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Regeneration capacity of introduced varieties of clematis on different modifications of the nutrient media

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Abstract

It is shown, that the regeneration potential of the studied clematis varieties depends on the modification of the nutrient medium, that is, on the content of the components, present in it, as well as on the varietal affiliation of plant.

Keywords: nutrient media, regeneration capacity, clematis, varieties.

Introduction

Plant regeneration is a key moment in all methodology of cell and tissue culture. Without regeneration, research in *in vitro* culture is meaningless, because the final stage of this work is ultimately plant regeneration. That is why a huge number of publications are devoted to this problem, in which the results of experimental studies of the authors are presented, obtained in the study of factors that influence on this process.

Issue of depending on the banana regeneration from ratio of hormones in the nutrient medium, and the effect of genotype on this process is devoted to work Banerjee and Sharma (1988). The authors conclude that the addition of the nutrient medium 0,2 mg L^{-1} IAA (indoleacetic acid) and 0,2 mg L^{-1}benzylaminopurine (BAP) promotes regeneration of shoots banana, while replacing by BAP kinetin reduced the rate of regeneration.

Influence of hormonal additions on the regeneration of sugar beet in culture *in vitro* is devoted to work Cacas and Lasa (1987). It is shown that the best growth of explants was observed on the Murashige-Skoog medium containing 4,4mkM BA (benzyladenine), 0,5 mkM and 0,3 mkM GA_3 (gibberellic acid).

A detailed study on the regeneration of forest species (*Pinus sylvestris* L., *P. Zheffreya*, *P. strobes* L., *Pseudotsuga menziesii* (Mirbel) Franco, *Robinia pseudoacacia* L.) was held Bara Magdalena (1986). Explants were top and the median parts of the 5-7-week-old seedlings. According to the author, the active shoot formation occurred on medium containing at benzyladenine concentration 2-2,5 mg L^{-1}, 2-γ-dimethylaminopurine– 0,5-0,9 mg L^{-1}naphthylene acetic acid – 0,02-0,04 mg L^{-1}.

Soroka (2004) studied the regeneration processes of the two hybrid genotypes of flax on nutrient media N_6 and LMA-1 at various concentrations of BAP. It was shown that the growth and development of better callus occur in the medium at a concentration of BAP 2 mg L^{-1} compared to 4 and 6 mg L^{-1}. Regeneration of shoots and roots was observed in only genotype F_1 6-8 clustered × M 22 and did not depend on the concentration of BAP in the medium and on the medium itself.

Kurenina et al. (2001) conducted studies of the process of regeneration of red clover *Trifolium pratense* L. to obtain regenerated plants. The authors found, that the optimal combinations of phytohormones in the regeneration process were (mg L^{-1}): BAP – 4.0; NAA – 0.1, kinetin – 2.0 and BAP – 4.0; NAA – 0.05; kinetin – 1.0 for a number of varieties (VIC –7, Early– 2, Arlington, Altyn, C7 –11, RP150).

Issue of plant regeneration in cell and tissue culture is devoted to extensive literature (Smirnov et al., 1986; Tuskan, 1990; Kalyaeva et al., 2000; Seetharam et al., 2002; Sharad et al., 2004; Ghanti et al., 2004; Lemesh et al., 2006; Duong et al., 2007; Orlovskaya et al., 2008; Sarmast et al., 2009; Lebedev and Shestibratov 2010; Concioiu et al., 2010; Byadovsky, 2011; Muhametvafina and Akhmetov, 2011; Ružić et al., 2012; Cüce et al., 2013; Kunyi et al., 2013; Kakarla et al., 2014, Nqobile et al., 2015). However, for each species or plant cultivars, this problem is solved experimentally.

The physiological state of the explant, its age are of paramount importance in the regeneration processes occurring in the culture of cells and tissues. Thus, experimental studies of cereals and other crops have shown that in juvenile tissues of the explant, compared with mature ones, selective cells are differentiated only partially and are not completely involved in special functions (Haydu and Vasil, 1981; Conger et al., 1983; Wenzier and Meins, 1986).

