In vitro multiplication of cherry rootstock Krymsk®5 (VSL-2)

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Abstract: *This article presents new aspects regarding the technology of sweet cherry rootstock Krymsk®5 (VSL-2) by in vitro multiplication. Stages of initiation, multiplication, rooting, and acclimatization have been researched. In order to explant proliferation were used standard nutrient medias: MS, DKW, WPM; and growth regulators: BAP, Kin, GA_3, IBA in several concentration and variation. In different phases the research results have been proven on the basis of number of experimental indications (bud bursting days, bud proliferation percent, occurrence of callus percent indications, length of shoots, rooting rate, quantity and length of roots).After 35 days, the highest shoots were observed in DKW media, 3.16±0.13 cm. The DKW nutrient media is found the most suitable among four nutrient media for in vitro bud proliferation, callus development, shoot multiplication of Krymsk®5 explants. The most progressive results were obtained in DKW media with BAP -1.0 mg/l and Kin - 0.5 mg/l concentration with 92 % shoot proliferation percentage. In rooting stage, ½MS media supplemented with 3 mg/l IBA provided 1/8 rooting ratio, 3.1sm length roots, and 96 % rooting percentage.*

Key Words: *Cherry, Krymsk®5, acclimatization, in vitro rooting, rootstock, nutrient media.*

1. INTRODUCTION:

Sweet cherry is considered to be the most favorite fruit overcoming with the centuries. Sweet cherry is assignable among other fruits with its taste, maturation period and specific fascination. These factors make sweet cherry one of the world leading fruit in sphere of production and export. With its smooth appearance, light colour and wonderful taste sweet cherry attracts strongly the fruit consumers.

Meanwhile, sweet cherry has strict requirement to soil and climate. It prefers fertile soil with well drainage and a bit sandy soils. Nevertheless, cherry insufferable with salted or high humidity soils. It grows weak and slowly in the lands with sandy surface.

Rootstock and varieties are regarded as the main source of horticulture production. Therefore, basing on the rootstock and varieties it is necessary to increase healthy plants multiplication and to improve them by applying modern technologies. According to FAO STAT 2017 data, Top 5 the biggest producers of sweet cherry are Turkey 627K tons, USA 398K tons, Iran 140K tons, Uzbekistan137K tons with their production scale. The intensive plantation with semidwarf and dwarf rootstock system are applied in all upon listed countries. Nowadays, cherry harvested area is 9.8K hectares in Uzbekistan with 137K tons of production quantity, however, in 2015 quantity was 90K tons [5]

The most efficient way of producing rootstock is *in vitro* micro propagation. Nevertheless, there are not purely general methods and nutrient media, which affordable to each cultivar, it varies with organic and inorganic elements and their concentrations [1, 14]. The most vital ingredient of nutrient media is cytokine [10]. Therefore, rich BA (Benzyl Adenine) nutrient media occurs high scale shoot proliferation. The function of BA consists of reducing apical growth activity, which promotes bud shoot proliferation and partially or fully stopping root proliferation [9]. Contrary, it is estimated that Indole-3-butyric acid (IBA) activates root proliferation sensitively [8].

This survey aimed to investigate more efficient and appropriate concentration of growth regulators to estimate higher proliferation rates.

2. MATERIALS AND METHODS:

All experiments were conducted on Krymsk5 rootstock using four types of nutrient media. For the experiments the plants were selected and brought from central exp. station of institute to the laboratory in March, 2017. The leaves of the brought shoots were removed without any damage to side and top vegetative points.

Surface sterilization. The explants were kept for 60 minutes under running water and then were hold on96% ethanol for 30 seconds. Consequently, explants were mixed on magnetized blender with 800 ml distilled water and 200

ml 1% sodium hypochlorite for 20 minutes. Following, all plant materials were washed under distilled and autoclaved water for four times in order to eliminate any chemical remains.

Culture media. All research works on Krymsk®5 were performed using four different MS, MSm, DKW, WPM media. BAP, Kin, IBA and GA_3were used as plant growth regulators, agar was used as gelling agent, in carbon source were 18 g/l sugar +12 g/l glucose, and the pH of the media was adjusted to5.8 with using of 1N HCl and KOH before adding the gelling agents. The culture media were prepared using stock solutions of microelements, macro elements, plant growth regulators and vitamins. All the necessary components were added to the media before autoclavation.

