

EFFECTS OF GROWTH REGULATOR AND SUCROSE CONCENTRATION FOR *IN VITRO* DEVELOPMENT OF *SOLANUM TUBEROSUM* L, CULTIVARS

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Abstract.

The aim of this research was to compare the influence of hormone-free medium with media supplemented with growth regulators and different concentrations of sucrose on induction and development of microtubers in potato. Explants of Christian and Désirée cultivars were multiplied by subculturing nodal segments on growth regulators – free medium Murashige, T., and Skoog, A., 1962. For experiments single node stem segments were excised and transferred to six tuberization media. The cultivars showed wide percent of tuberization ranging from 15-68% for Christian and aprox. 50% for Désirée cultivars. Sucrose and growth regulators also influence the size of tubers. Medium V₆, containing 10% sucrose + 1.0 mg/l Zeatin + 0.5 mg/l β-indolylacetic acid (AIB), gave the highest rate of tuberization and the largest tubers size.

Key words: *in vitro*, cultivar Christian, Désirée, tuberization, tubers size, highe rate, sucrose, growth regulators, zeatin, β-indolylacetic acid.

INTRODUCTION

The potato is one of the most important crops in the world, numerous researches have been improved the tissue culture techniques for the mass propagation of germ/free and clonal plants (Estrada, R., et al., 1986; Agud, E., et al., 2009; Cachiță – Cosma, D, 2007). In the recent years seed production programs have been developed in which the first multiplication steps are speeded up by using either *in vitro* plantlets (Ahloowalia, B. S, 1990), or microtubers (Agud, E., et al 2009; Levy D., et al., 1996). There are many studies concerning the effect of growth regulators on tuberization of potato (Ravnikar. M., Gogala, N., 1990; Butiuc, Keul, A, et al., 1997/1998; Agud, E., 2010). Microtubers formed on potato plants growing *in vitro* are very convenient for the maintenance and handling of disease-free material (Seabrook J. E.A., Coleman, S., 1988; Zăpârțan, M., 1992:). *In vitro* formation of microtubers is closey associated with hormonal changes, auxins, cytokinins, and gibberellins(Butiuc, A.L., et al. 1996; Agud, E. 2011) have all been used to increase tuber formation. In regards possible economical advantages, adders reported a scale/up culture using jar fermentor techniques, the technique is also valuable for studying the physiology of tuberization, because the tuber/inducing tissues and the developing tubers can be mass propagated under experimental conditions(Akita M., and Takayama, S., 1988).

In this study we compare the hormone/free medium with different media with growth regulators and sucrose in the meaning of in vitro tuberization of the potato cultivars Christian and Désirée.

MATERIALS AND METHODS

In vitro potato stock plants *Solanum tuberosum* L, Christian and *Désirée* cultivars were maintained by subcultivation of nodal explants on hormone/free MS medium. For experiments single-node explants have been cultivated on MS (Murashige, T., and Skoog, A., 1962) medium supplemented with different concentrations of sucrose and/or Z (zeatine) and AIB (β -indolilacetic acid). You can see the composition of the medium in table 1.

Table 1

Medium for tuberization <i>in vitro</i> the potato cultivars			
Variants	Basal Medium	Sucrose concentration(%)	Growth regulator concentration (mg/l)
V ₁	MS	4%	-
V ₂	MS	8%	-
V ₃	MS	10%	-
V ₄	MS	4%	1.0mg/l Z + 0.5mg/l AIB
V ₅	MS	8%	1.0mg/l Z + 0.5mg/l AIB
V ₆	MS	10%	1.0mg/l Z + 0.5mg/l AIB

(MS=medium after Murashige, T., and Skoog, A., 1962; Z=zeatine; AIB= β -indolilacetic acid)

The culture were preserved under vegetation chamber conditions, the illumination with fluorescent tubes, light intensity 87.5 $\mu\text{mol}/\text{m}^2/\text{s}$, 16/8 hours photoperiod and temperature 27/28°C. The cultures have been evaluated after 4 and 8 weeks respectively.

RESULTS AND DISCUSSION

The following six variants of medium have been tested concerning number of tubers/explant, size of tubers and percent of tuberization. The influence of sucrose concentration and growth regulators on the number of tubers/explant of potato after 4 weeks and 8 weeks of culture is showed in fig. 1. The medium Murashige, T., and Skoog, A.,(MS) with 4% sucrose and without growth regulators doesn't ensure tuber induction even after 8 weeks of culture.

