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In vitro Morphogenesis of Ornamental Shrubs *Camellia japonica* and *Hydrangea macrophylla*

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Abstract

The effects of genotype, explant source and basal media on shoot growth and callus formation of the mature explants of *Camellia japonica* and *Hydrangea macrophyla* were studied. Apical and lateral buds showed higher growth potential compared to meristem in the both species. The best results in three cultivars of *C. japonica* were obtained on WPM supplemented with 2 mg/l TDZ + 0.5 mg/l Kn + 1 mg/l GA_3. Similarly, best result was observed in two cultivars of *H. macrophylla* on MS supplemented with 1 mg/l BAP + 0.1 mg/l NAA + 1 mg/l GA_3.

Introduction

Stock plants of perennial crops as a source of cuttings usually lose their vigor after many years of constant propagation. More so there is possibility that these plants may be infected by bacteria and fungi during field and greenhouse propagation. On the other hand, micropropagation is becoming an effective tool for production of elite genotypes and rejuvenation of plant material. Cuttings derived from micropropagated plants usually produce roots easily and grow vigorously.

In vitro techniques can provide reliable conservation and propagation of valuable germplasm as well as reduce or eliminate the load of microorganisms which may cause serious diseases (Sharma and Agrawal 2012, Kolomiets et al. 2014). Ornamental industry applies micropropagation for wide range of plant species however, for many perennial trees and shrubs vegetative *in vitro* propagation is still a problem. Several problems in micropropagation of trees and shrubs take place, such as low multiplication rate, high value of phenolic and

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other compounds and heavy contamination due to presence of endophytes. Such factors as genotype, explant type and size, media composition have significant effect on micropropagation success (Malyarovskaya 2014). Understanding the role of these factors is important for development successful protocols for cultivar micropropagation of ornamental trees and shrubs.

Camellia japonica is one of perennial ornamental species with more than 32,000 cultivars of great ornamental value used as outdoor shrub and as potted home plants (Vela et al. 2013) and has a high nutrition value (Lee et al. 2014). In earlier published articles on *C. japonica* propagation by somatic embryos (San Jose et al. 2016), juvenile seedlings (Mondal 2014) as well as meristems and bud explants (Vieiteza et al. 1989) were developed. However, there are only a few publications with many missing details and clonal *in vitro* propagation remains problematic for *C. japonica* (Mondal 2014).

Hydrangea macrophylla is another perennial ornamental shrub grown commercially as potted plants, as outdoors bushes and as cut flowers with large, round flowerheads of various colors ranging from white to purple, pink and red (Malyarovskaya and Karpun 2008). Very little has been reported on the micropropagation and morphogenesis of *Hydrangea in vitro* (Abou Dahab 2007), and as with many woody species, the development of an efficient regeneration protocol in *Hydrangea* has not yet been successful (Liu et al. 2011).

The aim of this work is to study effects of culture media composition, genotype and explant on growth of *C. japonica* and *H. macrophylla* cultivars, which are established as outdoor ornamental plants on the Black Sea Coast of Western Caucasus.

Materials and Methods

Young shoots of several cultivars of *Camelia japonica* (cv. Reine des Beautes, cv. Lelie, cv. David Bocchi) and *Hydrangea macrophylla* (cv. Draps Wonder, cv. Madame Hamard) were obtained from the field collection of the Institute (Sochi, Russia). Shoots were washed in running tap water for 20 min, soaked in $KMnO_4$ pink solution for 30 min. Decontamination procedure was conducted by the protocols of Malyarovskaya 2012, Kolomiets et al. 2014. Apical and lateral buds and meristems with 2 - 3 primordia were isolated and used as explants.

Explants were placed on MS and WPM (Lloyd and McCown 1980) basal medium for initiation of growth and further multiplication. Media were supplemented with 25 g/l sucrose and solidified with 2.5 g/l phytagel. To study the effect of PGR on growth of explants media were supplemented with BAP, Kn, NAA, GA_3, TDZ. The chosen combinations were selected according to

previous investigations. The pH of medium was adjusted to 5.7 before autoclaving at 12°C for 20 min.

Explants were placed on the medium filled in 100 ml glass jars (one explant per each jar) and incubated in light chamber under fluorescent tubes with photoperiod 16/8, light intensity 3000 lux and temperature 24 ± 2ºC

Effects of basal medium and PGR were observed after 30 - 60 days in culture. The percentage of callus formation, shoot growth and no responded explants were recorded. All experiments were conducted in three replicates with 30 explants per each treatment. Statistical data were analyzed using ANOVA test and differences were considered as significant at $p \leq 0.05$.

