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REGENERATION POTENTIAL OF DIFFERENT TYPES OF EXPLANTS FROM INTRODUCED VARIETIES OF RHODODENDRONS IN ASEPTIC CULTURE

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Abstract

The paper presents data on the regenerative capacity of different types of explants in 6 introduced species of rhododendrons under conditions of sterile culture. It is shown, that the apex of seedling has the largest regeneration potential, giving the maximum output of the plants-regenerants per explant. It allowed to recommend it as the primary explant for clonal micropropagation of studied varieties of rhododendrons.

Keywords: regeneration potential, introduced varieties of rhododendrons, aseptic culture.

Introduction

One of the factors, that has a great influence on the process of plant micropropagation, is an explant, his physiological state. The physiological state of explant closely is related with the age of the mother plant, the organ, from which explant was isolated, and also is related with the time of year.

Stage of development of explant has priority importance in the regeneration process, occurring in the culture of cells and tissues.

The results of experiments, received by Belonogova and Raldugina (2006) as forinvestigation of the regeneration of shoots on the cotyledon explants of flax (*Linum usitatissimum* L.), showed, that the optimal for shoot formationratio of growth regulators in the nutrient medium for regeneration was depended on the age of the seedlings, from which explants were taken. For explants of earlier age was optimal the lower concentration of BAP and the absence of auxin. With increasing of age of explants, conversely, were required higher concentrations of BAP and NAA presence. Similar results for flax were obtained by other authors (Mundhara and Rashid 2001-2002, Wilhemova et al., 2004).

Based on the results of experimental studies of Averyanova et al. (2002) was established the dependence of callusogenesis of diploid tissues of lily of the valley from the age and nature of the explant. It is shown, that the production of callus from mature diploid tissue of lily of the valley is difficult, whereas the young diploid tissue, containing a sufficient number of meristematic cells are capable to form, morphogenic callus, and besides type of morphogenesis (rhizogenesis), as the authors believe, is strictly determinated and weakly depends on exogenous phytohormones, used in the experiments, conducted by them.

An analysis of the literature on the regenerative capacity of juvenile and mature explants gives grounds to believe, that there are two aspects of this problem. On the one hand, the experimental data, obtained by numerous researchers, indicates a high regenerative ability, inherent to juvenile explants (Halperin 1986, Hanter 1979, Cheng 1975, Sommer and Brown 1974,Champhel and Durzan 1976, McCown and Amos1979, Von and Eriksson 1978, Dublin 1980, Durand and Boudet 1979, Lu Chin et al. 1990, Christopher andRajam1996, Ashrafuzzaman et al. 2009, Dabauza and Pena 2001, Deepika and Kanwar2010, Memon 2012, Naz et al. 2012, Kumar and Reddy 2012,Grozeva and Velkov2014, Wagh et al. 2015) and on the other hand − there are studies in favor of the mature explants (Clog et al. 1990, Lac and Ahuj 1989, Mondal et al. 1990, Purohit and Kukda 2004,Rathore et al. 2004, 2007, Papafotiou 2008).

This convinces us that it is only experimentally possible to determine the morphogenability of those or other explant, regardless of our knowledge of its physiological state, that is, the degree of maturity. In our opinion, of great interest is the study of the regenerative capacity of different types of explants from introduced varieties of rhododendrons. The study of this question will allow to determine the type of explants, having high regenerative capacity and giving the maximum output of regenerated plants and to recommend it as a primary for culture in vitro.

The aim of investigation was to study the regenerative capacity of different types of explants from introduced varieties of rhododendrons under conditions of sterile culture.

Materials and Methods

In the experiment explants were parts of the plant from vegetative and generative organs and various by age: juvenile and mature (see Table).

