Development of clonal micropropagation technique for *Gloxinia hybrida* hort. “Strawberry Ice-Cream”

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Abstract. The reported paper presents outcomes of clonal micropropagation of ornamental variety *Gloxinia hybrida* hort. “Strawberry Ice-Cream”. In the study conditions supporting in vitro implanting leaf blade fragments are developed with an outcome of 97 % viable explants. An optimal composition of the Murashige and Skoog growth medium is suggested; it contains BAP (0.5 mg/l) and IAA (0.2 mg/l) and brings about efficient adventive sprout regeneration. Implantation takes place in a growth medium; no root stimulating hormones are applied. The survival of regenerative plants is reported to be high after transplanting ex vitro.

1. Introduction

To date, plant tissue and organ culture is widely used all over the world in commercial flower growing. This technique has a number of advantages over traditional plant propagation, such as output of genetically homogenous planting stock; production of virus-free plants owing to meristem culture; a high propagation coefficient; a possibility of year-round works and efficient use of working areas for young plants production; and easy transportation of tube stock plants to any destination [1].

Clonal micropropagation makes it possible to reduce a growth period of plants till they are ready for selling, e.g. 1 – 1.5 months shorter for foliage begonias and ornamental flowering saintpaulias, gloxinias, and 3 – 4 months less for chrysanthemums, lilies, carnations, and orchids. Propagating a new variety, several millions of plants can be output annually; in 2 – 3 years of growing a planting stock of high quality is produced. It might take over 10 years given that conventional methods are used [2].

On the world scale a majority of ornamental flowering plants are grown with the help of clonal micropropagation techniques. The Netherlands is a West-European leader in the field of clonal micropropagation (about 70 laboratories are involved into micropropagation production). This fact is related to cultivation of ornamental plants – a global market niche traditionally dominated by the Netherlands. Approximately a half of cut flowers and ornamental flowers exported worldwide are output in this country [3]. Among most important groups propagated in vitro in the Netherlands there are pot plants, orchids, and bulbous plants. Recently, Russia has an extensive experience in the micro-cloning most demanded cultures. Biotechnology laboratories are opened almost in all scientific and research institutes, selection centers with the purpose to improve a valuable planting material and promising varieties as well as propagate them via micro-cloning [3, 4].

Our study aimed at developing a clonal micropropagation technique for *Gloxinia hybrida* hort. “Strawberry Ice-Cream” in culture in vitro. To achieve the purpose our study sought for a production method of a sterile culture, an optimal composition of the growth medium supporting adventive shoot regeneration and implantation in vitro, conditions of transplanting regenerative samples ex vitro.
2. Materials and Methods
The technique of plant tissue and organ culture is adapted to the clonal micro-propagation [5].

Our study is focused on a double-flowered variety of *Gloxinia hybridra* hort. “Strawberry Ice-Cream” selected in the Netherlands; its fascinating big double flowers have the colour of strawberry juice and a white petal brim. The plant has a strong medium length flower-bearing stem and a compact shrub. The variety tends to flowering in bouquets and grows in the collection of South-Ural Botanical Garden-Institute, Ufa.

For experiments we selected undamaged leaves of generative plants, washed them in a mild soap solution for 15 minutes and in flow tap water for 30 minutes. Then the surface of a planting material was treated with sterilizing 0.1 % diacid and 70 % ethanol solutions in the aseptic laminar flow cabinet. The exposure time to sterilizing agents was set 0.5 to 20 minutes. Finally, the planting material was washed in sterile distilled water for 15 minutes. As explants we took 2-4 cm² fragments of leaf blades with a midrib.

Explants were placed into the growth medium in test tubes or retorts. Growth media were prepared and sterilized as recommended [5, 6]. The Murashige and Skoog growth medium (MS) was used for cultivation [7], its composition and concentration were modified with growth stimulators 6-benzylaminopurine (BAP), kinetin, indole-3-butryc acid (IBA), and 3-indoleacetic acid (IAA). Plants were reproduced in such controlled conditions as a 16-hour photoperiod, a temperature of 24°C, and air humidity of minimum 70 %.

