Genetic Analysis of Some Morphological Traits in Egyptian Clover (*Trifolium alexandrinum* L.)

Tejveer Singh
Indian Grassland and Fodder Research Institute, India

D. R. Malaviya
Indian Grassland and Fodder Research Institute, India

P. Kaushal
Indian Grassland and Fodder Research Institute, India

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Tejveer Singh*, D. R. Malaviya, P. Kaushal
ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India
*Corresponding author e-mail: tejveersinghbhu@gmail.com

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Introduction

Trifolium alexandrinum, commonly known as Berseem or Egyptian clover (2n=2x=16) is an important winter forage legume in India, Pakistan, Turkey, Egypt and Mediterranean region. It is characterized by three ecotypes *viz*; Mescavi, Fahli and Saidi. Amongst these, Mescavi type is most popular. In India it is cultivated in about 2 million hectare area.

The major impediment in genetic improvement of berseem is the lack of appreciable genetic variability (Verma and Mishra, 1995; Roy *et al*., 2004; Malaviya *et al*., 2005). Although limited, variability for desirable traits at intraspecific (*T. alexandrinum* var Fahli and var Saidi) has been reported, considerable variability exists at interspecific level. Affinity of *T. alexandrinum* was tested with different wild *Trifolium* species (Malaviya *et al*., 2005) and was successfully hybridized with *T. apertum* for transfer of desirable traits such as late maturity and root rot resistance (Malaviya *et al*., 2004). In the segregating population of the cross, novel morphological traits such as black seed coat colour were also observed (Malaviya *et al*., 2012). *T. alexandrinum* var Fahli is single cut type and has several desirable characteristics like self compatibility (even not requiring tripping), good plant height, better seed yield, apical branching, etc.

For incorporation of desirable genes in the improved genepool of beseem, the study of inheritance pattern of traits under consideration is a prerequisite. Therefore, present study was undertaken to study the inheritance pattern of pentafoliate leaf, dark green colour of leaf, black seed coat colour and regeneration capacity.

Materials and Methods

Plant materials: Two inbred lines, one derived from wide hybridization between *T. alexandrinum* × *T. apertum* and another developed from *T. alexandrinum* ecotype Fahli, were crossed (including reciprocal) during *Rabi* 2012-2013. A single F_1 was self-pollinated and seeds for F_2 population were harvested during *Rabi* 2013-2014. Each F_2 population was space planted during *Rabi* 2014-2015.

Leaflet number: Leaflet number was recorded from leaves developed on first to eighth node of main branch. Based on leaflet number of leaf from fourth to eight node, each plant was categorized as trifoliate, pentafoliate or multifoliate. If all four leaves possessed three or five leaflet plant were categorized as trifoliate or pentafoliate respectively, remaining plants were placed in multifoliate category.

Foliage colour: Leaves of all F_2 plants were classified as dark green or green depending upon the intensity of foliage colour recorded by Royal Horticultural Society (RHS) colour chart.

Regeneration: Arial part of each plant was harvested (defoliated) at opening of first flower. If regrowth appeared then plant was categorized as possessing regeneration capacity, otherwise marked in non regeneration capacity.

Results and Discussion

The inbreds used as parents in present study showed variation for different morphological traits (Table 1). Study of F_1 showed that genes for green foliage colour and regeneration capacity are dominant over dark green foliage colour and non-regeneration capacity. However, for leaflet number the F_1 showed multifoliate leaf, indicating the absence of dominance effect of both pentafoliate as well as trifoliate leaf. Result of reciprocal cross indicates that traits are under control of nuclear gene.

In F_2 generation, the frequency distribution of plants having normal foliage colour and dark green colour showed a ratio of 3:1 respectively, suggesting a single recessive gene control of dark green colour. Similarly, regeneration capacity was found under single dominant gene control (Table 2). The gene symbol proposed for foliage colour is *Fc* and its recessive allele *fc* is responsible for dark green colour of foliage; and gene symbol *Rg* is proposed for regeneration capacity.

In F_2 generation variation for leaflet number was observed (Fig. 2). Frequency of leaflet number is presented in Table 3. It indicates that gene(s) responsible for pentafoliate leaf are expressed after seedling stage. Frequency of plants with pentafoliate leaf is about 7% in both F_2 populations indicating that ≥2 genes controlling pentafoliate leaf formation (Fig. 1).