Table. Regeneration potential of different types of explants from introduced varieties of rhododendrons

Type of explant	Variety					
	R.brachycarpum	*R.ponticum*	*R.discolor*	*R.catawbiense*	*R.japonicum*	*R.smirnowii*
	Quantity of regenerants per explant, pieces.					
Epicotyl	0±0	0±0	0±0	0±0	1±0	0±0
Hypocotyl	0±0	0±0	0±0	2±1	1±0	0±0
Stem	1±0	2±0	0±0	1±0	2±1	0±0
Root	0±0	0±0	0±0	0±0	0±0	0±0
Cotyledons	1±0	3±1	4±2	4±1	6±1	5±1
Apex seedling	6±2	10±2	9±3	7±2	12±3	8±2
Buds of not lignified shoots:						
apical	3±1	4±2	4±2	5±1	9±2	5±2
lateral	2±1	3±1	3±1	4±2	6±1	3±2

Notation: At the buds from lignified shoots (apical, lateral), and also of parts of the flower (petals, sepals, ovary, stamens, pistils) the number of regenerants per explant was zero for all 6 varieties of rhododendrons.

For the isolation of juvenile explants were used sterile seedlings of 6 varieties of rhododendrons: *Rhododendron brachycarpum* D.Don. (Syn. *Azalea brachycarpa*D.Don), *Rhododendron ponticum* L., *Rhododendron discolor* Franch., *Rhododendron catawbiense*Michaux, *Rhododendron japonicum* (A.Gray) Suring), *Rhododendron smirnowii* Trautv. In order to obtain sterile seedling, seeds of listed varieties of rhododendrons were sterilized in 0,1% solution of sodium diacid for 8 minutes, previously soaking them in 70° ethanol for 5 seconds. After sterilization the seeds were washed in three portions of distilled water for 15 minutes in each. The apical and lateral buds of rhododendrons were sterilized by the same way.

Epicotyl, hypocotyl, stem, root, cotyledons, apexes of seven-day sterile seedlings and also sterile apical and lateral buds were planted on agar nutrient medium Anderson (Anderson 1975), containing 4 mg L^{-1}IAA, 15 mg $L^{-1}$of 2-ip supplemented with 80 mg L^{-1} adenine sulfate, 1 mg L^{-1}thiamine and cultivated at 25° C, illuminance 4000 lux, 16 hour photoperiod.

The calculation was made on the basis of 10-15 explants for each variety. The figures in the table are arithmetic mean values with their standard errors.

Results, Discussion and Conclusion

An analysis of the material, presented in the table, gives grounds to assume that for each type of explant is characterized by a certain regeneration potential, depending on the species of plant and its degree of maturity, that isof physiological state of explant. At studied varieties of rhododendrons apex of seedling has maximum regenerative potential. Thus, the leader in the number of regenerants per explant (apex of seedling) should be considered as *Rhododendron japonicum* (12 pcs.), then the *R. ponticum* (10 pcs.), *R. discolor* (9 pcs.) *R. smirnowii* (8 pcs.), *R. catawbiense* (7 pcs.), *R. brachycarpum* (6 pcs.).An intermediate position on this indicator occupy the cotyledons, apical and lateral buds, isolated from not lignified shoots. At *R. japonicum* was received 6 regenerants per explant from cotyledons, 9 − from the apical buds and 6 − from lateral buds, at *R. smirnowii* − 5, 5 and 3, at *R. catawbiense*− 4, 5 and 4, *R. discolor* − 4, 4 and 3, *R. ponticum* − 3, 4 and 3 *R. brachycarpum* − 1, 3 and 2, respectively.

An entirely different picture was observed in root, stem, epicotyl, hypocotyl. Regeneration potential of these explants is zero for the vast number of varieties. A similar result was obtained for the buds from not lignified shoots and parts of the flower.

The lack of ability to regeneration of these explants confirms the generally recognized fact, that different parts of the same plant have unequal ability to morphogenesis. So, for some bulbous was noted the high regenerative capacity of the pair of scales of bulbs in comparison with other organs: leaves, stem, root (Halperin 1986). At crocus high regenerative potential is characterized for the ovary (Fakhari and Evans 1989), at lilies − for the scales of bulbs (Churikova et al.1991), in French bean − for the young leaves (Kamal and Praven 1991), at the gladioli − for terminal buds of renewal (Rumynin et al. 1990), at a hybrid of mountain ash − for the apical buds (Suvorova et al. 1990).

Study of the regenerative capacity of different types of explants in 6 introduced species rhododendrons allowed us to determine the type of explants, having high regenerative capacity, giving the maximum output of the plants regenerated perexplant and recommend it as a primary for micropropagation studied species of rhododendrons.

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