THE INITIATION OF A TROPIC SHRUB SPECIA *PSIDIUM GUAJAVA*

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Abstract. Because this tropical fruit is not so popular in Europe, we sis try he initiation of an tropic shrub of *Psidium guajava* it was possible to make, using them seeds from the matured fruit of guava. The fruit is originally from Egypt – Alexandria. Those seeds were dry and before using them, they were kept in sterile water few hours, after that it was performed the sterilization process, and they were inoculated in 4 different experimental variants.

Because them germination process was start late, after 2 months from inoculation, observations were made to the level of the germinated seeds, didn't shown any infections, but the best results were noticed only on variant V_1 (BM basic medium - MS with BA (1 mg/l) + IBA (1 mg/l)), where the germination capacity it was more bigger.

Finally, we did noticed that after the end of this experiment, the best medium culture for the generation of stemlets with many leaves is V_1 and V_3, but for the root development only V_2 showed a very good result. Kept in good light intensity, humidity and optimal temperature conditions, the experiment showed good results, what made this research possible.

Keywords: *Psidium guajava*, vitrocultures, growth regulators, fruits, C vitamin

INTRODUCTION

Guava – *Psidium guajava* (Fam. *Myrtaceae*) is a tree that can breed up to 10 meters in height, and has evergreen leafs. Its trunk is covered with a thin crust, that carries drops periodic and then are regenerated.

The **guava** tree has the white flowers, and the fruits have oval form and when ripe ditch arrive at a length of 12 cm. The fruit of **guava** has a greenish color, and the core is white or pink (**Fig. 1**). The crust is not comestible, like as the seeds, which can be white or pink as well.

Its aroma is similar to strawberries. It is a C vitamin abundant fruit and is recommended against anemia, and to those who suffers of spasmophylia or hepatic affections [1].

Figure 1. The development of the gawafa flower and fruit

Internationally is known as: goyave (France), guave (Germany), guaiva (Italy), guayaba (Spain), goiaba (Portugal), guyava (Israel, gawafa (Africa), amrud (Hindi), koya (Tamil), malaka (Burmese), farang/ma kuai (Thailand), jambu batu (Malaysia), jambu biji/jambu klutuk (Indonesia), bayabas (Philippine), fan shi liu (China), banjiro/guaba (Japan), kuawa (Hawaii), guave de Chine (Réunion) (*Psidium guajava* -- Family Myrtaceae)

From the oldest times, the guava was found through the archaeological parts of Peru, flapping the 800 B. C. This was, probably, firstly cultivated there, but this prevalent then over towards North, which Mexico was with 200 years B. C. For European, first date, met the fruit, when this fruit arrived in Haiti, where it was called "guayavu". Soon, the Spanish and Portuguese sailors extended prevalent of this tropical fruit tree to other regions.

In the 17th century, it was well founded in India and Southeast Asia, where it remained very popular. It is also cultivated in Hawaii, Florida, and California.

The Indians from West Arawakia called this fruit "guayaba" and they gave it to Columbus to spread it. The first appearance of this fruit was in 1526 and it is characterized by a lot of seeds, which are suggested to be the most useful "instruments" for the plant prolonging.

Guava is very rich in C vitamin and represents a good source of niacin, potassium and dietetic fiber. They are remarkable for their astringency quality and are very used when diarrhea is a problem. Therefore they are consumed temperately, avoiding the constipation.

To form, it looks like a plum, guava can touch the minimum size of 1 inch (2.5 cm) and, if elder, it can achieve 4 inch (10 cm) in diameter, but it can vary in size, form, and color, even to the main species.

When the fruit is elder, with a plum form, the white one are considerate the most tasty. Most fruits of gawafa have a thin skin, and during on ripening (maturation) they turn into a pallid yellow. The cores vary from the white as far as a purple till dark red color. The fruits contain a number of strong seeds (**Fig. 2**), comestible and extremely smelling with an acid very sweet aroma, likewise with one of quince. The matured guava is very delicious when eated fresh, but also could be consumed as syrup or jam.

There are 150 guava varieties, which can have various forms: the form of egg or plum, with greenish, white, yellow or red skin, which can be glare and holey. Sometimes, the have an apple or plum form. The diversity of cognate species like *Psidium cattleianum* (Strawbwerry) or *Carrley* (guava), are considered the best and largely most cultivated. One of these trees, native from Brazil, form roundly reddish gnaws.

The most common variety is from North America, the kind Beaumont, found in a big commercial variety. It looks like a pallid yellow lemon, with a smooth skin covering a pinky core. Another variety, the pineapple guava, isn't a true guava, but rather a feijoa.

There are also other varieties of guava, but they have less aroma, and only when the fruit is fresh [4].

The interest which this species stirred, as the fructiferous shrub, caused us to research the possibility of *Psidium guajava* vitrocultures initiation, and to study the possibility of these species micropropagation *„in vitro"*.

