



ACCLIMATIZATION OF CHERRY ROOTSTOCK KRYMSK-5 (VSL-2)

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ABSTRACT

This article presents new aspects regarding the influence of nutrient media on acclimatization of cherry rootstock Krymsk-5 (VSL-2). Experiments are carried out for estimating the effect of nutrient media: MS, mMS DKW, WPM on acclimatization of cherry plantlets. The shoot length and width are measured periodically. The highest mean shoot length of Krymsk-5 in acclimatization were observed in DKW media: $4,55 \pm 0,22$ cm after 30 days, and followed by $118,87 \pm 2,56$ cm growth after 160 days in transferring to the field. The DKW nutrient media is found the most suitable among four nutrient media for acclimatization development of Krymsk-5 explants.

1. INTRODUCTION

The effective application of techniques of micropropagation of fruit plants are common in today's world horticulture. These techniques are most efficient for rootstock propagation [1,2]. There is not unique nutrient media for micropropagation of all plants; they differ in inorganic salt and organic molecules composition [3]. Acclimatization is defined as the climatic or environmental adaptation of an organism, especially a plant, to a new environment. Substantial numbers of in vitro cultured plants do not survive transfer from in vitro conditions to the greenhouse or field environments. Plantlets or shoots that have been grown in vitro have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and nearly optimal conditions for plant multiplication. Plantlets are often developed within the culture vessel under low light intensity, aseptic conditions, and on a medium containing ample sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high levels of humidity. These conditions result in the formation of plantlets of abnormal morphology, anatomy and physiology.

2. MATERIALS AND METHODS

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 25°C temperature. The containers kept closed for 4 days in order to provide high humidity. The containers are ventilated with air by opening the containers. The containers are opened frequently for a short time in the beginning of acclimatization, after they are opened for longer period. The peat and seedlings are watered frequently in the beginning of acclimatization, after they watered rarely. High humidity provides fungal contamination; the seedlings must be treated with Topas® and Khom® fungicide solutions to prevent the contamination. The high light intensity are provided for autotrophic growth of seedlings, these are cultured in mixotrophic mode during in vitro propagation. The seedlings were incubated in the growth rooms at 23 ± 1 °C, 6500-lux light intensity and 16 hours daylight / 8 hours darkness photoperiod.



3. RESULTS AND DISCUSSION

Growth dynamics on acclimatization period of Krymsk 5 plantlets were studied which were micropropagated in different nutrient media. The highest shoot growth length (4.55 ± 0.22 cm) was established in DKW media propagated plantlets while the lowest

shooting length (3.95 ± 0.14 cm) was established plantlets which was propagated in standard MS (MS(st)). Krymsk-5 explants which planted in modified MS (MSm) and WPM showed shooting length of 4.05 ± 0.18 cm and 4.21 ± 0.15 cm respectively (Table 1).

Table 1

Growth dynamics of Krymsk-5 plantlets in acclimatization room (cm).

N _o	Nutrient media	day1	day5	day10	day15	day20	day25	day30
1	MS(st)	$3,08 \pm 0,13$	$3,31 \pm 0,13$	$3,56 \pm 0,14$	$3,74 \pm 0,14$	$3,78 \pm 0,14$	$3,92 \pm 0,14$	$3,95 \pm 0,14$
2	DKW	$2,96 \pm 0,14$	$3,19 \pm 0,14$	$3,48 \pm 0,15$	$3,65 \pm 0,15$	$4,10 \pm 0,20$	$4,46 \pm 0,22$	$4,55 \pm 0,22$
3	MSm	$3,03 \pm 0,15$	$3,15 \pm 0,13$	$3,53 \pm 0,15$	$3,62 \pm 0,18$	$3,81 \pm 0,14$	$3,98 \pm 0,19$	$4,05 \pm 0,18$
4	WPM	$2,98 \pm 0,13$	$3,10 \pm 0,18$	$3,38 \pm 0,18$	$3,61 \pm 0,17$	$4,05 \pm 0,21$	$4,13 \pm 0,21$	$4,21 \pm 0,15$

Field (*in vivo*) growth dynamics of different media micropropagated plantlets were studied according to two aspects: shoot growth and shoot width.

The Shoot height. According to the results, the highest rates were demonstrated by plantlets, which were

developed in DKW media ($118,87 \pm 2,56$ cm), while the lowest rates were mentioned at standard MS ($92,87 \pm 3,59$ cm). Modified MS and WPM were demonstrated an average results: $108,82 \pm 2,62$ cm and $112,75 \pm 3,23$ cm respectively (Table 2).

