The use of phytohormones to increase the efficiency of potato propagation in nodal cuts culture

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Abstract. Micropropagation of potato *in vitro* by periodic cutting of plants and passaging them to new nutrient media is the most common approach to obtaining large volumes of planting material in mini-tuber production systems. The article summarizes research data on the effect of gibberellic acid, auxins, and cytokinins on the rate of plant growth and internode formation of potato microplants *in vitro*. According to the results published in the scientific literature, the greatest efficiency in the process of series cutting of plants can be achieved by using media with a predominance of gibberellic acid in combination with auxins. The use of cytokinins is not relevant within this technology framework.

1 Introduction

The technology of micropropagation is widely used in potato seed material production at the stage of planting material multiplication for transplantation into cultivation systems to obtain mini-tubers. The beginning of this approach was the work of Stewart and Caplin [1], in which the introduction of potato into the culture of isolated tissues was first reported. Nevertheless, active research concerning the development of shoots of potato microplantss started, apparently [2], only in the late 70s. Studies of those years did not show any advantages in terms of increasing the yield of microclones of introducing into the medium auxins and cytokinins available at that time [2]. To date, nevertheless, an opinion has been formed that the stimulating effect of plant growth regulators (PGR) is sufficient to consider as a factor of microclonal reproduction effectiveness in serial cuttings. The results presented in the scientific literature, at the same time, are quite contradictory. This applies to both the PGR names, their concentrations, and the ratio. This is, clearly, due, among other things, to the well-known variety dependence of the potato reaction to the composition of nutrient media with this cultivation method. In turn, the rate of internode formation and their growth directly affects the efficiency of the plant reproduction process in industrial production of microclones [3]. The purpose of this work is to systematize the known data on the effect of exogenous PGRs on the growth and development of potato microplants *in vitro*.

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2 Materials and Methods

The review includes studies aimed at identifying the PGR effect on the length of shoots and the number of internodes of potato microplants *in vitro*, as the main targets affecting the cutting rate. The problems of the effectiveness of multiple stem regeneration initiation based on various explants, as well as callusogenesis, are not the subjects of the present review. When searching for information, the method of visual graphs of the Connected papers service was used (https://www.connectedpapers.com /). The search for scientific articles was also carried out through the databases Google Scholar, Research Gate, Science Direct, Elibrary

3 Results and Discussion

The introduction of potato into culture to obtain primary microplants is carried out both on the basis of apical meristems, and by direct and indirect regeneration of shoots, using various explants [4]. Series cutting of potato microplants are used at further stages to obtain large volumes of planting material .

Auxins and gibberellins are hormones that promote cell division and growth. Deficiency of gibberellic acids leads to dwarfism of plants, as well as to suppression of auxin transport [5]. In turn, defects in auxin transport also lead to dwarfism, by themselves. At the same time, auxins contribute to the accumulation of bioactive gibberellins, while they themselves reduce their accumulation through the suppression of biosynthesis and deactivation [6]. At the same time, exogenous gibberellins in the absence of auxins (cuttings without tops) contribute to the degradation of DELLA proteins responsible for suppressing gibberellic acid (GA) signaling, which contributes to the elongation of shoots [7], which may occur at the initial stages of cuttings growth after passaging.

3.1 Monohormonal media

In one of the first papers on this topic, Roca and co-authors [8] used an MS medium with the addition of 0.2 mg/l GA3 for passaging of single-node segments at the final stage of obtaining potato plants for greenhouse conditions. The concentration of 0.25 mg/l GA3 in the MS medium in [9] induced an increase in the stem length and the number of internodes on the 30th day of observation by one and a half times in comparison with the hormone-free medium, and increased these parameters by 10% in comparison with the media containing 0.1 and 0.5 mg/l GA3. In a similar study [10], the increase was 86% and 15-30%, respectively. These results are consistent with the data of the study [11], where the level of GA3 0.25 mg/l allowed to obtain plants with the maximum number of internodes – 1.1-1.5 times higher than on the medium without PGR, and increased the height of the plants by 1.5-2 times.

In [12], the medium with 0.25 mg/l GA3 was at a disadvantage both in the number of internodes and in the length of stems to monohormonal media with auxins (1 mg/l NAA or IAA or IBA) and variants with a combination of auxins at the same concentrations with GA3.

High concentrations of GA3 (1, 2, 3, 4, and 5 mg/l) were used in the work [13], where a statistically significant increase in stem length (by 18%) was noted only when the level of 3 mg/l was reached, the maximum length (38% higher than the MS medium) was recorded at a concentration of GA3 4 mg/l. A further increase in PGR concentration led to a decrease in the length to the level of the hormone-free medium. The presence and concentration of GA had no effect on the number of internodes.