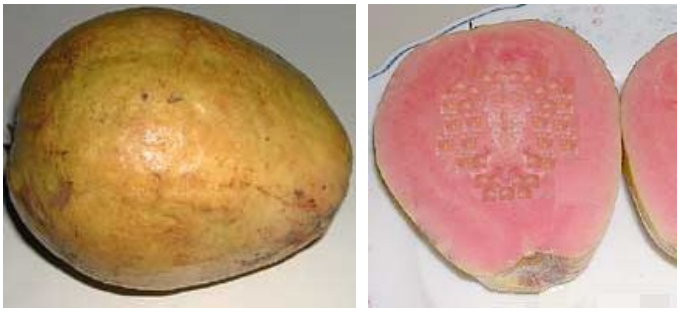


Figure 2. The fruit and seeds of guava

MATERIALS AND METHODS

For the initiation of *Psidium guajava* vitrocultures, we collected 2-3 mm length seeds from a matured guava fruit. The seeds of this guava fruit were sterilized by submersing them in ethylic alcohol (96º), for only 1 minute, and then resubmersing them in a Natrium hypochlorite solution (0.5 %) for 15 minutes, 1:2 water. Afterwards they were washed in repeated baths, with sterile water, 5 minutes / each. Finally, these fragments were put on sterile filter paper, closed in sterile Petri dishes. After that, in the perimeter of laminar flux hood with sterile air, in aseptic conditions, we proceeded to inoculation "in vitro" on the surface of the culture media (**Fig. 3**).

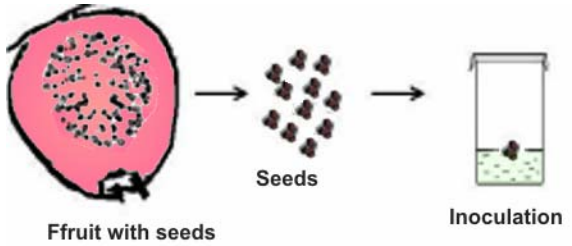


Figure 3. The inoculation method of the *Psidium guajava* seeds

The culture substratum consisted in a basic culture medium (BM) Murashige-Skoog (MS) (1962) [2] (BM - MS) with macroelements and Fe EDTA, Heller microelements, mineral mixture with vitamins: pyridoxine HCl, thiamine HCl and nicotinic acid (1 mg/l each), m – inositol (100 mg/l), sucrose (30 g/l), agar-agar (7 g/l), with pH = 5.8.

In the basic medium culture (BM) growth hormones were added, resulting the following experimental variants:

- V_0 – witness variant (control) – BA medium - MS without growth hormones;
- V_1 – BM medium - MS with BA (1 mg/l) + IBA (1 mg/l);
- V_2 – BM medium - MS with BA (1 mg/l) + NAA (1 mg/l);
- V_3 – BM medium - MS with BA (1 mg/l) + IAA (1 mg/l).

where:

- BA – benzyladenine;
- IBA – β–indolebutyric acid;
- NAA – α-naphtylacetic acid;
- IAA – indole-3-acetic acid.

The culture media were portioned in 8 x 3 cm recipients, each one containing 5 ml medium. The cultures media sterilization was made by autoclaving at 121ºC temperature, during 30 minutes. After their cooling, the post-autoclaving process was performed at those explants inoculation.

After inoculation, the recipients with the inoculs (were covered with a polyethylene folia, immobilized to the mouth bottles with elastics) were placed on shelves, to a varied temperature (between 20-24ºC) and a 16 h light / 24h photoperiod, with 1700 lux light intensity, emitted from white fluorescent tubes.

In this experiment, we collected seeds, from a maturated fruit, in December. After their sterilization, in axenic conditions, they were inoculated "in vitro", 25 seeds / variant, on a basic medium culture (BM) MS (1962), with an adding of growth hormones - in order to brought to these levels - the formation of roots and stemlets.

After 2 months from inoculation, observations were made to the level of the germinated seeds, to bio-measure the following parameters: the length of stemlets regenerated from seeds, the formation of ramifications, and the elongation of the developed sprouts.

RESULTS AND DISCUSSIONS

In this experiment, the *Psidium guajava* seeds were standstill increasing better after 2 months, presenting a

slow evolutions, and very differed, on three of experienced variants.

After 2 month of inoculation, the seeds, at all of experimental variants, didn't show any infections, the lowest evolution being noticed to the witness variant V_0 (BM basic medium - MS without growth hormones), V_2 (BM basic medium - MS with BA (1 mg/l) + NAA (1 mg/l)) and V_3 (BM basic medium - MS with BA (1 mg/l) + IAA (1 mg/l)) the clones presenting a very low regeneration capacity. The best results were noticed only on variant V_1 (BM basic medium - MS with BA (1 mg/l) + IBA (1 mg/l)), where the germination capacity was much bigger.