Table-2

In vivo conditions shoot height results indicators of in vitro micropropagated Krymsk 5 plantlets (cm)

N _o	Nutrient media	Day 1	Day 20	Day 40	Day 60	Day 80	Day 100	Day 120	Day 140	Day 160
1	MS(st)	$21,52 \pm 0,49$	$25,52 \pm 0,69$	$32,67 \pm 1,20$	$40,62 \pm 1,75$	$59,10 \pm 2,47$	$67,60 \pm 3,14$	$80,50 \pm 3,80$	$87,47 \pm 3,52$	$92,87 \pm 3,59$
2	DKW	$32,37 \pm 1,36$	$36,45 \pm 1,23$	$44,27 \pm 1,26$	$52,42 \pm 1,48$	$71,30 \pm 2,48$	$78,75 \pm 3,48$	$99,23 \pm 2,52$	$116,14 \pm 2,76$	$118,87 \pm 2,56$
3	MSm	$39,30 \pm 1,14$	$41,73 \pm 1,01$	$46,70 \pm 1,04$	$51,73 \pm 1,27$	$68,55 \pm 1,91$	$79,40 \pm 1,99$	$98,80 \pm 2,63$	$104,00 \pm 2,48$	$108,82 \pm 2,62$
4	WPM	$35,45 \pm 1,30$	$38,57 \pm 1,18$	$44,32 \pm 1,17$	$49,47 \pm 1,37$	$68,07 \pm 2,22$	$84,62 \pm 2,76$	$103,72 \pm 2,91$	$112,03 \pm 3,13$	$112,75 \pm 3,23$

The shoot width. The highest mean value of shoot width was observed in DKW media, $10,68 \pm 0,21$ mm. The shoots cultured in WPM and mMS followed after, the mean shoot widths were

$9,36 \pm 0,28$ mm and $8,55 \pm 0,25$ mm, respectively. The lowest mean value of shoot width was observed MS media, $7,66 \pm 0,23$ mm (Table 3).

Table 3

In vivo conditions shoot width results indicators of in vitro micropropagated Krymsk-5 plantlets (mm)

N _o	Nutrient media	Day 1	Day 20	Day 40	Day 60	Day 80	Day 100	Day 120	Day 140	Day 160
1	MS(st)	$2,09 \pm 0,05$	$2,39 \pm 0,06$	$2,88 \pm 0,08$	$3,46 \pm 0,11$	$4,81 \pm 0,13$	$5,05 \pm 0,15$	$5,57 \pm 0,14$	$6,75 \pm 0,22$	$7,66 \pm 0,23$
2	DKW	$2,43 \pm 0,10$	$2,88 \pm 0,10$	$3,84 \pm 0,11$	$4,75 \pm 0,13$	$5,83 \pm 0,17$	$6,44 \pm 0,19$	$7,10 \pm 0,15$	$8,46 \pm 0,21$	$10,68 \pm 0,21$
3	MSm	$2,43 \pm 0,10$	$2,94 \pm 0,10$	$3,92 \pm 0,09$	$5,24 \pm 0,13$	$5,60 \pm 0,14$	$6,13 \pm 0,14$	$6,79 \pm 0,16$	$7,81 \pm 0,21$	$8,55 \pm 0,25$
4	WPM	$2,53 \pm 0,15$	$3,07 \pm 0,14$	$4,00 \pm 0,12$	$4,85 \pm 0,14$	$5,90 \pm 0,15$	$6,36 \pm 0,15$	$6,76 \pm 0,17$	$7,81 \pm 0,19$	$9,36 \pm 0,28$



4. CONCLUSION

According to research results, it is established in vitro micropropagation of Krymsk 5 the most effective nutrient media was DKW. DKW was followed by WPM and the lower results were obtained in standart MS nutrient media in vitro micropropagation of Krymsk 5. Well-developed root system plays a vital role in acclimatization process, which provide good basis for adaptation to new conditions. Due to careful treatment in acclimatization period, persistence of plants was 90%. Based on results of the survey the media protocol to micropropagate of Krymsk 5 can be established in industrial production scale.

Abbreviations

MS (st)–standard Murasige and Skoog Media (Murasige and Skoog, 1962)

MSm - modified Murasige and Skoog

DKW– Driver and Kuniyuki Walnut (Driver and Kuniyuki 1984)

WPM - Woody Plant Media

LITERATURE

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