment[2]. Plants on the whole just have to endure whatever the habitat offers[3] but they show significant phenotypic differentiation in response to variable environment[4,5]. Nevertheless, plant species and their populations appeared to be stable entities even in spatially and temporally changing environment[6].

Any alteration in the environment can be regarded as disturbance[7] that can reduce the growth and performance of the plant[8]. Disturbance seems to occur in all ecosystems even in stable environments some levels of disturbances are always operative[9]. Disturbance can produce several physiological and genetical changes in plant populations which predominantly morphological effects[10,11].

The development of different phenotypes is a remarkable property of plants conferring their broad adaptive capability to accommodate spatially and temporally disturbed environment[12]. Therefore, morphologically differentiated populations are characteristic of disturbed habitats and have been reported for some plant species[13,14]. Thus, differentiation of plant population in their immediate environment seems to have an adaptive significance to cope with the variability of habitat[15].

However, the phenotypes are not just an aggregate of morphological and genetical characters but they emerged from the interaction of a particular developmental plan, the genotype with a particular environment[2]. Moreover, the modification of these plans is under genetic control[1].

The variability of morphological character expression can indicate differentiation of plant populations in a particular habitat. Similarly, the genetic variability can be distinguished from superimposed phenotypic variation in plants on the basis of their transplantation to common garden conditions, which is a fundamental technique of revealing genetic variation in populations[16]. So the differences that are attributable to habitat can be recognized.

Trifolium alexandrinum L. is an important forage crop, which can be grown in a wide array of habitats. Early investigations have revealed differentiation of various morphometric attributes in relation to various environments[17]. However, a few studies have closely examined the differentiation in *T. alexandrinum* in

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response to disturbance regimes. Genetic variability for a wide array of characters, accessibility to sampling sites, feasible analysis of biometric traits and clonal propagation made this species an experimental plant.

The present study was carried out to test the hypothesis that populations of *T. alexandrinum* are differentiated with respect to their habitat conditions. Therefore, the present work aims to reveal:

- Differentiation in *T. alexandrinum* populations under different habitat conditions
- To study alteration of the character expressions that has a significant role in the adaptation of the species to environmental fluctuations.
- To resolve environmental and genetic components of responses of the species.
- To understand the underlying phenomenon involved in the adaptation of this species to a spatial and temporal disturbed habitat.

MATERIALS AND METHODS

Collection and cultivation of experimental material study sites: Fields were visited to observe the cultivated populations of *Trifolium alexandrinum* around Multan, Pakistan (32°20'N 71°82'E). Two contrasting habitats were selected for sampling. The first provenance was sampled from an undisturbed field and was designated as an undisturbed population. The field was located inland, therefore, it appears that it is free from any sort of environmental disturbances such as air pollution due to automobiles or industrialization. Moreover, due to an inland location there were no trampling or grazing regimes. Field was irrigated by canal watercourses thus there was no disturbance because of strong water currents.

A vast field located in the vicinity of Pak-Arab Fertilizer Factory, Multan was selected as disturbed site. The provenance sampled was exposed to various kinds of disturbances, mainly air pollution (NH_3, Sulpher, oxides of carbon, smog etc.). Moreover, the major source of irrigation water for this field was an ice factory effluent, which contains considerable amount of salts. Thus, this population was inhabiting an area disturbed by both air and water pollution.

Sampling procedure and collection protocols: The sampling was done from both of the provenances during April, 2003 when plants have inflorescence on them. Each individual plant was carefully identified and regarded as genet. The whole genet was up rooted along with extended stolons and with adherent soil. Twenty genets were collected from each of two habitats. Each genet was given a number and referred to its respective population. Collection was carried out at considerable distances (10 m apart) in order to ensure the collection of an individual plant. All genets collected were put in properly labeled paper bags and brought to the laboratory.

Biometric character analysis: Ten out of twenty plants from each habitat were analyzed for various morphometric attributes by taking consistent measurements. The detail of measurements and characters analyzed is as follows:

Characters	**Measurement details**
1- Biosynthetic characters	
a. Fresh weight (g)	The soil was removed from each genet and their fresh weights were taken using a digital Balance (Chyo Balance Corporation
b. Dry weight (g)	Each sample was dried at 90°C for 72 h in an oven (Memmert Germany, T.V. 400, Capacity 53 L) and then weight measurements were taken as above.
2-Leaf characters	
a. Leaflet number	The total number of leaflets on each plant was counted.
b. Leaflet length (cm)	The leaflet length was measured from its tip to the point of its attachment to the petiole with a centimeter ruler. All middle leaflets of trifoliate leaves on each plant were thus measured.
c. Leaflet width (cm)	The maximum width of the middle leaflets was measured from trifoliate leaves on each plant.
d. Petiole length (cm)	Petiole length was measured from its point of attachment to main axis to the base of middle leaflet.
3. Internodal distance (cm)	The maximum distancebetween nodes was measured.

