EFFECT OF NUTRITIVE MEDIA ON SOLANACEAE SP.
REPRODUCTION IN VITRO

D. DIMANOV¹, V. MASHEVA¹ and D. DIMITROVA²
¹ Tobacco and Tobacco Products Institute, BG - 4108 Markovo, Bulgaria
² Plant Genetic Resources Institute “K. Malkov”, BG - 4122 Sadovo, Bulgaria

Abstract


It has been researched the possibility for in vitro N. tabacum sp., S. tuberosum sp., S. lycopersicum sp. Cultivars at a different nutritive mediums. A positive effect of liquid nutrient media on micropropagation in vitro in three Solanaceae species, compared to solid agar medium was established. The best for micropropagation in vitro in the varieties of three species - N. tabacum, S. tuberosum and S. lycopersicum is nutritive medium B. The importance of genotype in the reproduction in vitro has been demonstrated. The unified systeme for micropropagation in vitro - based on nutritive medium B of varieties of three family Solanaceae species was created.

Key words: nutritive media, in vitro reproduction, cultivars

Introduction

Growth and development of plants in vitro is determined by the following factors - genotype, nutritive medium, physical factors - light, temperature, pH, concentration of O₂ and CO₂, organic substances, phytohormones, etc. The culture medium is essential for growth and development of plants (Annela and Kahn, 2010).

For gelling of the medium in the in vitro cultivation is most commonly used agar. It is a polysaccharide of high molecular weight and high gelling ability. It is important to know that the gelling agent is the most expensive component in solid nutrient media (Murashige and Skoog, 1962). There are studies on the influence of agar on the development of plants in vitro conditions. It was found that low concentrations of agar facilitate rooting of apple (Werner and Boc, 1980). The literature indicated to increase stem cell proliferation from the standpoint of the physical condition of the medium with agar concentration of 0.48 to 1% (Lundergan and Janick, 1980; Kassim, and al., 2010). It should be borne in mind that it contains organic and inorganic residues (Teixeira da Silva, et al., 2005).

Therefore, some authors recommend the use of liquid culture media. For example in N. tabacum (cv. Xanti) growth and proliferation increase in the use of liquid culture medium (Stevenson et al., 1982). The same is recommended in planting some trees (Piek, 1987; Kassim et al., 2010) and vine (Dimanov et al., 2001).

Similar findings have been established in our previous studies (Dimitrova and et al., 1992).

The aim of this study is to trace the growth and development of varieties of tobacco, potatoes and tomatoes - representatives of the family Solanaceae cultivated in vitro in medium MS (Murashige and Skoog, 1962) and medium B (Dimanov and Atanasov, 1986) in two versions - solid (agar) and liquid.

Materials and Methods

Attempts are reproduced in laboratories of plant tissue cultures, with TTPI - Markovo and PGRI- Sadovo.
For source material used plant explants - cultivars by Tobacco \((N. tabacum)\): Virginia 89, N. alata, Krumovgrad 90 and line Ri F\(_{10} \) (Plovdiv 7 x L.2); Potatoes \((S. tuberosum)\): Vivaks, Bobar, Estima, Maranka, Ponto and Nela; Tomatoes \((S. lycopersicum)\): Monalbo, P2046, l1104.

**Nutritive mediums:** MS - basic 5 mg / l IBA; Medium B - (Dimanov, 1987) – from microelements in Heller (1953) is removed AlCl\(_3\), sucrose is increased to 20g / l. Contains 0,1 mg / l IAA

**Biometric indicators:** Dynamics of growth - root system and stems were reported in 10 days in 1 month. Plant explants were plated in tubes with 10 ml of culture medium at 10 plants in four replications. The material is placed in a growth-chamber at 25-27\( ^\circ \)C, photoperiod 16/8 h light / darkness, light 3000 lux and 75% humidity.

**Results and Discussion**

Induction of rizogenez in vitro and the rate at which the process goes, it is essential for overall development of plants. The cultivars rooting of study species and varieties depend on the conditions of cultivation (mentioned in Materials and methods, as it is essential from composition of the medium). In tracing the reaction of the varieties of \(N. tabacum\) in the initial period of cultivation experienced some varietal differences in the versions of the medium. The results presents in Figure 1 shows that most favorable to the rooting medium is B - liquid. The average percentage of rooted plants is 84%. Varietal differences were observed in the range – 88% for variety Krumovgrad 90 and 81% in explants from \(N. alata\). Both versions of the medium B – liquid and solid, rooting percentage was significantly higher than the corresponding for MS. The difference between the B-liquid (MS liquid is 6.25% and 4.75% for B-solid) MS solid.

