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IN VITRO REGENERATION INTRODUCTION IN DORMANCY STATE AS A WAY OF POST-ACEPTIC ADAPTATION

За використання культури тканин у розмноженні рослин дуже важливою та відповідальною є проблема їх постасептичної адаптації, тому що за посадки неадаптованого матеріалу втрати можуть становити до 100 %. Водночас, у природі відомий такий спосіб пристосування рослин для уникнення несприятливих факторів як входження рослин у стан спокою. Вивчаючи ефективність введення *in vitro* у стан спокою регенерантів двох філогенетично віддалених видів (*Solanum tuberosum* та *Hosta*), встановлено ефективність цього прийому. Рослини картоплі сортів Подолянка і Червона рута, що пройшли стан спокою, мали довший вегетаційний період та забезпечували вищу урожайність. У регенерантів хости сорту Паульс Глорі з розсади без кореня прижилося 37,8 % рослин, приживлюваність розсади із коренем становила 56,6 % і найбільший відсоток приживлюваності (87,2 %) становив у варіанті з рослинами, які пройшли стан спокою. Подібна закономірність встановлена й по сорту Патріот.

Таким чином, на рослинах картоплі та хости встановлено позитивний вплив введення регенерантів *in vitro* у стан спокою на постасептичну адаптацію.

Ключові слова: культура тканин, розсада, адаптація, *Solanum tuberosum, Hosta*.

Cloned micro propagation allows you to rapidly propagate plants almost all species of the multiplication factor 1 to 1000 and more [1]. Obtained biological material is planted after *in vitro* in natural conditions or under glass in the form of seedlings. This method is convenient and easy to use. However, despite the advantages it has some weaknesses. Namely, it's impossible to pursue a gradual (during the year) material accumulation; seasonality and availability of peak periods; the cost of creating a microclimate; non compact and significant injury during transport. A seedling after planting requires a certain period of engraftment and acclimatization *in vitro.* During this time loss of regenerated plants can be 50-100% [2, 3].

It's connected with the fact that the conditions which are formed in aseptic autotrophic cultivation, such as low water potential of nutrient (ten times lower soil), high humidity, transpiration intensity is close to zero [4] leads to a loss of stomata ability [5]. With a sharp movement of plants *in vitro* to natural intensity reaches a very high level, which can cause loss of 100% of seedlings [6]. So, the problem of rehabilitation of the natural conditions remains relevant.

At the same time, in nature are known mechanisms the occurrence of plants dormancy, which allow us to overcome the adverse conditions and start the life cycle of plants from the beginning in the form of a new organism: seeds or unit of vegetative reproduction. Germination of seeds and tubers begins with the first stage of organogenesis and during the life cycle of plants are adapting to environmental conditions. As it well known, conditions determine the peculiarities of the formation of organs and tissues of the growing organism, in which laid the adaptation for these conditions [7]. Searching of methods of using the rest of plants-regeneration, which would improve the post-aseptic adaptation, were the aim of our research.

Research methods. For research were cultured *in vitro* two phylogenetically distant species of plants, such as *Solánum tuberósum* (varieties of the Podolyanka and The Chervona Ruta) and *Hosta* (varieties of the Patriot, Pauls Glori). We used nutrient medium of Murashige and Skoog. We sampled of 30 plants. Post-aseptic cultivation was carried out on substrate in a humid chamber.

Results and discussion. For such types of material we need different conditions of cultivation cuttings, different time, different costs of electricity and consumables. One or another method of accelerated reproduction is characterized by such a quantity as the multiplication factor. For example, one test-tube plant of potato in propagation by cuttings for approximately 1 month, you can get 5-7 regenerated plants, two months - 30-40 plants, for the three months - 150-200, for four - months 450-550 plants and 10 months - more than half a million plants. In case of receipt of micro tubers this figure will be about 4-5 times less because one test-tube plant depending on the type of plant 1,3 - 2,1 pcs micro tubers [8]. For almost a lack of seasonality in production multiplication factor is also directly correlated with the period of cultivation of regenerator (Fig.1).