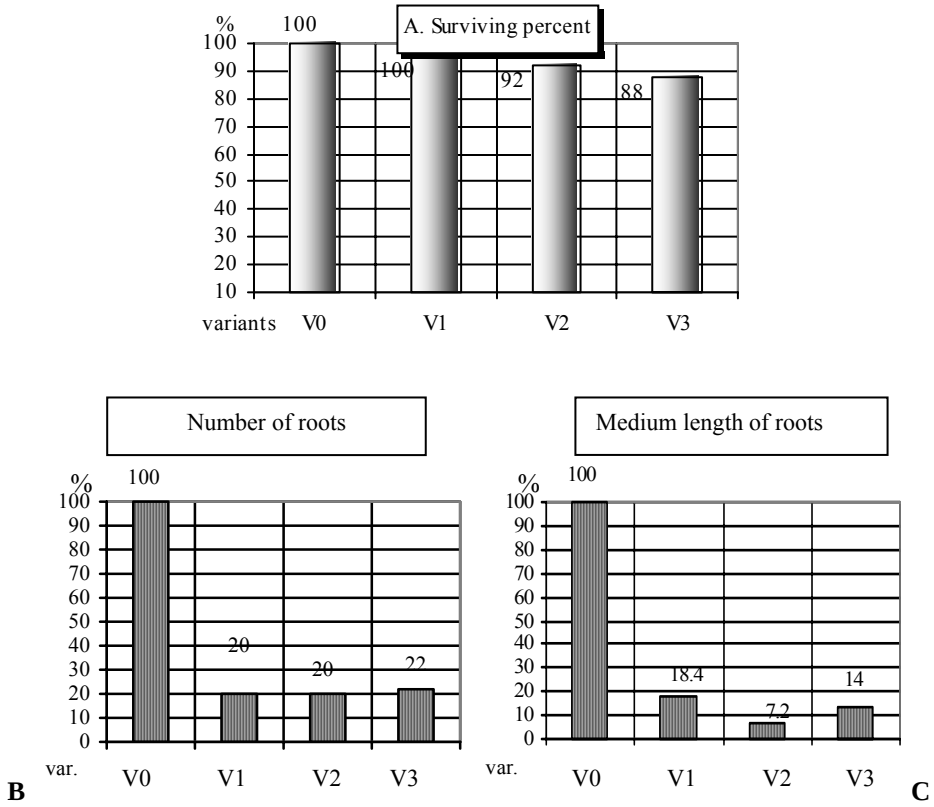


Figure 4. The comparative evolution of *Psidium guajava* vitroplantlets, at *8 weeks* after inoculation „in vitro" on MS (1962) simple medium culture and with an add of different growth hormones, where: the clones of the variants V_0 – witness medium (lot control) – without growth hormones, V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l); V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l); V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), values being expressed in percentage values obtained in report with the bio-measured values to vitroplantlets of the lot witness (V_0), values considered as the 100%.

We presents in **Fig. 4**, the bio-measured data, obtained at *2 months* after the *Psidium guajava* vitroplantlets establishment.

At *60 days* after the *Psidium guajava* culture establishment, on MS (1962) [2] medium culture, the *surviving percent* (**Fig. 4A**) of the clones was 100% on the witness variant V_0 (BM basic medium - MS without growth hormones) and variant V_1 (BM basic medium - MS with BA (1 mg/l) + IBA (1 mg/l)), on variant V_2 (BM basic medium - MS with BA (1 mg/l) + NAA (1 mg/l)) we obtained 8% deficit, respectively on V_3 (BM basic medium - MS with BA (1 mg/l) + IAA (1 mg/l)) a 12% deficit in comparison to witness variant V_0.

Concerning the number of roots (**Fig. 4B**), the clones generated roots on each experimental variant, and the roots length (**Fig. 4C**) varied, thus on the variant V_1 (BM basic medium - MS with BA (1 mg/l) + IBA (1 mg/l)) developed 0.6 mm length roots, and on the other experimental variants they formed 0.4mm roots, presenting merely deficits on all experimental variants, thus the values have adequate deficits of 82,6% on variant V_1 (BM basic medium - MS with BA (1 mg/l) + IBA (1 mg/l)), 86% on variant V_3 (BM basic medium - MS with BA (1 mg/l) + IAA (1 mg/l)), respectively on variant V_2 (BM basic medium - MS with BA (1 mg/l) + NAA (1 mg/l)) a deficit of 93,8% beside the control lot V_0.

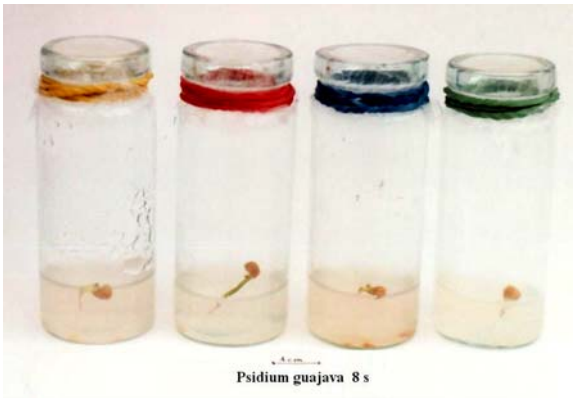


Figure 5. *Psidium guajava* vitroplantlets, at *60 days* after the „in vitro" inoculation

