STUDY REGARDING TO *ASPARAGUS OFFICINALIS* L. CALUS REACTION, SUBCULTURED ON ASEPTIC MEDIUM WITH VARIOUS GROWTH REGULATORS

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Abstract. In our studies, we investigated the effects of some growth regulators, in aseptic conditions, on obtaining Asparagus officinalis plantlets from calus, after 12 weeks of its inoculation.

The most recomanded culture media, for obtaining a high number of multimodal stems was MS basal medium, with a mixture of 1mg/l BA and 1 mg/l IBA, which inducing a considerably calusogenesis, and a lot of regenerated plantlets from it, but finally, this medium-after 12 weeks of culture initiation, induced plantlets vitrification.

Keywords: *Asparagus*, calus, hyperhidria, vitrification, auxine, cytokines

INTRODUCTION

The vitrification or hypherhidria is the process wherewith, in certain condition, aerial organ of the plant become translucent, from bring about the water infiltration in intercellular spaces of these, replacing the air from them. In kind, thus of phenomenon is spent seldom, especially to be greenhouse salad [1].

In aseptic condition the hyperhydric vitroplantlets appear spontaneously, from high temperature, or either from the presence oy ethlylene, in big amounts in the bottles with co-cultures(much more clones per vessel), emanation produced from suprapopulation. The hyperhydric vitroplantlets suffer morphological transformations which consist in: hypertrophiated recurved, translucent (glassily) rugose leaves, sometimes wavy and cracky, having a malformerd growth, as a rule they can have big size and an ethylene appearance [1].

In experiments which make the object of present work, proposed us to investigate the effect of the growth regulators, inserted on the vitroculture media in varied concentrations of hormones, designed medium for *Asparagus* calus vitrocultivation. The practiced calus subculture, it was performed in sight regeneration to his level of many neoplantlets.

MATERIALS AND METHODS

The used-up vegetable material in this experiment consisted from *Asparagus officinalis* calus, the Jersey Knight F1 kind, after *12 weeks* of vitroculture, inoculated in bottles of 7,5 cm height and 4 cm in diameter, fitoclones breed in a photoperiod regime of 1700 lucs at 16/24 hours in light, the ambient temperature corresponding to 23°C±2°C the day and 20°C±2°C at the night [3].

The subculture of calus, achieved through the prick-out of a calus in age *12 weeks*, fragmented in piece of its 1 cm, which they were subcultivated, 20 clones per variant, on a jellified basis medium culture Murashige-Skoog (MS, 1962) [2] modified by us, with an addition of growth rehulators and vitamins(thiamin HCl, pyridoxine HCl, nicotinic acid(1mh/l each of them), mesoinositol 100 mg/l, sucrose 20 mg/l (instead of 30g/l in the original recipe) and agar-agar 7 g/l.

Thus, depending on growth regulator ads in the medium, they were organized in many experimental variants, as follows:

- V1 – MB Murashige-Skoog (MS, 1962) with addition of 2mg/l 2,4 diclorfenoxiacetic acid (2,4-D);
- V2 – MB Murashoge-Skoog (MS, 1962) with a mixed addition of 1 mg/l bensiladenine (BA) and 1 mg/l indolilbutiric acid (IBA).

After the medium culture preparation, they were sterilized through autoclavation, to pressure of 1 atmosphere, for 25 minutes.

To point put the differences of calus reaction subcultivated on those two variants of media culture, the calus, depending on the nature and concentration of the growth regulators present in vitrosubstrate after *4, 8* and *12 weeks* from these subcultivation, they did the prolusion concerning the evolution of vitrocultures, at *8*and *12 weeks* from prick/outs operation, determining: the calus size, the number of regenerated stems from those level, stems length, the number of knots, the number of ramification, finally evolving the degree of those vitrifies.

The resulted data we represented through histograms and tables.

RESULTS AND DISCUSSIONS

After the prelevated calus subcultivation from the initial vitroculture, on medium culture with varied growth regulators, these were differentiated in dependency of media nature on which these were subcultivated, also and in dependency of the previously applied layers type in the primary culture.

At *4 weeks* from the *Asparagus officinalis* Jersey Knight F1 calus subcultivation, the *survival percentage* of caluses was 100% on both variants.

