



## Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

### THE USE OF THE DRIVER-KUNIYUKI NUTRIENT MEDIUM FOR MICROPROPAGATION OF ROOTSTOCKS OF LC-52 (*Cerasus vulgaris* x *Cerasus fruticose*) AND GIZELA 6 (*Peisica vulgaris* x *Cerasus canescens*) STONE FRUIT CROPS

Salman Magomedovich Khamurzaev<sup>1</sup>, Ibragim Musaevich Bamatov<sup>2</sup>, Elina Minerovna Butsaeva<sup>3</sup>,  
Sergey Vasilievich Sibiryatkin<sup>4\*</sup>

<sup>1</sup>FGBOU VO Chechen State University, 364051, Russia, Grozny, Sheripov Str., 32

<sup>2</sup>ResearchLaboratory", FGBU VO Chechen State University, 364051, Russia, Grozny, Sheripov Str. 32

<sup>3</sup>Centre for Collective Use" of the Federal State Educational Establishment for the Chechen Republic State University, 364051, Russia, Grozny, Sheripov str., 32

<sup>4</sup>Laboratory of Biotechnology NPF OOO "Sady Chechni", 366318, Russia, CHR, Kurchaloy district, v. Tsentoroi

Received – December 20, 2017; Revision – March 28, 2018; Accepted – June 16, 2018

Available Online – June 20, 2018

DOI: [http://dx.doi.org/10.18006/2018.6\(3\).623.627](http://dx.doi.org/10.18006/2018.6(3).623.627)

#### KEYWORDS

Rootstock

Micropropagation

Nutrient medium

Driver-Kuniyuki  
mediumstone fruit

#### ABSTRACT

The article presents an overview of the use of different nutrient media during in vitro micropropagation of plants. Further, article also provides information regarding the effect of Driver-Kuniyukinutrient medium on the proliferation and micropropagation of the LC-52 and Gisela-6 clonal rootstock of stone fruit. Result of study revealed that use of the Driver-Kuniyuki nutrient medium have significant effect on micropropagation of LC-52 and Gisela-6 clonal rootstocks of stone fruit and increase the size by 1.15 cm for the LC-52 rootstock and 0.5 cm for the Gizela-6 rootstock per passage.

\* Corresponding author

E-mail: [itdf.79@mil.ru](mailto:itdf.79@mil.ru) (Sergey Vasilievich Sibiryatkin)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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## 1 Introduction

Economic viability of a fruit production enterprise is directly linked to orchard productivity and management efficiency. To increase productivity and efficiency requires tree survival, managed vigor and increased marketable yields over the expected life span of the orchard. The intensive technology of cultivation of fruit and berry crops is impossible without a sanitized planting material. Production of virus free plant is based on various predefined international rules and inter and intra country exchange of such virus-free planting material is possible only under quarantine certificates. Therefore, the development of the seed biotechnology elements will allow entering the international market of fruit crop seedlings.

Based on the available literature it can be conclude that mineral composition of artificial nutrient media for clonal micropropagation of stone fruit crops and their rootstocks has been not yet properly determined (Benmahioul et al., 2012; Scientific and methodological recommendations for micropropagation of stone fruit crops, 2014; Mitrofanova, 2014; Kostyuk, 2017). Further, most widely used medium for mass multiplication of stone fruit tree is Murashige & Skoog (1962). Along with MS media, various other tissue culture media such as Gamborg, Lee-Fossard, White, Heller, WPM has been used for micropropagation of stone fruit and obtained good results (White 1954; Gamborg 1975; Murashige & Skoog 1962; Nitsch & Nitsch, 1969). Arena (1992) compared micropropagation efficacy of MS and Lee-Fossard medium and reported that MS media have significantly higher propagation rate as compared to Lee-Fossard (Arena, 1992).

For micropropagation of stone fruit of rootstock, basic composition of MS medium has been modified by some researcher (Cheng, 1979; Werner 1980; Zayova et al., 2010). Werner (1980) decrease in the concentration of mineral salts at the time of apple rootstock M 7 micropagation and reported that it was twice effective than the normal concentration (Werner, 1980). Similar result was reported by Zayova et al. (2010) when they carried out in vitro micropropagation of *Valeriana officinalis* at half mineral concentration of MS media (Zayova et al., 2010).

Some other nutrient media was also used for the micropropagation of various root stock of stone fruits. Shornikov (2008) successfully used the Quoirin and Lepoivre medium for micropropagation of *Eleutherococcus sps.*, *Actinidia sps* and *Schisandra chinensis*. For in vitro propagation of rhododendrons Filipenya (2009) used Wood Plant Medium (WPM) with 3% sucrose and phytohormones at different concentrations and found significant results.

