11.3 Genetic Transformation in *Avena sativa* L. (Oat)

D.A. Somers¹, H.W. Rines¹,², K.A. Torbert¹, W.P. Pawlowski¹, and S.K.C. Milach¹,³

1 Introduction

Genetic transformation of cultivated hexaploid oat (*Avena sativa* L.) was first reported in 1992 (Somers et al. 1992). Since that time, the oat transformation system has been significantly improved. Current applications of transformation to oat improvement are focused on investigating mechanisms of resistance to barley yellow dwarf virus and fungal pathogens. This chapter reviews the key factors leading to the development of a routine transformation system for oat. The current status of oat transformation will be presented with consideration of selection systems and transgene inheritance in regard to eventual practical applications of transformation to oat improvement.

2 Development of the Transformation System

2.1 Totipotent Target Cells – Friable, Embryogenic Callus

Regeneration of fertile oat plants from tissue culture was first reported in 1976 (Cummings et al. 1976; Lorz et al. 1976). Immature embryos were used as the tissue culture explant and organogenic callus was thought to arise from the embryonic axis, most likely the mesocotyl (Cummings et al. 1976; Bregitzer et al. 1989). Progress in development of regenerable oat tissue culture systems was substantial during the 1980s (Heyser and Nabors 1982; Rines et al. 1992). However, protoplast culture systems, which were pursued by a number of researchers (Galston 1983; Tiburcio et al. 1986; Hahne et al. 1989), were not developed to the point where fertile plants could be routinely regenerated. Therefore, regenerable oat callus or suspension culture cells were clearly the most appropriate source of totipotent target cells for transformation. Ac-
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cordingly, further extensive improvements of the oat callus culture system from its original state were essential for the development of oat transformation technology. A specific genotype, termed GAF, that routinely produces highly regenerable callus, was selected from the progeny of a cross of the cultivar Garland by an accession of the wild oat *A. fatua* (Rines and Luke 1985). GAF and a daughter line derivative, GAF/Park, continue to be the genotypes most amenable to culture initiation and thus the transformation system (Bregitzer et al. 1989; Torbert et al. 1995). The observation and characterization of somatic embryogenesis in oat callus initiated from these genotypes and development of methods to routinely establish friable, embryogenic callus were key steps in the development of this culture system for oat transformation (Bregitzer et al. 1989, 1995; Rines et al. 1992). In the culture system currently used for oat transformation, callus is initiated from immature embryos isolated from GAF or GAF/Park lines on MS medium (Murashige and Skoog 1962) containing 2 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D). Friable, embryogenic sectors are visually selected from the initially produced callus within 2 to 3 months after culture initiation and are continually selected upon subculture for development of uniformly friable, embryogenic callus. Production of sufficient callus to initiate transformation experiments usually takes an additional 3 months of selective subculturing and bulk-up. Plant regeneration is initiated on MS medium containing 2 mg/l α-naphthaleneacetic acid and 0.2 mg/l benzylamino purine. This medium favors shoot primordia formation and proliferation (Bregitzer et al. 1989). Shoots are subsequently rooted on MS medium without phytohormones (Rines and Luke 1985).

A flaw inherent in all plant transformation systems, including oat, which utilize tissue cultures as sources of totipotent target cells, is the undesirable recovery of tissue culture-induced genetic variation in regenerated plants. Tissue culture-induced changes undoubtedly contribute to reduced plant regeneration frequency and plant fertility. Furthermore, the duration of the capacity of a tissue culture to regenerate fertile, normal plants is critical to the successful development of a transformation system. Extensive characterization of cytogenetic, qualitative, and quantitative genetic changes observed in progeny of oat plants regenerated from tissue culture has been conducted (McCoy et al. 1982; Dahleen et al. 1991; Rines et al. 1992). The frequency of cytogenetic variation in regenerated plants varies with genotype and increases with culture age. Although no studies have been conducted which examine the frequency of culture-induced genetic variation in plants regenerated from friable, embryogenic callus of the GAF and GAF/Park lines, the frequency of tissue cultures producing fertile plants declines as culture age increases. Friable, embryogenic oat callus has been shown to regenerate fertile plants for longer than 2 years (Bregitzer et al. 1989). However, in the current transformation system, newly initiated callus is used only for 3 to 12 months following initiation in an attempt to minimize culture-induced variation and maintain regenerated plant fertility. Use of these younger tissue cultures results in regeneration of plants from about 36 to 73% of transgenic tissue cultures (Tables 1, 2). Plants produced from about 50% of the plant-regenerating tissue cultures are fertile. Fertility among regenerated plants varies from tissue cultures that produce