



Research review paper

Potential applications of cryogenic technologies to plant genetic improvement and pathogen eradication



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ABSTRACT

Rapid increases in human populations provide a great challenge to ensure that adequate quantities of food are available. Sustainable development of agricultural production by breeding more productive cultivars and by increasing the productive potential of existing cultivars can help meet this demand. The present paper provides information on the potential uses of cryogenic techniques in ensuring food security, including: (1) long-term conservation of a diverse germplasm and successful establishment of cryo-banks; (2) maintenance of the regenerative ability of embryogenic tissues that are frequently the target for genetic transformation; (3) enhancement of genetic transformation and plant regeneration of transformed cells, and safe, long-term conservation for transgenic materials; (4) production and maintenance of viable protoplasts for transformation and somatic hybridization; and (5) efficient production of pathogen-free plants. These roles demonstrate that cryogenic technologies offer opportunities to ensure food security.

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Sustainable development of agriculture

Requirements and necessary actions

The global human population was only about 3.3 billion 45 years ago, but is now more than 7 billion and is expected to exceed 9 billion by 2050 (Braun, 2010). Such a rapidly increasing population has resulted in considerable challenges to the world food security. Significant increases by approximately 70% in food production are necessary to meet the future demand (Braun, 2010; Germanà, 2011). Sustainable development of agricultural production by breeding more productive cultivars using both traditional breeding (Yuan, 2001) and genetic transformation (Qaim, 2010), and by increasing the productive potential of existing cultivars through the use of pathogen-free plants (Waterworth and Hadidi, 1998) will hopefully meet this demand.

Access to diverse plant germplasm is a prerequisite for breeding more productive cultivars (Ronald, 2011). However, the rapid loss of habitats and ecological changes, mainly due to urbanization, industrialization, salinity and desertification (Jackson and Kennedy, 2009; Ronald, 2011), have been/are causing the extinction of plant species at an alarming rate (Esquinas-Alcázar, 2005; Jackson and Kennedy, 2009). Global warming, which has increased the mean temperature of the Earth's surface by approximately 0.8 °C from the early 1900s to 2011, with an increase of about 0.6 °C in the last 30 years (America's Climate Choices, 2011), has further worsened the situation (Jackson and Kennedy, 2009). It is estimated that about 50,000 plants, accounting for more than one-fourth of all plant species in China, are at present time endangered or facing extinction (Huang, 2010). This loss in valuable germplasm limits the materials available to breeders for use in breeding programs (Esquinas-Alcázar, 2005). Conservation of diverse genetic resources will assure valuable genes are available for breeding new cultivars, thus enhancing food security (Wang et al., 2012a).

Traditional breeding

Traditional cross breeding has played a central role in increasing the yields of crops (Yuan, 2001). For example, rice (*Oryza*), the largest staple crop globally, is the major food source for more than half of the world's population (Zhang et al., 2010). Poor rice production has long threatened food security in China, which has the largest population in the world with more than 1.3 billion people. Breeding programs have increased rice yield and productivity since 1996 (Zhang et al., 2010). In 2013, a new global record for rice yields was set at 14.8 t/ha using the 'super' hybrid rice 'Y liangyou 900' (China Daily, 2013). This yield was more than 3 times that of traditional rice cultivars used 20 years ago and may improve food security not only in China, but also in the world (Zhang et al., 2010).

Genetic transformation

Newer biotechnology approaches, such as genetic transformation, offer a complementary strategy to conventional breeding programs (Qaim, 2010). Since the first report on transgenic plants was published in the early 1980s (Zambryski et al., 1983), various transgenic plants have been obtained, including those that are resistant or tolerant to

herbicides (Schahczenski and Adam, 2006), insect pests (Qaim, 2010), diseases (Delteil et al., 2010; Harfouche et al., 2011), low temperatures (Guo et al., 2009; Harfouche et al., 2011), drought (Harfouche et al., 2011), and salinity (Chen and Polle, 2010; Harfouche et al., 2011), as well as possessing an improved nutrient quality (Ye et al., 2000). The first commercial cultivation of genetically modified (GM) crops was in 1996 (Qaim, 2010). Since then, areas used to grow GM crops have consistently increased at unprecedented rates and covered 134 million ha worldwide by 2009 (ISAAA, 2009; James, 2010). Cultivation of GM crops has brought great benefits to the farmers by increasing the yield and at the same time reducing the costs (James, 2010), and also has the potential to improve the nutritional content of foods, such as rice (Ye et al., 2000).

Production of pathogen-free plants

Plant pathogens such as viruses can be transmitted from generation to generation in vegetatively propagated staple crops such as potato, sweet potato and cassava, and horticultural crops such as banana, apple and citrus (Wang et al., 2011; Waterworth and Hadidi, 1998). Pathogen-induced diseases have for a long time threatened the sustainable production of agricultural and horticultural crops (Wang et al., 2011; Waterworth and Hadidi, 1998). Experimental data showed that total tuber yield and marketable tuber yield (tubers > 85 g) of plants derived from *Potato leafroll virus* (PLRV)-infected seed tubers decreased by at least 60% and 88%, compared with those from healthy tubers (Hamm and Hane, 1999). *Potato virus Y* (PVY) infection of seed tubers caused a 49% and 65% reduction in total and marketable yields as compared with healthy plants (Hane and Hamm, 1999). An epidemic occurrence of papaya dieback in 2002, a phytoplasma-induced disease, led to total crop failure in south-east Queensland and Western Australia (Streten and Gibb, 2006). Citrus Huanglongbing (HLB), a gram-negative bacterium-induced disease, widely spread in most of the citrus-growing areas in Asia, Africa, and in both North and South America, has been recognized as the most damaging disease in the global citrus industry, and kills many thousands of citrus trees every year (Bovè, 2006). In practice, the use of pathogen-free plants is now an effective method to control these pathogen-induced diseases (Wang et al., 2011; Waterworth and Hadidi, 1998).

Cryopreservation for vegetatively-propagated species

Conventional conservation

Field genebank and in vitro conservations (Reed et al., 2004a) are among the major conventional methods used to conserve plant species that are vegetatively propagated or that have seeds that are not amenable to seed storage techniques. Field genebanks are expensive to maintain due to the high costs of labor and land (Reed et al., 2004a; Sakai and Engelmann, 2007). Furthermore, field-conserved plant genetic resources are threatened by attacks of insects and pathogens, and by natural disasters such as extremely low or high temperatures and drought, which may result in massive plant germplasm losses (Reed et al., 2004a; Sakai and Engelmann, 2007). In vitro conservation is labor intensive, requires specialized skills and techniques, and is subject to the risk of

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