EXOGENOUS CARBOHYDRATE UPTAKE BY TOMATOES EXPLANTS CULTIVATED IN VITRO FOR MASS PROPAGATION

Tina Oana Cristea, Maria Prisecaru, Silvia Ambarus, Maria Calin, Creola Brezeanu, Marian Brezeanu, George Florin Şova

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INTRODUCTION

The development of procedures for the efficient regeneration of plants from cultured cells, tissues and organs are a prerequisite for the application of in vitro culture techniques to plant genetic manipulation and crop germplasm enhancement.

The efficiency of propagation technique in vitro is affected by a large number of factors, including the growth conditions of the donor plants, the explant stage, the explant plating density, the type of stress treatment applied, the culture medium composition, etc.

Among them the availability of carbon in culture medium, dependent on the type of carbohydrate utilized play an important role. The carbohydrate is a major component of culture medium because it provides the energy necessary for growth and development processes of microspores and also acts as an osmotic regulator. The most frequent type of carbohydrate is sucrose. The studies publishes results in which the embryo yield enhances in the presence of maltose, glucose, fructose or mannitol as carbon source.

In spite of the importance attained by somatic organogenesis and embryogenesis and also of the many studies that have been conducted on these developmental process, there are still many aspects that are not fully understood.

Among those features, the involvement of exogenous carbohydrate on determining the conversion of somatic onto embryogenic tissues, and on allowing progression and maturation of somatic embryos, are far away from being completely comprehended. Part of these difficulties relies on the frequent appearance of contradictory results when studying the effect of a particular stimulus over a specific stage in somatic embryogenesis. Recent progress achieved on understanding the interaction between exogenously added carbohydrates over the regeneration processes, together with the involvement of sensitivity of the tissues to particular hormone groups, might help to clarify the occurrence of divergent patterns in tissue culture at tomatoes. The aspects described above, emphasizing on the effect of the type of carbohydrate and its concentration during the different phases of somatic embryogenesis, will be reviewed in this paper.

MATERIAL AND METHODS

Plant growth conditions

The explants were collected from valuable mother plants maintained at Vegetable Research Station Bacau in controlled conditions. Young shoots of 1.5 -2 cm length were excised from actively growing plants.

Sterilization

The defoliated shoots were first washed in tap water and the sterilized in 0.1% HgCl2 for 15 minutes, and 3 rinses in sterile distilled water.

Culture techniques

The shoots were then utilized as donor source for explants. The apexes of ~ 1,5 cm were excised and inoculated on Murashige -Skoog, 1962 culture basal medium supplemented BAP – 8.9 µM and IAA – 1.1 µM containing 3, 6, or 13% (all w/v) of sucrose, fructose, glucose and maltose (Table 1).

Table 1. Experimental variants for the determination of most beneficial type and concentration of carbohydrates

No. crt.	Variant	Type	Concentration %
1.	V1	sucrose	3
2.	V2		6
3.	V3		13
4.	V4	glucose	3
5.	V5		6
6.	V6		13
7.	V7	fructose	3
8.	V8		6
9.	V9		13
10.	V10	maltose	3
11.	V11		6
12	V12		13

To all these variants 8 g/l agar was added. The pH of the medium was established at 5,8 before the autoclavation at 121^0C for 25 minutes.

Cultures were incubated at 24±1°C under 16 hr photoperiod of 3000-lux light intensity.

The cultures were transferred at a 3 weeks interval on fresh media, for a period of 90 days.

Observation of shoot multiplication and growth were recorded at weekly intervals. After three weeks, shoots of above 3 cm length were harvested and subcultured on the same medium.

Rooting and acclimatization

After 3 to 4 weeks, when regenerated shoots reached a length of more than 4.0 cm, they were separated and transferred on MS basal medium supplemented with 2.7 µM NAA for rooting. The rooted plantlets were transferred to the hydroponics conditions in bottles and hardened by maintaining a high humidity (90% RH) during first week of hardening, which was gradually decreased and it resulted in more than 95% survival of plantlets.

After acclimatization, the regenerants were planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to a green house.

RESULTS AND DISCUSSIONS

As highlighted by the literature, for the growth of plant tissues the carbon source serves as the energy and osmotic agent for various energy requiring processes that can occur at the expense of available metabolic substrates for the growth and root initiation. Due to the presence of low levels of corbon dioxide concentration present in vitro conditions, an appropriate type and concentration of sugar is needed to promote seed germination and regeneration of plant (Faria et al. 2004).

Thus, the main purpose of the present study was to test different carbohydrate sources as sucrose, fructose, glucose, and maltose at different concentrations as promotors for shoot generation, elongation and rooting. In order to determine the best carbohydrate source and concentration for maintenance and proliferation, sucrose was substituted with fructose, glucose, and maltose.

The results obtained (presented in table 2) demonstrate without doubt that among the different carbohydrates used, maltose is the most efficient in inducing multiple shoot number, followed by sucrose, glucose and fructose.

Maltose has been reported to be effective in preventing hyperhydricity and helps in production of adventitious shoots [12]. The maximum mean shoot number (22.0 ± 0.10) was recorded at 3% maltose, with maximum frequency of shoot regeneration (78%).

Maltose aids as both a carbon source and as an osmaticum, compared to sucrose there is a gentler rate of extracellular hydrolysis, it is taken up more gradually, and hydrolysed intracellularly more slowly (Fig. 1).

