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IN-VITRO PRESERVATION OF POTATO (*SOLANUM TUBEROSUM* L.) GERMPLASM

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Abstract

Meristan tip culture of potato (*Solanum tuberosum* L.) were established on MS medium containing BA and NAA and multiplied on hormone free MS medium. Potato culture survival and growth was significantly influenced by storage period and the incubation temperature, whereas stress levels significantly affected shoot growth where its effect on survival was non-significant. A temperature of 25°C and longer storage period adversely affected the culture survival.

Introduction

Germplasm considered as an essential natural resource contain the genes that direct the development of living organisms. Different combination of genes give rise to the genetic diversity which form the basis for crop improvement (Paul, 1989). As germplasm collections continue to increase in size and number, there is need for efficient preservation of these plant materials in a manner that they are both secure from loss and yet available for use in crop improvement programmes (Jarret & Florkowski, 1990).

Grout & Henshaw (1978) stated that seed storage is applicable to many species as a method of conservation, but is less suitable for general use with potato, because of strong heterozygosity in many cultivars and various degree of sterility. Much of the potato germplasm is conserved by tuber propagation, which is not ideal since valuable accessions are exposed to a wide range of environmental hazards both in field and store. Similarly, Thieme (1992) has regarded *in vitro* conservation as a practicable alternative to field collection, especially for crop plants that can be vegetatively propagated such as potato. Westcott *et al.*, (1977) obtained successful storage of potato cultures for 6 month using minimal or enriched media and growth inhibitors.

At the National Agricultural Research Centre, Islamabad, *in-vitro* preservation laboratory of Plant Genetic Resources Institute (PGRI), was established with the objective to conserve the vegetatively propagated crops and fruit trees by the use of *in-vitro* preservation techniques. The present report describes the in-vitro preservation of potato using the minimal growth techniques.

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Materials and Methods

Single nodes of potato (*Solanum tuberosum* var. Desire Red were disinfected using 1% sodium hypochlorite solution and rinsed three times with sterile distilled water. The meristems of approximately 1.0 mm were excised with the aid of a dissecting microscope and cultured on Murashige & Skoog's (1962) nutrient medium containing 1.0 mg/l benzyl adenine (BA) and 0.05 mg/l naphthalene acetic acid (NAA) with a pH adjusted to 5.8. Cultures were incubated at 25 ± 2°C constant temperature with a 16 h photoperiod. MS modified medium was used for the multiplication of the established cultures without using any hormones. A single nodal cutting after 3-4 weeks was again subcultured to obtain a large number of plantlets.

Minimal growth medium was used for *in-vitro* preservation experiment by modifying MS medium with the addition of 0,1,2 and 3% w/v mannitol as osmotica. A single nodal cutting was placed per tube. Two temperature regimes of 17°C (as low) and 25°C (as high/control) were used as incubation temperature. Each treatment consisted of 22 replicates. The data at intervals of 0, 0.5, 1,2,3,4 and 6 months was recorded for percentage survival of the cultures and shoot growth (mm) as the percentage of growth over the initial length of explant. Data was analyzed using 3 factorial ANOVA for:

Factor A = 2 temperatures 17°C and 25°C,
Factor B = Osmotic stress 4 levels 0,1,2 and 3% w/v mannitol, and
Factor C = Storage period from 0-6 months.

Results and Discussion

In-vitro survival of the preserved potato plantlets declined significantly with the increase of storage period after 4 months. Osmotic stress (0-3% w/v mannitol) showed no significant effect on the plantlet survival, whereas the culture survival was significantly higher at the lower temperature regime. At 6 months period it declined to 62.5% at 17°C temperature regime and to only 33.8% at 25°C temperature regime (Fig.1). A reduction in temperature from 22 to 6°C resulted in an average survival rate of 29%. in shoot cultures of 6 *Solanum* spp. However, a switch to a cycling temperature regime of 12°C day/6°C night increased the average survival rate to 83% as reported by Westcott (1981).

The effect of temperature regime at various stress levels remained non-significant, whereas some encouraging interaction of these stress levels were observed after four months storage period. In mannitol free media, the survival at 25°C and 17°C was 57.14% and 81.82%, respectively, after six months. However, it may be noted that within each temperature regime survival was higher when cultures were not subjected to stress, even then the percentage survival of cultures with 3% osmotica at 17°C was higher (63.64%) than at 25°C with any stress level i.e., from 0 to 3% osmotica, indicating the suitability of lower temperature for obtaining higher survival of stored cultures (Fig.1). Le & Collet (1988) reported that they successfully cultured potato nodal cuttings in a solidified agar medium at a temperature of 2-4°C and with a photoperiod of 12h. All 20 cultivars under study were successfully stored for at least 12 months.

