1 Introduction

Using cold test methods, the germination, emergence, and percentage emergence were studied in cold soil or at room temperature following cold treatment (Kovács 1961; Pesev 1970; Herczegh 1978; Eagles and Hardacre 1979; Szundy and Kovács 1981a,b; Eagles 1982; Stamp 1984; Nagy et al. 1984). The soil usually originated from a field of maize monoculture so that the conditions in the experiment could be close to those in the field. This means that the seeds and embryonic plants were affected simultaneously by cold and by the pathogenic microorganisms of the soil. This explains why the differences between hybrids were less than within hybrids (Rush and Neal 1951). In an experiment of this type, one cannot decide the effect of cold only. Dickson (1923) reported that maize seeds and seedlings were destroyed by Gibberella saubinetii (Mont.) Sacc. at 8 °C, but seeds emerged without damage at 24 °C. This condition after treatment at 8 °C resulted in low physiological activity in seeds and embryos; they were also attacked by fungi for a long time. The development of maize seeds after cold treatment was determined by the interaction between pathogen fungi and temperature and not by the maize genotypes. The effects of cold treatment on maize leaves were studied by Baker et al. (1983) and Long et al. (1983). The degree of damage was greater at a lower temperature and after a longer period of cold treatment. After cold treatment, leaves photosynthesized at 20 °C less effectively than the control at 20 °C. Creencia and Bramlage (1971) noted that the growth of cold-treated plant leaves is slower at 21 °C than in the control. The primary roots of maize grown in nutrient solution at 5 °C elongate at about 1% of the rate found at 20 °C but cell proliferation is still possible at 5 °C, and the maximum elongation rate achieved by roots during the recovery period was less than that of roots which had not experienced cooling (Barlow and Adam 1989). Leaves and roots in general show damage after a cold period, but not enough data are available in the literature on embryonic plants.

The effects of low temperature on emergence time were studied by Miedema (1979) on 21 maize genotypes. The emergence time was in significant correlation with the dry matter of the shoot. Contrary results were obtained when the cold tolerance of 34 inbred lines was studied by Mock and McNeill (1979) in a field
experiment. The cold tolerance of maize seedlings was not correlated with their vegetative vigor. The in vitro growth of seedlings at 13 °C was studied by Christeller (1984) in embryos dissected from maize seeds. This method was capable of showing genetic differences in maize seedling growth. There were no significant differences between the growth of embryos in intact seeds and embryos growing in culture media at 13 °C. This means that the results originating from in vitro sterile embryo culture can be used to understand the cold tolerance of the embryonic growth of maize lines under cold conditions. The question arises as to how maize seedlings from a maize line assortment and A188 maize tissue cultures react to cold treatment near 0 °C under sterile conditions without pathogens. Spontaneous somaclonal variation in plants recovered from tissue culture has been documented for many species (see Bajaj 1990). Variability among cultured cells may result in positive and negative changes in many agronomic traits. But is this also true of cold tolerance? Does somaclonal variation provide plant breeders with new sources of genetic variation for cold tolerance?

In this chapter three topics are described:

1. Study of some selected lines containing good, medium, and poorly cold-tolerant genotypes in sterile embryo culture. (a) Measuring growth of embryonic plants at 13 °C; (b) measuring tolerance to cold treatment at 4 °C during growth at 13 °C.
2. Study of the reaction of A188 genotype tissue culture to cold treatment at 4 °C.
3. Study of some cold tolerance traits in Sc, somaclones originating from A188 inbred line calli in field experiments in early sowing.

2 Material and Methods

Experiments were performed in the phytotron and experimental field of the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár.

Cold Tolerance in Seedlings. In embryoculture 15 inbred lines were used: 156, A619, B37, B73, B125, C103, C123, Mo17, N6, OH509A, SzG25, TSPT, W64A, W117, and W153R. Cobs were collected 6 weeks after self-pollination, and the seeds were disinfected with 5% sodium hypochlorite for 5 min and 0.1% HgCl₂ for 3 min in a laminar flow sterile chamber. During and after the disinfection steps, the seeds were rinsed three times in sterile water. Embryos were dissected from the seeds and ten embryos were put on 30 ml Murashige and Skoog (1962) medium containing 3% sucrose without hormones, the scutellum side being placed down; 20 embryos were studied in each of the four replications. Seedlings were grown for 4 weeks at 13 °C in the dark in a G-30 type Conviron chamber. This time growth differences between genotypes were noticeable. If the temperature was not low enough and the periods used not long enough, the treatment was less effective in discriminating between genotypes for differences in growth in cool environments (Hardacre and Greer 1989). The material was