Common garden transplantation: Twenty clay pots (18 cm internal diameter) were filled with 1.5 kg of garden compost and were labeled for genet number and population. Ten genets from each population were transplanted to these pots immediately after their collection from the fields. Pots were placed in a

Randomized Block manner in a glass house 25±°C day and 18±°C night temperature and 12 h day length.

The watering regimes were kept same for all pots and plants were allowed to establish under uniform conditions for two weeks then whole plants were removed and morphometric attributes were studied again.

Statistical analysis: Mean and standard error were calculated for each parameter both for field and common garden cultivation. Following Schlichting[18] data were subjected to a nested two-factor analysis of variance in order to elucidate differences between genotypes and two environments as well as to reveal genotype environment interaction using MS Excel, 2000.

RESULTS

Fresh weight: Overall mean values (Table 1) showed that the genotypes from an undisturbed habitat produced greater fresh biomass while, plants from disturbed population consistently exhibited lower mean values for fresh weight. The responses of plants did not vary after their transplantation to common garden conditions. Table 2 depicted that genets from undisturbed provenance produced consistently greater fresh biomass and individual plants of a disturbed population exhibited smaller mean fresh weight under common conditions of growth.

Analysis of variance of the data for field collection (Table 3) revealed a marked contrast ($P<0.001$) between two environments. However, the genotypes did not show any significant variability for fresh biomass. Statistical analysis of the data also indicated that the two populations became uniform when they were grown under garden environment. Similarly, the expressions of genets were also found to be insignificantly variable.

Dry weight: Results presented as mean values (Table 1) for dry weight revealed that genotypes of an undisturbed population exhibited greater dry biomass. All genets of a disturbed environment showed consistently lower means for this attribute. Genotypes of an undisturbed provenance behaved in a similar manner when they were grown under common garden conditions and showed greater biomass production (Table 2).

Statistical analysis of the field data (Table 3) indicated that the two environments varied considerably ($P<0.001$). However, the genetic component of the variability was lower as genets did not differ significantly under field conditions for this attribute. It is also evident from ANOVA (Table 3) that genotypes did not show any distinction after their cultivation in common garden.

Table 1: Overall mean values (±S.E) for various biometric attributes in populations of *Trifolium alexandrinum* for field collection from two contrasting habitats during April, 2003

Biometric characters	Undisturbed population	Disturbed population
Fresh weight (g)	19.24±3.15	6.75±0.72
Dry weight (g)	3.45±0.59	1.43±0.23
Leaflet number	18.45±1.07	19.25±1.38
Leaflet length (cm)	2.07±0.10	2.05± 0.23
Leaflet width (cm)	0.71±0.05	0.52±0.03
Petiole length (cm)	3.09±0.18	3.4±0.18
Internodal distance (cm)	4.79±0.31	4.98±0.28

Each mean value is across ten genets each replicated twice

Table 2: Overall mean vales (±S.E) for various biometric attributes in populations of *Trifolium alexandrinum* after cultivation in common garden

Biometric characters	Undisturbed population	Disturbed population
Fresh weight (g)	8.52±0.57	8.44±0.48
Dry weight (g)	2.49±0.35	2.42±0.19
Leaflet number	12.15±1.44	16.05±1.30
Leaflet length (cm)	2.11±0.13	2.37±0.11
Leaflet width (cm)	0.45±0.04	0.82±0.19
Petiole length (cm)	3.43±0.50	1.76±0.13
Internodal distance (cm)	4.27±0.74	5.67±0.24

Each mean value is across ten genets each replicated twice

However, peculiarity between two provenances became invariable under uniform conditions of growth.

Leaflet number: Table 1 indicated that genets which were sampled from a disturbed site exhibited greater leaflets number as compared to its contrasting normal population. The overall expression for leaflet number was consistent for both provenances in the field and under uniform conditions (Table 2). Under field conditions no significant variability between and within population was observed for this character expression. However, significant differences ($P<0.05$) became evident on their cultivation in the garden. On the other hand, genotypes did not exhibit any differentiation when they were shifted to the pots from their original environment (Table 3).

Leaflet length: Leaflet length measurements (Table 1) revealed that the genotypes of an undisturbed site had significantly longer leaflets than individuals of a disturbed environment. When genotypes were grown under uniform conditions, their response changed significantly and leaflets of disturbed plants showed considerable elongation (Table 2)

ANOVA presented in Table 3 for field data revealed significant variability between genotypes ($P<0.01$) and a significant genotype environment interaction ($P<0.05$). The differences between two types of habitats became indistinguishable under common environment.