Regenerative plants were transplanted into containers with a soil substrate and kept in the vegetation chamber.

3. Results and Discussions
A state of an explant is one of key factors when implanting into culture *in vitro*. Here, particular attention is to be paid to the physiological age and size of an explant.

Our observations showed mature developed and grown leaves of gloxinia were more capable to morphogenesis than young and growing old leaves.

Another important factor of successful micro-propagation is an explant size. Bigger explants of gloxinia were viable longer, a leaf tissue did not shrink as a consequence, and more meristematic zones developed.

As known, micro-propagation begins from selecting and disinfecting an explant to obtain an aseptic culture. Explants of gloxinia leaves had gentle and soft tissues, a planting material was disinfected in aseptic conditions as given in Table 1; accurately, trying to avoid any damages.

| Disinfection solution | Exposition, min. | Number of sterile explants, % | Number of viable explants, % |
|-----------------------|------------------|-------------------------------|-----------------------------|
| 70% ethanol 0.1% diacid | 0.5              | 80                            | 80                          |
| 70% ethanol 0.1% diacid | 8                |                               |                             |
| 70% ethanol 0.1% diacid | 0.5              | 97                            | 97                          |
| 70% ethanol 0.1% diacid | 10               |                               |                             |
| 70% ethanol 0.1% diacid | 0.5              | 100                           | 30                          |
| 70% ethanol 0.1% diacid | 20               |                               |                             |

From the data of experiments it is apparent the exposition time in the first diacid-containing disinfection solution is short because a percentage of sterile explants are low. Using the third disinfection solution, we obtained less viable explants in spite of a high percentage of sterility. An optimal disinfection variant appears to be the exposure of a planting material in 70% ethanol solution for 0.5 min. and 0.1 % diacid solution for 10 min. A maximal number of viable and sterile explants are a product of this disinfection variant.
A lot of species representing Gesneriaceae family: Saintpaulia, Cape primrose, Episcia, Gloxinia, etc. regenerate plants from leaf blades, sprigs, stems. In traditional vegetative propagation one Gloxinia leaf gives one plant. Using the tissue culture technique, mass propagation of a particular Gloxinia variety is possible through inducing the adventive bud formation de novo from small leaf segments, i.e. origination of organized structures immediately from specialized epidermal and sub-epidermal tissues, leaf mesophyll, etc.

Under appropriate disinfection conditions all explants of Gloxinia hort. “Strawberry Ice-Cream” are capable for regeneration after aseptic treatment. Adventive buds form from plant tissue due to supplementing growth regulators in different amounts to the nutritive medium. In most cases, this process takes place in culture media containing either cytokinin or its combination with auxin. As cytokinin BAP or kinetin is used, IAA is frequently a source of auxin [8]. Sterile Gloxinia explants were introduced into five growth medium variants MS (Table 2). First morphological transformations were observed during the first cultivation week. Explants became thicker, especially on the cut zone. In two weeks first adventive sprouts developed. In a month of cultivation without a passage sprouts formed 2 to 4 leaves (Fig. 1).

**Table 2.** The effect of growth regulators on the morphogenesis of Gloxinia hybrida hort. “Strawberry Ice-Cream” in conditions in vitro

| Growth regulators, mg/l | Morphogenesis type                                      |
|-------------------------|--------------------------------------------------------|
|                         | Continuous regeneration of sprouts (hemmogenesis)      |
| (- - - -)               | Growth of a single sprout with a lot of roots (hemmorogesis) |
| 0.5 - 0.2 -            | Continuous regeneration of sprouts (hemmogenesis)      |
| - - 0.5 0.5           | Growth of roots (rhizogenesis)                         |
| 1.0 1.0 1.0 -          | Continuous regeneration of sprouts (hemmogenesis), into weeks after their formation we observe vitrification |
| 0.5 - 0.5 -            | Continuous regeneration of sprouts (hemmogenesis)      |

