African Journal of Biotechnology Vol. 9 (52), pp. 8955-8957, 21 December, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Short Communication

Cryopreservation of embryonic axes of maize (*Zea mays L.*) by vitrification protocol

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Accepted 27 April, 2010

A storage protocol at cryogenic temperature was established for embryonic axes of maize using a basic vitrification protocol with direct immersion in liquid nitrogen (-196ºC). The response of isolated embryonic axes of five maize genotypes to plant vitrification solution (PVS2) at different concentrations was studied. Recovery after cryopreservation ranges from 47.5% - 75% among genotypes. PVS2 at 50% and 100% concentrations gave comparable high recovery rate.

Key words: Cryopreservation, maize, embryonic axes, vitrification.

INTRODUCTION

Cryopreservation which refers to preservation in liquid nitrogen (-196 c) offers one of the best method for long-term preservation of plant genetic resources without change. There are several cryopreservation techniques available such as slow cooling, rapid freezing, vitrification and encapsulation-dehydration methods. The success of the first two protocols has been very limited as it seems to be genotype-specific, while the encapsulation-dehydration protocol is complicated, time consuming and produce low level of recovery growth (Zhao et al., 2001). The vitrification technique with or without encapsulation offers various advantages in terms of greater recovery and its simplicity as a protocol (Matsumoto et al., 1994, 2002).

Maize (Zea mays L.) is the third most important cereal crop in the world after wheat and rice (FAO, 2006). It provides about 20% of the world calories and 15% of all food crop protein (National Research Council, 1988). In Nigeria, maize is important as food stuff for the population and equally serves as feedstuff for livestock. Seed banking of maize at low temperatures and low moisture content usually allows the preservation of plant material for long period of time (Cromarty et al., 1982). However, loss of viability can occur with prolonged storage, depending on the species (Robert and Ellis, 1984). This suggests the need to explore other options like cryopreservation that could improve maize seed longevity and thus, improve the conservation of this crop. This study is conducted with the aim of developing a cryopreservation protocol for maize

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MATERIALS AND METHODS

Maize genotypes (ACR SY NY, DT SR CO, POOL 16 DT, TZE COMP3 AND TZL COMP1) were used in this study. Embryonic axes were aseptically excised from surfaced sterilized seeds. The embryonic axes were pre-cultured for three (3) days on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 0.7 M sucrose. They were then transferred to the different levels of plant vitrification solution (PVS2) as presented in table 1 for 30min.

The embryonic axes after treatment with the PVS2 were transferred to cryovials and immersed directly in liquid nitrogen (LN). After 1 h storage in LN (-196°C), samples were retrieve and thawed at room temperature. The samples were then rinsed with sterile washing solution and then cultured on MS medium with 0.7M sucrose and incubated under dark condition for 3 days. They were then transferred to MS medium and incubated under light to resume growth.

Embryonic axes in batches of 15 per genotype were treated with three (3) levels of the PVS2 and a control treatment was run through all the steps of the experiment but without PVS2 and freezing in liquid nitrogen. The experiment was repeated four (4) times. Take off was measured by counting the number of embryos that show signs of growth seven days after culturing, while survival and recovery were taken at 14 days after culturing by counting the number of embryos that survived and recovered as normal plants, respectively. The data collected was analyzed as means and

Table 1. The composition of PVS2 used in the study.

Component	0%PVS2*	50% PVS2	100% PVS2	150% PVS2
MS Basal salts	0.4%	0.4%	0.4%	0.4%
Sucrose	0.7M	0.7M	0.7M	0.7M
Ethylene glycol	0%	7.5%	15%	22.5%
Glycerol	0%	15%	30%	45%
DMSO	0%	7.5%	15%	22.5%

*Control.

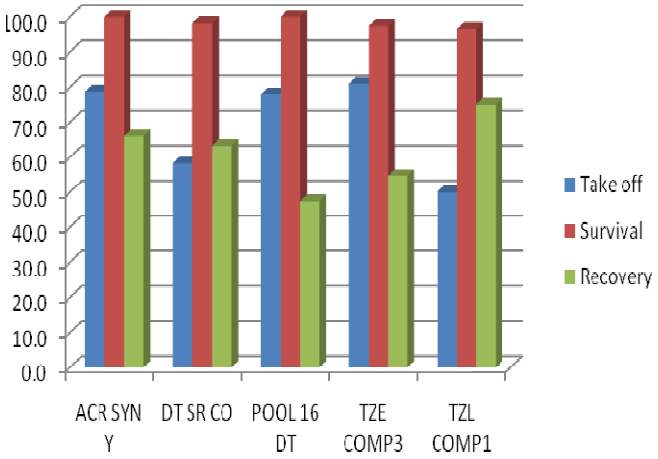

Figure 1. Response of maize genotypes to vitrification treatments.

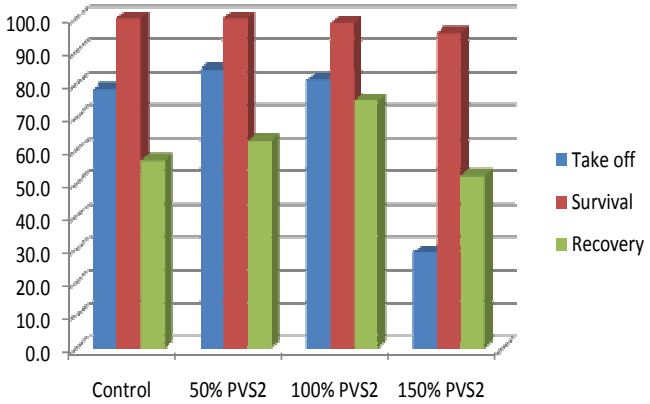

Figure 2. Effect of plant vitrification solution (PVS2) treatment on takeoff, survival and recovery of maize embryo.

percentages.

RESULTS AND DISCUSSION

Results obtained from this study indicate the genotypes responded to the PVS2 treatment. TZL COMP1 had the highest percentage recovery (75%) followed by ACR SYN Y and DT SR CO, which were similar to TZE COMP3. The lowest recovery of 47.5% was recorded by POOL 16 DT (Figure 1). The effect of the different concentration of the PVS2 was significant. Take off embryo axes was hindered by 150% PVS2 treatment (Figure 2). Similarly the recovery of normal plants was lowest at 150% PVS2. In this study both take off and recovery were optimum at 50% and 100% PVS2. In this study, embryonic axes of maize survived even the highest level of PVS2 treatment. This suggests that using either 50% or 100% PVS2 is ideal for cryopreservation of maize germplasm.

For successful cryopreservation, it is essential to avoid lethal intracellular freezing which occurs during rapid cooling in liquid nitrogen. Thus, specimens to be preserved have to be sufficiently dehydrated to avoid intracellular freezing and thus, vitrify upon rapid cooling. Vitrification is a transition phase of an aqueous solution from a liquid into an amorphous glassy solid or glass at the glass transition temperature while avoiding ice crystallization. It is reasoned that the high recovery and survival recorded in this study is associated with the dehydration of the embryos by pre-culture on high sucrose medium.

Conclusion

Embryo axes of elite maize genotypes were used as explants material. Dehydration and plant vitrification pretreatments prior to cryopreservation were studied to enhance recovery of normal plants after storage in liquid nitrogen. Maize embryos survived up to 150% PVS2 while both take –off and recovery were relatively better at 50% and 100% PVS2. The genotypes responded positively to the treatments. Results showed that the cryopreservation protocol by vitrification has the potential for improving the conservation of maize germplasm.

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