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FACTORS AFFECTING *Camelia japonica* L. CLONAL MICROPROPAGATION

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Abstract

These studies aimed at studying the factors that influence the processes of organogenesis in tissue culture and organs of *Camellia japonica in vitro*. It is shown that high concentrations of 6-BAP – 2.5 and 3 mg/l cause rapid growth of callus tissues at the base of microcuttings. However, decrease at the concentration of phytohormones: 6-BAP from 2.0 mg/l to 0.5 mg/l in combination with indole acetic acid from 0.5 mg/l to 0.1 mg/l induced the growth of microshoots in WPM medium. It is noted that, variant from the culture medium with phytohormones of cytokinins-and-adenine type (6 BAP and zeatin) at a concentration of 2.0 and 1.0 mg/l, respectively, and 0.1 mg/l of naphthyl acetic acid was the most appropriate for accelerating the growth of microshoots obtained from explants of mixed varieties while the variant of the culture medium containing 6 BAP 2, 0 mg/l, naphthyl acetic acid – 0.5 mg/l and gibberellic acid – 1.0 mg/l was the best for the cultivar *Reine des Beautes.*

Key words: *Camellia japonica*, in vitro, micropropagation, phytohormones, microshoots

Camellia japonica genus relates to tropical and subtropical woody plants from East Asia. According to some researchers, in 2000, over 325 species belonged to this genus (Mondal et al., 2002). Some species are widely used as valuable ornamental evergreen and flowering plants; they are widely used in interior landscaping and creations of exhibitions in parks.

Ever-increasing interest in ornamental camellias (*C. japonica* L., *C. reticulata* Lindley, etc.) allowed breeders to create numerous cultivars having the improved ornamental floral qualities and growth characteristics. It became necessary to develop effective methods for their propagation.

The first initiator in breeding camellias on *in vitro* culture was Lammert (1950). Clonal propagation of these species was generally carried out by apical flower and young nodal segments with dormant axillary buds (Samartin et al., 1984; Vieitez and Vieitez, 1983, Carlisi and Torres, 1986). Cultivation of meristems on nutrient media resulted in induction of callus formation (Crézé, 1980). Positive results were obtained using parts of herbaceous (young) *Camellia japonica* stems as explants (Malyarovskaya, 2012).

Seglie L. et al. (2012) also developed methods of *Camellia japonica* clonal micropropagation (Seglie et al., 2012). They studied various types of explants (apical and axillary buds, apical shoots) of 19 genotypes, among which 4 cultivars – *California, General Coletti, Doctor Burnside* and *Charles Cobb* are often used for commercial purposes. They also evaluated the effect of different growth regulators, such as 6-BAP, gibberellic acid, etc., on the induction of morphogenesis. According to the research, they have found that the induction of *Camellia japonica* morphogenesis was influenced by various factors, which primarily included varietal characteristics, type of explant and combination of different growth regulators. However, a deterrent factor in developing methods for clonal propagation was a low multiplication coefficient of various cultivars, ranging from 18% to 39%. Thus, a slow increase of microshoots on *in vitro* culture together with serious difficulties in rooting of *in vivo* microplants was recorded.

Despite the experimental work in the study of morphogenesis and regeneration of *Camellia japonica* plants *in vitro*, still systems of obtaining and maintaining these plants in tissue culture are not developed. This is due to the lack of clear and well-reproducible biotechnological methods for obtaining, maintaining and breeding valuable cultivars of the given ornamental culture.

MATERIAL AND METHODS

In the experiments, there apical and axillary buds as explants were used, as well as shoots with apical and axillary buds of *C. japonica* model cultivar called *Reine des Beautes*, and the cultivars mix № 1; the isolation of explants was conducted during two periods: in early April after flowering of the plants, and in May, during young shoots growing phase.

Before the sterilization, the plant material was washed in a soapy solution and kept in a solution of K_2MnO_4 (30-40 minutes) with continuous stirring, fol-

lowed by 15% sodium hypochlorite during 10-15 minutes, and finally washed three times with sterile distilled water (Malyarovskaya, 2012).

The studies included a mineral base of nutrient medium (WPM) with various concentrations of plant growth regulators called 6- benzylaminopurine (6-BAP) at a concentration of 2, 2.5 and 3 mg/l, and IAA – 0.5 mg/l, with addition of 0.6% agar-agar. To accelerate microshoots growth, two options of nutrient media were studied: first – with the addition of 6-BAP and zeatin at concentration of 2.0 and 1.0 mg/l respectively and naphthylacetic acid – 0.1 mg/l; second option contained 6 BAP – 2.0 mg/l; NAA – 0.5 mg/l and gibberellic acid – 1.0 mg/l.

The solution of the nutrient medium was adjusted to pH 5.7 and autoclaved during 20 minutes at a temperature of 120 ºC. Primary explants and microshoots were cultivated with light intensity of 5000 lux, 16 hour photoperiod and temperature of 24 ± 1 ºC.

Aseptic techniques and methods of plants clonal micropropagation on *in vitro* culture were carried out according to Butenko (1999) and Cherevchenko et al. (2008).

RESULTS AND DISCUSSION

The studies found that the induction of new formations was effected by phytohormones in various ways (Table 1).

During cultivation on nutrient media variants with high concentration of 6 – BAP from 2.5 to 3 mg/l, formation of a compact callus of light green colour was recorded at the microshoots base. The resulting callus was separated from the base, and separated into segments, placing on nutrient medium with the reduced content of 6-BAP – 2 mg/l. After 30 days, embryogenic zones were observed in callus tissue, but they did not develop further.