Results and Discussion

In our study, when MS and WPM basal media were compared, the average shoot length was not depended significantly on basal media (data are not presented). But in case of *C. japonica* leaves were greener on WPM so this medium was used in the following experiments.

The medium previously used for *Hydrangea* was usually based on MS salts and some authors reported that WPM was not appropriate for this ornamental shrub (Abou-Dahab 2007). So it was interesting for us to test WPM for explant development.

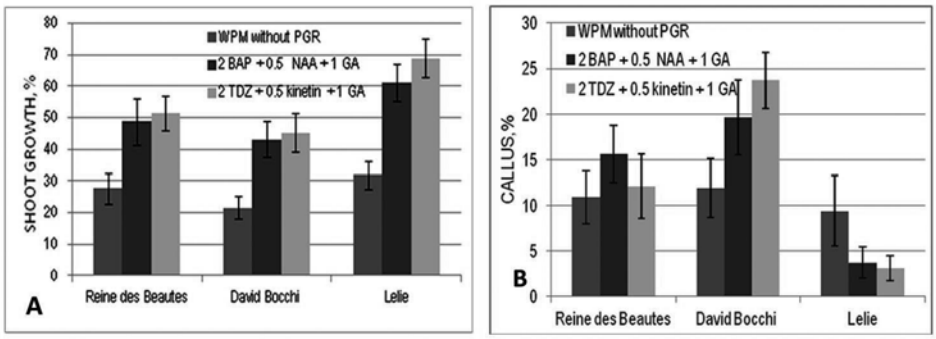

Fig 1. The effect of growth regulators (mg/l) for *in vitro* development of *Camelia japonica* buds in culture medium.

On the other hand, in case of *C. japonica* previously reported that WPM is the best growth medium (Vieitez et al. 1989). However, other authors found that MS and half strength of MS were best media for *C. japonica* micropropagation (Carlisi and Torres 1986). So it can be concluded that different responses were probably genotype-dependent (Mondal 2011).

Addition of two combination of PGR affected significantly on explants growth and showed better morphogenesis in both species. In case of *C. japonica* the best response of buds (43.1 - 68.9 %) was obtained in both PGR combinations compared to control (21.7 - 31.9 %) (Fig. 1A). The tested PGR combinations did not result adventitious shooting, so multiplication of *Camellia* was only possible by increasing internodes number.

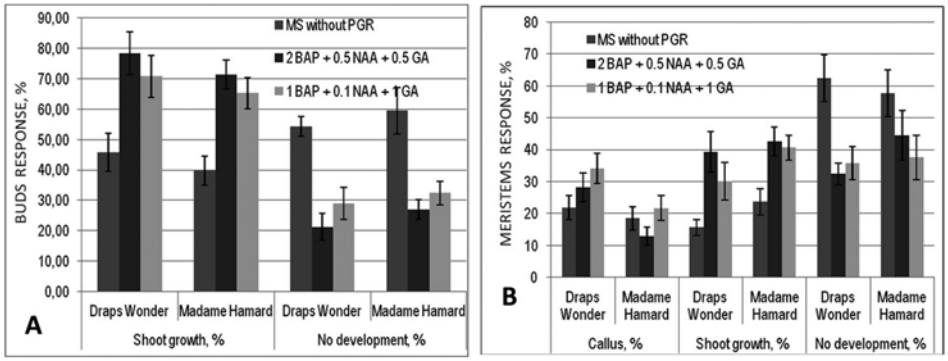

Fig. 2. Effect of growth regulators (mg/l) for *in vitro* response of buds (A) and meristems (B) of *Hydrangea macrophylla* in culture media.

Solid callus was formed in basal portions of *Camellia* explants. The callus seemed to have inhibitory effect on further shoots development. Effect of PGR on callus percentage was affected by cultivar (Fig. 1B). Both PGR combinations significantly increased callus formation in cv. David Bocchi, decreased callus formation in cv. Lelie and showed no effect on callus formation in cv. Reine des Beautes compared to control. So, as it was previously supposed, different responses are probably more genotype-dependent than PGR-dependent (Mondal 2011).