Leaflet width: Table 1 showed that genotypes sampled from an undisturbed had broader leaflets. All genets from the disturbed habitat showed invariably less expanded

Table 3: Analysis of Variance (mean squares) for various biometric attributes in populations of *Trifolium alexandrinum* for field collection from two contrasting habitats and after cultivation in common garden during April, 2003

Biometric characters	M.S G	Significance	M.S. E	Significance	M.S. I	Significance
Field collection						
Fresh weight(g)	62.05	1.46 n.s.	779.50	18.43***		
Dry weight (g)	2.91	2.63 n.s.	20.32	18.33***		
Leaflet number	25.93	0.39 n.s.	01.21	00.18 n.s	39.76	0.60 n.s
Leaflet length (cm)	0.63	4.05**	00.001	00.009 n.s	00.63	4.03**
Leaflet width (cm)	0.038	1.27 n.s.	00.38	12.57***	00.03	1.11 n.s
Petiole length (cm)	0.41	0.68 n.s.	00.96	01.57 n.s	01.26	2.05*
Internodal distance (cm)	1.99	2.12*	00.15	00.16 n.s	01.32	1.41 n.s
Common garden cultivation						
Fresh weight (g)	9.98	1.56 n.s	00.03	00.005 n.s		
Dry weight (g)	1.04	1.86 n.s	01.23	02.19 n.s		
Leaflet number	29.9	1.07 n.s	152.10	05.45*	45.6	1.63 n.s
Leaflet length (cm)	0.16	0.91 n.s	00.14	00.77 n.s	00.41	2.39*
Leaflet width (cm)	0.38	7.62***	01.34	26.31***	00.4	7.94***
Petiole length (cm)	2.58	8.22***	24.50	78.07***	02.9	9.25***
Internodal distance (cm)	4.74	5.18***	19.69	21.51***	07.7	8.41***

n.s = Non-significant

*,**, *** = significance at 0.05, 0.01 and 0.001% levels of Probability, respectively

$M.S_G$ = Mean square Genets, $M.S_E$ = Mean square Environment, $M.S_I$ = Mean square Interaction

leaflets. However, a significant change for leaflet width was observed in the common garden (Table 2). For this expression, the responses of populations were contrary for field and common garden cultivation. The plants from an undisturbed field showed almost 50% decline in overall leaflet width under uniform conditions as compared to genotypes of disturbed environment.

The statistical analysis of field data (Table 3) supported a striking contrast (P<0.001) between two environments. ANOVA for common garden cultivation revealed a significant variability between the genotypes (P<0.001) from each habitat. In addition, a significant (P<0.001) genotype environment interaction became evident from statistical analysis.

Petiole length: Overall length of petioles was greater in the disturbed population (Table 1) while shorter length in plants of the normal populations. However, the difference for petiole morphology was found to be statistically non significant (Table 3). A significant decline petiole length was observed when genets of disturbed habitat were grown in common garden (Table 2). However, by contrast, the petiole morphology of the normal population was comparable both for field (3.04 cm) and garden cultivation (3.45 cm). Analysis of variance Table 3 depicted a significant genotype environment interaction (field collection P<0.05 and garden cultivation P<0.001). The genotypes of the two contrasting habitat retained their distinction after transplantation to uniform environment (P<0.001).

Internodal distance: It is obvious from (Table 1) disturbed provenance showed significantly (P<0.05) greater overall internodal distance as compared the normal population. The genets of the disturbed site also showed longer internodes under uniform growth conditions (Table 2). Statistical analysis of the data for common garden cultivation revealed significantly (P<0.001) differentiated internodes (Table 3). The genetic component of variability was considerably (P<0.001) higher as genets were found to be distinct in common garden cultivation. Moreover, genotype environment interaction was also found to be highly significant (P<0.001).

DISCUSSION

This work considers the differentiating morphological responses of *Trifolium alexandrinum* populations collected from two varied habitats. Character expressions varied considerably between populations from diverse habitats. Similarly, a marked contrast between two environments also became evident from this study. Biometric traits of plants growing in a normal habitat were compared with plants of a disturbed site and various modifications of morphological attributes were reported. Using common garden technique, those alterations that were attributable to particular habitat conditions were identified.

Fresh and dry biomass production in crops can be used as a predictor for plant growth and performance under various environmental conditions[8]. The results indicated that the two populations produced significantly different biomass. Greater fresh biomass production was reported for the population, which was sampled from a normal habitat while the reverse was true for a disturbed population. Thus, it appears that biomass production is sensitive to disturbance regimes and environmental stress had considerably influenced the biosynthetic traits.