The variant V₂ ensure the in vitro tuberization, each cultivar forms only one tuber after 4 weeks and in case of Christian cultivar we obtained 2 tubers after 8 weeks of culture. We observed that the increasing of sucrose concentration determines the increasing of the number of tubers/explant.

Whether the medium is supplemented with 8-10% sucrose, 1.0mg/l Zeatine and 0.5mg/l AIB, we obtain in average, 2 tubers/explant.

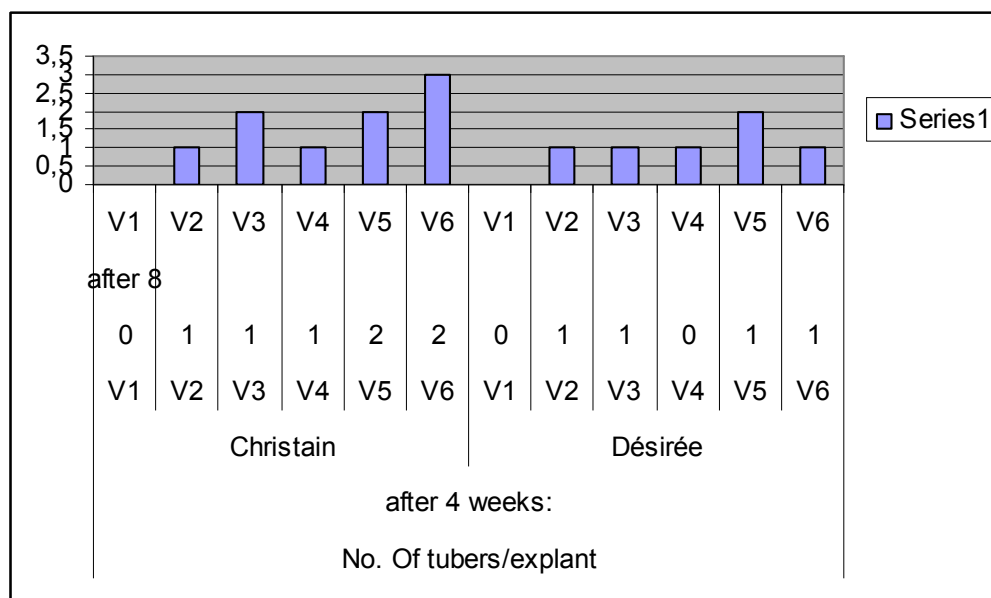
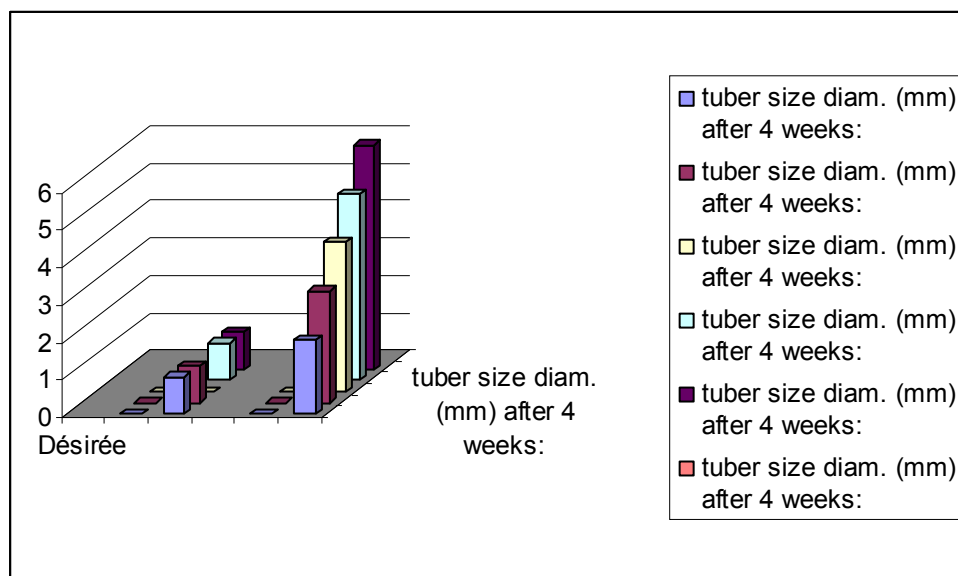