In what look the calus diameter (**Fig. 1B**), subcultivated for *4 weeks* on variant V1 (MS medium with an addition of 2 mg/l 2,4-D) they generated calus with sizes contained between 1,1-1,2 c, and on variant V2 (MS medium with a mixed addition of 1mg/l BA and 1mg/l IBA) caluses with sizes contained between 1,2-1,4 cm. After *8 weeks* from the administration regimes of culture, it has been noticed a growth of vitroplantlets peaks with at most 5 knots, on variant of culture V2 (MS medium with a mixed addition of

1mg/lBA and 1mg/l IBA).Morphologically, at the superior level knots of *Asparagus* stems, to variant of culture V2 (MS medium with a mixed addition of 1mg/l BA and 1mg/l IBA), have generated 1-2 ramifications (**Fig. 2E**).

Thus, the cultivated minicuttings on V1 (MS with an addition of 2 mg/l 2,4-D) variant, they generated calus to the level base of stems, calus became gradually senescent, respectively of plantlet with most little stems, the stems length **(Fig. 2C)** contained between 0,2-0,5 cm, and on V2 (MS with a mixed addition of 1mg/l BA and 1mg/l IBA) variant, generated maximum 5 stems length falling between 0,8-1,43 cm, but with eldest number of knots to these level, touching the maximum values of 6 knots per plantlet **(Fig. 2D)**: and in what look the number of ramifications **(Fig. 2E)** on this variant, the explants generated 1-2 ramifications.

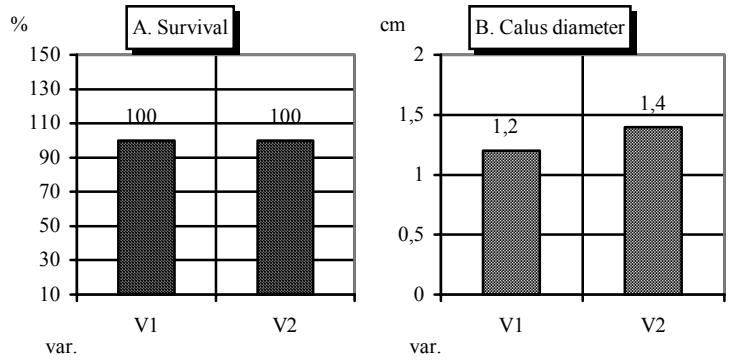


Figure 1. The survival percentage (A) and the calus diameter (B) of *Asparagus officinalis* Jersey Knight F1 vitroplantlets, at *4 weeks* of"in vitro" calus subcultivation, on Murashige-Skoog (1962) (MS) media with an addition of growth regulators, where: V1 – MS medium with an addition of 2 mg/l 2,4-D, repectively V2 – MS medium with a mixed addition of 1 mg/l BA and 1 mg/l IBA

