トウモロコシembryogenicカルスの超低温保存（予報）

UPADHYAY, M.P., M. ISHIKAWA, P. TANDON, Y. KUNIHIRO, K. OZAWA, K. NAKAJIMA (Nat'l Inst. Agrobiol. Res.) Improvement in frequencies of embryogenic calli and their cryopreservation by vitrification.

For easier maintenance of maize embryogenic calli (type II, friable), we attempted to improve the frequency of embryogenic calli by modifying subculture medium and to cryopreserve them by vitrification method. Earlier (Ohuchi et al 1991), plant regeneration was possible, but difficult to grow the cryopreserved calli on subculture media.

Embryogenic calli of *Zea mays* L. derived from immature embryos of a hybrid maize variety (A188 x B73 imbred lines) were maintained on semi-solid modified N6 (AA) medium supplemented with various compounds at a 10 day subculture intervals at 25°C in the dark. At each subculture, the rate of friable embryogenic calli was scored from 1 (>90%) to 9 (<20%) and the fine globular somatic embryos (friable calli) were selectively transferred to a new medium. For cryopreservation, 6-7 day old calli cut into 2-4 mm pieces were used either after preculture, after pretreatment or directly. Preculture consisted of serial transfers from 0.3, 0.5, to 0.7 M sucrose containing N6 medium (2 day each). Pretreatment was done by soaking them either in I-YC cryoprotectant (Ishikawa et al 1991: suc. 10%, DMSO 10%, Glyc. 5%) for 30 min or in 1/4 strength vitrification solution for 45 min followed by 1/2 solution for 15 min at 23°C. For vitrification, Sakai's method was followed with some modifications: 2, 3, 5, 10 min exposure at 23°C to either PVS2 or L or L+ (Tandon & Ishikawa unpublished).

The gowth media supplemented with 1 mM thioproline (with 2 ppm 2,4-D) favoured the growth of highly embryogenic calli (Fig. 2). They once turned whitish, but after several transfer, regained the normal appearance which was even better than media with 2,4-D alone (Fig. 1). The proportion of non-embryogenic calli and matured somatic embryos were higher when the growth media contained either 2 ppm MDBA or higher concentration of sucrose (6, 10% with 2,4-D at 2ppm)(Fig. 1). The filter sterilized modified N6 medium (2ppm 2,4-D) exhibited relatively poorer growth of embryogenic callus than the autoclaved medium.

Maturation of embryos was apparent with the increasing concentration of sucrose in preculture medium. Cryopreservation by vitrification tended to change the colour of the callus and slowed down their growth, however, the embryogenesity was fairly maintained. After regular transfers, the callus regained their yellow colour and the rate of growth (Fig. 3).

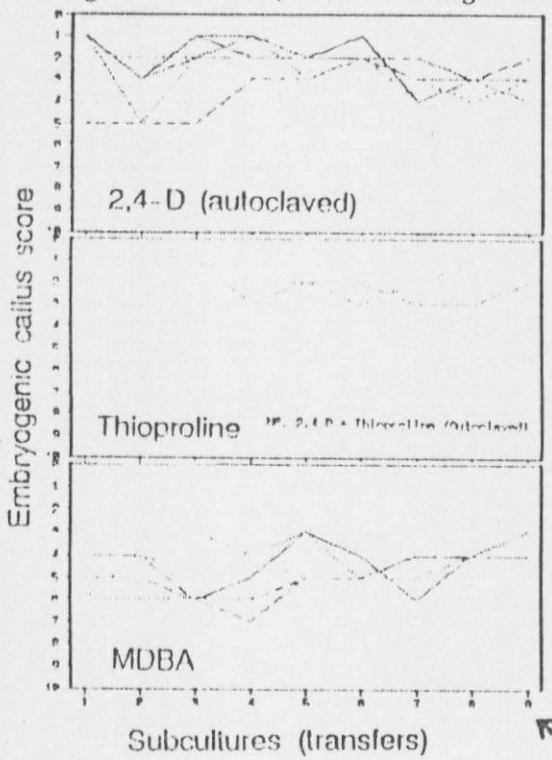

Fig. 1 Embryogenic callus score under various treatments.

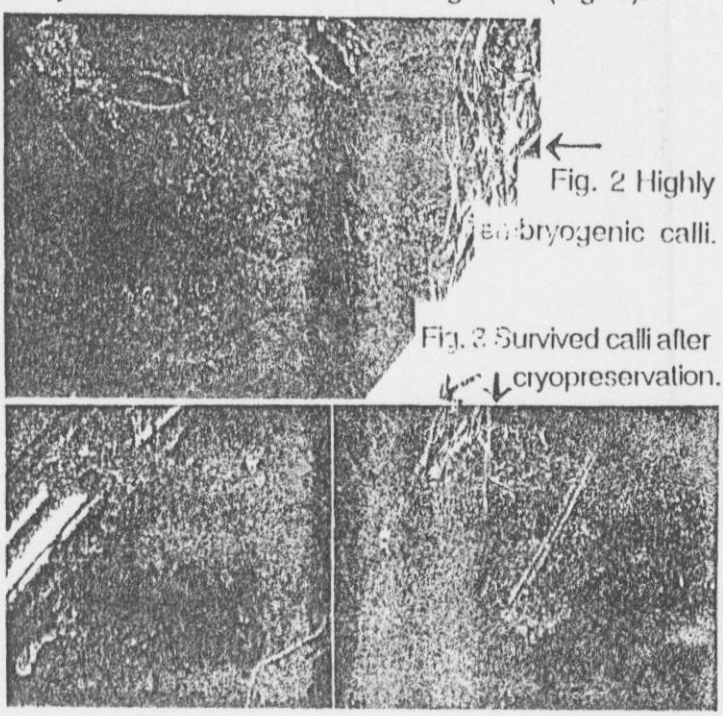

Fig. 2 Highly embryogenic calli.

Fig. 3 Survived calli after cryopreservation.