Volume 15(1), 227- 232, 2011
JOURNAL of Horticulture, Forestry and Biotechnology

Preliminaries results concerning the *in vitro* multiplication of *Ginkgo biloba* species

Radomir Ana – Maria[1*], Tudor Radu C. M.[1]

[1]National Research & Development Institute for Biotechnology in Horticulture Stefanesti, Sos. Bucuresti-Pitesti, no. 37, CP 117713, Arges, ROMANIA

*Corresponding author. Email: radomir.anamaria@yahoo.com

Abstract This article presents the realizations of the technology of producing biological material with rapidly clonal multiplication with reference at the phases of in vitro initiation and multiplication. The growth of *Ginkgo biloba* explants was influenced by the period of explants sampling and by the composition of culture medium. The explants sampled from the herbaceous shoots a year old cropping at the end of the summer have the best behavior. They have registered 80% explants growth on culture medium MS supplemented with 20 mg/l benzyladenine. As one goes along the concentration of benzyladenine decreased, has been found a diminution of the number of explants growth until 25% and a progresive growth of the length of the shoots obtained. For the *Ginkgo biloba* multiplication proved to be efficient the concentration of 1 mg/l kinetine. The lenght of the microshoots was influenced by the type of citokinine and the dimensions achieved was directly proportional to multiplication rate.

Key words

Ginkgo biloba, in vitro initiation, explants, multiplication, culture media

Ginkgos are very large trees, normally reaching a height of 20 – 35 m, with some specimens in China being over 50 m. *Ginkgo biloba* currently occurs in the wild only in China. It has also been commonly cultivated in other countries for medical purposes. In the last years the medical plants are more important because are used like base material for obtaining active substances for pharmaceutical industry. In the present in our country ascertained a tendency for returning at phytotherapy, who lead at the extinctions of plantations of *Ginkgo biloba*. Also, the Ginkgos were planted for ornamental purposes. Considering the medicale and ornamentale importance we took the initiative of *in vitro* propagation of *Ginkgo biloba* in order to obtain rejuvenated planting material, in good phytosanitary condition.

Material and Method

For the initiation phase the biological material consisted of nodale segmentes long as 2 cm sampled from the herbaceous shoots a year old. The sampling was made in active growing stage (spring - summer) and in vegetative repose (autumn – winter).

Sampling of explants and the explants transfer on the multiplication culture media was made in sterile conditions, on a hood with laminar air flow (fig. 1).

Figure 1. Sampling of explants

The desinfection of biological material was made with ethanol 94% for 10 minutes, after which the material was transferred in a solution of calcium hypochlorite concentration of 6% for another 20 minutes – treatment applied on shoots in vegetative repose phase. For the shoots in the active growing stage, the treatment was reduced to half of the initial time.

The inoculums passed through 5 variants of culture medium, different in concentrations of benzyladenine (tab.1).

Table 1

The components of culture media used for the growth explants of *Ginkgo biloba*

Components (mg/l)	**V.1**	**V.2**	**V.3**	**V.4**	**V.5**
Macroelements	MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS
NaFeEDTA	32	32	32	32	32
Benzyladenine	-	5	10	15	20
Mio-inositol	100	100	100	100	100
Thiamine	0,5	0,5	0,5	0,5	0,5
Dextrose g/l	40	40	40	40	40
Agar g/l	7	7	7	7	7

Legend: MS = MURASHIGE - SKOOG (1962)

Before autoclaving, the pH registered in a culture medium was adjusted to 5.6-5.8.

For the growing stage of explants were used test-tubes obturated with polyethylene foil.

The surgical type instruments used were sterilized in the drying stove, at 120^{o}C temperature for 2 hours. The culture mediums were first sterilized by autoclaving at 120°C temperature for 20 minutes.

During the phases of the *in vitro* initiation and multiplication, in the growing room we have ensured controled conditions (photoperiod of 16 hours, temperature between 22-24^{o}C) (fig.2).