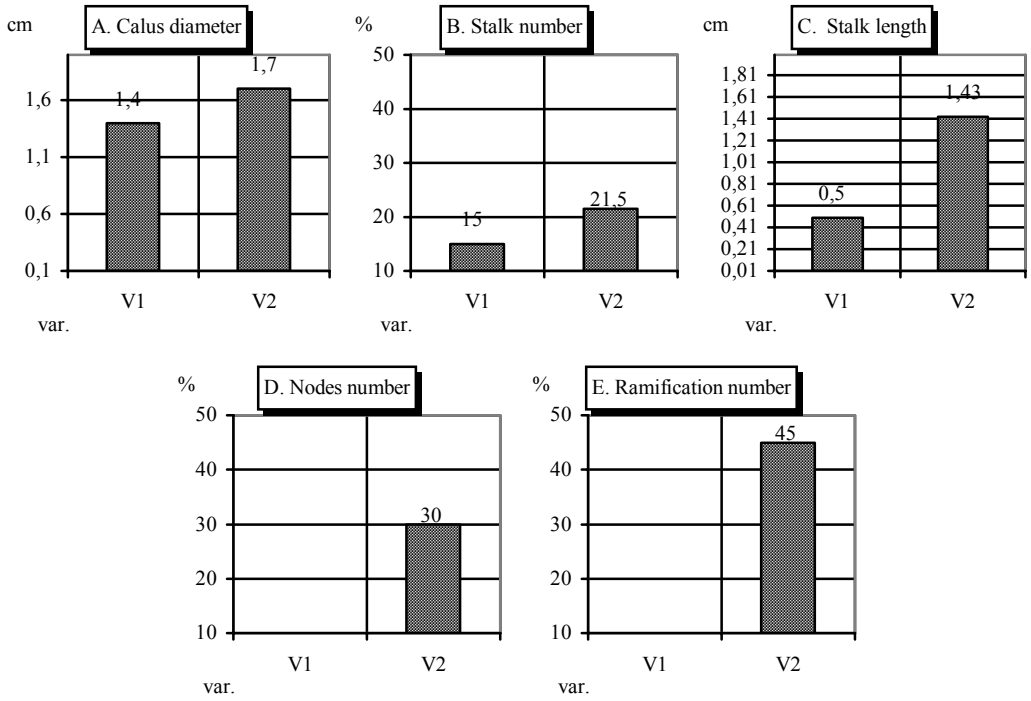


Figure 2. The comparative evolution of *Asparagus officinalis* Jersey Knight F1 vitroplantlets, at *8 weeks* of "in vitro" calus subcultivation, on Murashige-Skoog (1962) (MS) media with growth regulators, where: V1 – MS medium with an addition of 2 mg/l 2,4-D, respectively V2- MS medium with a mixed addition of 1mg/l BA and 1mg/l IBA.