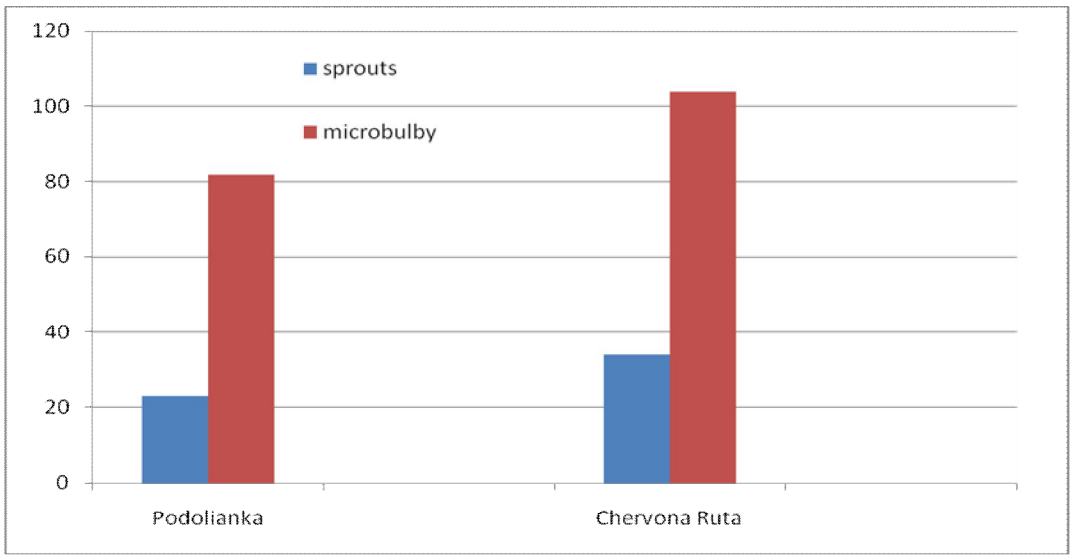

Fig. 1. **Duration of period of cultivation regenerators in dependence on the method of speed-up reproduction,** days

Expenses of time for growing of one plant *in vitro* (sprouts) comparatively with micro tubers were smaller and depending on the studied varieties were: variety Podolianka - 23 days, variety Chervona Ruta - 34 days. Much longer time was needed for micro tubers of variety Podolianka - 82 days, variety Chervona Ruta – 104 days.

At the time of planting seedlings and micro tubers in greenhouses there were found differences in the ontogeny of plants. (Table 1). Seedlings were landed in greenhouses with already formed in certain extent the aboveground part, and micro tubers needed an average of 18 days for the appearance of the first steps. At planted out regenerators defined a different number of main stems of shrubs and different depth of laying of stolons. Plantings which were formed by seedlings were characteristic by single stem and shallow laying stolons (1,5cm).

Table 1 – **Features of ontogenesis of *Solánum tuberósum* in greenhouses, depending on the initial seeding material**

Type of initial seeding material	Quantity, unit		Depth of laying of stolons, cm	Vegetation period, days	Weight of minitubers, g
	shoots	stolons			
Variety of Podolianka					
Seedlings	1,1	2,1	1,5	78	1,68
Microtubers	2,3	4,3	5,7	94	2,74
LSD $_{0,05}$	0,1	0,3	0,2	3	0,08
Variety of Chervona Ruta					
Seedlings	1,3	2,8	2,3	104	1,93
Microtubers	2,9	5,3	6,3	122	3,06
LSD $_{0,05}$	0,1	0,2	0,3	5	0,10

At the same time bushes of potatoes which grown out micro tubers formed two or more stems. Seedlings of both varieties compared to micro tubers had a shorter period of cultivation. In our opinion, it could be due to two factors:

1. Slow down and acceleration of plant growth as a result of difficult post-aseptic rehabilitation plants after culture *in vitro*.
2. Test-tubes plants are started their vegetation period after planting them in greenhouse with already formed stem.

