

# Micropropagation of Hardy Hibiscus (*Hibiscus Moscheutos* L.)

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**Abstract.** Hardy hibiscus (*Hibiscus x moscheutos* L.) is gaining popularity as a landscape plant and the accelerated increase in the volume of planting material is of particular relevance. The work investigated the effect of the concentration of 6-BAP (0.5, 1.0, 1.5 mg / L) on the shoot formation of hibiscus of the following varieties: cv. Cranberry Crush, Fantasia, Fireball, Jazzberry Jam. It was revealed that the concentration of 6-BAP 0.5 mg / L is optimal for the proliferation of microshoots of *Hibiscus x moscheutos* L. With the increase in the concentration of 6-BAP, the number of formed microshoots decreases.

## 1 Introduction

The genus *Hibiscus* has more than 250 species, including annual and perennial herbaceous forms and ending with small woody shrubs [1]. Representatives of various species of the genus *Hibiscus*, due to their attractiveness, are used for landscaping for various purposes, including in coastal areas with a high salt content, and some species are used as raw materials for the preparation of medicines [2-4].

Hardy hibiscus (*Hibiscus x Moscheutos* L.) is one of the species that is a promising crop for the landscaping objects for various purposes [5].

Hibiscus plants have beautiful flowers with a diameter of 5-30 cm with the bright color of petals, differ in a variety of shapes and colors of leaves, size of bushes, are characterized by a long flowering period (up to 3 months). The mature plants grow from 0.6 to 2.4 m in height, depending on the variety and growing conditions. Stems usually die off in the autumn, and new shoots appear in the spring [5, 6].

Production of Hardy hibiscus is carried out by dividing the bush, green cuttings and sowing seeds. Seed propagation is rarely used, because plants do not retain all the characteristics of the original variety, and plants of interspecific hybrids may not form seeds at all. Moreover, breeding for sterility of *Hibiscus x moscheutos* L. is currently underway, since sterile hibiscus plants have a longer flowering period, and unsightly fruits develop and remain on the stems at the end of the flowering season, which significantly reduces the decorative value [7, 8]. Reproduction by dividing the bush and green cuttings have

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difficulties in terms of the need to grow large numbers of mother plants, as well as the relatively low rooting rate of cuttings.

In this regard, clonal reproduction is the especially relevant method of reproduction of Hardy hibiscus [9]. Microcloning of valuable varieties, rootstocks, scions, unique forms from a minimum amount of starting material, in comparison with the traditional vegetative propagation method, has a number of advantages: the ability to reproduce plants all year round regardless of the season, large-scale production in small areas and the high reproduction rate [10]. This method of reproduction is relevant when preserving endangered plant species [11].

The publications on clonal micropropagation of hibiscus *in vitro* are extremely rare. There are some results on the reproduction of *Hibiscus syriacus* L. [12], *Hibiscus sabdariffa* L. [13], *Hibiscus srosa-sinensis* L. [1, 14]. However, there is no information on *in vitro* cultivation of Hardy hibiscus.

Therefore, the improvement of the method of clonal micropropagation of Hardy hibiscus (*Hibiscus x moschetous* L.), in particular, the determination of the time of initiation into *in vitro* culture, the optimal composition of the culture medium, cultivation conditions, adaptation, etc., is of particular relevance.

## 2 Materials and methods

The studies were carried out in the conditions of the selection and biotechnological laboratory of Federal State Budget Scientific Institution «North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making». For the initiation into the *in vitro* culture we used varieties of Hardy hibiscus (*Hibiscus x moschetous* L.) such as *cv. Fantasia*, *cv. Fireball*, *cv. Jazzberry Jam*, *cv. Cranberry Crush*, as the most promising and demanded for the agricultural landscape purposes due to its resistance to the complex of unfavorable summer stress factors (high summer temperatures, lack of air and soil moisture, dry wind phenomena, increased insolation, etc.) [14].

To optimize the technological process, in the first decade of December, 4-year-old mother plants of hibiscus from open ground were transplanted into individual containers with the soil mixture consisting of peat, soil and sand in a 1: 1: 1 ratio. Further, the plants with a closed root system were grown under artificial conditions at a temperature of 22-24 ° C, humidity 60-75%, illumination 1500 Lx. This technique was used to reduce the infectious background during the selection of explants, as well as to awaken the buds earlier.

The segments of cuttings with apical and axillary buds were used as explants. For the disinfection of explants, freshly cut shoots were segmented and washed under running water for 1.5-2 hours. After that, they were washed repeatedly with distilled water. Then, under sterile conditions, the explants were processed in 70% alcohol for 1 minute, followed by washing in sterile water and disinfection in a solution of chlorine-containing tablets "OKA-TAB" (0.5% active chlorine, exposure 12 minutes). After that, explants were washed five times in sterile distilled water. With this method of processing, the outcome of sterile explants on average for the varieties was 85%.