Culture conditions. The cultures were incubated in the growth chambers at 23±1 °C, 7000 Lux light intensity and 16-hour photoperiod. The experiments were conducted in four variations and three repetitions.

*Multiplication.*In this stage, growth regulators BAP, Kin and GA_3were used in 0.0 mg/l, 0.5mg/l, and 1.0 mg/l concentrations for each of them (Table №1). The incubation period was 4 weeks. After which explants with higher results were subcultured for further surveys.

In vitro rooting. Explants with 1.5-2.0 centimeters shooting were planted into MS, ½MS, DKW and WPM nutrient media in order to root proliferation. In this stage, each nutrient media contains IBA with different concentration and 6.5 g/l agar for solidification.

Acclimatization. Before acclimatization process, all in vitro rooted plantlets were gently washed in distilled water to eliminate any nutrient media remnants. Consequently, explants were planted to plastic containers with peat filled cell trays, after which all containers were covered by plastic glasses and then, were maintained in acclimatization room with 25ºC temperature and 55-60 % air humidity for 2-3 weeks.

Statistical analysis. Traced research was provided in four variations and three repetitions. B.D.Dospekhov analytical placement and Dispersion method was applied in analyses of each repetitions [3]. Accuracy was achieved due to comparison of control variation with others.

3. RESULTS AND DISCUSSIONS:

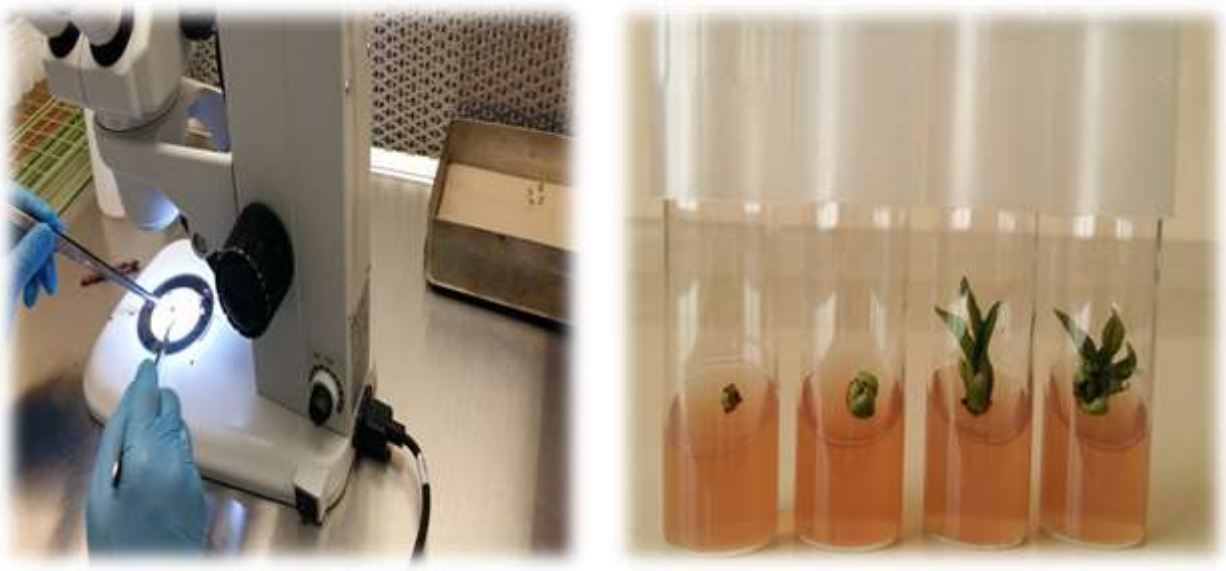
The *in vitro* multiplication of Krymsk®5. In preliminary studies, the in vitro shoot regeneration is observed within two weeks in DKW media containing 1.0mg/l BAPand 0.5mg/l Kin. The bud proliferation rate was 92 %. The short micro shoots developed by influence of different concentrations of plant growth regulators. The bud proliferation rate was low, 5.07 %, when 0.5 mg/l Kinwas added to culture media (Figure 1 and Table 1). The selection of the most suitable cytokine type and concentration for culture media is the most important issue in large-scale production of high quality plants [13]. However, somatic regeneration protocols have been established for several cherry cultivars [4, 7, 11, 15]. The proper use of exogenous cytokines is significant in shoot proliferation in plant micropropagation [6, 12].