To exclude further appearance of callus tissue at camellia microshoots base, the plants were transplanted onto mineral medium with low content of phytohormones: 6-BAP – 0.5 mg/l, IAA – 0.1 mg/l, and kinetin – 0.1 mg/l. This led to positive results; induction of callus formation was not observed subsequently.

In the next experiment the effect of different concentrations of phytohormones on microshoots growth was studied. During the investigations it was established that the cultivars mix had more microshoots after cultivation during 2.5 – 3 months than *Reine des Beautes* cultivar had; it relates to both first and second variants, and resulted in 1.7 and 1.0 cm, respectively (Table 2).

As it is known, different hormone compositions applied in various concentrations do not equally influence morphogenesis processes in cultivars even within the same species.

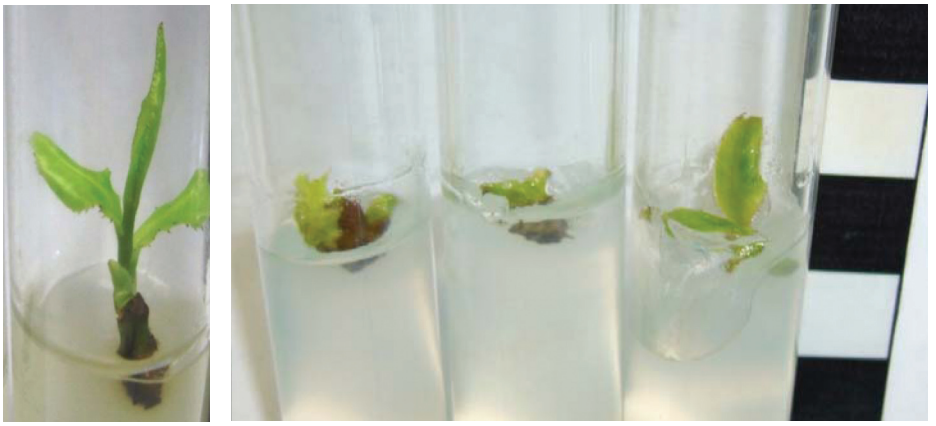
Thus, apparently, the nutrient medium option intensified by phytohormones of cytokinin type (6-BAP and zeatin) increased the rate of cells division, which contributed to more active growth of *Camellia japonica* microshoots. Thereby, depending on the concentrations and ratios of phytohormones in nutrient medium,

Table 1. Induction of *C. japonica* 'Reine des Beautes' new formations depending on phytohormones concentration

Concentrations of growth regulators, ml/l	Number of explants, piece	Type of new formations, %			Infected, %
		callus	direct organogenesis	frequency of shoot formation	
6-BAP – 2 IAA – 0.5	120	5.8 ± 0.9	0	54.5 ± 5.7	39.7 ± 4.1
6-BAP – 2.5 IAA – 0.5	120	33.7 ± 1.9	2.4 ± 0.4	12.1 ± 2.7	51.8 ± 6.3
6-BAC – 3 IAA – 0.5	120	51.4 ± 4.8	7.7 ± 1.2	3.6 ± 0.8	37.3 ± 3.9

Table 2. Effect of different growth regulators concentrations on microshoots growth

Concentrations of growth regulators, ml/l	Cultivar, cultivars mix	Number of microshoots, piece	Height (cm) during introduction to the culture	Height (cm) after three months of cultivation
Variant 1 6 BAP – 2.0; Zeatin – 1.0; NAA – 0.1	*Reine des Beautes*	25	0.5 ± 0.09	0.6 ± 0.1
	Cultivars mix	25	0.9 ± 0.2	1.7 ± 0.08
Variant 2 6 BAP – 2.0; NAA – 0.5; Gibberellic acid – 1.0	*Reine des Beautes*	25	0.5 ± 0.06	0.8 ± 0.03
	Cultivars mix	25	0.5 ± 0.04	1.0 ± 0.2



a) **b)**

Fig. 1. Camellia japonica microshoots
a) after 5 months of cultivation; b) micropropagation.

the growth of microshoots averaged from 0.1 to 0.8 cm for the whole research period. On the basis of three years of research, we can conclude that the growth and development of *Camellia japonica* microshoots requires a longer time period in comparison with the other woody plants. In this case, our results are consistent with findings of other investigators, such as Kato (1986) and Seglie et al. (2012).

For the study period the induction of adventitious shoots was not observed. It means that *C. japonica* propagation is possible only due to the microshoots growth and increase of internodes number on them (Figure 1).

It is necessary to add that the more often subcultivation was carried out, the more active secondary infection was. Reapplied sterilization did not lead to any positive results and the microshoots became infected and died.

CONCLUSIONS

As a result of the research it can be concluded that high concentrations of 6-BAP – 2.5 and 3 mg/l cause rapid growth of callus tissues on microshoots base. However, lowering the phytohormones concentration: 6-BAP from 2.0 mg/l to 0.5 mg/l at the ratio with IAA from 0.5 mg/l to 0.1 mg/l on WPM nutrient medium induced the growth of microshoots.

It is noted that, the first variant from the culture medium with phytohormones of cytokinins-and-adenine type (6-BAP and zeatin) at concentration of 2.0 and 1.0 mg/l, respectively, and 0.1 mg/l of NAA was the most appropriate for accelerating the growth of microshoots obtained from explants of the cultivars mix, while the second variant of the culture medium containing 6-BAP – 2.0 mg/l, NAA – 0.5 mg/l and gibberellic acid – 1.0 mg/l was the best for the cultivar *Reine des Beautes.*

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