Research by Rajasekaran et al. (1987) showed that tissues of juvenile explants *Pennisetum purpureum* Schum (immature embryo, young leaf or inflorescence) contained high doses of indolylbutyric acid and abscisic acid and had a morphogenic ability, and mature parts of leaves, which lacked morphogenic ability, were characterized by a relatively low content of endogenous regulators of growth. According to some authors (Hesemann and Schroder, 1982; Beaulieu and Bendich, 1985; Halperin, 1986; Churikova et al., 1991; Hunter, 1979; Cheng, 1975), with an increase in the age of the leaves from which the explant was isolated, disturbances in the content of nuclear DNA may occur, which leads to the loss of the morphogenic ability of the explant.

It is generally accepted that different parts of the same plant have different morphogenetic ability (Cheng, 1975; Clod et al., 1990). Explants selected from juvenile organs have a greater regenerative capacity compared to those from mature tissues (Clod et al., 1990; Smirnov et al., 1986). Despite this, regenerated plants can be obtained from mature leaves, buds, roots, stems, and parts of a flower by organogenesis or somatic embryogenesis. This convinces us that only experimentally it is possible to determine the regenerative capacity of one or another explant, regardless of our knowledge of its physiological state, i.e. degree of maturity.

An extensive literature is devoted to the issue of plant regeneration in cell and tissue culture (Ashrafuzzaman et al., 2009; Deepika and Kanwar, 2010; Memon, 2012; Grozeva and Velkov, 2014; Naz et al., 2012; Wagh et al., 2015; Chakradhar and Pullaiah, 2014; Cheruvathur et al., 2015; Sweety and Rahman, 2016; Shete et al., 2017; Yandia et al., 2018; Fikadu and Tileye, 2019;Choudhari et al., 2020; Dereje et al., 2020; Grozeva and Nankar, 2020). Unfortunately, we have not found information on the regeneration of introduced clematis varieties *in vitro*.

Therefore, the study of the regeneration capacity of introduced varieties of clematis on various modifications of nutrient media will make it possible to determine the optimal composition of the nutrient medium for this physiological process to proceed under sterile culture conditions. The nutrient medium is the substrate on which all go morphogenetic processes, characteristic for the explant, introduced into the culture *in vitro*, take place.

Further studies were focused on studying the role of the nutrient medium in the clonal micropropagation of the studied plants. The nutrient medium is the substrate on which go all morphogenetic processes, characteristic of the explant, introduced into culture *in vitro.*

Proceeding from this, we carried out comprehensive studies aimed at studying the regeneration potential of introduced varieties of clematis depending on the modification of the nutrient medium, that is, on the

content of hormonal supplements in the nutrient medium, macro- and microelements, vitamins, sucrose, meso-inositol, etc.

Materials and Methods

The objects of study were 5 introduced varieties of clematis: “Patricia Ann Fretwell”, “Fujimusume”, “Asagosumy”, “Wildfire”, “Pink Flamingo”. The experiments were carried out on two types of MS (Murashige and Skoog, 1962) and Anderson (Anderson, 1975) nutrient media, represented by 6 different modifications (Table 1). As explants, we used microcuttings of introduced varieties of clematis “Patricia Ann Fretwell”, “Wildfire”, “Fujimusume”, “Asagosumy”, “Pink Flamingo”, introduced into a sterile culture. The registration of regenerants (shoots) per explant was counted on the basis of 10 explants for each variety.

The results of the experimental data were processed statistically and are presented in Table 2. The figures in the table are arithmetic means with their standard errors.

Results and Discussion

Analysis of the material, presented in Table 2 gives reason to consider, that the regeneration potential of the studied plants depends on the modification of the nutrient medium, that is, on the content of the components, present in it, as well as on varietal affiliation of plant.

Comparative analysis of the regeneration potential of introduced clematis varieties ("Patricia Ann Fretwell", "Fujimusume", "Asagosumy", "Wildfire", "Pink Flamingo") showed, that the largest number of shoots (regenerants) per explant was formed in two varieties: "Patricia Ann Fretwell" and "Pink Flamingo" on MS medium of the 4^{th} modification and amounted to 5 pieces for each variety (Table 2). The smallest number of regenerants per explant (1 piece) was observed on Anderson's medium of the 5^{th} modification in all varieties without exception. On the media of the remaining modifications (1st, 3rd, 6th), the studied varieties took an intermediate position in terms of this indicator (Table 2).