In case of *H. macrophylla* two combinations of growth regulators significantly increased shoot growth. Bud growth percentage was 65.3 - 68.4 compared to control 37.9 - 45.7 in cv. Draps Wonder and Madame Hamard, respectively (Fig. 2A, 3A). Addition of PGR did not affect significantly on callus formation of *H. macrophylla*. Callus was observed only from meristems in both cv. Draps Wonder and Madame Hamard and its percentage was 12.9 - 34. Callus percentage was also more genotype-dependent than PGR-dependent (Fig. 2B). *In vitro* flowering occurred in *H. macrophylla* shoots on MS supplemented with 1 mg/l BAP + 0.1 mg/l NAA + 1 mg/l GA (Fig. 3B). In other studies on *Hydrangea quercifolia* authors used BA and concluded that propagation technique did not consistently influence response to PGRs (Cochran et al. 2014). In other studies MS with BA (2

mg/l) (Abou-Dahab 2007) and MS supplemented with BA (0.25 mg/l) were found to be the best medium for *Hydrangea macrophylla* with the highest multiplication rate.

Effect of explant on morphogenesis success was also significant. Lateral and apical buds were better explants of *C. japonica* than meristems with the higher percentage of morphogenesis (43 - 65). There were only 2.1 - 2.3 % meristems developed into shoots and after 30 days in culture they died (data are not shown).

In case of *Hydrangea macrophylla* shoot growth appeared from the apical and lateral buds after 8 - 10 days in culture. The best morphogenesis was observed using buds as explant on MS supplemented with PGR (Fig. 2A, B).

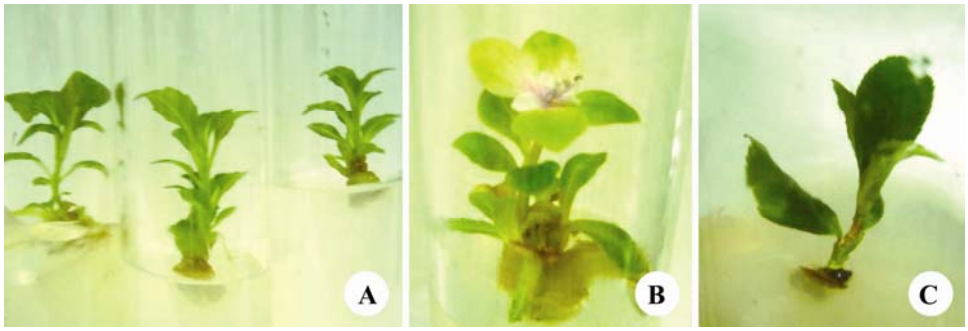

Fig. 3. A. Shoot growth from buds of *H. macrophylla* cv. Draps Wonder after one month on MS. B. *In vitro* flowering of *Hydrangea* shoots. C. *C. japonica in vitro* morphogenesis from buds.

Present data confirmed: despite the fact that plants can be rejuvenated and grow better through meristem culture there were many constraints to establish reliable micropropagation from meristem of perennial ornamentals (Prakash 2009).

Significant differences between cultivars were observed during the propagation of *C. japonica*. Cultivar Lelie showed the highest percentage of bud growth (68.9) compared to Reine des Beautes (51.4%) and David Bocchi 45.2% (Fig. 1A). On the other hand, cultivar David Bocchi showed the highest percentage of callus formation (23.7) compared to Reine des Beautes (12.1) and Lelie (3.1) on WPM supplemented with 2 mg/l TDZ + 0.5 mg/l Kn + 1 mg/l GA (Fig. 1B).

Cultivar effect in *H. macrophylla* was not such evidential. There were only significant difference between the two cultivars in meristem growth but not in bud growth (Fig. 2B). The rate of callus formation of cv. Draps Wonder (34.1%) was significantly higher than in cv. Madame Hamard (21.7 %) in presence of

PGR. On the other hand meristem growth was better in cv. Madame Hamard (23.7%) than in cv. Draps Wonder (15.7%).

To summarize the results it can be concluded that explant type, genotype and growth regulators affected significantly shoot growth and callus induction of *Camellia japonica* and *Hydrangea macrophylla*. Best results in *C. japonica* were obtained using bud explants on WPM supplemented with 2 mg/l TDZ + 0.5 mg/l Kn + 1 mg/l GA. *In vitro* proliferation of *H. macrophylla* was also affected by PGR and explant type. Best results in *H. macrophylla* were observed using buds placed on MS with 1 mg/l BAP + 0.1 mg/l NAA + 1 mg/l GA. This study will help to understand the role of different factors in tissue initiation in culture of ornamental shrubs.

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