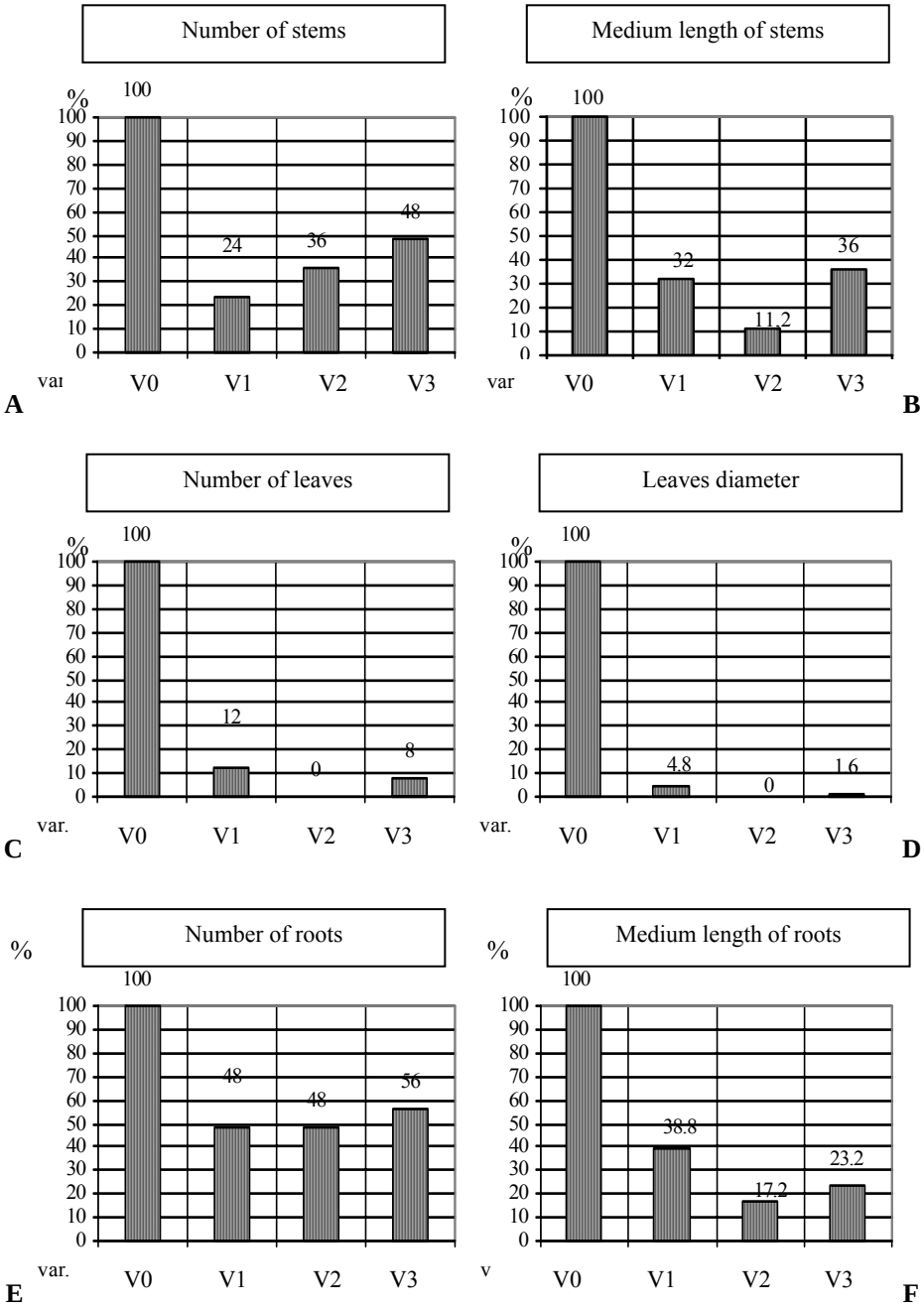


Figure 6. The comparative evolution of *Psidium guajava* vitroplantlets, at *9 weeks* after inoculation „in vitro" on MS (1962) simple medium culture and with an add of different growth hormones, where: the clones of the variants V_0 – witness medium (lot control) – without growth hormones, V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l); V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l); V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l)

At *9 weeks* (**Fig. 7**) *after Psidium guajava* culture initiation, we could notice a progressive growth and development of vitrocultures on all experimental variants. Thus, concerning the evolution of guava vitroplantlets, after the effectuation of bio-measurements at *8 weeks*, we noticed that after *1 week* they start to generate stems, leaves and roots, and on variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) each plant generated adventive roots.

The *stems lenght* (**Fig. 6B**) varied between 0.1 and 0.7 mm, on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with a deficit of 64%, on V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) with 68%, respectively on V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with 89,8% beside V_0.

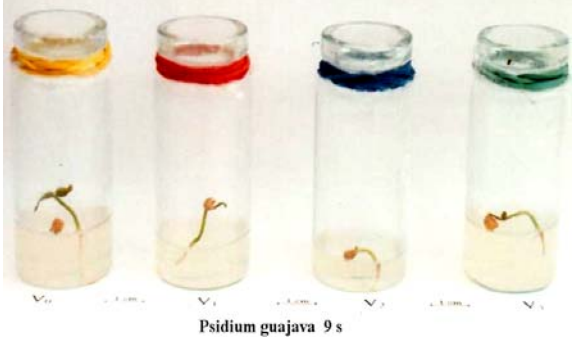


Figure 7. *Psidium guajava* vitroplantlets, at *9 weeks* after the „in vitro" inoculation