Of the studied 6 different modifications of nutrient media, only on media of two modifications (2^{nd} and 4^{th}) is characterized by a relatively high regeneration potential for the studied varieties of clematis (Table 2.). These two modifications of nutrient media, containing macro- and microsalts according to MS, as well as other components (Table 1) can be used for the regeneration of the studied varieties of clematis, and the 5^{th} modification, containing macro- and microsalts according to Anderson and some supplements, for depositing sterile cultures of clematis (Table 1).

Conclusion

An analysis of the results of experimental studies obtained on the study of the regeneration potential of introduced varieties of clematis on six modifications of nutrient media differing in the content of macro- and microsalts, hormonal supplements, showed that the best for this process were the media of the 2^{nd} and 4^{th} modifications, containing in its composition is macro- and microsalts according to MS, as well as hormonal supplements (0.4 mg / l indolylbutyric acid and 1 mg / l benzylaminopurine−2^{nd} modifications; 0.4 mg / l indolyacetic acid and 1.5 mg / l benzylaminopurine− 4^{th} modifications). The regenerative ability of introduced clematis varieties depends on the content of hormonal supplements in the nutrient medium and the plant genotype. The maximum number of regenerants per explant (5 pieces) was obtained for two clematis varieties ("Patricia Ann Fretwell", "Pink Flamingo") on MS medium of the 4^{th} modification, supplemented with 0.4 mg / l IAA, 1.5 mg / l BAP ; minimum - (1.0 pcs) on the Anderson medium of the 5th modification.

Thus, as a result of studying the influence of the composition of nutrient media on the regeneration potential of introduced clematis varieties, we were able to assess the complex effect of the components contained in nutrient media on this process and recommend the media of the 2^{nd} and 4^{th} modifications for the regeneration of introduced clematis varieties ("Patricia Ann Fretwell", "Fujimusume", "Asagosumy", "Wildfire", "Pink Flamingo", medium of 5^{th} modification- for depositing of sterile cultures of these varieties.

Table 1 − Composition of nutrient media for studying shoot formation in introduced varieties of Clematis

Component, mg/l	Modification of medium, №					
	1	2	3	4	5	6
Macrosalts on MS	f.n.	f.n.	f.n.	f.n.	-	f.n.
Microsalts onMS	f.n.	f.n.	f.n.	f.n.	-	f.n.
Macrosalts on Anderson	-	-	-	-	f.n.	-
Microsalts on Anderson	-	-	-	-	f.n.	-
Mesoinositol	100,0	100,0	100,0	100,0	100,0	100,0
Adenine sulphate	-	-	-	-	80	-
Tiamine (B_1)	0,1	0,1	0,1	0,1	0,4	0,1
Pyridoxine (B_6)	0,5	0,5	0,5	0,5	-	0,5
Nicotinic acid (PP)	0,5	0,5	0,5	0,5	-	0,5
Glycine	0,2	0,2	0,2	0,2	-	0,2
Indolylacetic acid	-	-	0,4	0,4	-	-
Indolylbutiric acid	0,4	0,4	-	-	-	-
Benzylaminopurine	0,5	1,0	0,5	1,5	-	-
Saccharose, g/l	30,0	30,0	30,0	30,0	30,0	30,0
Agar g/l	6,0	6,0	6,0	6,0	6,0	6,0
pH	5,8	5,8	5,8	5,8	5,8	5,8

Notation: «-» − component is absent in the medium , f. n. – full norm

Table 2 − Shoot formation in introduced varieties of clematis depending on modification of nutrient medium

Modification of medium, №	Quantity of regenerants on one explant, piece				
	"Patricia Ann Fretwell"	"Fujimusume"	"Asagosumy"	"Wildfire"	"Pink Flamingo"
1	3,0±1,0	2,0±0,4	3,0±1,0	2,0±0,6	3,0±0,6
2	4,0±1,0	3,0±0,6	4,0±0,6	3,0±0,5	3,0±1,1
3	3,0±0,3	2,0±0,6	2,0±0,1	2,0±0,7	3,0±0,5
4	5,0±2,0	3,0±0,9	4,0±1,7	3,0±1,1	5,0±0,5
5	1,0±0,4	1,0±0,2	1,0±0,3	1,0±0,1	1,0±0,2
6	3,0±0,6	3,0±1,1	2,0±0,3	2,0±0,2	3,0±0,4

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