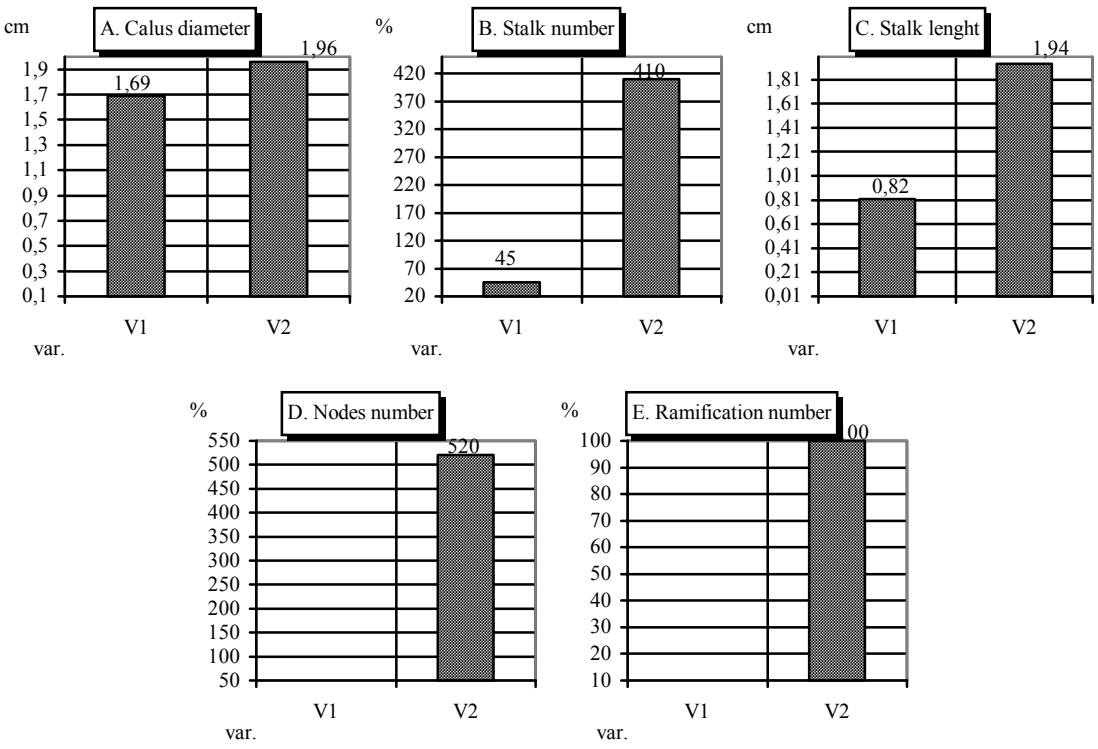


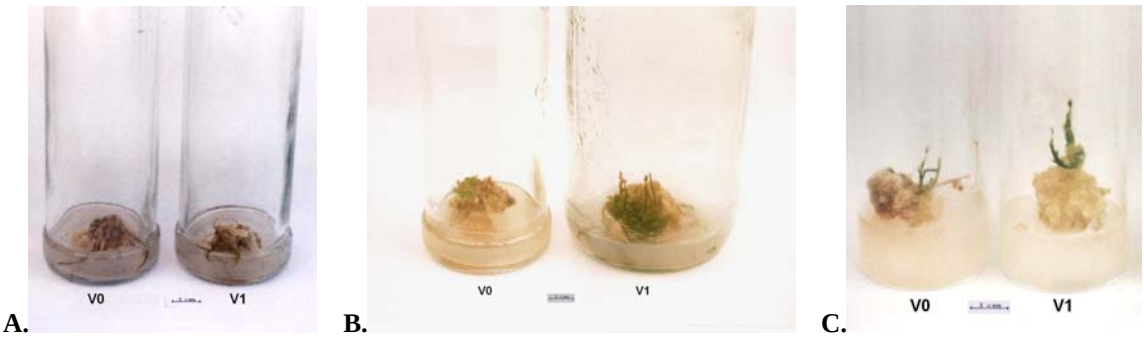
Figure 3. The comparative evolution of *Asparagus officinalis* Jersey Knight F1 vitroplantlets, at *12 weeks* of "in vitro" calus subcultivation, on Murashige-Skoog (1962) (MS) media with an addition of growth regulators, where: V1 – MS medium with an addition of 2 mg/l 2,4-D, respectively V2 - MS medium with a mixed addition of 1mg/l BA and 1mg/l IBA.

The vitrification degree be still very raising, at *12 weeks*, from these, without roots for the supply with mineral substances, becoming gradually senescent (**Fig. 3A, Table 1**), the size of calus at variant V2 (MS with a mixed addition of 1 mg/l BA and 1 mg/l IBA) presented the high values, values between 1,5-1,9 cm, comparative with variant V1 (MS with an addition of 2mg/l 2, 4-D), where these they varied between 1,4-1,8cm.

Conversely, on medium culture V2 (MB with a mixed addition of 1mg/l BA and 1 mg/l IBA) were strong vitrified, where 1-6 normal plants were regenerated just as the result of submersion in the hormonal mixture, the explants had a morphological superior development: a number of ramifications (**Fig. 3E**) with 17 copies, with 9 knots and 2 ramifications.

Table 1. The vitrification degree of *Asparagus officinalis* Jersey Knight F1 vitroplantlets, expressed with "+" as much they were, the transparency degree is much more also, and "-" is the absence of vitrification.

Nr. of plants	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
V1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V2	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-



A. **B.** **C.**

Figure 4. The comparative evolution of *Asparagus officinalis* Jersey Knight F1, vitroplantlets:**A**.normal aspect of vitroplantlets at *4 weeks;***B** normal aspect of vitroplantlets of vitroplantlets at *8 weeks;***C** normal aspect of vitroplantlets at *12 weeks*, subcultivated"in vitro" , on Murashige-Skoog (1962) (MS) medium culture with an addition on varied growth regulators, where:V1-MS medium with an addition of 2 mg/l 2,4-D, respectively V2-MS medium with a mixed addition of 1 mg/l BA and 1 mg/l IBA.

As per presented data from the **table 1**, the vitrification degree of *Asparagus officinalis* Jersey Knight F1 vitroplantlets registered 50, 5% from total number of plants from the respective vitroculture.

On V2 (MS with an addition of 1mg/l BA and 1mg/l IBA) medium the plantlets were well regenerated (**Fig. 4**).

CONCLUSIONS

- Most indicate the enviroment of culture for subcultivation of calus and obtain of a big number of stems and of knots, he proved to be variant V2 (MS with a mixed addition of 1mg/l BA and 1mg/l IBA) carry inducts caulogenesis, raising considerable the clones surviving chances.
- Also, he proved that on variant of culture V2(MS with a mixed addition of 1mg/l BA and 1 mg/l IBA) he inducted the phenomenon of explants vitrification.

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