Propagation of Canadian rose 'John Franklin' *in vitro* culture

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Abstract. Many researchers are interested in optimizing the clonal micropropagation of ornamental plants. It is known that the varietal specificity of the studied crops makes it necessary to select and optimize the composition of the nutrient medium for each stage of propagation *in vitro*. The purpose of our research was to identify the effect of the composition and concentration of nutrient hormones on multiple shoot formation, root development in regenerating plants of the rose 'John Franklin' *in vitro*. A technology has been developed for obtaining a large amount of self-rooted planting material of a winter-hardy decorative rose variety *in vitro* culture. Clonal micropropagation was performed using vegetative buds as explants. The multiple of shoots is achieved by activating the axillary meristems of the shoots. It has been shown that the efficiency of micropropagation of the 'John Franklin' rose increases on the Murashige and Skoog nutrient medium containing BAP at a concentration of 1.0 mg/l and IAA – 0.5 mg/l. This medium provides high quality regeneration of micro-shoots with a propagation coefficient equal to 5.2. The optimal modification of the nutrient medium at the rooting stage was revealed, and the expediency of using IBA with a concentration of 0.5 mg/l as auxin was noted. The selected conditions allow you to get a larger number of roots on the shoot – 5 pcs. and their greatest length is 51.5 mm.

1 Introduction

The role of floral and ornamental plants increases every year in landscaping, landscape architecture and floristry. One of the main crops that is preferred is the rose (Rosa L.) [1]. Modern garden roses are divided into 10 groups, the most popular of which in recent years are Canadian park roses. Canadian park roses are a unique series of varieties bred in the second half of the 20th century in Canada specifically for cultivation in cold and harsh climates. These are, as a rule, vigorous and spreading bushes, blooming with small flowers. Canadian roses are a godsend for the climate of Siberia and the Urals. Created for growing in harsh conditions, Canadian roses are almost disease-free and undemanding in care, easily adapting to low temperatures, heat and high humidity. Representatives of this group prefer sunny areas, nutritious, well-drained soils with neutral or weak acidity. Canadian roses can withstand temperatures as low as -40°C. Even if in winter their shoots freeze slightly (to the level of snow), then in the spring they recover quite quickly. This is the most important

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advantage of these attractive flowers. Canadian roses bloom from early summer until frost sets in.

The unpretentious variety 'John Franklin', which was obtained as a result of interspecific hybridization, is very popular among domestic and foreign flower growers. Occupies one of the leading places in landscaping. Planting material is in demand in the modern market. Traditional propagation methods do not satisfy the demand for a promising variety. In this regard, the development of technologies that accelerate the production of large quantities of planting material is relevant. Currently, an alternative method of rose propagation can be clonal micropropagation [2].

The purpose of our study was to identify the influence of the composition and concentration of hormones in the nutrient medium on the multiplication of shoots and root development in regenerated Canadian rose 'John Franklin' plants under *in vitro* conditions.

2 Materials and methods

The object of research on rose propagation *in vitro* was the 'John Franklin' variety, which belongs to the Canadian park variety according to the International Classification [3]. Stem sections with vegetative buds were used as explants when introduced into *in vitro* culture. Explants were taken from young bushes growing in the exhibition areas of the Botanical Garden. Selected kidneys were washed in a 3% aqueous Nika disinfectant solution and rinsed with tap water. Next, under aseptic conditions, surface sterilization was carried out using diacid at a concentration of 0.1% and ethanol 70%. The exposure to diacide was 20 minutes, ethanol - 1 minute. The preparation of materials, culture media and work under aseptic conditions was carried out according to generally accepted methods [4, 5, 6]. The buds were placed on a hormone-free medium containing mineral salts according to the prescription of Murashige and Skoog (MS) [7]. After the leaves appeared, the explants were transplanted into MS nutrient medium for induction with the addition of 25 g/l sucrose and growth regulators cytokinins and auxins.

The propagation process was studied under controlled conditions: 16-hour photoperiod, temperature 24°C, air humidity of at least 70%. For shoot formation, the method of activating axillary meristems was used. Subcultivation was carried out after 25-30 days, during which the number and length of shoots and roots were taken into account. When rooting regenerated shoots, MS medium was used, diluted by half in macro- and microsalts and with a reduced sucrose content. Indolyl-3-acetic acid (IAA) and indolyl-3-butyric acid (IBA) were used as an inducer of rhizogenesis.

3 Results and discussion

As a result of experiments on inoculation of the buds of the rose 'John Franklin', we found that exposure to 0.1% diacid solution for 20 minutes and 70% ethanol solution for 1 minute provide up to 72% of non-infected live explants.

Careful selection of growth regulators of the nutrient medium and identification of their optimal concentrations makes it possible to increase the efficiency of clonal micro-propagation: the formation of the maximum number of micro-shoots capable of rooting [8]. Therefore, an important stage of the work is to study the effect of exogenous growth regulators on the process of morphogenesis.

Most studies on the clonal propagation of commercial rose varieties are aimed at optimizing the composition of nutrient media, especially the selection of the composition of the mineral base [9]. Often, one medium is suitable for one variety and completely unsuitable

for another, so researchers modify the nutrient media, choosing the optimal composition for those plants with which they work [10].

