I.1 Somatic Embryogenesis in Wheat

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1 Introduction

Wheat is the most intensively bred species in the world. It is second to rice in world production, which in recent years has approached 550 million metric tonnes per year (Young et al. 1990). The bread wheats (Triticum aestivum L.) are divided into four main categories, based on the protein content of the grain. Hard red spring (11 to 18% protein) and winter (9 to 15% protein) wheats are used primarily for bread. Soft red winter (8 to 12% protein) and white (8 to 11% protein) wheats are used primarily for muffins, noodles, cakes, crackers, and cookies. Durum wheat (Triticum durum L.) is used primarily for macaroni and pasta. While an extremely adaptive crop, wheat is still subject to many diseases, which makes genetic engineering an appealing biotechnology for wheat improvement.

Somatic embryogenesis is used in wheat improvement programs via (1) transformation protocols (Vasil et al. 1992; Weeks et al. 1993); (2) generating somaclonal variants (Hashim et al. 1990); (3) cloning sterile derivatives from wide hybridization (Tabaeizadeh and Campeau 1992); and (4) encouraging chromosomal translocations, eliminations, and other aberrations in sterile or fertile derivatives obtained from wide hybridization (Larkin et al. 1990). The use of somatic embryogenesis in transformation protocols is appealing because thousands of unique transgenic wheat plants (obtained by insertion of genes at different loci) could potentially be produced. Such plants could then be screened for agronomically important temporal and/or spatial variation in transgenic expression. While transformation has been accomplished (Vasil et al. 1992; Weeks et al. 1993), poor efficiency in maturing and germinating somatic embryos is a serious impediment to the mass production of somatic embryogenesis-based transgenic plants.

Aberrant somatic embryos of wheat were first recognized in tissue cultures by Ahloowalia (1982), and Oziash-Akins and Vasil (1982). Numerous reports followed these early studies and have been reviewed (Carman and Campbell 1990; Scott et al. 1990). This chapter summarizes early findings, reviews recent studies, presents concepts amenable to further experimentation, and provides a brief protocol for somatic embryo production and germination.

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2 Somatic Embryogenesis

The frequency of somatic embryogenesis in wheat is affected by a number of factors discussed below.

2.1 Donor Plants and Explants

Genotypic variation in frequency and intensity of embryogenic responses from various wheat explants is well documented (Carman and Campbell 1990; Scott et al. 1990). It is perhaps best described as variation in genetic plasticity for embryogenic competence such that poorly responding genotypes respond favorably under a narrow range of explant and medium conditions, while superior genotypes respond favorably under a broad range of explant and medium conditions (Carman et al. 1988; Carman 1990; Redway et al. 1990b).

Kaleikau et al. (1989a,b) crossed highly regenerative wheat lines and monosomic, ditelosomic, and compensating nullisomic-tetrasomic wheat lines to study the genetic basis for embryogenic competence. Segregation ratios suggested that a major regeneration gene is located on chromosome 2D and that minor regeneration genes are found on 2A and 2B. Previously, Galiba et al. (1986) showed that genes on wheat chromosomes 7B, 7D, and 1D are important to regeneration, and Lazar et al. (1987) reported that regeneration from callus cultures was enhanced when wheat addition lines contained rye chromosomes 6 or 7.

Breadth of genotypic plasticity for embryogenic response is more fully experienced when donor plants are grown under favorable conditions. Variation in growth conditions may cause embryogenic responses from immature embryo explants to range from 5 to 50% for individual, poorly responding genotypes and from 60 to 100% for individual, superior genotypes. Growth conditions that decrease the frequency of embryogenic responses include heat or water stress, fungal or insect infestations, poor lighting, or poor plant nutrition. In general, we have found that the percentage of favorably responding explants from greenhouse-grown plants occurs in the winter when plants are produced under supplemental lighting. In contrast, our poorest results occur when such plants are produced in the summer, when it is difficult to maintain moderate daytime greenhouse temperatures (J.G. Carman, unpubl.).

Donor plant growth conditions affecting explant competence are poorly understood. We have evaluated effects of temperature. Two sets of plants from each of two wheat lines, a poorly responding line (Yaqui 50) and a superior line (PCYT 10), were grown in growth chambers at constant temperatures of 15 and 25 °C, when donor plants were grown at 25 °C, Yaqui 50 and PCYT 10 calli produced 105 and 309 somatic embryos per g (fr. wt.), respectively. These numbers were 258 and 676, respectively, when the donor plants were grown at 15 °C. Hence, continuous, moderately high temperatures during donor plant growth contribute to poor embryogenic response. Endogenous hormone levels in wheat caryopses during embryo differentiation were not strongly correlated with the donor plant temperature treatments or subsequent embryogenic responses (Hess 1992).