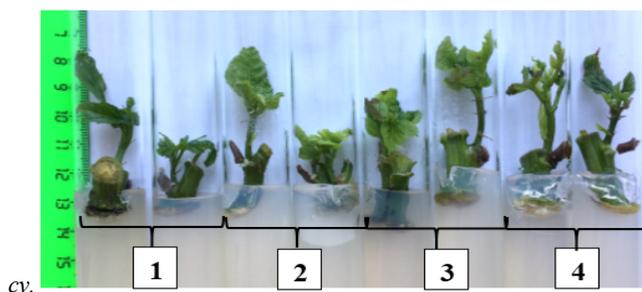
**Table 1.** Outcome of sterile explants of *Hibiscus x moschetous* L.

Variety	Common number of explants, pcs	Sterile explants outcome, %
<i>CranberryCrush</i>	63	89
<i>Fantasia</i>	65	85
<i>Fireball</i>	60	79
<i>JazzberryJam</i>	70	87

For the initiation into the *in vitro* culture, the culture medium according to the DKW prescription (Driver-Kunijuki, 1984) was used, containing 6 g / l agar-agar, 30 g / l sucrose, 0.3 mg / l 6-benzylaminopurine (6-BAP) «Sigma Aldrich». The autoclaving was carried out at 121 ° C for 20 minutes, medium pH 5.9. The microshoots were subcultured on the fresh culture media every 3-4 weeks.

### 3 Results and discussion

The growth of microshoots of Hardy hibiscus began in the second week of cultivation. By the end of the third week, the passage of mericlones were carried out on the fresh culture medium for the further reproduction (Figure 1).



1 –*cv.* Cranberry Crush; 2 –*cv.* Fantasia; 3 – *cv.* Fireball; 4 – *cv.* Jazzberry Jam.

**Fig. 1.** *Hibiscus x moschetous* L. explants (3 weeks after introduction into culture).

At the stage of multiplication of shoots, the culture media with different contents of 6- (BAP) were tested: 0.5, 1.0, 1.5 mg / L. (Table 1).

**Table 2.** Influence of 6-BAP Concentrations on the Multiplication of Hardy hibiscus.

Variety	Concentration of 6-BAP, mg / l		
	0,5	1	1,5
<i>Cr.crash</i>	2,53±0,18	2,17±0,31	1,9±0,19
<i>Fantasia</i>	3,67±0,21	3,5±0,22	2,67±0,21
<i>Fireball</i>	2,33±0,21	2,17±0,17	2±0
<i>Jazzberry Jam</i>	4,33±0,21	3,67±0,21	3±0,26
Significance		**	
T-test	5%	0,60	
	1%	0,79	

According to the Table 2, it can be seen that the multiplication factor of Hardy hibiscus depends on the concentration of the hormone in the culture medium and the variety specificity in *in vitro* culture. The largest number of hibiscus shoots, depending on the variety, was obtained on the culture medium containing 6-BAP 0.5 mg / l. With the increase in the concentration of 6-BAP, the number of formed microshoots were decreased.

At the stage of multiplication, the microshoots of *cv.* Fireball, *cv.* Cranberry Crush had the lowest multiplication factor, the plants were chlorotic and gradually dried out, so it was not possible to carry out the rooting stage and adaptation of microplants of these varieties.

The grown plants were rooted after 5-6 subcultures. For the rooting microshoots of *cv. Jazzberry Jam* and *cv. Fantasia* varieties, we used DKW hormone-free culture medium. The root formation of microplants began in the second week of cultivation. The outcome of rooted plants was 100%. The microplants of *cv. Jazzberry Jam* variety had more root hairs than *cv. Fantasia* (Figure 2).



**Fig. 2.** Rooted hibiscus plants *cv. Jazzberry Jam* –1, *cv. Fantasia* – 2.

The rooted plants were planted in the containers with a sterile substrate consisting of garden soil, peat, vermiculite, sand in a ratio of 1: 1: 0.5: 0.5. The planted plants were adapted under illumination of 1500 Lx, humidity 85% and temperature 24-25 ° C. Conditions with high humidity were maintained until the appearance of the first new leaf for the better survival of microplants. The outcome of hibiscus plants adapted to *ex vitro* conditions was 83%, in average for varieties (Figure 3).



**Fig. 3.** *Ex vitro* adapted hibiscus plants *cv. Jazzberry Jam*.

## 4 Conclusion

At the stage of multiplication of microshoots of Hardy hibiscus (*Hibiscus x moschetous L.*), the largest number of shoots was formed on the culture medium with the 6-BAP concentration of 0.5 mg / l. With the increase in the concentration of 6-BAP, the number of formed microshoots was decreased. Hibiscus varieties such as *cv. Jazzberry Jam* and *cv. Fantasia* reproduce well in *in vitro* culture and have a high adaptive ability to *ex vitro* conditions (83%). For the hibiscus varieties such as *cv. Fireball* and *cv. Cranberry Crush* the

conditions of the studies were not favorable: at the stage of multiplication, they showed signs of chloroticity, drying out, which was leading to the death of microshoots.

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