Figure 2. Aspect from the growing room

For the initiation of the experiments, in the *in vitro* multiplication phase, the biological material was represented by explants who were obtained in the initiation phase on the next culture media: macro and microelements Murashige - Skoog (1962), vitamins Murashige – Skoog (1962), 20 mg/l benzyladenine, 100 mg/l mio-inositol, 0,5 mg/l thiamine, 32 mg/l NaFeEDTA, 40g/l dextrose and 7g/l agar.

The culture media used in the micropropagation phase are complex composition with differente types and concentrations of phytohormones (tab. 2).

Table 2

The components of culture media used for the micropropagation of *Ginkgo biloba* explants

Components (mg/l)	V.1	V.2	V.3
Macroelements	MS	MS	MS
Microelements	MS	MS	MS
Vitamins	MS	MS	MS
Mio-inositol	100	100	100
Benzylaminopurine	1	-	-
Kinetine	-	1	-
TDZ	-	-	1
NaFeEDTA	32	32	32
Glucose g/l	40.000	40.000	40.000
Agar g/l	7.000	7.000	7.000

Legend: MS = MURASHIGE - SKOOG (1962)

The observations were realized weekly and the explants were passed on fresh cultures media when appearing the vitrification phenomenon or oxidatives processes.

Results and Discussions

The growth of *Ginkgo biloba* explants was influenced by the period of explants sampling and by the composition of culture medium.

Therefore we have discovered that the explants sampled in vegetative repose period was infected in percentage of 100% with funguses or bacteria.

The explants sampled from the herbaceous shoots a year old cropping at the end of the summer have the best behaviour. The differences registered at the initiating percentages were influenced by the composition of culture media, especially by the concentration of benzyladenine. The maximum values (80% explants growth) were obtained in the presence of 20 mg/l benzyladenine (fig. 3).