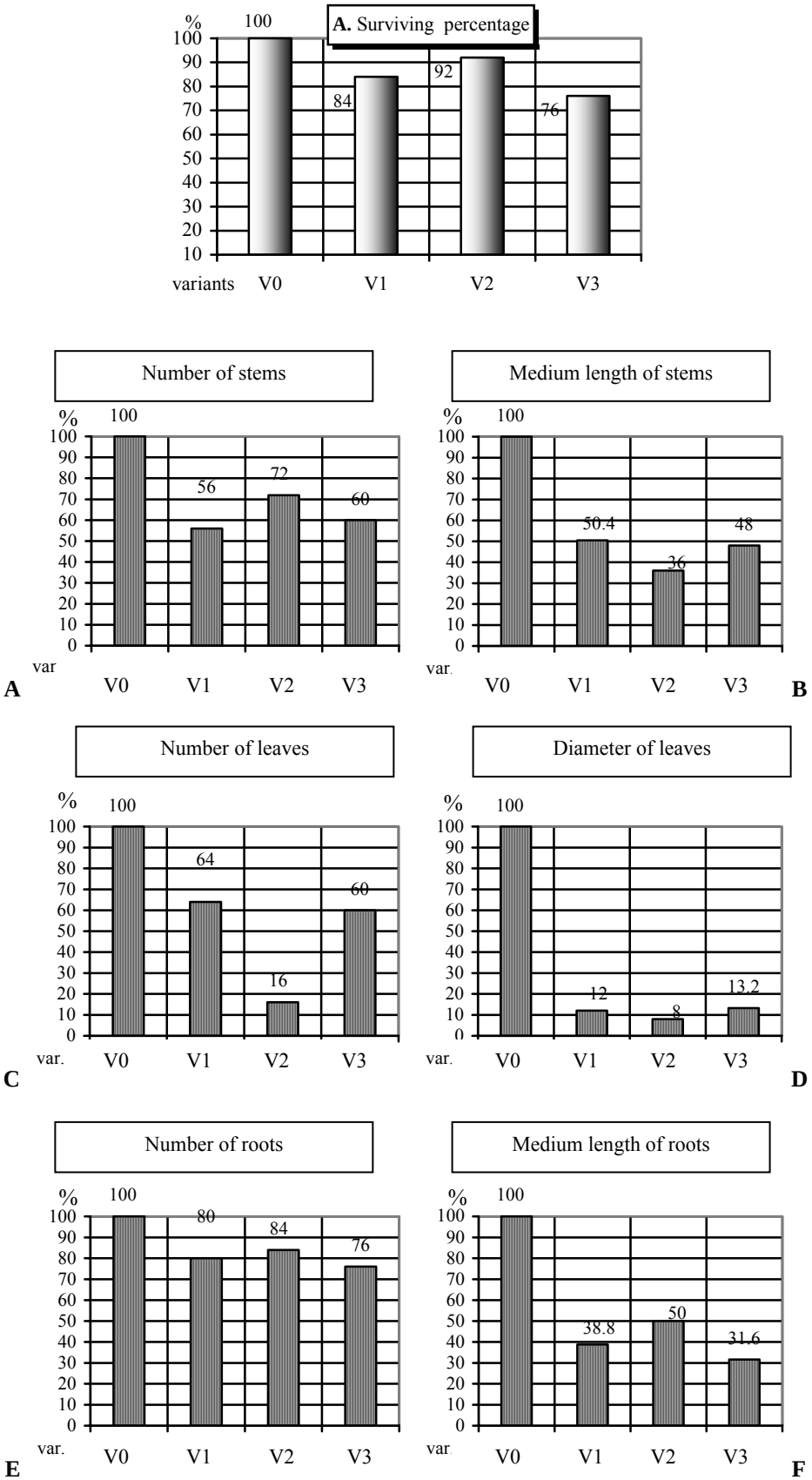


Figure 8. The comparative evolution of *Psidium guajava* vitroplantlets, at *10 weeks* after inoculation „in vitro" on MS (1962) simple medium culture and with an add of different growth hormones, where: the clones of the variants V_0 – witness medium (lot control) – without growth hormones, V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l); V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l); V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l).

The *caulogenesis* was manifested on all experimental variants, generating 1-2 leaves, except variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l). *The leaves diameter* (**Fig. 6D**) varied around 0.1-0.3 mm, thus on variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) it represented 4.8%, respectively on V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) the value was 1.6%.

Regarding the *risogenesis* of the guava vitroplantlets, *the roots length* (**Fig. 6F**) varied between 0.1 and 1.5 mm, on the witness variant they were much more developed. Thus, them length showed values to the witness variant like 18.4% on V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l), 14% on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), respectively on variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) 7.2%.

At *10 weeks* (**Fig. 9**) of vitroculture, the *Psidium guajava* explants, presented infections on V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), thus the *surviving percentage* presented only deficit beside the witness variant, on variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) they reached each values of 84%, respectively on the variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) 76%.

The explants *stems length* (**Fig. 8B**) varied between 0.2-1.5mm, the biggest stems were found on V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) with 50.4%, respectively on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with 68,4%.

The vitroplantlets that generated the highest number of leaves were on the variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), the *number of leaves* (**Fig. 8C**) varied between 2 and 4 leaves, thus on variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) has achieved value of 64%, on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), a value of 60%, the biggest deficit was present on the variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with 84% in comparison to the witness lot.

The leaves diameter (**Fig.8D**) varied between 0.2-0.5 mm, thus on the variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) represented 12%, respectively on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) a value of 13.2%, except variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with only 8%.

Regardless to the *risogenesis* of the guava vitroplantlets, the longest roots were noticed on the witness lot, these were 0.1-2.5mm length, and the *roots length* (**Fig. 8F**) presented the most nearest values beside the witness lot on the V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with 50%.

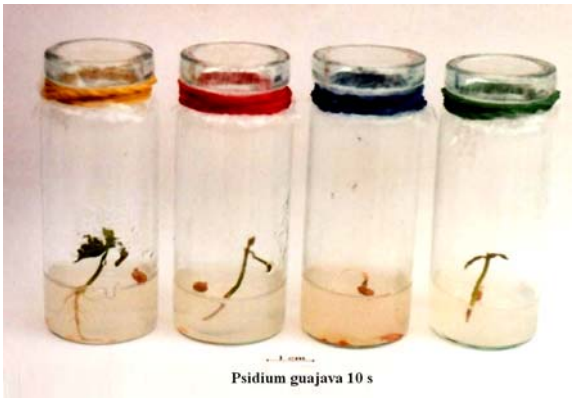


Figure 9. *Psidium guajava* vitroplantlets, at *10 weeks* after the „in vitro" inoculation

At *11 weeks* (**Fig.11**) after the guava culture initiation, *the surviving percentage* (**Fig. 10A**) of vitroplantlets presented deficits beside the witness variant V_0, because the infections, thus on variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) we get 92% values nearest to witness variant V_0, on variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) 84%, respectively on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) a value of 88%.