Optimal growth media supporting the formation of sprouts are variants containing BAP (0.5 mg/l) and IAA (0.2 and 0.5 mg/l) (Table 2). In these nutritive media the highest regeneration coefficient is obtained. Approximately 50 sprouts form per 1 cm² leaf explant during one month after visual regeneration begins. Further, we observed continuous budding, explant tissue did not die. Adventive sprouts tend to form even more intensively in the growth medium MS containing an equal concentration of BAP, kinetin, and IAA (1.0 mg/l). However, after 6 weeks of cultivating explants in this medium we observed partial vitrification of forming sprouts. Moreover, stems got thicker; leaves elongated and tended to deformations because of morphological transformations in tissues and cell expansion related to water stress. One of the reasons inducing such transformations is supposed to be low transpiration caused by abnormal evolution of mouth-like openings and exodermis structure. To solve the problem of vitrification we reduced a BAP concentration in the medium to 0.5 mg/l or transplanted them into a hormone-free nutritive medium Murashige and Skoog (MSO), where sprouts developed a normal morphology over two weeks of cultivation. 4 weeks later bigger sprouts formed a root system.

In aseptic conditions of laminar flow cabinet a conglomerate of sprouts was taken out of culture tubes, sprouts with formed roots were separated; sprouts without roots were transplanted for rhizogenesis into the medium MS containing IAA (0.5 mg/l) and IBA (0.5 mg/l). During 3 – 4 weeks micro-sprouts developed roots, and a habit of regenerative plants expanded (Fig. 2). There was no significant difference in the development of plants in growth media with and without hormones. A growth medium containing auxins at the stage of rooting brought forward the rhizogenesis by 1 – 2 weeks compared with a hormone-free nutritive medium.
Figure 1. Adventive sprout regeneration of *Gloxinia hybrid a hort.* “Strawberry Ice-Cream” in a growth medium MS, 0.5 mg/l BAP and 0.2 mg/l IAA over 1 month.

Figure 2. Adventive sprout regeneration of *Gloxinia hybrid a hort.* “Strawberry Ice-Cream” in a growth medium MS, 0.5 mg/l IAA and 0.5 mg/l IBA over 3 weeks.

In the process of further growing regenerative plants nodules formed and developed. All *Gloxinia* plants grown in the hormone-free medium continued forming branch sprouts (Fig. 3), although less numerous than in the propagation medium. This fact indicates a high morphogenesis potential of *Gloxinia*.

Figure 3. Evolution of regenerative plants *Gloxinia hybrid a hort.* “Strawberry Ice-Cream” in a hormone-free growth medium MS.

Figure 4. Cultivation of plants *Gloxinia hybrid a hort.* “Strawberry Ice-Cream” in a soil substrate.

In the next propagation phase plants were transplanted into the soil substrate and kept in the vegetation chamber. *Gloxinia* plants with a well-developed root system were transplanted into containers with a soil blend (Fig. 4) made up of garden mold, organic matter, and bank sand taken in equal amounts. First two weeks plants *ex vitro* were covered with a horticulture fleece “Agrotex”. The fleece “Agrotex” is breathable, maintains humidity and protects from temperature fluctuations, furthermore, it is not heavy to cause plants bending. The survival ability of plants was as high as 90 – 95%. Over 3 weeks regenerative plants were transplanted into individual vegetative pots (Fig. 5). Transplanted *Gloxinia* samples developed forming flower buds and started flowering 1 – 1.5 months later (Fig. 6).
Conclusion

A clonal micropropagation technique of Gloxinia hybrid a hort. “Strawberry Ice-Cream” is developed. Double-flowered Gloxinia varieties are slowly reproducible. If reproduced through leaf cuttings, one leaf gives one sprout only. A plant starts flowering in 1.5 years. In culture in vitro one medium-sized leaf provides on average 10 – 12 fragments (2 cm²). Further, more than 100 sprouts are regenerated on each explant placed into a growth medium. Owing to up to date biotechnology methods, clonal micropropagation, in particular, one leaf gives above one thousand identical plants per 6 months, making a propagation time one year shorter.

References

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