Further, it has been reported that types and concentration of nitrogen source in nutrient media effect the rate of tissue multiplication. According to Pronina (2008) reduction in ammonium concentration accelerates the rate of rhizogenesis in apple rootstocks (Pronina, 2008). Similar results was reported by Naija et al. (2009) when they made changes in the mineral concentration of MS medium. Contradictory findings were reported by Solovykh (2010), when nitrate concentration increased two fold at the time of proliferation, it gave higher number of adventitious shoots in raspberries, blackberries and raspberry-blackberry hybrids explants (Solovykh, 2010).

Thus, scientists did not agree on the optimal composition of the nutrient medium. At the same time, the search for the optimal nutrient medium and its mineral composition for specific propagated plants are the need of present condition. The Driver-Kuniyuki nutrient medium (Driver & Kuniyuki, 1984) is one of those developed for the propagation of rootstocks of nut cultures and gave excellent results in the propagation of walnuts rootstock (Ibragimov 2013). A distinctive feature of this medium is higher amount of calcium ions, as well as a smaller amount of chloride, which significantly affects the growth and development of in vitro plants. There are also minor differences in the composition of trace elements and the amount of iron chelate introduced into the medium. Ibragimov (2013) has successfully used this nutrient medium for propagation of walnuts rootstock.

In the modern gardening, micropropagation of rootstocks of stone fruit crops plays an important role in large scale production of stone fruit production. Further, modern rootstocks Gizela-6 exclusively propagate in vitro. It has been well reported that during micropropagation, response of propagated plants are variety-specific and are also dependent on mineral vitamins and phytohormones composition of the nutrient medium. Therefore, present study has been conducted for optimization the micropropagation process of Gisela-6 and LC-52 rootstocks. Based on the foregoing, the following objectives were set: identify the patterns of growth and development of rootstock of Gisela-6 and LC-52 on Driver-Kuniyuki nutrient medium, and the growth of these two on Driver-Kuniyuki nutrient medium compared with the nutrient media used by other authors.

## 2 Materials and Methods

The research was carried out in the laboratory of biotechnology NPF LLC "Sady Chechni". Objects of the study were clonal rootstocks of LC-52 (*Cerasus vulgaris* X *Cerasus fruticose*) and Gizela-6 (*Peisica vulgaris* X *Cerasus canescens*) stone fruit crops. Attempt to culture RVL-1 (*P. cerasus* X *P. maackii*) and VSL-2 (*P. fruticosa* X *P. Lannesiana*) root stock was also taken in present study but these two cannot multiplied on Driver-Kuniyuki

and other nutrient medium that's why these two were not used for further study.

The method recommended by Popov (1979), and Dzhigadlo (2005) were used for the tissue culture. Further, method proposed by Dospikhov (1979) and Raikov (2012) was used for the variance analysis.

The size of the apex introduced on the culture medium was 0.3-0.5 mm. The plant was sterilized with an aqueous solution of the Belizna commercial preparation in the proportion of 1:3 with an exposure of 4 minutes and subsequent washing in distilled sterile water 5 times per 5 minutes each. The explants were isolated under aseptic conditions in the VP-12 laminar boxes under the binocular microscope (MBS-1) with 7-times magnification using a scalpel and tweezers.

The explants were cultured in 20x140 mm or 20x200 mm tubes at a temperature of  $24 \pm 2$  C and with the 16-hour illumination with an intensity of 2-2.5 thousand lux. The research was conducted in 4 replications (10 tubes per one replication). Various concentrations of macro- and microelements, vitamins, as well as phytohormones such as cytokinin 6-benzylaminopurine (6-BAP) and auxin  $\beta$ -indolylacetic acid (IAA) were tested during the work.

### 3 Results

To test the effectiveness of the nutrient medium, four nutritive media viz., Murasige and Skoog, Driver-Kuniyuki, Quoirin and Lepoivre and WPM media were tested in present study. In case of explant survival and establishment, MS medium shows superiority over the Driver-Kuniyuki media but this difference are not significantly different from the LC-52 and Gizela-6 rootstock cultured on the Driver-Kuniyuki nutrient medium (Figure 1).

