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REGENERATION FROM LEAF EXPLANTS OF BULGARIAN RASPBERRY CULTIVARS AND ELITES

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ABSTRACT

The development of efficient regeneration system from various explants is a very important part of the biotechnology programs for crop improvement.
Four extensively grown Bulgarian raspberry cultivars and two elite hybrids were tested for regeneration along a period of eight weeks. Leaf explants and petioles were used on ten culture media combinations. In most cases, regeneration became visible after 3 weeks of culture. Genotype specific procedures for adventitious shoot formation both from leaf segments and petioles were developed. Most surprisingly, high regeneration response was achieved without exogenous application of plant growth regulators and with slight changes in the vitamin compositions. Bulgarski rubin, Samodiva and Elite 1 appeared to be useful candidates for further biotechnology procedures.

Introduction

The development of regeneration procedures from various plant organs allows the establishment of useful genetic variation. Such procedures are an obligatory prerequisite of numerous biotechnology and classical breeding programs. This is particularly true for perennial crops such as the small fruits or fruit trees where the classical breeding process is rather extensive and prolonged.

As a rule, the raspberry production all over the world is based on local varieties and hybrids. Thus, breeding programs involving biotechnology procedures have been established in many countries involving genotypes of various origins (4, 7, 15, 16). Studies with Bulgarian cultivars and elites have not been published so far.
The aim of the present study was to develop useful protocols for regeneration from leaf explants and petioles.

Materials and Methods

Plant material

Genotypes
Several high-quality and high yielding varieties and elites of Bulgarian origin were

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid; 2-ip – N-isopenthenylamino purine; BAP -6-benzyladenine; GA – giberellic acid; IAA –indole-3-acetic acid; IBA – indolebutyric acid; TDZ - thidiazuron; MS – Murashige & Skoog plant tissue culture medium.

tested for regeneration ability. The Bulgarski rubin, Shopska alena, Samodiva and Lyulin cultivars are grown extensively in the country (1). Elite 1 and Elite 3 were selected for improved performance and disease resistance among several breeding combinations.

Explants and sterilization

To initiate cultures, initial explants were taken form 1-year old shoots of 2-years old mother plants grown under controlled conditions in greenhouse. Standard procedure for sterilization was applied. Leaf segments and petioles of approximately 0.5x1 cm were taken from well developed leaves of 3 weeks old in vitro plantlets and used as explants for regeneration.

Culture media

Media for initiation and maintaining

Cultures were initiated on MS (9) (Duchefa) with 0.1 mg/l IBA, 0.3 mg/l BAP and 0.1 mg/l GA3 and transferred to MS basal medium. Rooting took place on MS with 0.3 mg/l IBA. Regular subculture was performed every 3 weeks.

Media for regeneration

Several combinations of plant growth regulators were added to MS basic formula (Table 1). The regeneration was estimated for 8 weeks period once per week. At least 35 explants per genotype were used for every media composition.

Culture conditions

The cultures were grown under controlled conditions – about 23ºC at 16:8 light/dark period and under 2500-3000 lx light supplied by white-light Osram white-light lamps.

Results and Discussions

The possibility to obtain explants from in vitro maintained raspberry plants ensured the major advantage to use uniform, standardized and available all the year long initial material in our trials.

The development of genotype-specific procedure for in vitro regenerations is one of the obligatory prerequisite for biotechnology approach to improve and fasten the traditional plant breeding programs. There are numerous genotype-dependent regeneration procedures available in most raspberry producing countries (2, 4, 7, 8, 13, 15, 16). The significant variability and genetic diversity of genus Rubus and the specific requirements of the raspberry production in our country serve as important preconditions for the development of regeneration procedures for Bulgarian genotypes.

Regeneration from leaf segments

All genotypes tested showed differences in their morphogenesis on the various combinations of plant growth regulators. The adventitious shoots developed directly on the explants. Small amounts of yellowish-green embryogenic callus were formed occasionally. We were not able to achieve regeneration or even callus induction on variants B and F1 (**Table 1**). Thus, these variants are not presented in our further figures.

Dynamics

In most cases, the regeneration started after three weeks of culture. The only exception was the culture of Bugarski rubin on variant A where moprhogenic response appeared after two weeks (**Fig. 1**). Mucha later, after five weeks of culture, started the regeneration on medium F2. Despite of the uniformity of the initial material and the culture conditions, the regeneration showed steady increase till the very end of the experiments.