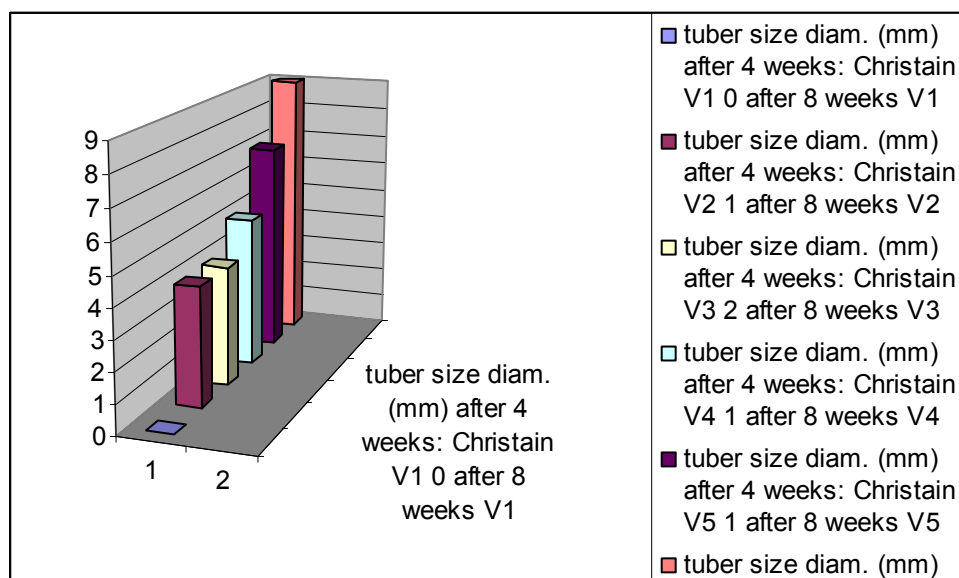
Fig. 1 Influence of growth regulators and sucrose concentration on tubers induction (after 4 and 8 weeks)

The results regarding the size of tubers, which can be observed in Fig. 2. A, B and C, are very similar with the results that we obtained in case of tubers number. Whether the medium contains growth regulators and increasing concentrations of sucrose, the size of tubers is also increasing. It means that growth regulators as auxins and cytokinins ensure the growth and development of tubers.

A.



B.



C.

Fig. 2 Influence of growth regulators and sucrose concentration on tubers size, after 4 and 8 weeks (A, B, and C)

Generally, the media that contain only sucrose ensure the formation of small tubers (3 mm diameter). On V₄ and V₆ media the size of tubers is increasing, especially in case of Désirée cultivar. We observed that Christian cultivars requires high concentrations of sucrose 10% for tuberization and in the same time Désirée cultivar requires only 8% sucrose. The medium with contain growth regulators and 8 - 10% sucrose ensure the highest percent of tuberization.

Cytokinins are commonly included in media for in vitro tuberization of potatoes but their role is still obscure. Cytokinins are essential for lateral bud outgrowth. We tried to find the role of auxin in combination with cytokinins on the in vitro tuberization of potato, because it is was reported a considerable decrease in stolon rooting when tuberazion was induced indicating that root growth could interfere with tuberization in whole plants. To alter this tendency of shoot formation, the induction of radial cell enlargement, the increase of cell number and subsequent increase in cell volume is required. Sucrose 8 - 10% was sufficient to induced these processes and tuberzation. The Christian and Désirée cultivars formed several tubers in the medium by thickening of the apex of stolons, most of the tubers have been formed directly on the explant but some of were formed at the apex of offshoots.

CONCLUSIONS

1. The maximum number of tubers/explant was obtained on the medium supplemented with growth regulators and 8% sucrose.
2. The size of tubers increases on the medium with higher concentrations of sucrose 8% and 10% in all cases.
3. The highest value of percent of tuberization we obtain at Christian cultivar on the medium with high concentration of sucrose (10%).
4. The percent of tuberization for Désirée cultivar are increasing proportionally with sucrose concentration in the medium.

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