I.13 Regeneration of Plants from Protoplasts of Trititrigia (Triticum sect. trititrigia)

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1 Introduction

Trititrigia (Triticum sect. trititrigia) is an artificially produced perennial wheat from the hybridization of durum wheat (T. durum, 2n=28) and intermediate wheatgrass (Elytrigia intermedia=Elymus hispidus=Agropyron intermedium=Thinopyrum intermedium, 2n=42), which both belong to the tribe Triticeae in the family Gramineae. Durum wheat, the main tetraploid type of wheat, is mainly grown in relatively drier regions, embracing the Mediterranean basin, India, the former USSR, and the low rainfall areas of the great plains in the USA and Canada (Feldman 1976).

Intermediate wheatgrass is a tall-growing grass with moderately vigorous creeping rhizomes, and cultivated as a useful forage in temperate regions, particularly during the relatively cool periods of spring and early summer. As a member of the tribe Triticeae, it is related to cultivated cereal crops including wheat (Triticum spp.), barley (Hordeum spp.), rye (Secale cereal L.), and forage grasses such as Elymus and Sitanion. Elytrigia intermedia gains the high regard of plant breeders because it carries genes for resistance to wheat streak mosaic and barley yellow dwarf virus (Brettell et al. 1988), resistance to the three major rusts of wheat (Cauderon et al. 1973), and salt tolerance (Dewey 1960; McGuire and Dvorak 1981). Salt-affected lands cover about 950 million hectares of the earth’s surface (Shannon 1982); therefore, the significance of incorporating desirable genes from wheatgrass genome into wheat or other cultivated crops cannot be overemphasized.

Although kinds of gene transfer such as from wheatgrass into wheat are accessible via sexual hybridization, those into phyllogenetically unrelated crops like maize or rice are difficult of access by the conventional method. Recently, highly developed biotechnology including gene manipulation and somatic hybridization, has proved powerful in the transfer of genes between unrelated organisms. However, culture of the protoplasts in Triticum species remained recalcitrant in plant regeneration until recently when Vasil et al. (1990) and

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Sun et al. (1990) in wheat, and then Wang et al. (1990) in trititrigia found the breakthrough.

This chapter describes the regeneration of soil-grown plants from the protoplasts isolated from cell suspension cultures of trititrigia, a perennial wheat which plays an important role in wheat breeding.

2 Establishment of Embryogenic Cell Suspensions

The embryogenic cell suspensions were established from a friable embryogenic callus of trititrigia, a hybrid of *Triticum durum* Desf. (2n=28) and *Elytrigia intermedia* (Host) Nevski (2n=42). The original embryogenic callus, induced from the immature inflorescence of trititrigia on MS (Murashige and Skoog 1962) plus 2 mg/l 2,4-D, was compact and nodular and showed green spots on its surface (Fig. 1a). When placed into liquid suspension culture media, it failed to grow and finally died. Eighteen months later, to improve its quality for suspension culture, the callus was transferred to CIM medium (Table I) and incubated at 26 °C day and 22 °C night with a 9/15-h light/dark photoperiod under 1300 lx, and subcultured at 15–20 day intervals. After 4 months of selective subcultures, a friable, fast-proliferating and totipotent callus without green spots (Fig. 1b) was selected.

From this callus, a fine homogeneous cell suspension was established in S4 medium (Table I). At the beginning, about 1 g of the callus was placed into 30 ml of S4 medium in a 100-ml flask, and cultured on a rotary shaker at 125 rpm at 26 °C in dim light with a subculture cycle of 4 days. After 3 months of selective subculture, a finely dispersed and embryogenic cell suspension (Fig. 2a) was finally established. The majority of the embryogenic cell clusters were composed of 100–200 spherical and cytoplasmic cells. The growth curve (Fig. 3) without subculture showed that the suspension cultures doubled within the first 3 days in the liquid medium as measured by the increase in the fresh weight of the medium-removed cells, the initial 0.669 g of the suspension cells in 50 ml of medium proliferated continuously for 18 days, and finally grew to 8.755 g, which was

![Fig. 1a,b. Two types of embryogenic callus induced from immature inflorescences of trititrigia, observed under a dissection microscope. a The compact and nodular callus with green spots (arrow heads) b The friable and fast-proliferating callus selected from the compact type of callus. Bar 571 µm. (Wang TB, unpubl.)](image-url)