In our opinion, forming of less numbers of stolons of plants from seedlings is also depending on post-aseptic adaptation of plants. Seedlings needs a certain period of ingrowth, during that time turgor is decreasing and it's also significant stressful factor. [9]. It's known that stress inhibits the growth, and hence, it's inhibits the formation of more numbers of vegetative organs and makes vegetation period shorter. In turn, it's impacted on productivity of plants. In both varieties of plants, which were grown in greenhouse from micro tubers, was set more numbers of forming of mini tubers.

Thus, the introduction of the potato *in vitro* at dormancy (formation of micro tubers) improves post-aseptic adaptation.

Another variety of plant, in which studied the effect of the introduction of plants in vitro in the dormancy, were regenerators of two kinds of *Hosta*.

We compared three kinds of seedlings:

1. Regenerators with roots and without entering into dormancy.
2. Regenerators without roots and without entering into dormancy (Fig. 2).
3. Regenerators which were introduced into dormancy (Fig. 3).

Fig. 2. **Morphogenesis *in vitro* seedling *Hosta* depending on cultivation conditions:**
1 – medium with an excess of cytokinin; 2 – medium with an excess of auxin.

Fig. 3. **Introduction of plants of *Hosta in vitro* in dormancy:**
1 – before dormancy; 2 – after dormancy: a) without removal of dead leaves;
в) after the removal of dead leaves.

The research found differences of plants which were cultivated in vitro, as well as differences of plants during of post-aseptic development. Seedlings with root systems, as well as without them differed during the aseptic cultivation.

Thus, when growing plants without roots on artificial nutrient medium with an excess of cytokinin (benzylaminopurine 2,5 mg/l), were formed plants with 2-3 stems and large leaves. However, rhizogenes was almost absent. Only in some plants were formed roots 1-2 with length 50-10 mm. Seedlings that grown on media with an excess of auxin (Indole-3-butyric acid 4 mg / l), by contrast, had extensively developed roots, but inferior to the development of organs of assimilation. Seedling plants *in vitro* which was released from dormancy, were characterized by less developed assimilation system (size of leaf plate) and little root system (3-5 roots with length 2-3mm) but in comparison with other variants there were a greater number of stems.

Morphological features that were detected in aseptic conditions also were manifested at ex vitro conditions (table 2). It means that seedling plants which were formed root systems in vitro also during the planting of culture under cover they had the greatest number of roots: 7,4 pc per plant at the variety of Patriot and 8,1 pc per plant at the variety of Pauls Glory.

Table 2 – **Effect of introducing regenerants *in vitro* in dormancy on post-aseptic ontogenesis of seedlings of *Hosta* at 60th day**

Variant of seedling		Got accustomed, %	Amount of roots, pc	Amounts of stems, pc	Weight of plant, g
Variety of Patriot					
It wasn't in dormancy	Without a roots	43,57	5,3	1,9	0,5
	With a roots	59,4	7,4	1,2	0,9
Passed the dormancy		91,6	6,9	6,1	3,6
$LSD_{0,05}$		4,1	0,3	0,3	0,4
Variety of Pauls Glory					
It wasn't in dormancy	Without a roots	37,8	6,2	1,7	0,6
	With a roots	56,6	8,1	1,4	1,2
Passed the dormancy		87,2	7,6	5,2	3,9
$LSD_{0,05}$		5,3	0,4	0,3	0,5

The highest number of stems had the plants which were emerged from dormancy. Plants of this species with more stems for 60 days of cultivation increased their weight. At the first days of growth they had little and rolled plates of leaves. But over time these plants of both varieties dominated by plants of the other options several times in size and weight. Also plants of variety Patriot which grown from material that hasn't been at dormancy had weight 0,5 grams (without roots), 0,9 grams (with roots) and 3,6 grams (plants which were at dormancy).

The establishing of plants, as well as Morphogenesis of stems and roots, is one of the main indicator of post-aseptic adaptation. There wasn't a clear difference between seedlings and micro tubers of potato at engraftment of aseptic material and there was a clear difference in development, at the same time the indicator of ingrowth of Hosta was very different at variants. In particular, at the variety of Pauls Glory from seedlings without roots established 37,8% plants, establishing of seedlings with roots was higher - 56,6% and the higher establishing of seedling was at plants which were at dormancy - 87,2%. A similar pattern is set to the variety of Patriot.