Table 1

Effect of different concentration and combination of plant growth regulators on in vitro establishment of Krymsk®5 explants

DKW mediafortified withgrowth regulator (mg/l)			Days taken for bud burst	Bud proliferation percent
BAP	**Kin**	**GA_3**		
-	0.5	-	16-19	5.07
-	-	0.5	18-22	0.00
0.5	1.0	-	14-17	67.12
-	0.5	1.0	13-16	54.08
1.0	0.5	-	12-14	92.10
1.0	-	0.5	13-16	80.15
0.5	-	0.5	15-19	51.08
-	1.0	0.5	18-22	25.57
1.0	-	-	14-17	72.50
0.5	-	-	14-18	33.44
-	0.5	-	19-22	16.25
-	1.0	-	17-20	40.13
$CD_{0.05}$				0.42
SE±				1.41

Values in parenthesis are arc sine transformed values.
CD = Critical difference
SE = Standard error



A B

Figure 1.
A) The procedure of in vitro establishment of Krymsk®5 into culture.
B) The bud bursting and shoot proliferation.

After 29 days, the callus proliferation rates showed 100%, 72.57%, 65.56%, 62.45% basal DKW, MS, modified MS and WPM media, respectively. The best result for callus proliferation was revealed in DKW media (Table 2).

Table 2

Effect of different nutrient media on callus proliferation of Krymsk®5 explants (%)

Nutrient media	Basal MS	DKW	Improved MS	WPM
Period	**X±Sx**	**X±Sx**	**X±Sx**	**X±Sx**
5 days	3.20±0.10	5.54±0.10	4.53±0.13	2.69±0.41
8 days	7.93±0.25	15.86±0.18	11.03±0.21	4.99±0.35
11 days	14.90±0.38	25.29±0.16	22.40±0.31	10.21±0.29
14 days	24.13±0.25	38.32±0.22	29.93±0.21	17.26±0.37
17 days	35.83±0.25	51.93±0.52	36.60±0.29	26.47±0.31
20 days	47.07±0.31	69.10±0.23	42.00±0.26	35.67±0.45
23 days	54.97±0.19	78.89±0.41	49.23±0.67	43.36±0.29
26 days	66.27±0.32	89.46±0.52	59.12±0.56	53.46±0.47
29 days	72.57±0.21	100.00±0.00	65.56±0.49	62.45±0.36

The in vitro multiplication of Krymsk®5 explants was carried in growth chambers. The development dynamics of micro shoots length of Krymsk®5 explants was experimented in different nutrient media. After 35 days, the highest shoots were observed in DKW media, 3.16±0.13 cm. The shoots cultured in modified MS media followed after, the shoot length was 2.20±0.06 cm. The lowest shoots were observed in WPM and basal MS media, 1.50±0.06 cm and 1.31±0.04 cm, respectively (Table 3).

Table 3

The development dynamics of microshoots length of Krymsk®5 explants in growth chamber

Nutrient media	MS	DKW	MSm	WPM
Periods	**X±Sx**	**X±Sx**	**X±Sx**	**X±Sx**
3 days	0.86±0.04	1.53±0.06	0.84±0.02	0.75±0.05
5 days	0.86±0.04	1.67±0.06	0.96±0.02	0.75±0.05
8 days	0.95±0.04	1.76±0.06	1.05±0.03	0.75±0.05
11 days	1.09±0.04	1.76±0.06	1.24±0.04	1.01±0.05
14 days	1.17±0.03	1.76±0.06	1.34±0.05	1.18±0.05
17 days	1.20±0.04	2.08±0.07	1.41±0.05	1.33±0.05

