1 Introduction

Mutants resistant to chemical stress such as antibiotics, amino acid analogs, and phytotoxins, are considered to be excellent materials for research on plant cell genetics. To obtain such mutants, it has been shown that selection of mutants in vitro is a very efficient method, because large cell populations can be screened easily and the cell culture itself produces genetical variability (Larkin and Scowcroft 1981; Sun et al. 1983; Oono 1984). However, there are some problems in the cell culture method to maintain the mutant cell lines or to obtain whole plant mutants: the regenerative capacity of cultured cells is lost with increasing time in culture (Widholm 1977), instability in the chromosome number is frequently observed in cultured cells or regenerated plants (Karp et al. 1982), and variant traits selected from the cultured cells are not always expressed in the whole plants (Kool 1982; Miflin et al. 1983). An alternative method, the selection of mutants from mutagenized population, has been proposed as a practical method for establishing a genetic marker useful both in the whole plant system and in the cultured cell system (Hasegawa and Inoue 1983; Miflin et al. 1983).

Hydroxy-L-proline (Hyp), a proline analog, exists in the plant cell wall as a constituent of glycoprotein (Sadava and Chrispeels 1971; Roberts 1979; Hood et al. 1988), but it severely inhibits the growth of both the seedling and the callus (Cleland 1963; Widholm 1976; Kueh and Bright 1981; Hasegawa and Inoue 1983). Mutants resistant to Hyp have been selected from mutagenized M2 population in barley (Kueh and Bright 1981, 1982) and in rice (Hasegawa and Inoue 1983). In potato, Hyp-resistant plants were regenerated from Hyp-resistant callus (van Swaaij et al. 1986, 1987). Hyp-resistant cell lines were also isolated in carrot (Widholm 1976). Furthermore, cell lines resistant to azetidine-2-carboxylic acid, another proline analog, were isolated in carrot (Nielsen et al. 1980), in Nicotiana sylvestris (Breiman et al. 1982), in rice (Nielsen et al. 1986), and in Nicotiana plumbaginifolia (Ye et al. 1987).

In higher plants, proline analog-resistant mutants can be a useful material not only for research on proline biosynthesis, but also for the study of stress resistance, because proline is proposed to play an important role in plants grown under stress. Free proline accumulation is frequently observed in plants under stress. Evidence

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that proline-accumulating mutants or cell lines themselves show salt or freezing resistance has been demonstrated in some plants (Riccardi et al. 1983; van Swaaij et al. 1986, 1987).

In this chapter, we describe the selection technique of rice mutants resistant to Hyp from an M₂ population. Biochemical and genetical characteristics of the selected mutants both at the whole plant and callus levels are also described. (For more information on various types of mutants in cell cultures of rice, see Chap. VI.2, this Vol.).

2 Isolation of Hyp-Resistant Mutants

In rice, the growth-inhibiting effect of Hyp and the selection technique for isolating Hyp-resistant mutants were demonstrated by Hasegawa and Inoue (1983). In a rice variety Nipponbare (japonica type), seedling growth was inhibited by Hyp at the concentrations of 5 × 10⁻⁵ M or higher. Presoaking the seeds in distilled water increased the extent of the inhibition. From these results, it is recommended that for the efficient mutant screening the seeds presoaked in water for 4 days should be cultured in the presence of Hyp at the concentration of 2 × 10⁻⁴ M or higher.

Actual mutant screening was carried out as follows. The M₂ seeds mutagenized by gamma-rays, ethylene imine (EI), ethyl methanesulfonate (EMS), and sodium azide (NaN₃) were allowed to germinate in distilled water for 4 days and then the germinated seeds were transferred to the culture in the presence of 2 × 10⁻⁴ M Hyp. After culturing with Hyp for 14 days, seedlings grown, as well as those cultured with distilled water, were selected (primary screening, Fig. 1A). The selected seedlings were cultured with nutrient solution plus 2 × 10⁻⁴ M Hyp for 14 days or more. Until this stage, well-grown seedlings were selected as resistant variants (secondary screening, Fig. 1B). From about 90,000 seedlings screened, 27 resistant variants were selected (Table 1). Of the selected variants, 25 produced the M₃ seeds.

In M₃ seeds obtained as an M₂ plant progeny, Hyp resistance was examined as in the M₂ seeds. Of the progenies of 25 variants, one did not show Hyp resistance in M₃. Finally, 24 variants selected in M₂ were identified as real Hyp-resistant mutants and the mutant lines (HYP lines) were established.

Table 1 also shows the comparison of effectiveness of mutagens in inducing Hyp-resistant mutation. EI induced most of the mutants (22 out of 24). The frequencies for Hyp-resistant mutants in M₂ were 2.5 × 10⁻⁴ and 1.6 × 10⁻³ per M₂ seedlings for 0.2 and 0.4% EI for 2 h, respectively, and were about one-tenth of those for chlorophyll mutations. EMS and NaN₃ each produced one mutant, although considerable amounts of chlorophyll mutants were obtained. No real mutants were obtained from the population mutagenized by gamma-rays.