II.7 Cryopreservation of *Hordeum* (Barley)

JUN-HUI WANG and CHUN-NONG HUANG

1 Introduction

1.1 Distribution and Important Species

Barley is the fourth most important cereal after wheat, rice and maize. Although its distribution is generally similar to wheat, barley can be grown in much drier and colder regions than wheat. It is distributed mainly over the middle latitudes of the earth especially of the northern hemisphere. Russia, Canada, United States, some European and Asian temperate countries are the world's leading producers of this crop.

The genus *Hordeum* is in the Triticeae tribe in the Poaceae and contains about 30 species. Only three subspecies (*distichon*, *hexastichon* and *intermedium*) of *H. vulgare* L. are cultivated. *H. spontaneum* and *H. argriocrithon* are their wild relatives. Among the other wild forms, *H. bulbosum* L., *H. murinum* L., *H. marinum* Huds., *H. brachyantherum* Nevski., and *H. brevisubulatum* (Trinius) Link are comparatively important in agriculture and genetics. For instance, *H. bulbosum* is employed to produce haploid barley plants. Wild species are annual or perennial, with chromosome number of 2n = 14, 28 or 42, and show many valuable traits in stress tolerance and disease resistance, which may be used to improve cultivated barley.

1.2 Routine Methods for Germplasm Conservation

Both cultivated and wild species have a vast number of varieties, which grow over broad environmental ranges. More than 1600 ecological or genetic accessions have been collected just for *H. spontaneum* (von Bothmer et al. 1992). The China National Academy of Agriculture together with some provincial subunits have collected 13,116 germplasm accessions of this plant (Gao and Ma 1996). However, a very limited number of cultivated varieties are adopted in modern agriculture, while others that may be of less importance at present are underutilized and may be completely lost. Resources of wild species are being eroded with expanding industrialization and environmental alteration. Therefore, germplasm conservation of this plant is increasingly important for...
its biodiversity. In recent years, some Triticeae species have become endangered despite the fact that their seeds are orthodox like barley. *Elymus foliosus* is no longer in existence, while *Elymus grandis* and *Psathyrostachys huashanica* are threatened with extinction (Lu 1995).

Barley seeds are desiccation-tolerant and can be stored by routine approaches. Barley seeds have been used to study longevity and equations have been developed to predict longevity under various conditions of temperature and moisture content (Ellis and Roberts 1980). Under conditions recommended for use in genebanks (ca. −18°C), longevities can be estimated to be many decades. However, there are few experimental data for such conditions. Practically speaking, in jars or pots with some desiccants, they can retain their germination capacity for 5–8 years under ambient temperatures, whereas in modern air-conditioned seed banks, the duration may reach about 30 years (Gao and Ma 1996). However, like other cereal seeds, barley seeds are very susceptible to mildew during room temperature storage if they are not dried sufficiently.

### 1.3 Necessity of Cryopreservation

Cryopreservation is an attractive technique for long-term conservation of plant germplasm. This technique has several unique advantages such as providing great longevities and enhanced safety. Reviews (Bajaj 1976, 1979; Withers 1978, 1987; Sakai 1986; Steponkus 1985; Engelmann 1991; Steponkus et al. 1992; Blakesley et al. 1996; Wang and Huang 1998b) and books (Kartha 1985; Bajaj 1995) detail preservation of plant materials at cryogenic temperatures (ca. −130 to −196°C).

Cryogenic storage not only benefits long-term storage of both desiccation-tolerant and desiccation-sensitive seeds, but also is important in plant biotechnology. It may aid germplasm preservation of vegetatively propagated plants, maintain the morphogenetic potential of cultured cells, and facilitate regeneration from young explants. Most transgenic cereal plants are obtained via transformation of immature embryos (Christou et al. 1991; Vasil et al. 1993; Ritala et al. 1994; Wan and Lemaux 1994), whose developmental stages restrict the efficiency of experiments.

Regeneration of green plants from barley suspension cells and protoplasts was less efficient compared with other major cereals and it was not until the 1990s that significant advances were attained (Jähne et al. 1991; Yan et al. 1991). However, the genotypic specificity and reproducibility of protocols developed in individual laboratories remain problems. Protoplast isolation and subsequent plant regeneration can be achieved from primary calluses that are derived from immature embryos or microspores (Kihara and Funatsuuki 1995; Salmenkallio-Martilla and Kauppinen 1995; Stöldt et al. 1996). So far, establishment of protoplast-producing and embryogenic barley cultures is still a time-consuming job (Singh et al. 1997).

Cultured barley cells often lose totipotency drastically and also produce a high ratio of albino regenerants (Wang et al. 1992; Huang et al. 1993). It is