Table 2. Effect of different type and concentration of carbohydrates over the regeneration processes “in vitro”

No. crt.	Variant	Shoot regeneration frequency	Mean number of shoots/explant
1.	V1	70	19.20 ± 0.16
2.	V2	67	17.0 ± 0.04
3.	V3	60	14.20 ± 0.01
4.	V4	58	8.60 ± 1.43
5.	V5	43	8.20 ± 0.24
6.	V6	40	7.20 ± 0.14
7.	V7	42	7.09 ± 0.09
8.	V8	30	6.41 ± 0.02
9.	V9	35	4.39 ± 1.09
10.	V10	78	22.0 ± 0.10
11.	V11	72	20.8 ± 0.02
12	V12	60	18.4 ± 0.42

Fig. 1. Explants cultivated on media with maltose

Still, sucrose also obtained very good results, as it promoted a shoot regeneration frequency of 70%, with a maximum number of shoots per explant of 19.20 ± 0.16. Our results strengthen the literature findings, that state that sucrose is broken down during autoclaving and converted to glucose and fructose by the action of invertase (Pierik 1987). Glucose is then utilized first and followed by fructose. On the other hand, on media supplemented with fructose, the explants had a low morphogenetic reaction, the shoots had a yellowish colour and most of them degenerated (Fig. 2 and 3)

Fig. 2. Explants cultivated on media with fructose

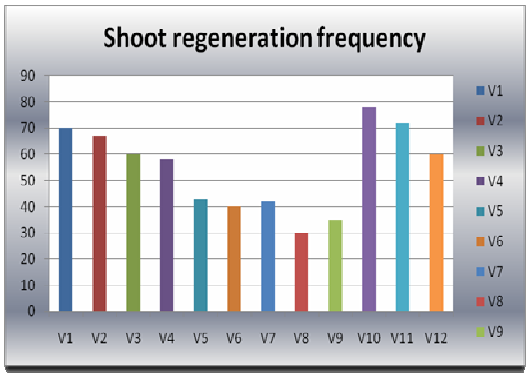

Fig. 3. Responses of explants on mediawith different type and concentration of carbohydrates

The subculivation of shoots on fresh media, allowed the continuation of regeneration process, as on the base of the newly formed shoots, small outgrowth appeared (Fig. 4).

Gradually the shoots that were at the best stage of development were inoculated on rooting medium, which should allow the initiation and development of roots.

Part of them which already had roots were directly transferred to hydroponic condition for acclimatization (Fig. 5).

Fig. 4. Fully formed neoplantule

After transfer to rooting media, in 4-5 days the shoots already developed roots which allowed us to transfer it on hydroponics medium. Before their transplantation the root system of the plantlets were continuously washed with tap water.

Then the plantlets were transferred to different hydroponic variants: simple tap water, addition of Previcur (an accredited substance utilized for fungus control on vegetable plants) in concentration of 0,15%.

The plants were kept about four days covered with a plastic foil, in the culture room. Subsequently, they were day by day acclimatized to room atmosphere.

Fig. 5. Plants in hydroponic culture

After acclimatization, the regenerants were transferred to the greenhouse and grown to maturation.

CONCLUSIONS

In the present study the impact of different carbohydrates and their concentrations on *in vitro* regeneration of tomatoes was tested. In order to identify the best carbohydrate source and concentration for the initiation and proliferation of shoots *in vitro*, sucrose was subtituted with fructose, glucose and maltose. The culture medium was basal medium Murashige-Skoog, 1962 supplemented with BAP – 8.9 µM and IAA – 1.1 µM containing 3, 6, or 13% (all w/v) of sucrose, fructose, glucose and maltose.

The results obtained show that maltose, rather than sucrose is the most suitable carbohydrate source with the most effective concentration of maltose of 3% (w/v), functioning optimally as both an osmotic regulator and a carbon source for tomatoes explants cultivated *in vitro*.

ABSTRACT

In order to investigate whether the type of carbohydrate used as a carbon source might affect the efficiency of regeneration, a complete screening of the main types of carbohydrates in different concentrations were tested.

The study was conducted at Vegetable Research and Development Station Bacau, in The Laboratory of Tissue Culture. The explants were collected from valuable mother plants maintained at Vegetable Research Station Bacau in controlled conditions.

The results obtained demonstrate without doubt that among the different carbohydrates used, maltose is the most efficient in inducing multiple shoot number, followed by sucrose, glucose and fructose.

The maximum mean shoot number (22.0 ± 0.10) was recorded at 3% maltose, with maximum frequency of shoot regeneration (78%). Sucrose also obtained very good results, as it promoted a shoot regeneration frequency of 70%, with a maximum nember of shoots per explant of 19.20 ± 0.16.

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AUTHORS ` ADDRESS

CRISTEA TINA OANA, CALIN MARIA, AMBARUS SILVIA, BREZEANU CREOLA, BREZEANU MARIAN, SOVA FLORIN - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, Bacau, code: 600388, e-mail: tinaoana@yahoo.com

PRISECARU MARIA - „Vasile Alecsandri", University of Bacau, Faculty of Science, Department of Biology, Marasesti Street, no. 157, Bacau, e-mail: prisecaru_maria@yahoo.com