Furthermore, responses of the individual plants were consistent in each type of habitat hence; a marked contrast between two habitats also became evident. Likewise, Similar responses of populations were observed for dry biomass production. The reduction in fresh and dry biomass in disturbed habitat clearly signifies environmental constraints on plant growth. The reduction in growth under environmental stress is in lines with Bradshaw and McNeilly[19].

The leaf is an important photosynthetic organ of the plant and several changes in the leaf number, morphology and physiology have been reported in different plant species in relation to the heterogeneity of the environment[7]. The study revealed that total number of leaves produced on individual plants did not vary considerably and formation of leaf was not influenced by each kind of habitat. It has been argued that certain changes occur in the leaf morphology after a long exposure of plants to a set of environmental conditions though some attributes can change quickly[7]. The exposure of plants to varied habitats did not result in any differentiation for leaflet number in these populations.

Since, leaf is a module[2] and it seems that plans for the modular development are under genetic control[1]. Moreover, common garden cultivation has revealed significant differences between populations but all genets of a population showed consistent morphology for leaf number. Therefore, the variability for leaflet number appears likely to be genetically controlled. Thus, the exposure of genets to varied habitats did not result in any change for this attribute in *Trifolium* plants.

Environmentally induced changes in plant phenotypes can be identified from a significant genotype environment interaction[18,20]. Similarly, genetically based differences can be revealed by common garden cultivation where phenotypes can become invariable[10,16].

The changes for leaflet length thus can be attributable to the specific set of environmental conditions as a significant GxE interaction was observed for this expression. It seems that different plant phenotypes have developed in relation to their original habitat. Changes in leaf morphology have also been well reported in other species[21].

With regard to leaflet width, populations showed significant variability while growing in field but their expression altered significantly after their growth under uniform conditions. Although, both leaflet length and width are foliage characteristics but showed differentiated responses in relation to environmental situations and there seems to exists no associated change in leaf length and width.

Many workers[3,21] have reported significant adaptive changes in the leaf morphology attributable to changing habitat conditions. Although, for this study any adaptive advantage in leaf morphology is not very clear but it can be assumed that longer leaves of the species might possess greater amount of leaf tissues (Bokhari, 2004, Personal communications). Such leaves can be assumed functionally more efficient but further anatomical evidences are required to reach any firm conclusion. Nevertheless, these findings are in close agreement with other studies[22,23] that reported diverse morphology of leaves under varied environmental regimes.

The petioles of the *Trifolium* species are regarded as the most plastic organ and their morphology can alter very quickly under changing environmental situations[24]. A significant genotype environment interaction revealed that the changes in petiole morphology are due to environmental disturbance. The habitat related changes of petioles are further supported by common garden cultivation where genetic variability appeared as significant component when environmental constraints were removed. Thus, differentiation of petioles is comparable to other species of the genus *Trifolium*[25,26]. While considering the internodal distance, the responses of plants were comparable to petiole morphology. Again, it seems that this character expression has alerted under the influence of disturbance.

Terrestrial plants, which are both modular and sessile, have to cope with the variability of environment[7]. Plant populations might show an increase in their fitness to accommodate spatial and temporal fluctuations of the environment[27]. The fitness is achieved either by variable gene pool or by altered morphological responses of plants. Species that are differentiated morphologically are regarded as flexible species and phenomenon is known as response flexibility, which has both genetic and environmental components[7].

This study indicated that the genetic variability is lower in the species as genotypes did not show distinctive morphologies under uniform conditions of growth for most of the attributes. Thus, the alteration of responses of seems to be environmentally induced rather than variable genetic pool for ***T. alexandrinum.***

Accordingly, response flexibility seems be attained by the plasticity of some traits and acclimation of others[7]. Present study indicated that leaf attributes; leaflet length and petiole morphology appeared to be plastic characters and represented environmental component while acclimation seems to occur for biosynthetic traits, leaflet number and leaf width thus appeared to be genetically planned.

The increase in leaflet and petiole length coupled with an increased internodal distance presumably give the plants a maximum aerial exposure so that it could perceive more light as the presence of fumes of effluent gasses appears to hamper light penetration in the disturbed habitat. Therefore, modifications of these characters seem to be adaptive and advantageous for plants.

Thus, it can be concluded that *T. alexandrinum* appears to hold increased fitness for growing under a wide array of habitat disturbances. The species has accommodated environmental fluctuations both by plasticity and acclimation of morphological attributes. Hence, response flexibility can be considered as an underlying phenomenon for species existence and maintenance under strong environmental pressures.

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