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**Analysis of seed production traits in interspecific hybrids
between
Trifolium repens (white clover) and *Trifolium uniflorum***

A thesis submitted in partial fulfilment of the requirements for the
degree of

Doctor of Philosophy
in
Plant Breeding and Genetics

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New Zealand

Muhammad Naeem

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I dedicate this effort to my homeland

PAKISTAN

Analysis of seed production traits in interspecific hybrids between *Trifolium repens* (white clover) and *Trifolium uniflorum*

Trifolium repens L. (white clover) is an important component of New Zealand's pastoral and herbage seed industries. It has delicate shallow roots vulnerable to drought and pest attack. *T. uniflorum* is a wild species, adapted to dry environments, with deep woody roots but poor vegetative growth and only 1-3 florets per head. Hybridization was done to incorporate the drought tolerance and root characteristics of *T. uniflorum* into white clover. The primary hybrids (F_1 and BC_1F_1) showed poor seed production. The project aimed to analyse this problem and improve seed production by further hybridization and selection.

The hybrids expressed high variation for almost all seed production traits. Heads per plant, florets per head and seeds per floret were important determinants with moderate to high heritabilities. The derived traits seeds per head, florets per plant and seeds per plant expressed low-moderate to moderate heritabilities. Flowering pattern showed high heritability. No negative correlations between seed production and root traits were found. Whereas nodes per stolon favoured head production, stolon production had a negative effect on nodes per stolon. No negative associations between head production, persistence and foliage production of the hybrids were observed. No cytoplasmic influences were evident.

The hybrids showed, higher self fertility and a wider gap between potential and actual seed yield than white clover. The BC_1F_1 generation produced low numbers of heads per plant and seeds per floret. Intercrossing coupled with recurrent selection improved variation, fertility and realization of potential seed yield. The second backcrossed and intercrossed generations had high seeds per floret and plant. Backcrossing of selected hybrids effectively recovered the white clover phenotype and created new variation. The BC_2F_1 and BC_3F_1 generations expressed the better head production ability of *T. uniflorum* combined with the bigger head size of white clover.

Seed production capability of the hybrids was successfully restored. More genetic variation was created in the hybrids for future breeding and selection endeavours.

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Abbreviations and terminologies

The abbreviations and terminologies used are defined and explained below.

F₁: Filial 1 generation, produced by hybridization between white clover and *T. uniflorum*.

BC₁F₁: First backcrossed generation, produced by crossing between white clover and F₁.

BC₂F₁: Second backcrossed generation, produced by crossing between white clover and BC₁F₁.

BC₃F₁: Third backcrossed generation, produced by crossing between white clover and BC₂F₁.

BC₁F₂: Second intercrossed generation, produced by crossing among BC₁F₁ hybrids.

BC₁F₃: Third intercrossed generation, produced by crossing among BC₁F₂ hybrids.

BC₂F₂-BI: Second backcrossed and intercrossed generation. The BC₁F₁ hybrids were backcrossed with white clover to produce BC₂F₁ progenies. In the subsequent year, crosses were made among these BC₂F₁ hybrids to produce BC₂F₂ hybrids.

BC₂F₂-IB: Second intercrossed and backcrossed generations. Crosses were made among BC₁F₁ hybrids to produce BC₁F₂ progenies. In the subsequent year, these BC₁F₂ hybrids were backcrossed with white clover to produce BC₂F₂.

Interspecific hybridization: A type of wide hybridization in which plants representing two different species are crossed to produce F₁ hybrids.

Backcrossing: Crossing of a hybrid with one of its parents (recurrent parent). In this study, the recurrent parent was always white clover. The backcrossed nature of the hybrids is represented by the abbreviation “BC”. For example, the F₁ hybrids between white clover and *T. uniflorum* were backcrossed with white clover to produce BC₁F₁ hybrids.

Intercrossing: A synonym of cross breeding used for crossing between different breeds, varieties, or species. In the current study, this term was used for crossing of the hybrids with each other. The letter “F” in the name of a generation is used to show intercrossing status of the hybrid. For example, the BC₁F₁ hybrids were intercrossed among themselves to produce BC₁F₂ generation. The pollination of a plant with its own pollens was described as selfing.

Recurrent selection: The process of selection for particular traits repeated after each cycle of hybridization, in order to bring improvement and stability in the traits. The target in the current study was improvement in seed production traits.

Family: In this study, the term “family” was used to denote a group of plants having at least one identifiable common parent. For example, all plants belonging to the ‘family A’ were the progeny of plants having white clover cultivar ‘Aran’ as their recurrent parent.

System of naming the families: Families of generation BC_1F_1 , BC_2F_1 and BC_1F_2 are named after the white clover cultivar used as the recurrent parent in hybridization with the F_1 in the development of these generations. For example, in the BC_1F_1 generation, plants A_1 and A_2 are members of same family (family A) as they had cultivar “Aran” involved as recurrent parent in their production. The BC_1F_2 plants produced by intercrossing of A_1 and A_2 were named as family A. Families of BC_3F_1 and BC_1F_3 had unique parental compositions and represented by their unique family codes.

Generation: A group of families at the same stage of a breeding strategy belonged to the same generation. For example, families A and B of generation BC_1F_1 were products of the first backcrossing of F_1 hybrids with white clover cultivars Aran and Barblanca, respectively.

Direction of a cross: Every individual cross was set out as “female parent x male parent”. For example, “white clover x *T. uniflorum*” indicates that white clover was used as the female parent and *T. uniflorum* was used as the male parent.