The best results we get on the witness variant where we obtained vitroplantlets with the *stems length* (**Fig. 10C**) between 0.5-1.9 mm, and on variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) the most vigorous plantlets, them length being between 0.9-1.8 mm, presenting an increase with 2%, respectively variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with an increase of 3,2% beside V_0, excepting V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with a deficit of 28%.

The vitroplantlets from the witness variant generated 2-8 leaves, and on the variant V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_3 – BM-MS medium with an adding of BA

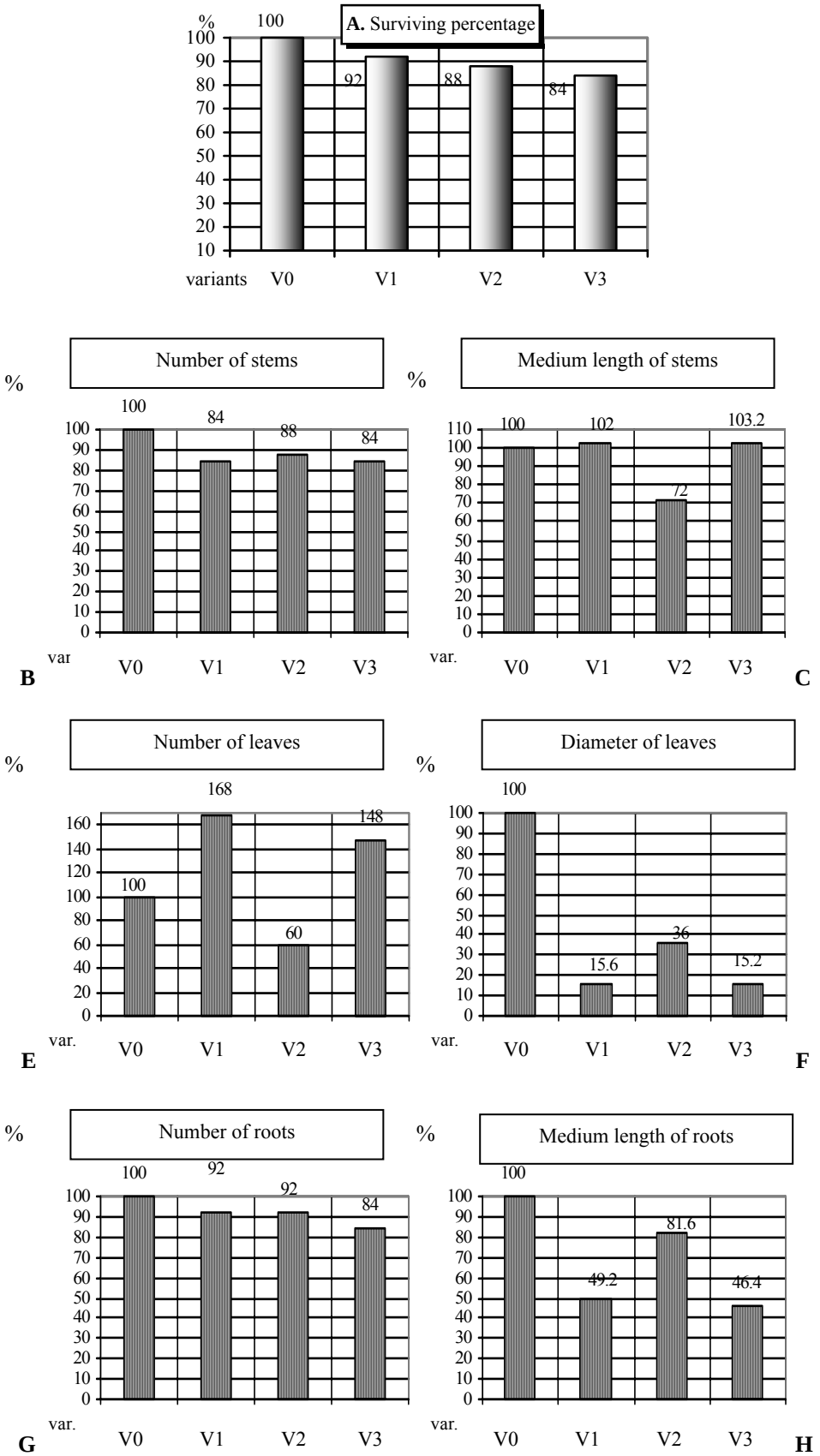


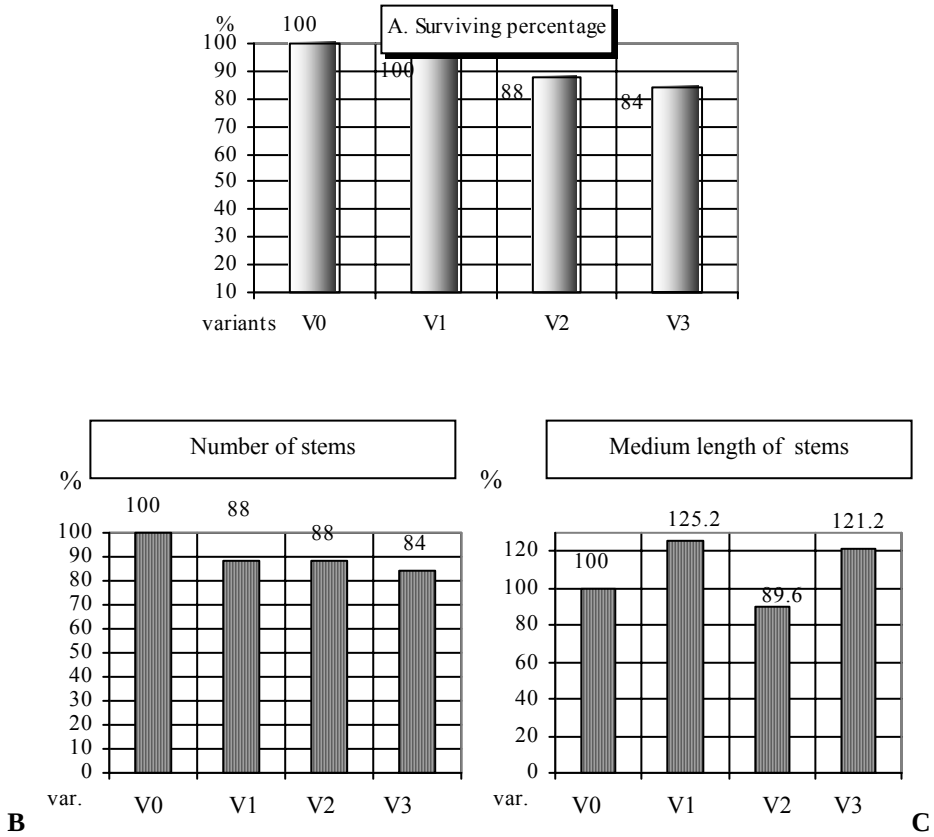
Figure 10. The comparative evolution of *Psidium guajava* vitroplantlets, at *11 weeks* after inoculation „in vitro" on MS (1962) simple medium culture and with an add of different growth hormones, where: the clones of the variants V_0 – witness medium (lot control) – without growth hormones, V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l); V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l); V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l).

(1 mg/l) and IAA (1 mg/l) 2-6 leaves each, the *number of leaves* (**Fig. 10E**) on the variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) present a decrease of 40%, beside the witness lot, the increases were noticed on V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) with 68%, respectively on variant V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with an 48% increase.

The diameter of leaves (**Fig. 10F**) showed that the most leaves were visible on the witness variant, their dimension being between 0.2-0.7 mm, and the other variants were between 0.1-0.4 mm.

Only the vitroplantlets from the witness variant generated 1-2 roots, and the rest explants generated only one each. The most vigorous roots same as from the witness variant were those from variant V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) presenting a value of 81.6%.

Figure 11. *Psidium guajava* vitroplantlets, at *11 weeks* after the „in vitro" inoculation