Regeneration rate

Low BAP concentrations (variants B and F1) resulted in no regeneration. The ap-

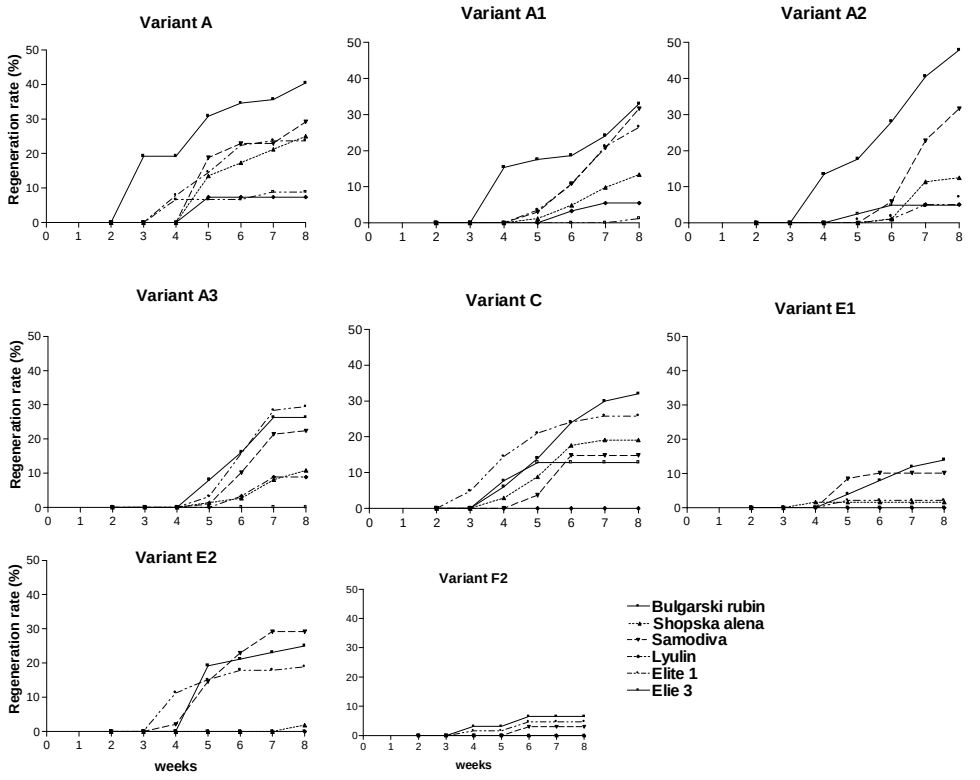

Fig. 1. Dynamics of the adventitious shoot formation from leaf explants of Bulgarian cultivars and elites on various culture media.

plication of 1 mg/l BAP (variants E1 and F2) resulted in very low regeneration rate (**Fig. 2**). The highest BAP concentration used in our experiment (2 mg/l) (variant E2) influenced positively the response of Samodiva and Bulgarski rubin only. Thus, we could conclude that BAP had low to moderate influence on the morphogenic ability of the tested Bulgarian genotypes. Previous experiments with raspberry-blackberry hybrids showed similar results (2, 13).

The effect of BAP was less pronounced than that of TDZ. This was particularly true for variants A and A2 where Bilgarski rubin reached almost 50% regeneration efficiency (Fig. 2). Even at very low concentrations 0.22 mg/l) (variant C) the response of this cultivar was significant. At all TDZ concentrations Samodiva and Elite 1 responded quite well, too.

The strong effect of the same cytokinin was confirmed in various Rubus genotypes (15) as well as in other fruit species (3, 5, 6, 10, 11, 12, 14, 17).

The effect of one or other type of plant growth regulator should always be considered in combination with other type – in our case, this the combination of cytokinins with auxins. The highest regenera-

TABLE 1

Combinations of plant growth regulators tested for regeneration efficiency from leaf explants and petioles of Bulgarian raspberry cultivars and elites.