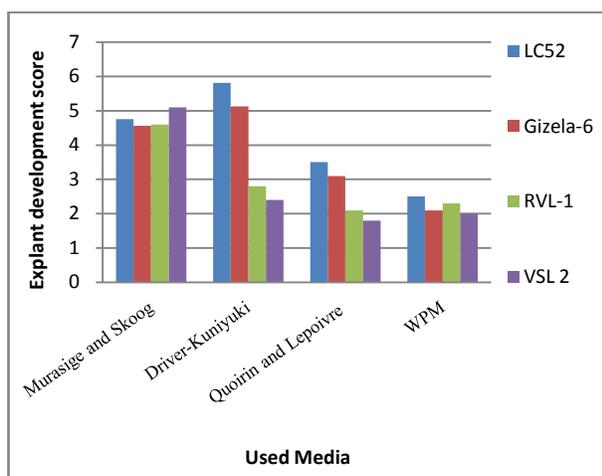


Figure 1 - Degree of development of initial explants on various nutrient media.

Further, growth and establishment of various stone fruits explants on Quoirin and Lepoivre and Anderson media were significantly lower than the Murasige and Skoog media which used as control that's why these two media were not used for the next level study. Further, it has been reported that explants inoculated on Anderson and Quoirin and Lepoivre medium failed to developed the green pigment in microspheres, which led to the death of plants or initiate smaller microshoots. From these results, it can be conclude that these two media did not favor the growth of callus tissue, from which, in subsequent observations, the development of single fascia-sprouted shoots was noted. We classified this phenomenon as a phenotypic change in the cultivated plant.

The Driver-Kuniyuki nutrient medium for the clonal rootstocks of the sweet cherry, in particular, LC-52 and Gizela 6 rootstock had virtually not affect the number of shoots in the conglomerate at the same concentration of MS medium, but at the end of study period (4 weeks) Driver-Kuniyuki nutrient medium had significant effect on the shoot height and it showed superiority over the MS media (Table 1, Figure 2 & 3). Micropropagation of other two clonal rootstocks (RVL-1 & VSL-2) on the Driver-Kuniyuki and other nutrient medium was insignificant as compared to MS medium that's why these two not used for further study. Similar results were reported by Sibiryatkin(2017).

Measurements of microshoots at the proliferation stage showed, on average, an increase in shoot growth by 0.5 cm per passage

Table 1 Influence of the mineral composition of the nutrient medium on the in vitro development of the LC-52 and Gizela 6 rootstock

Medium	Microshoots set out (pcs.)				Average increase in shoots (cm)		
	Growth dynamics, cm		Growth dynamics, cm		in 2 weeks	in 4 weeks	
	2 weeks	4 weeks	2 weeks	4 weeks			
	number of shoots	average length of shoot (cm)	number of shoots	average length of shoot (cm)			
Murasige and Skoog	35	1.9	2.90	2.83	4.76	0.95	2.81
Driver-Kuniyuki	35	1.9	2.93	3.22	5.81	1.08	3.96
Gizela 6							
Murasigeand Skoog	35	1.8	1.80	2.67	4.56	0.82	2.67
Driver-Kuniyuki	35	1.8	1.86	3.15	5.13	0.96	3.14
HCP <sub>0.5</sub>							0.72



Figure 2a LC-52 root stock after the Murashige and Skoog medium on the 30th day after the ex vitro adaptation.



Figure 2b LC-52 root stock after the Driver-Kuniyuki medium on the 30th day after the ex vitro adaptation.



Figure 3a Development of shoots of Gizela rootstock on the MS medium (right) and Driver-Kuniyuki (left) nutrient media.



Figure 3b –The development of LC-52 rootstock shoot on the Murashige and Skoog (right) and Driver-Kuniyuki (left) nutrient media

(Figure 3a & 3b). The collection of data on the use of the Driver-Kuniyuki nutrient medium in the micropropagation of the LC-52 and Gizela-6 clone rootstock is currently cumulative and requires a careful analysis for assessing its influence on the growth and development of microshoots during proliferation. However, preliminary data allow recommending this medium for wider use in the process of studying and optimizing the technology of obtaining a sanitized planting material of the "basis" category.

#### 4 Discussion

Thus, the use of the Driver-Kuniyuki nutrient medium in micropropagation of rootstocks for stone fruit crops is promising. Its effectiveness is due to better development of microshoots at the same concentration of cytokinins in the nutrient medium, a large increase in shoots per passage and a large yield of plants suitable for in vitro rooting. Proceeding from the foregoing, it can be recommended to use this nutrient medium more widely for micropropagation of rootstocks of stone fruit crops when obtaining a planting material of the "basis" category.

#### Conclusion

The use of the Driver-Kuniyuki nutrient medium in micropropagation of clonal rootstocks of the LC-52 and Gizela-6 stone fruit crops makes it possible to obtain a larger increase on average by 1.15 cm for the LC-52 rootstock and 0.5 cm for the Gizela-6 rootstock per passage.