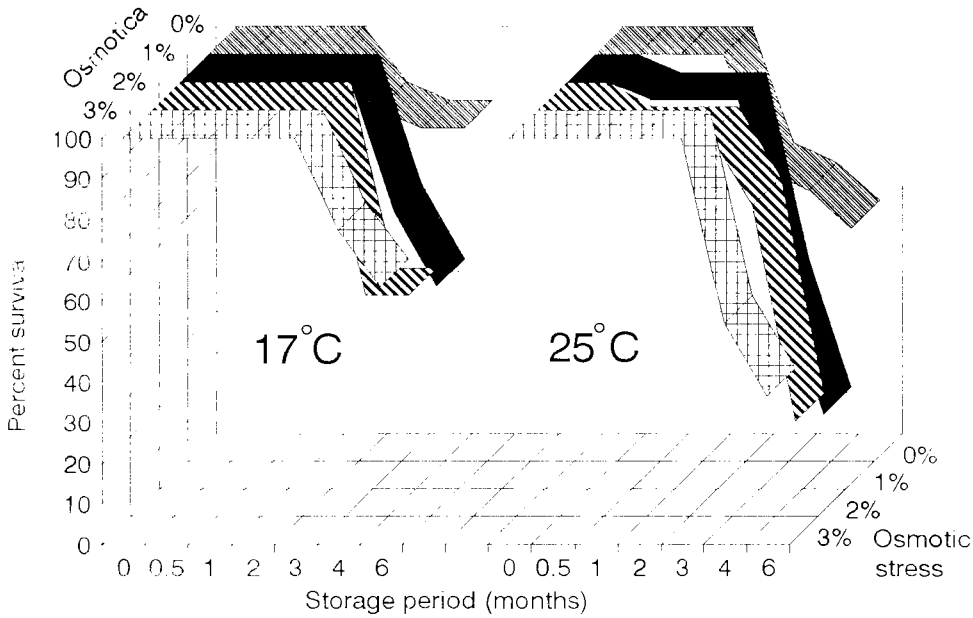

Fig.1. Effect of temperature and osmotic stress on the survival of *in-vitro* stored potato cultures.

The shoot growth was significantly reduced at the lower temperature regime 17°C as compared with 25°C. Similarly, the growth of shoots was also significantly reduced with increase in the osmotica (Fig.2), but the corresponding growth at each osmotic stress level was always found reduced at the low temperature regime. The plantlets showed significant increase in growth over the storage period and likewise the increase in growth was always found significantly reduced at the lower temperature. At every stress level the reduction in growth with the progression of storage period was more significant at higher osmotica, whereas the growth of control (0% mannitol) was at par with 1% mannitol. However, it was observed that reduction in growth 23.2% (2194 mm) due to lowering of temperature alone (0% osmotica) was more than the reduction in growth at 25°C and 3% osmotica 20.8% (2264 mm). The growth was only 1696 mm (40.65% reduction) in case of 17°C with 3% mannitol at 6 months period (Fig.2). The results would suggest that 17°C was more suitable for the storage of potato cultures *in-vitro* in medium containing either no osmotica as a high percentage of survival of 81.8% was obtained alongwith significant reduction of the plantlets shoot growth (except 3% osmotica with 17°C). There is need to study the conservation of shoot-tips at lower temperature at appropriate day length to confirm the findings of Lee & Collet (1988). Goodwin *et al.*, (1980) found that potato shoots cultured on solid medium without hormones produced aerial tubers about 12 weeks after inoculation. Similarly, in the present study also the cultures kept at 17°C without osmotic stress produced aerial stem tubers at a later stage which can also serve as storage organs. Schilde-Rentschler *et al.*, (1985), have also suggested that tubers produced *in vitro* can be used for potato germplasm storage and distribution.

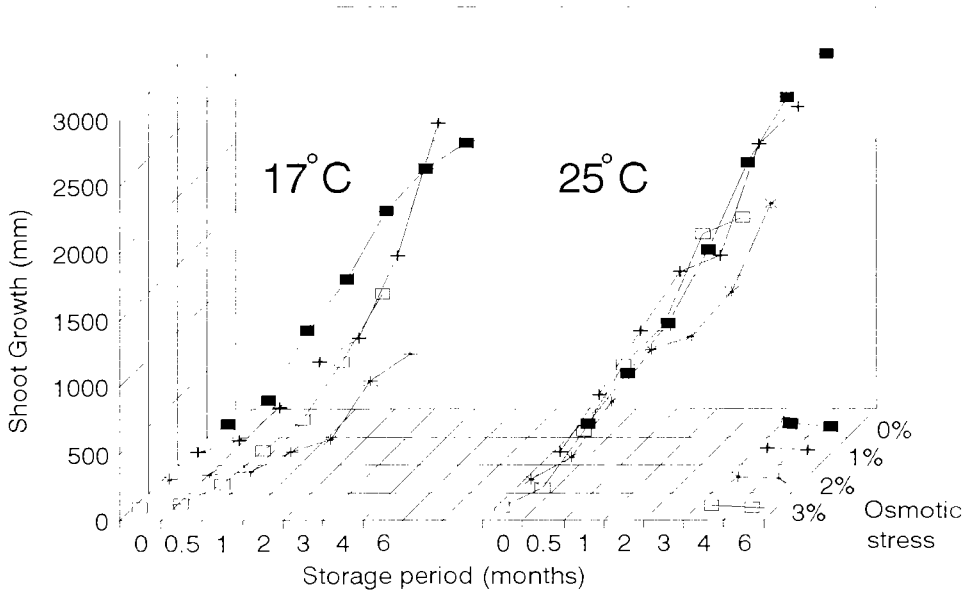

Fig.2. Effect of temperature and osmotic stress on the shoot growth of *in-vitro* stored potato cultures.

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