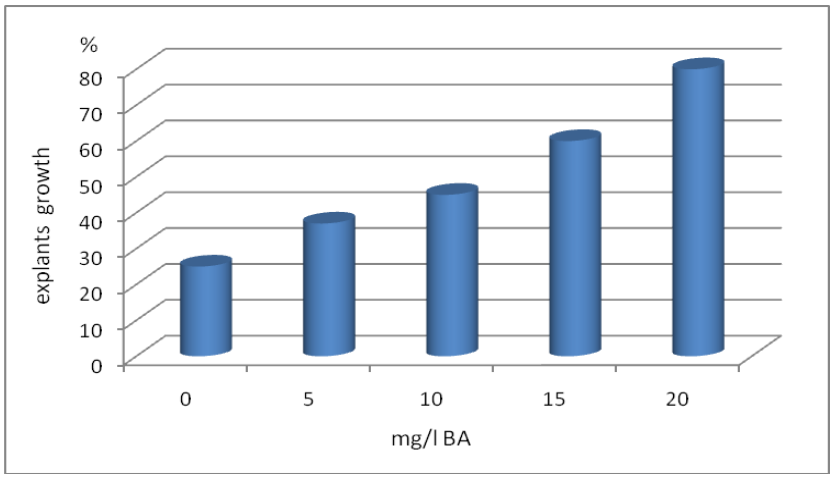

Figure 3. The influence of BA over the growth of *Ginkgo biloba* explants

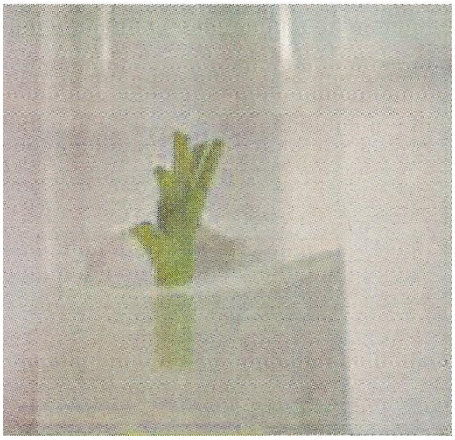

Figure 4. Apex caulinar sampled from the herbaceous shoots a year old

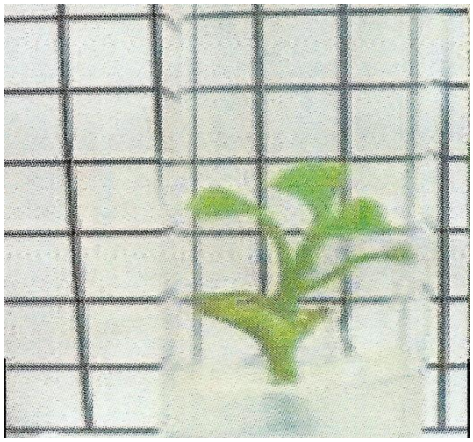

Figure 5. Shoot formed *in vitro*

The conclusion obtained from the research achieved during 6 weeks is that as one goes along the concentration of benzyladenine decreased, has been found a diminution of the number of explants growth until 25% and a progresive growth of the length of the shoots obtained.

For example, after 6 weeks incubation period, the length of the shoots varies between 1,9 cm on the culture medium with 20 mg/l BA and 4,8 cm on the culture medium without BA(fig. 6).

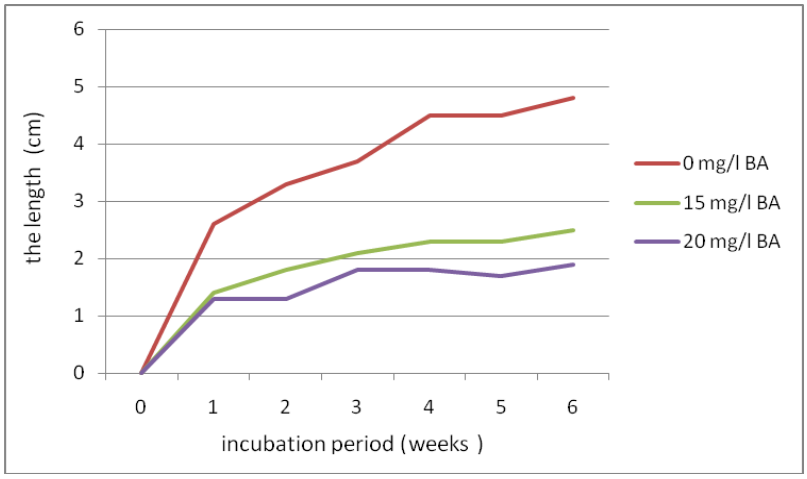

Figure 6. Variation of the length of the *Ginkgo biloba* shoots during 6 weeks incubation period on the culture medium with different concentrations of benzyladenine

Between the culture media tested for *Ginkgo biloba* micropropagation a positive influence over caulogenesis and growth of microshoots has 1 mg/l kinetine, when multiplication rate was 4 microshoots/explant. Microshoots have on the average 2,4 cm and they can be easy individualized for the realization a new subculture.

On the culture medium with 1 mg/l benzylaminopurine was obtained 3 microshoots around an explant, but they not gowing in lenght (1,2 cm) and for that reason the individualization was realized with difficulty, but after transfer many was necrosed.

The presence of TDZ (1 mg/l) in culture medium produced the proliferation of the callus at the base of the shoots. It obtained only 2 microshoots/explant multiplication rate; these have 0,5 cm long, abnormales leafs, with short petiole and lamina foliare turned.