20 days	1.20±0.03	2.42±0.12	1.50±0.05	1.37±0.05
23 days	1.21±0.04	2.69±0.11	1.59±0.05	1.43±0.06
26 days	1.31±0.04	2.75±0.12	1.71±0.06	1.46±0.06
29 days	1.31±0.04	2.97±0.12	1.85±0.06	1.50±0.06
32 days	1.35±0.04	3.11±0.12	2.02±0.06	1.56±0.06
35 days	1.42±0.03	3.16±0.13	2.20±0.06	1.59±0.06

The in vitro rooting of Krymsk®5. The most proper nutrient media for in vitro rooting was ½MS media fortified with 3 mg/l IBA, the rooting success showed 96%. The mean number of roots was 8, the mean root length exhibited 3.1 cm. The lowest rates of rooting success, mean root number, mean root length were observed in hormone free WPM nutrient media (Figure 2 and Table 4).

Table 4

The effect of nutrient media and IBA concentration on rooting parameter of Krymsk®5 plantlets

Nutrient media	IBA concentration (mg/l)	Mean root number	Mean root length (cm)	Rooting success (%)
MS (st)	0	2±0.14	0.7±0.03	10±0.52
MS (st)	1	4±0.16	1.2±0.03	28±0.57
MS (st)	3	7±0.17	2.0±0.02	92±0.53
½MS	0	3±0.13	0.9±0.02	30±0.58
½MS	1	4±0.18	1.5±0.03	35±0.51
½MS	3	8±0.12	3.1±0.03	96±0.56
DKW	0	2±0.17	1.1±0.02	20±0.65
DKW	1	4±0.15	2.0±0.03	56±0.57
DKW	3	2±0.12	1.2±0.02	35±0.51
WPM	0	0±0.00	0±0.00	0±0.00
WPM	1	4±0.11	1.9±0.03	69±0.62
WPM	3	3±0.15	1.4±0.02	52±0.64

A **B**

Figure 2.
A) The in vitro development of root systems of Krymsk®5 plantlets in MS, ½MS, DKW, WPM culture media.
B) The measurement of root length of Krymsk®5 plantlets in DKW culture media

The in vitro acclimatization of Krymsk®5. Acclimatization is defined as the climatic or environmental adaptation of an organism, especially a plant, to new environmental conditions[15]. After 7 days, the rooted Krymsk®5 plantlets were removed from nutrient media and transplanted to peat in trays (Agrobalt®, 250 liters). During the first four days, the boxes are kept closed for establishment of high humidity. Further high humidity is not favorable for Krymsk®5 plantlets. The acclimatization success of Krymsk®5 plantlets showed 90%. The low humidity, high light

intensity, autotrophic nutrition, septic condition must be provided for successful acclimatization of Krymsk®5 plantlets. Though the general technology is applied for in vitro acclimatization, the development of specific methods of in vitro hardening is significant for each plant type.

4. CONCLUSION:

The DKW nutrient media is found the most suitable among four nutrient media for in vitro bud proliferation, callus development, shoot multiplication of Krymsk®5 explants. The in vitro rooting success of Krymsk®5 plantlets is observed in half strength MS media. The growth regulators performed different effects on Krymsk®5 explants. The bud proliferation of Krymsk®5 explants showed progressive tendency in nutrient fortified with 1.0mg/lBAP and 0.5mg/l Kin. The IBA with3mg/l concentration was the most effective for in vitro rooting of Krymsk®5 plantlets. Research concludes that, specific type and concentrations of nutrient media and growth regulators must be provided for development of Krymsk®5 explants. The humidity, high light intensity, autotrophic nutrition, septic condition must be provided for successful acclimatization of Krymsk®5 plantlets. The rooted Krymsk®5 plantlets successfully acclimatized in peat (Agrobalt®, 250 L).

The results of this research work provide development of in vitro propagation protocols for commercial production of Krymsk®5 (VSL-2) rootstock.

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Abbreviations:

BAP - 6-Benzylaminopurine
IBA - Indole-3-butyric acid
Kin - Kinetin
GA_3- gibberellin
MS - Murashige and Skoog Media (Murashige and Skoog, 1962)
MSm - modified Murashige and Skoog
DKW - Driverand KuniyukiMedia (Driver and Kuniyuki, 1984).
WPM - Woody Plant Media