Interspecific Hybridization in *Trifolium* Utilizing Embryo Culture Techniques

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**SPECIES** hybridization is a valuable tool for plant breeders, since it may extend the range of heritable variation useful in breeding and selection.

The results of crosses between species may be classified into three categories: (1) complete fertility with the production of plump, viable seeds; (2) complete sterility with no fertilization or seed production; and (3) intermediate situations wherein fertilization is effected, but during development of the seed the embryo aborts, and shrivelled, inviable seeds are produced. It was the purpose of this investigation to study this last condition, which appears to offer possibilities for enlarging the supply of heritable variation available for breeding work. Attempts were made to grow hybrid embryos to maturity by excising them from their ovules before abortion occurred, and then culturing them initially on artificial nutrient media.

Many of the common forage species of *Trifolium* do not behave as perennials under field conditions. Several other species of this genus are perennials, but have serious limitations as forage plants. It would be desirable by appropriate crossing and selection to combine the perennial habit with other desirable characters.

*Trifolium* is a genus previously thought to exhibit complete cross-incompatibility between species. However, recent investigations have revealed that a number of *Trifolium* species are sufficiently compatible to effect cross-fertilization. Among several of these combinations of species that exhibit fertilization, embryo abortion and seed inviability preclude successful hybridization. By utilizing embryo culture methods it is believed that hybrid *Trifolium* plants can be produced.

**REVIEW OF LITERATURE**

**Embryo Culture Techniques**

Hannig (4) in 1904 is recognized as the first investigator to excise immature embryos successfully. He obtained mature plants from embryos of *Raphanus* and *Cochelea* which were less than 2 millimeters long at the time of excision.

Much of the basis for culturing embryos and seed in vitro has been developed by Knudson (7). Studying the influence of maltose on growth of corn embryos, he successfully excised corn embryos and cultured them on a nutrient solution medium obtaining normal, fertile plants.

Laibach (9) in 1929 realized the possibility of obtaining hybrids from interspecific crosses which otherwise produced shrivelled, inviable seeds by excising immature hybrid embryos. He crossed *Linum austriacum* with *L. perenne* and, by means of embryo culture, was able to nurture the hybrid embryos to maturity.

Skirm (13) emphasized the use of embryo culture as an aid to plant breeding. He cultured interspecific embryos in *Prunus* and *Lilium*. When embryos were not fertilized, development was normal until fertilization occurred. Inter-specific hybrid embryos developed into viable intra-species embryos. This proved to be an important factor in determining the proper time for embryo excision, which was not successful if the embryo had started to rot.

Randolph and Cox (11) studied seed dormancy in varieties of bearded iris, in which the dormancy period lasted several months to several years. Attempts to break the dormancy period with various treatments such as chilling, leaching, and drying of seeds were only moderately successful. However, when iris embryos were excised and cultured on an artificial culture medium, they grew well and produced seedlings. A nutrient agar medium was devised and used successfully by several investigators on other species.

**Interspecific Hybridization in *Trifolium***

Several investigators attempted without success to effect interspecific crosses of *Trifolium*. Guravich (3) obtained few viable pollen grains, and reciprocal backcrosses of *T. ambiguum* and *T. hybridum* did not germinate on moist filter paper but when placed on a nutrient agar medium, a considerable number developed. He studied seed development of this hybrid for several months to several years. Attempts to break dormancy with various treatments such as chipping, chilling, leaching, and drying of seeds were only moderately successful.

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**Materials**

1.—*Trifolium ambiguum* M. B., Honey-lett's Clover, was first described by Marschall von Bieberstein in 1808 (1). Several reports from Russia have indicated that this species is a valuable forage legume with wide adaptation (8). It grows successfully around the Black Sea into the Caucasus and from the river valleys to sub-alpine regions of the Transcaucasian states. Under greenhouse conditions it possesses extensive rhizomes, perennial habit, high palatability, winter-hardiness, and is highly attractive to bees.