Figure 7. Multiples shoots formed *in vitro*

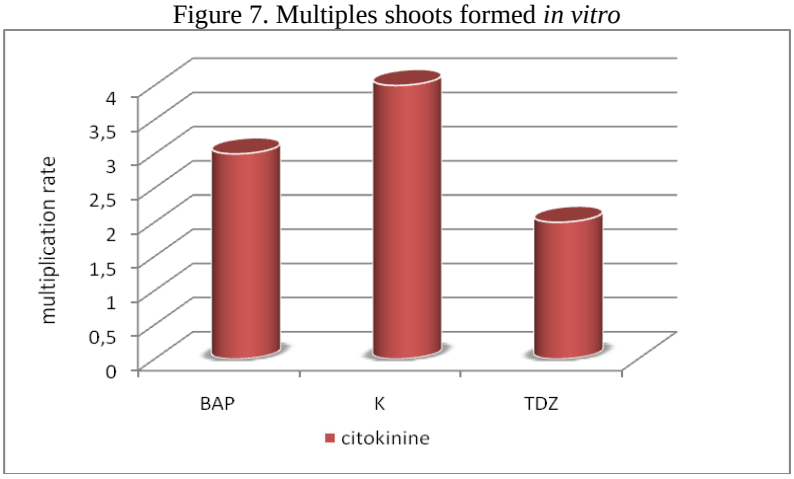

Figure 8. The influence of citokinines over the *in vitro* multiplication rate of *Ginkgo biloba* shoots

The lenght of the microshoots was influenced by the type of citokinine and the dimensions achieved was directly proportional to multiplication rate (fig. 9).

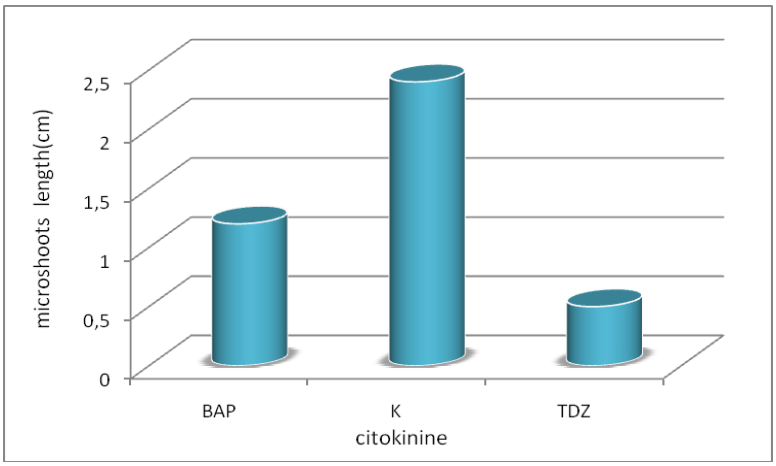

Figure 9. Variation of the length of the *Ginkgo biloba* microshoots depending the type of citokinine

Conclusions

- The growth of *Ginkgo biloba* explants was influenced by the period of explants sampling and by the composition of culture medium.
- The explants sampled from the herbaceous shoots a year old cropping at the end of the summer have the best behaviour. They have registered 80% explants growth on culture medium MS supplemented with 20 mg/l benzyladenine.
- As one goes along the concentration of benzyladenine decreased, has been found a diminution of the number of explants growth

until 25% and a progresive growth of the length of the shoots obtained.

- For the *Ginkgo biloba* multiplication proved to be efficient the concentration of 1 mg/l kinetine.
- The lenght of the microshoots was influenced by the type of citokinine and the dimensions achieved was directly proportional to multiplication rate.

References

1.Camper N.D., Coker P.S., Wedge D.E., Keese R.J.-1997- In vitro culture of Ginkgo. In vitro Cell. Dev. Biol.-Plant. 33, p. 125-127;

2.Montez - Lopez J.J., Rodriguez J.L. De La O.-2001- In vitro establishment and sprouting of axillary buds and shoot apex of ginkgo (Ginkgo biloba) Revista Chapingo Serie Horticultura, v.7, n.1,p. 49-59;

3.Nilton Mantovani, Magali Ferrari Grando, Wagner Campos Otoni E Tobias Pizzatto Grison-2007- Padroes de desenvolvimento de gemas caulinares in vitro x in vivo de Ginkgo biloba L., Revista Brasileira de Biociencias, Porto Alegre, v.5, supl.2, p. 594-596;

4.Rohr R.-1989- Maidenhair Tree (Gingko biloba L.),pp. 574-590. In: Biotecnology in Agriculture and Forestry, Bajaj, Y.P.S. (ed.). Vol 5. Trees II. Springer - Vearlag. Berlin Hindelberg. N. Y., London, Paris, Tokio;

5.Tommasi F., Scaramuzzi F.-2004- In vitro propagation of Ginkgo biloba by using various bud cultures. Biologia Plantarum.v.48, n.2, p.297-300.