Conclusions: we revealed, that introduction in dormancy of plants in vitro of *Solánum tuberósum* and *Hosta* improves their post-aseptic cultivation, so, there is a post-aseptic adaptation.

LITERATURE

1. Основи біотехнології рослин: навчальний посібник / В.В. Мацкевич, С.В. Роговський, М.Ю. Власенко, В.М. Черняк. – Біла Церква: БНАУ, 2010. – 156 с.

2. Гиголашвили Т.С. Особенности водообмена ассимиляционной ткани *Solanum* в условиях *in vitro*/ Т.С. Гиголашвили, В.Г. Реуцкий, О.И. Родькин // Физиол. и биохим. культ. раст. – 1997. – Т.29, №6. – С. 461-467.

3. Зеленіна Г.А. Мікроклональне розмноження та фізіологічні особливості *Arnica foliosa Nutt.*/ Г.А. Зеленіна // Вісник Одеського національного університету. – 2004. – Т. 9, Вип. 2. – С. 63-66.

4. Зеленіна Г.А. Морфогенез в культурі *in vitro* сегментів стебла та клональне мікророзмноження *Arnica chamissonis* Less. ssp. *foliosa* (Nutt.) Maguire: автореф. дис. на здобуття наук. ступеня канд. біол. наук: спец. 03.00.20 «Біотехнологія» / Г.А. Зеленіна. – Ялта, 2007. – 22 с.

5. Зеленіна Г.А. Мікророзмноження та особливості водного обміну *Arnica foliosa Nutt.* / Г.А. Зеленіна // Вісник Одеського національного університету. – 2005. – Т. 10, Вип. 5. – С. 7-11.

6. Деревинська Т.І. Проблеми і перспективи вирощування арніки на півдні України з використанням мікроклонального розмноження / Т.І. Деревинська, Г.А. Зеленіна, Н.С. Любимова // Збірн. наук. праць „Фальцфейнівські читання". – Херсон: Терра, 2005. – Т. 1. – С. 117–118.

7. Куперман Ф.М. Морфофизиология растений. Морфофизиологический анализ этапов органогенеза различных жизненных форм покрытосеменных растений / Ф.М. Куперман. – М.: Изд-во «Высш. школа», 1973. – 256 с.

8. Мацкевич В.В. Удосконалені методи оздоровлення картоплі від вірусів та використання отриманого матеріалу в первинному насінництві: дис. кандидата с.-г. наук: 06.01.14 / Мацкевич В'ячеслав Вікторович. – Київ, 2004. – 153 с.

9. Современная ботаника в 2 томах. Т 2. Глава 21. Регуляция роста и развития: гормоны растений. Глава 25. Внешние факторы и рост растений / Пер. с англ. – М.: Изд-во „Мир", 1990. – 344 с.

Введение регенерантов in vitro в состояние покоя как путь постасептической адаптации

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При использовании культуры тканей в размножении растений очень важная и значимая проблема – их постасептическая адаптация, поскольку при посадке неадаптированного материала потери могут составлять до 100 %. В то же время, в природе известен способ приспособления растений для предотвращения неблагоприятных факторов как вхождение растений в состояние покоя. Изучая эффективность введения регенерантов *in vitro* в состояние покоя двух филогенетически отдаленных видов (*Solanum tuberosum* и *Hosta*), установлена эффективность этого приема.

Растения картофеля сортов Подолянка и Червона рута, прошедшие состояние покоя, имели длительный вегетационный период и обеспечивали высокую урожайность. У регенерантов хосты сорта Паульс Глори из рассады без корня прижилось 37,8 % растений, приживаемость рассады с корнем составляла 56,6 % и наибольший процент приживаемости (87,2 %) составлял в варианте с растениями, которые прошли состояние покоя. Подобная закономерность установлена и по сорту Патриот.

Таким образом, на растениях картофеля и хосты установлено положительное влияние введения регенерантов *in vitro* в состояние покоя на постасептическую адаптацию.

Ключевые слова: культура тканей, рассада, адаптация, *Solanum tuberosum*, *Hosta.*

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