The first passages of cultivation showed that explants began to develop rapidly. After 2 weeks, the buds that had begun to grow were transferred to a propagation medium. We tested 4 variants of the MS nutrient medium, mg/l:1) BAP 1.0 + IAA 0.1; 2) BAP 1.0 + IAA 0.3; 3) BAP 1.0 + IAA 0.5; 4) BAP 2.0 + IAA 0.5.

The results of our experiments on buds cultivation have shown that morphogenetic processes *in vitro* are characterized by high intensity. A week after placing the explants on the MS nutrient medium, they increase in size. Along with their subsequent growth and development, multiple axillary buds were laid and further shoots developed from 37.4 to 65.5 mm long, depending on the medium variant (Table 1).

Table 1. The effect of nutrient hormones on the growth of micro-shoots of the rose 'John Franklin'.

Growth Parameters	**Hormone concentration, mg/l**			
	BAP 1.0 + IAA 0.1	**BAP 1.0 + IAA 0.3**	**BAP 1.0 + IAA 0.5**	**BAP 2.0 + IAA 0.5**
Shoot length, mm	42.6±1.0	48.5±1.3	65.5±1.3	37.4±1.8
Number of shoots, pcs./per explant	2.2±0.2	3.4±0.6	5.2±1.5	2.9±0.8

Analysis of changes in the length and number of shoots depending on the concentration of hormones in the nutrient medium revealed an increase in the value of their parameters with an increase in the concentration of IAA from 0.1 mg/l to 0.5 mg/l together with BAP 1.0 mg/l. An increase in the concentration of BAP (2.0 mg/l) had no effect on increasing the length and number of shoots. The largest number of additional shoots with a propagation coefficient equaling 5.2 was noted when cultivated on a nutrient medium with a BAP of 1.0 mg/l and an IAA of 0.5 mg/l (Fig.).

Fig. 1. Rose 'John Franklin' on MS nutrient medium containing BAP 1.0 mg/l and IAA 0.5 mg/l.

For rooting, micro-shoots were planted on a nutrient medium MS, diluted twice in macro- and microsols, containing auxins – IBA and IAA in concentrations from 0.25 to 1.0 mg/l. The frequency of rhizogenesis in all variants of the experiment was high and amounted to 74.4-78.0% (Table 2).

Table 2. The effect of auxins on the growth of rose roots 'John Franklin' at the *in vitro* rooting stage.

Growth Parameters	Hormone concentration, mg/l					
	IAA			IBA		
	0.25	0.5	1.0	0.25	0.5	1.0
The frequency of rhizogenesis,%	74.4±3.1	75.2±2.6	75.2±2.4	76.4±3.6	78.0±2.1	77.6±1.4
Number of roots, pcs.	3.1±0.6	4.6±0.4	4.8±0.2	3.9±0.8	5.1±0.2	4.9±0.5
Root length, mm	38.4±2.2	46.5±2.0	47.3±1.8	34.8±1.8	51.5±1.7	48.0±1.6

The analysis of the data presented in Table 2 allows us to emphasise that in all variants of the medium with auxins, intensive rooting of shoots and root growth were noted. The experiments made it possible to identify the optimal cultivation medium for this variety. The parameters of the length and number of roots were the highest at a concentration of IBA 0.5 mg/l in the nutrient medium.

4 Conclusion

As a result of the conducted research, the hormonal composition of the MS nutrient medium for the Canadian rose 'John Franklin' *in vitro* culture was optimized. It is demonstrated that the effectiveness of clonal micropropagation of the studied variety increases on the MS nutrient medium containing BAP at a concentration of 1.0 mg/l and IAA at a density of 0.5 mg/l. This medium provides a high propagation coefficient of 5.2 in combination with the high quality of regenerated shoots. The optimal MS nutrient medium at the rooting stage has been revealed. For rhizogenesis, it is advisable to use IBA at a concentration of 0.5 mg/l as auxin. The conducted research is the basis for the microclonal propagation of the rose 'John Franklin' and the production of mass planting material.

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References

1. A. K. Tolembetova, S. K. Turasheva, A. A. Imanbaeva, G. I. Ernazarova, Z. B. Serikova, Experimental Biology **73(4)**, 32-41 (2017)
2. O. A. Churikova, Plodovodstvo, semenovodstvo, introduktsiya drevesnykh rasteniy **21**, 286-288 (2018)
3. Modern Roses XI: The World Encyclopedia of Roses 638 (2000)
4. R. G. Butenko, Culture of isolated tissues and physiology of plant morphogenesis, 64-102 (1964)
5. V. F. Kalinin, V. V. Sarnatskaya, V. E. Polishchuk, Methods of tissue culture in plant physiology and biochemistry, 62-95 (1980)
6. N. V. Kataeva, R. G. Butenko, Clonal micropropagation of plants,14-36 (1983)
7. T. Murashige, F. Skoog, Physiol. Plant. **15(13)**, 473-497 (1962)
8. A. A. Zaripova et al, IOP Conf. Ser.: Mater. Sci. Eng. **941**, 012029 (2020)

9. M. G. Markova, E. N. Somova, Agrarnaya nauka Evro-Severo-Vostoka **63**, 2, 35-41 (2018)
10. T. G. Lekontseva, A. V. Fedorov, Agricultural Science Euro-North-East **23(6)**, 814-821 (2022)