Variant	Plant growth regulators mg/l						
	IBA	IAA	2,4-D	BAP	TDZ	2-iP	GA
A	0.5	-	-	-	2	-	-
A1	0.3	-	0.01	-	1	1	-
A2	0.1	-	-	-	2	0.3	-
A3	-	-	-	-	-	-	-
B	0.01	-	-	0.5	-	-	-
C	0.1	-	-	-	0.22	-	-
E1	0.1	-	-	1	-	-	-
E2	0.1	-	-	2	-	-	-
F1	-	0.1	-	0.1	-	-	0.1
F2	-	1	-	1	-	-	0.1

Note: The A3 variant is MS with no growth regulators and with modified vitamin composition - 1 mg/l thiamine HCL, 1 mg/l pyridoxine HCL, 1 mg/l nicotinic acid, 2 mg/l glycine, 0,5 mg/l Ca– panthetonatae, 0,005 mg/l cyanocobalamine, 0,025 mg/l biotin, 0,05 mg/l folic acid, 0,25 mg/l riboflavin)

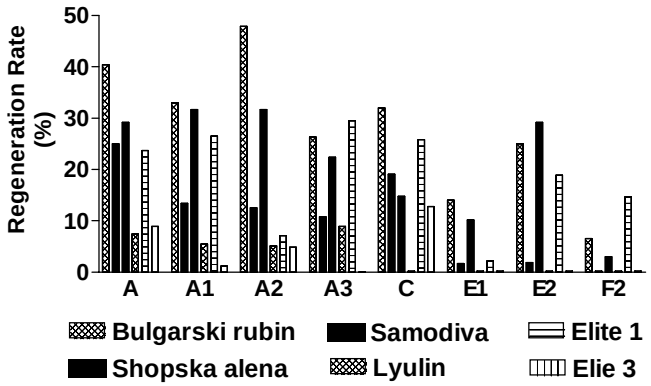

Fig. 2. Regeneration rates from leaf explants of Bulgarian cultivars and elites on various culture media.

tion rate (almost 50% for Bulgarski rubin) was obtained on variant A2 (Fig. 2) where cytokinins (TDZ and 2-iP) were combined with auxin (0.3 mg/l IBA). Significant although less pronounced regeneration rate was achieved when higher concentrations of IBA were combined with TDZ (variant A and A1).

Adding GA to various combinations of IAA and BAP (variants F1 and F2) we were able to induce only scarce regeneration in half of the genotypes tested (Fig. 2), obviously gibberellins were not of particular importance for the regeneration from raspberry leaf explants.

Significant regeneration rate was achieved on A3 variant where no growth regulators were added to MS basal medium and