In potato varieties \((S. tuberosum)\), the results presented in Figure 2 the influence of medium on the process of rooting were observed following varietal differences. The lowest percentage of rooting in Vivaks and Ponto varieties in medium MS - respectively 45 - 70% for liquid and 45-62% for the solid phase of the medium. The highest percentage of rooted plants is characterized by variety Bobar, as the best conditions for development of root system provides on medium B (liquid and solid) where the performance of 100% rooted explants.

The differences that occur between the variants tested on the respective phases - liquid (liquid and solid) solid c in the both mediums in \(S. tuberosum\) varieties are significantly B (MS-liquid – 13.28%) and B (MS-solid-12.15%). The results show that the most favorable in terms of rooting medium is in B-liquid, where the percentage rooted plants move in the range 90% - Vivaks and Ropta and 100% for all other varieties.

In the third representative of the family Solanaceaee, \(S. lycopersicum\), maintaining our established trend. Despite the low rate of rizogenez in all variants of the two mediums and relatively small varietal differences in medium B-liquid proved most favorable (Figure 3).

The results of comparison of both liquid and solid media are both very close to data \(N. tabacum\) -B liquid
Effect of Nutritive Media on Solanaceae Sp. Reproduction In Vitro

/ MS liquid – 6.25%; *S. lycopersicum* - B liquid (MS liquid - 6.66% and *N. tabacum* – B solid) MS solid – 4.75%, *S. lycopersicum* - B-solid (MS solid - 5.00%).

In conclusion we can summarize that we studied species of the family *Solanaceae*, B liquid medium is most suitable for process, rizogenez and vary between 70,66% (*S. lycopersicum*) to 97.14 % (*S. tuberosum*). Proliferation of the stem depends on several factors - good rizogenez, balanced nutritional medium providing sufficient nutrients and phytohormones for the normal growth of the stem. The survey this indicator is presented in cm, as readings are for 30-day period.

The growth of the stem in all variants - varieties and growing media began almost simultaneously. In the initial period of 5 -15 days visible differences in growth, stems in both medias are not observed. Between 15-30 days of setting the explants outlines differences not only in terms of culture medium, but also in response to the variety.

In Figure 4 is presented the influence of medium on the growth of the stem of varieties of *N. tabacum*.

When comparing the two culture medias MS (liquid and solid) and B (liquid and solid) can clearly faster growth on medium B. The average height of the stems by genotype ranged from 14.5 to 12.5 cm for varieties Krumovgrad 90 and Virginia 89 in B - liquid to 11.0 and 9.5 cm in MS-solid medium for the same varieties. For the other two options are similar results. Best proliferation of explants is recorded on the B -liquid medium.

The average height of the stems of potato varieties studied (*S. tuberosum*) is presented in Figure 5. After 15 day of explants, plating is account significant differences not only in terms of medium, but significant differences in responses of different varieties.

The average height of the stem varies from 7.0 cm at Nela variety of medium MS – solid to 18.0 cm in Estima, Bobar and Maranka in medim B-liquid. In all varieties, stem growth in liquid media B exceeds that of explants grown on solid medium. Although some authors recommend the use of liquid culture media (Stevenson et al., 1982 and Pierik, 1987) in Bobar, Estima, Maranka, Ropta and Ponto varieties explants cultured on solid medium B are in greater length than in MS-liquid. When comparing the two culture medias MS (liquid and solid) and B (liquid and solid) can clearly faster growth on medium B.

The results of this biometric indicator of tomato varieties (*S. lycopersicu*) are presented in Figure 6. Com-
pared with the variety differences resulting from other members of the family *Solanaceae* - tobacco and potatoes, of the stem length indicator in the options-varieties of tomatoes are less pronounced. When comparing the two culture media B and MS (liquids and solids) have a faster growth rate on medium B. Depending on the genotype variation in height was 9.5 cm for the first and third variety, and 8.5 cm for P 2046 in medium B-liquid, and 7.5 cm and 6.5 cm on medium MS-solid. Despite the slower growth of the stem with representatives of *S. lycopersicum*, confirmed our results found for tobacco and potato varieties that B-liquid media provides optimal conditions for expression of this indicator.

**Conclusions**

A positive effect of liquid nutrient media on micropropagation *in vitro* in three *Solanaceae* species, compared to solid agar medium was established. The best for micropropagation *in vitro* in the varieties of three species - *N. tabacum*, *S. tuberosum* and *S. lycopersicum* is nutritive medium B. The importance of genotype in the reproduction in vitro has been demonstrated.

The unified systeme for micropropagation *in vitro* - based on nutritive medium B of varieties of three family *Solanaceae* species was created.

**References**


Dimanov, D. and A. Atanasov, 1986. Development of a regenerative system of mesophilic protoplastic in *N. tabacum* (Virginia 89) and *N.alata*. *Genetics and Breeding*, 5: 447-449 (Bg).


Received April, 2, 2012; accepted for printing December, 2, 2012.