# FORAGE GRASS PLANT MODIFICATION THROUGH TISSUE CULTURE, HAPLOIDS AND DOUBLED HAPLOIDS

M. J. Kasperbauer

U.S.D.A., Agricultural Research Service, Florence, SC 29502-3039, USA

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## INTRODUCTION

Tall fescue (Festuca arundinacea Schreb.) is a widely-grown cool-season forage grass (BUCKNER and BUSH, 1979). It is wind-pollinated and generally self-infertile. Plants are highly heterozygous and improvement through conventional breeding is very slow. Therefore, use of tissue culture, haploids and doubled haploids offers an approach to accelerate the genetic improvement.

## METHODS

Most of the fescue cell and tissue culture research discussed in this report was done in coordination with the tall fescue breeding program of Dr. R. C. Buckner of the U.S. Dept. of Agriculture at Lexington, KY. We pre-selected candidate genotypes in field plots, cultured haploid plants, and then developed fertile doubled haploids. Details are outlined in KASPERBAUER et al., (1979, 1980) and KASPERBAUER and ENZENGA, (1985).

## RESULTS AND DISCUSSION

The collaborative research with Dr. Buckner began in the mid-1970's when he observed my progress with cell culture, plant regeneration, derivation of haploids and development of doubled haploids of Nicotiana tabacum that were fertile pure lines with characteristics selected during the haploid plant phase of the program

(KASPERBAUER and WILSON, 1979). The same goals were set for tall fescue and its hybrids.

Since haploid plants could be cultured from excised anthers of N. tabacum, the same approach was tried with fescue. However, no haploid plants developed from the excised fescue anthers. There were two possible reasons: (1) the nutrient medium may have lacked some ingredient needed for fescue, or (2) the fescue tissue was not suitable. Subsequently, a methodical approach was initiated and followed through successive stages that ended with fertile doubled haploid tall fescue plants. Field-grown plants that were pre-selected for winter hardiness and forage characteristics were used as tissue and anther sources.

Callus Establishment and Plant Regeneration from Somatic Tissue.

The basic medium previously used for N. tabacum was modified by using 2,4-D (2,4 dichloroplenoxyacetic acid) as the auxin. Callus establishment and maintenance were successful on a medium that contained 2 mg 2,4-D/liter, if the explant tissue was "young" (i.e., contained many dividing cells). Decreasing 2,4-D to 0.25 mg/liter resulted in regeneration of green plants from the callus as outlined in KASPERBAUER et al. (1979). The fact that fully-elongated stem tissue did not form any callus or regenerate plants proved to be a key to subsequent successful derivation of haploid plants with the aid of connected nurse tissue (i.e., anther-panicle culture).

Anther-Panicle Culture of Haploid Plants.

Rationale. When excised fescue anthers did not produce any callus or plants, we hypothesized that the mass of tissue in the excised anthers was too small. Further, since fully-elongated stem tissue did not form callus under our culture conditions, we decided to leave part of the panicle attached to the anthers to increase the tissue mass and to serve as a nurse tissue which would not form callus

or regenerate plants. The approach involved culturing appropriately staged anthers that were still attached to panicle (nurse) tissue. Any panicle tissue (other than the anthers) that had many dividing cells was excised and discarded. Haploid plants developed from immature gametes as discussed by KASPERBAUER et al. (1980).

Field evaluation. The haploid plants were increased through tillers and grown in replicated plots in the fescue breeding nursery The haploid lines differed in winter hardiness and forage characteristics. Observable differences between these infertile 21-chromosome lines supported the concept that each developed from a separate immature gamete. Some lines had leaf shape characteristics which later served as "markers" to evaluate doubled haploids.

Doubled Haploids Through Tissue Culture.

Previous experience with induced endomitotic divisions in somatic tissue excised from haploid N. tabacum plants was the basis for the approach with fescue. Again, considerable modifications of methods were needed, although success in both species involved regeneration of fertile doubled haploid plants from "aged" tissue from cytologically-verified haploid plants (KASPERBAUER and WILSON, 1979 and KASPERBAUER and EIZENGA, 1985).

After field evaluation of the infertile 21-chromosome haploid lines, young somatic tissue was excised from the base of rapidly elongating peduncles, and placed on callus establishment medium. After 3 weeks, the callus was transferred to fresh medium and left until the tissue began to senesce. The "aged" tissue was then placed on medium with a decreased 2,4-D level, and many normal green shoots emerged within 2 to 3 weeks. Root tip cytology showed that many of the regenerated plants were 42-chromosome doubled haploids. They had the same leaf markers as the "parent haploid" plants. The doubled haploids grew normally, were fertile and produced abundant seed that germinated into normal, vigorous plants.

## CONCLUSIONS

Well-planned and coordinated use of cell and tissue culture in conjunction with a conventional forage and/or turf grass breeding program can greatly accelerate derivation of fertile doubled haploid lines with specific characteristics. The doubled haploids can then be used as pure lines, crossed with other lines, and incorporated into the breeding program for conventional evaluation of forage, turf, and hardiness characteristics under field conditions.

## REFERENCES


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