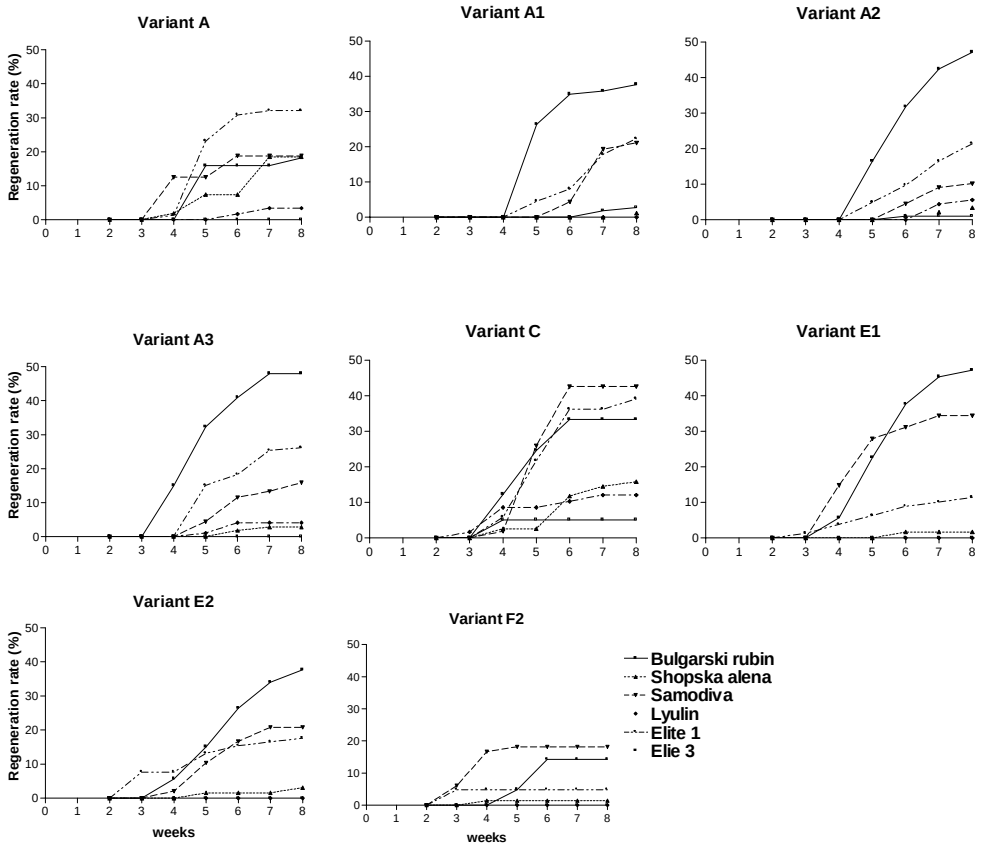

Fig. 3. Dynamics of the adventitious shoot formation from petioles of Bulgarian cultivars and elites on various culture media.

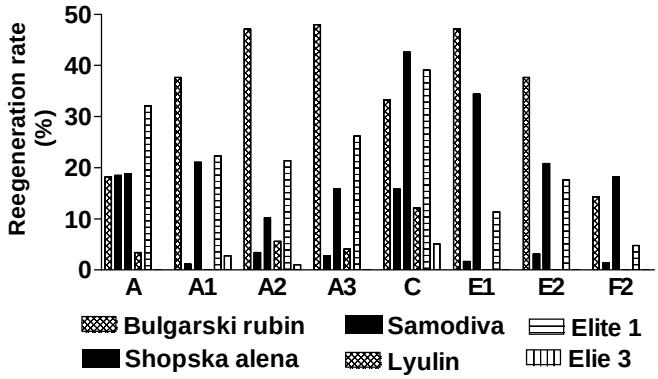

Fig. 4. Regeneration rates from petioles of Bulgarian cultivars and elites on various culture media.

the vitamin content was changed. With the exception of Elite 3, all tested genotypes reacted positively. The regeneration rate was especially pronounced for Elite 1 cultures (Fig. 2). This, we believe is the first report for such an alternative model of regeneration which could be of particular interest both for fundamental and practical studies.

The leaf explants of Bulgarski rubin (47.9%), Samodiva (31.7%) and Elite 1 (29.5%) showed highest regeneration ability on variants A2 and A3 respectively. The lack of regeneration or the very poor reaction of Lyulin and Elite 3 could be a consequence of unfavorable genotype x media combinations.

Regeneration from petioles

As already pointed out for the regeneration from leaf explants, we found genotype differences in morphogenesis from petioles, too. The adventitious shoots developed directly on the explants, most often at both ends. Callus formation was rare, similar to that on leaves. We were not able to achieve regeneration or even callus induction on variants B and F1 (Table 1) and they are not presented on the figures below.

Dynamics

With the exception of variant F2, the regeneration started after three or even four weeks of culture (**Fig. 3**). The regeneration showed steady increase along the whole period of culture after the initiation.

Regeneration rate

There were considerable differences in the morphogenic response of petioles, compared to the regeneration rates obtained from leaf explants. It appeared that genotype influenced more significantly the development of adventitious shoots. Thus, in most cases explants of Elite 3 showed no or very low regeneration (**Fig. 4**). On the other hand, the petioles of Bulgarski rubin were the most responsive ones on the predominant part of media variants. Highest regeneration rates were achieved with TDZ or BAP and, interestingly enough – on variant A3, where no plant growth regulators were added to MS. The regeneration from Samodiva petioles was quite high on variants C and E1, where IBA was combined with. Low amounts of BAP or TDZ (Fig. 4). Explants from Elite 1 plants reacted better on media with TDZ (variant A and C) and like Bulgarski rubin - to variant A3. The regeneration rates of Samodiva and Elite 1 petioles were higher then the respective response obtained from their leaf explants (Fig. 2 and 4). Only in the case of Bulgarski rubin, both explants reached similar regeneration rates. Similar observations were reported earlier (16) but contradictive results have been obtained, too (4, 7). Relatively moderate or low regeneration rates were achieved with petioles of the other genotypes tested.

In conclusion, we were able to develop genotype specific procedures for adventitious shoot formation both from leaf segments and petioles. Plants of Bulgarski rubin, Samodiva and Elite 1 showed high regeneration rate and plasticity. This makes them useful candidates for further biotechnology procedures. Most surprisingly, high regeneration response was achieved without exogenous application of plant growth regulators and with slight changes in the vitamin compositions. The regenerants obtained so far, were transferred in soil under control conditions for additional breeding studies.

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