#### Acknowledgments

The article was prepared in the framework of state task No. 37.10120.2017/NM.

#### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

#### References

Arena ME (1992) Factores and afectan la multiplicación in vitro de los brotes de portainjertos de Prunus (In Russian). Fyton 53: 29-39.

- Benmahioul B, Dorion N, Kaid-Harche M, Daguin F (2012) Micropropagation and ex vitro rooting of pistachio (*Pistacia vera* L.). *Plant Cell, Tissue and Organ Culture* 108: 353-358.
- Cheng TY (1979) Micropropagation of clonal fruit tree rootstocks. *Compact Fruit Tree* 12: 127-137.
- Shornikov DG (2008) Perfection of the technology of reproduction of rare garden plants in the culture of in vitro and evaluation of their potential for resistance to abiotic stressors (In Russian): the dissertation of the candidate of agricultural sciences. Michurinsk, Pp: 192
- Clonal micropropagation of stone fruit crops and their rootstocks: scientific and methodological recommendations (2014). Krymsk: FGBNU Krymskaya OSS SKZNIISiV., Krymsk, pp. 23
- Dospekhov BA (1979). Methodology of field experiment (with the basics of statistical processing of research results) (In Russian). Moscow: Kolos, Pp. 416
- Driver JA, Kuniyuki AH (1984) In vitro propagation of Paradox walnut rootstock. *HortScience* 19: 507-509.
- Dzhigadlo EN (2005) Methodical recommendations on the use of biotechnological methods in working with fruit, berry and decorative crops (In Russian). Oryol: GNU VNIISPK, Pp. 51.
- Filipenya VL (2009) Micropropagation of *Rhododendron* × *Hybridum* Hort (In Russian). *Physiology and Biochemistry of cultured plants* 41: 516-522.
- Gamborg OL (1975) The effect of amino acids ammonium of the growth of plant cells in suspens culture. *Plant Physiology* 45: 372-375.
- Ibragimov ZA (2013) Biotechnology and molecular genetic studies of *Juglans Regia*: condition and prospects. *Engineering Biology in the Modern World: II International Conference "Engineering Biology in the Modern World"* Maikop, December 22, 2012 - February 27, 2013, AGAU, Ganja, Pp. 47-65.
- Kostyuk MA (2017) Sterilization of explants in the production technology of healthy plum planting stock (In Russian). *Fruit growing and viticulture of the South of Russia* 44 : 186-194.
- Mitrofanova OV (2014) The use of biotechnological methods in the improvement of plants and reproduction of the virus-free planting stock of promising flower-decorative crops (In Russian). Collection of scientific papers of SNBS, 138: 5-56.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.
- Naija S, Elloumi N, Ammar S, Kevers C, Dommes J (2009) Involvement of polyamines in the adventitious rooting of micropropagated shoots of the apple rootstock MM 106. *In Vitro Cellular & Developmental Biology – Plant* 45: 83-91
- Nitsch JP, Nitsch C (1969) Haploid plants from pollen grains. *Science*, 163: 587-589.
- Popov YG (1979) Sanitation and reproduction of fruit and berry plants by the method of culture of meristematic apices: Methodical instructions, Moscow, Pp. 29
- Pronina IN (2008) Optimization of the process of rhizogenesis of rootstocks and apple and pear varieties in vitro (In Russian): the dissertation of the candidate of agricultural sciences. Michurinsk, pp. 158
- Raikov IA (2012) Perfection of micropropagation of interspecific forms of currant black and raspberries of remountant type (In Russian) The dissertation of the candidate of agricultural sciences. Bryansk, pp. 19
- Sibiryatkin SV (2017) Some aspects of introduction of new clonal rootstocks for stone fruit cultures in vitro (In Russian). *The Polythematical network electronic scientific journal of the Kuban State Agrarian University (Scientific Journal of KubSAU)*, 07. <http://ej.kubagro.ru/2017/07/pdf/42.pdf> (accessed December 17, 2017)
- Solovykh NV (2010) Induction of morphogenesis from somatic tissues of plants of the *Rubus* genus (In Russian). *Bulletin of MichGAU* 2: 104-110.
- Werner E (1980) In vitro propagation of Malling 7 apple rootstock. *Horticultural Sciences* 15: 509-510.
- White PR (1954) The cultivation of animal and plant cells. Ronald Press Company, New York, pp. 239
- Zayova E, Vassilevska-Ivanova R, Petrova M, Nedev T (2010) Micropropagation of *Valeriana officinalis* by tissue culture. *Comptes Rendus de L' Academie Bulgare des Science* s63: 1749-1756.