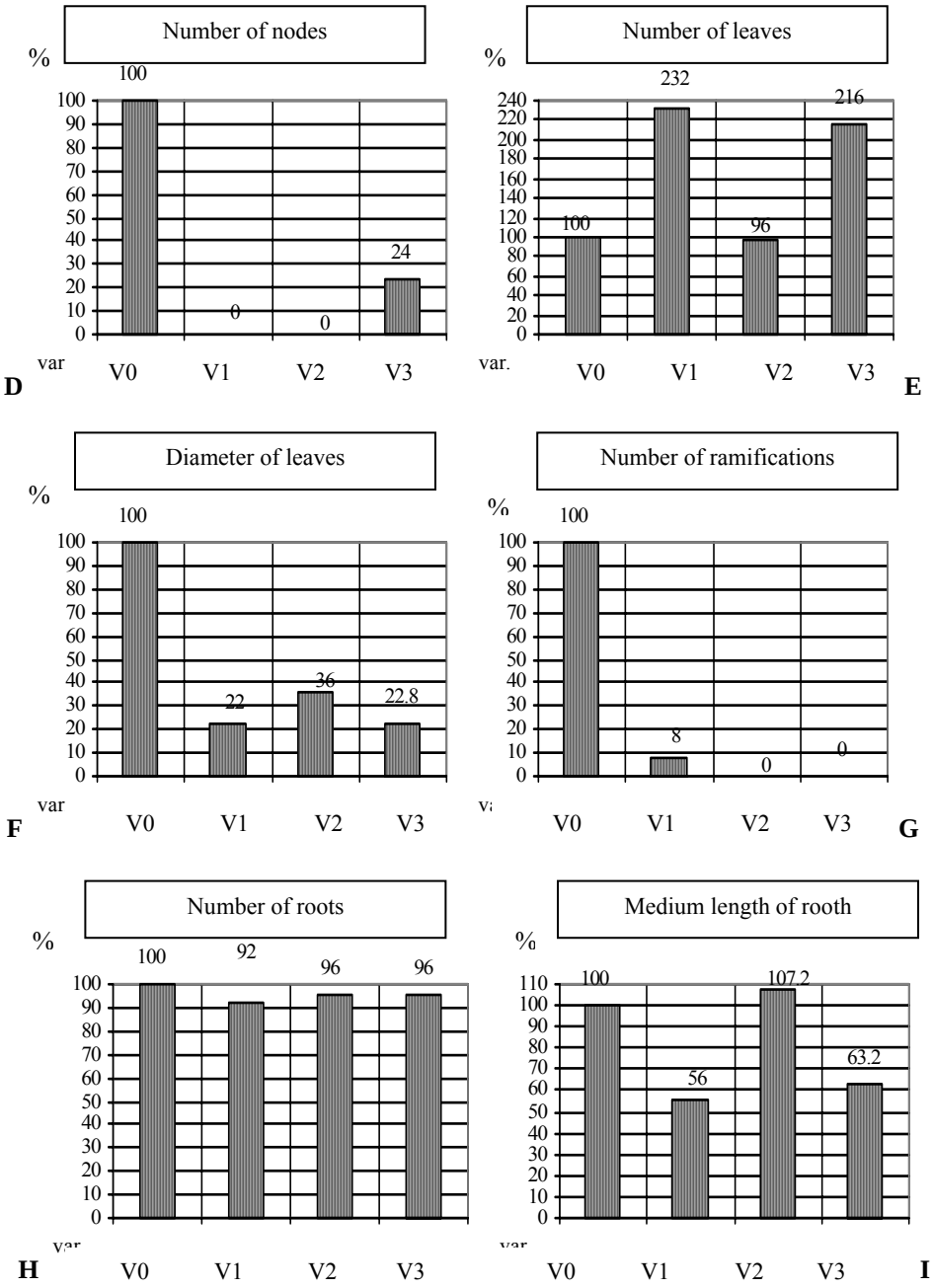


Figure 12. The comparative evolution of *Psidium guajava* vitroplantlets, at *12 weeks* after inoculation „in vitro" on MS (1962) simple medium culture and with an add of different growth hormones, where: the clones of the variants V_0 – witness medium (lot control) – without growth hormones, V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l); V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l); V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l).

At *12 weeks* (**Fig. 13**) after the guava culture establishment, the *surviving percentage* (**Fig. 12A**) registered only deficits an all experimental variants.

The stems length (**Fig. 12C**) of vitroplantlets, presented only deficits beside the witness variant V_0 only the V_3 – BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with a value of 89.6%, caused also some infections, thus, on the experimental variants we got increases of 25.2% on variant culture V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l), respectively on V_2 – BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with 21.2% increased value.

The most vigorous stems were generated on the control lot and on V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l), where the vitroplantlets had the *stems length* value between 1.1-1.9 mm.

Regardless to the *number of nodes* (**Fig. 12D**) the vitroplantlets generated each of them 2 nodes on V_0, respectively on the following 2 experimental variants V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_3– BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l)

The vitroplantlets from V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) generated 2-8 leaves, and on V_0 respectively on V_3– BM-MS

medium with an adding of BA (1 mg/l) and IAA (1 mg/l) 2-6 leaves, *the number of leaves* (**Fig. 12E**) on the culture variant V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) presented a small deficit of 4% beside the witness variant, the increases were noticed on the following variant cultures V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) with an increased value of 132%, respectively V_3– BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l) with 116%.

The *leaves diameter* (**Fig. 12F**) the most bigger were showed on V_0 and on V_3– BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), their dimension varying between 0.2-0.8 mm, and the rest of the variant cultures like V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) were 0.2-0.4 mm.

