Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):41-42, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

"*In vitro*" medium term preservation of different romanian landraces

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ABSTRACT In order to conserve important sources of variety as the landraces collected of four economically, alimentary and agriculturally important culture species, we developed a cheap protocol of slowing plants growth that uses *in vitro* cultures and low temperatures. Thus, as nutrients generator we used MS without growth regulators medium in the presence or absence of sucrose. The cultures were incubated in two growth rooms differing by temperature 16°C and 24°C, respectively. The results obtained on MS without sucrose medium and at temperatures of 16°C consisted of decreasing the regenerative capacity from 17% to 39% and slow down plants growth between 42% and 85% (see table).
Acta Biol Szeged 49(1-2):41-42 (2005)

KEY WORDS

Capsicum annuum
Pisum sativum
Phaseolus vulgaris
Allium sativum
landraces
in vitro cultures
medium term preservation

The elaboration of some vegetal cells, tissues and organs conservation methods is necessary to preserve the germplasm sources in the conditions of progressive extension of monoclonal cultures or selected genotypes. Most attempts to store cultures have had dual objectives of stabilizing experimental material in terms of special characteristics and to avoid the demands and risks of subculture. The term "slow growth" is used to cover "growth limitation", "inhibition" or "minimal growth" and other similar terms which imply a modification of the culture conditions, as well as naturally slowly growing material. One of the earliest experiments on slow growth involved callus cultures maintained under mineral oil (Abo-El-Nil 1977). In more recent years, atmospheric modification and growth at a reduced temperature and vitrification have been used (Wang et al. 2005) for long term plant cell preservation. However, the majority of investigations have concentrated upon the achievement of slow growth in shoot cultures (Badea et al. 1991). Prominent among technical approaches are the use of growth inhibiting chemicals (natural and synthetic hormones and osmotically active agents) and moderate reductions in the temperature to which cultures are maintained (Wang et al. 2005).

We took in our study 4 agricultural important species as bean, pea, garlic and pepper that from we have collected landraces from the small farms over the Western side of Romania and tried to set up the easiest and cheapest protocol for their medium term *in vitro* preservation.

Materials and Methods

The bean (*Phaseolus vulgaris*) - 10 Romanian landraces, pea (*Pisum sativum*) - 19 Romanian landraces and pepper (*Capsicum annuum*) - 50 Romanian landraces (three varieties: sweet pepper, green pepper and cayenne) initial material for "*in vitro*" culture was represented by seeds harvested in the same year. The seeds were sterilized following (Franklin et al. 1991; Badea and Sandulescu 2001) protocols inoculated for germination on free of hormones MS (Murashige and Skoog 1962) with normal sucrose concentration 3% and on MS without sucrose and incubated at two different temperatures, 16°C and 24°C respectively. These were the conditions used for all four species landraces taken in this study. The regeneration capacity was registered 5 days after embryos inoculation and the height of the plants was measured after 4 weeks of culture. To start garlic (*Allium sativum*) *in vitro* culture we used leaf discs of 1 cm length, isolated from 10 Romanian landraces garlic bulbs under aseptic conditions. The bulbs were initially sterilized according to (Bhojwani 1980) protocol and inoculated on the same media and under the same temperature conditions. Regeneration capacity was registered 5 days after inoculation and the regenerated shoots were transferred for slow growth experiment as described fore mentioned.

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Results and Discussion

In order to choose a storage method, the factors to take into account include practical requirements and logistical factors, suitability in principle for the culture system in question and efficacy and reproducibility of existing techniques. Regarding to the first aspect, available facilities influence the decision but more important are the storage demands in term of need for a self-renewal and accessible system. Cryopreservation clearly offers much longer periods of storage with minimal attention requirements. However, for shoots in particular, material in slow growth is much more readily returned to normal growth conditions or transported between users. Its storage habit is very similar to that of normal growth, whereas in cryopreservation the specimen is reduced to a relatively minute growing point (Badea and Sandulescu

Table 1. The effects of temperature conditions and carbon source presence in culture medium for *in vitro* growth and regenerative capacity of plants.

	T1				T2			
	M1 %		M2 %		M1 %		M2%	
Specie	HP	RC	HP	RC	HP	RC	HP	RC
Phaseolus vulgaris	100	100	80,54	93,48	73,86	83,09	54,71	75,35
Allium sativum	100	100	84,17	94,03	63,88	88,45	45,37	68,14
Pissum sativum	100	100	87,70	89	38,58	74,97	14,19	60,71
Capsicum annuum	100	100	79,45	97,28	61,23	90,17	44,02	82,44

T1 – growth room with 24°C temperature, T1 – growth room with 16°C temperature, HP – plantlets growth rate, RP – plantlets regenerative rate, M1 – MS with sucrose medium, M2 – MS without sucrose medium.

2001). These comparisons made us to decide on trying to develop a cheap and easy way to reduce *in vitro* plantlets growth. Thus for, the results showed that lack of sucrose and more over low temperatures decreased plantlets growth for all species studied. After 4 weeks of culture the results showed differences between the cultures incubated on MS without sucrose and on 16°C temperature conditions and the ones incubated on MS with sucrose and at 24°C with more than 17% or 42% for regenerative capacity or plants growth rate, respectively (see the table). Even if the conditions were the same for all species the differences in their response are variated, showing once more that *in vitro* response of plant cells is genotype dependent (Badea and Sandulescu 2001). Our study showed that some genotypes were more sensitive to low temperatures than to lack of sucrose, but also some of them grew very slow in both temperature conditions on medium without sugar. There have been various opinions on the beneficial effects of the various carbon sources on the growth of plants *in vitro*, but for the most sucrose is the carbohydrate of choice as carbon source for artificial conditions of plants culture, probably because it is the major transport sugar of many plants (Murashige and Skoog 1962). Thus, lack of this carbon source decrease cells and shoots proliferation comparing with the control, represented by the plantlets grown on MS with sucrose medium and 24°C temperature consitions. The highest regeneration rate on MS without sucrose was 83% for one of the bean landraces while the lowest was 36% for one of the pepper landrace comparing with the lowest regeneration rate of control which was 69% for one of the garlic landraces (data not shown). Of course, we can not compare the regeneration capacity between diffrent species, but even inside each specie all the measured parameters varied from one landrace to another and comparing with the control. The shoots proliferated on MS without sucrose medium had shorter internods, but the foliar leaf surface was higher comparing with the control that developed long stems with small and numerous leaves. Radicular system of control was better developed comparing with the plantlets obtained in the harshly conditions without sugar which roots were very short. The plantlets kept at low temperatures and more over without the carbon sourse should need more cloroplasts for photosynthesis to ensure the carbon necessary for living, comparing with the control that could take the suplimentary carbon from the medium. Thus the stressed cells save all the energy for surviving and the small plant dimenssions or reduced growth might be a response of stress. Under stress conditions plant cells synthesise reactive oxygen species like hydrogen peroxide or other oxigen free radicals (Gutteridge 1988) that are toxic for cells and can reduce their development. The results obtained in this study were helpful for our germplasm colection medium term preservation until we could use the long term preservation methods as cryopreservation in liquid nitrogen.

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