Table 1: Variability for different morphological traits in parental inbred lines

Traits	Inbred developed from *Trifolium alexandrinum* var Fahli	Inbred derived from wide hybridization
Leaflet number	Three	Five
Foliage colour	Normal green	Dark green
Seed coat colour	Yellow	Black
Regeneration capacity	Absent	Present
Days to flowering	Early	Late
Branching	Apical	Basal
Compatibility	Tripping independent self comptible	Tripping dependent self comptible

Table 2: Frequency distribution of F_2 plants in different classes

Trait	Classes	Observed	Expected frequency	Chi square value
Foliage colour	Dark green	54	52.25	0.059
	Normal green	155	156.75	0.019
	Total	209		**0.078**
Regeneration capacity	No-regeneration	58	51.75	0.755
	Regeneration	149	155.25	0.251
	Total	207		**1.006**

Table 3: Percentage of leaflet number on first to eighth node of main branch in F_2 population

Leaflet number		Percentage of leaflet number on first to eighth node						
	Node 1	Node 2	Node 3	Node 4	Node 5	Node 6	Node 7	Node 8
1	0.00	0.00	0.29	0.88	1.47	1.19	1.17	1.04
2	0.00	0.00	0.59	1.47	2.06	1.49	1.31	1.11
3	100.00	100.00	98.24	90.03	84.66	84.18	83.24	84.51
4	0.00	0.00	0.59	6.45	3.24	2.99	1.47	1.01
5	0.00	0.00	0.29	1.17	8.55	10.15	12.81	12.33

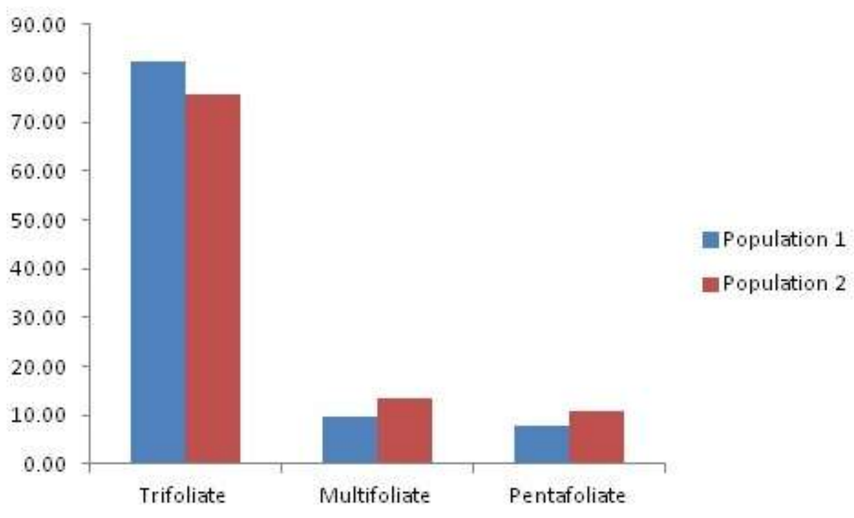

Fig. 1: Percentage of plants with trifoliate, pentafoliate and multifoliate leaf in two different (derived from direct and reciprocal cross) F_2 populations

Fig. 2: Variation observed in F_2 population for leaflet number and foliage colour

Conclusion

Study indicated that traits like regeneration capacity and foliage colour are governed by single major gene. Therefore, incorporation of these traits could be simple based on their dominance behaviour, and breeder could make selection in early segregating generations. However, genetic analysis of pentafoliate leaf indicates complex inheritance pattern. Therefore, for introduction of pentafoliate trait in improved gene pool of berseem could be made by selecting plants in advance generation that having large population size.

Study also indicates that variability present in *T. alexandrinum* var Fahli and generated through wide hybridization could be incorporated in cultivated berseem (*T. alexandrinum* var Mescavi) gene pool. Therefore, for genetic improvement of berseem it is recommended to develop desirable inbreds of *T. alexandrinum* var Fahli and enhance number of interspecific hybrids that posses desirable traits.

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