The number of ramifications (**Fig. 12G**) were each only 2 on V_0, respectively on V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l), their length (**Fig. 12 I**) varying between 0.2-0.3mm.

The most vigorous roots (**Fig.12 I**) were the same like on the witness lot, those from V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) with an increased value of 7.2%.

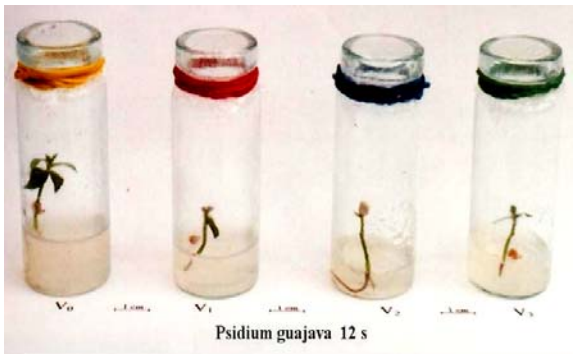


Figure 13. *Psidium guajava* vitroplantlets, at *12 weeks* after the „in vitro" inoculation

CONCLUSIONS

- In this experiment we presented the initiation of a widgeon tropical native shrub from Egypt, called *Psidium guajava* vitroculture. From our researches we found that the initiation of this specie vitrocultures is possible.
- In this experiment we have follow the initiation of the culture of *Psidium guajava*; the inoculations were made on 4 variants of medium culture; in first 2 months from inoculation, no infection or necrosis occurred to any experimental variants. The cultures on the witness medium V_0 presented a very good regenerative capacity, respectively on the variant culture V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l), we noticed that the germination of the guava seeds and the risogenesis process were active in all experimental variants.
- At *9 weeks* we have noticed a very good evolution of guava vitroplantlets, generating stemlets and roots, as well as adventive roots just on the variant of medium culture V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l), where on this variant the caulogenesis process it was not active.
- At *10 weeks* on V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) the caulogenesis process in the vitrocultures presented the longest leaves, in comparison to other variants.
- At *11 weeks* a very good evolution of the guava vitroplantlets could be observed on the variant V_0, and also a very good development on V_1– BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) and V_3– BM-MS medium with an adding of BA (1 mg/l) and IAA (1 mg/l), where they generated the most vigorous stemlets with many leaves, and on the control lot only, V_2– BM-MS medium with an adding of BA (1 mg/l) and NAA (1 mg/l) presented a very good risogenesis.
- At *12 weeks*, the vitroplantlets start to form nodes and to ramify on variant V_0 and V_1 – BM-MS medium with an adding of BA (1 mg/l) and IBA (1 mg/l) only.

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