The nonlinear nature of an effect of GA3 concentration increase in nutrient media on the size of shoots and the number of internodesof microplants may be associated with the

inhibitory effect of exogenous gibberellins on the biosynthesis of endogenous ones. We can talk about at least two peaks of activity: 0.25 mg/l [9, 10, 11] and 4 mg/l [13].

Along with GA3, auxins are involved in the regulation of division, elongation, and differentiation of plant cells [14]. There are a number of works where the effect of monohormonal media with IAA and its synthetic analogues NAA or IBA on the growth of potato microplants has been studied.

Exogenous auxins, apparently, have a more pronounced increase in their effect of induction of growth processes as their concentration increases, since they stimulate the synthesis of endogenous gibberellins [7].

The introduction of IAA at a concentration of 10 mg/l increased the stem length by 81.9% in comparison with the MS medium in the work [15]. A smaller, but statistically significant increase is also shown for media with 0.5, 2.5, and 5 mg/l IAA.

Graffor and co-authors [16] investigated the effect of several auxins (IAA, NAA and IBA) in monohormonal media at different concentrations (0, 0.05, 0.15, 0.25, and 0.35 mg/l). The maximum height of plants, twice higher than the control values, was achieved on the medium with NAA (0.15 mg/l), and the maximum number of internodes was recorded on the medium with IBA (0.35 mg/l): 38% higher than the control value.

3.2 Media with a combination of hormones

The idea of the need to introduce at least two phytohormones into the medium for the cultivation of microplants is a logical conclusion from the very nature of hormonal regulation of plant growth and development. The crossregulation of GA3 and auxins has long been of interest as PGRs inducing cell division [7].

The role of auxin and GA3 concentration and ratio in the regulation of potato microplant growth and development was considered in the work with varieties of Indian breeding [17]. The authors consecutively increased the concentration of GA3 and NAA by 2 times from 0.05 and 0.005 mg/l, respectively, to 0.4 and 0.04 mg/l, maintaining the ratio of 10:1. In all variants, an increase in stem length and the number of internodes was observed in comparison with the control. At the same time, the minimum values in this series were obtained on the medium with a maximum concentration of PGR, while the maximum values were recorded at the level of 0.1 mg/l GA3 and 0.01 mg/l NAA: the length of shoots by 50-60% and the number of internodes by 38-42% higher than the values obtained in the control. Nevertheless, the best results, 69-73% longer stems and 52-56% more internodes, were recorded on the medium with a ratio of GA3 and NAA 20:1, where the GA3 concentration was doubled while maintaining the NAA level (0.2 mg/l and 0.01 mg/l, respectively).

The study of different concentrations of GA3 and NAA while maintaining a ratio of 10:1 is also described in the work with the "Montecarlo" variety [18]. When comparing data from the medium with 0.1 mg/l GA3 and 0.01 NAA with the medium with twice the concentration, it was shown that the latter leaded to a longer stem length (8.7 cm) and higher number of internodes (5.1 pcs/plant). The remaining media 0.2 mg/l GA3/ 0.03 mg/l NAA and 0.2 mg/l GA3/ 0.04 mg/l NAA were also inferior to this medium. There was no compare with the variant with a hormone-free environment.

In [19], the length of stems and the number of internodes on the medium with a ratio of GA3 and NAA 5:1 (0.5 mmol and 0.1 mmol – corresponding to 1.73 mg/l and 0.186 mg/l) were higher than on a medium where the difference between phytohormone levels was 10:1 (0.5 μmol and 0.05 μmol). The ratio of 5:1 (0.5 mg/l and 0.1 mg/l) turned out to be the best variant in the work [20]: the were 34% more internodes and 60% longer stems, compared with the hormone-free medium. Also, this medium surpassed media with ratios of 1:1 (13% more internodes, 25% longer stems) (0.25 mg/l and 0.25 mg/l) and 2:1 (9% more internodes and 8% longer stems) (0.5 mg/l and 0.25 mg/l).

In the exploratory study [21], the ratio of 1 mg/l GA3 and 1 mg/l IAA did not significantly increase the number of internodes, while double increase in the concentration of GA3 (2:1 GA3:IAA) led to the formation of 30% more internodes per plant. There was no such effect as on media with an inverse GA3 to IAA ratio 1:2, 2:1.5, thus, as well as with increase in the concentration of both PGRs to 2 mg/l. Upon this the length of the stems increased by 20% on three media containing 1/2, 2/1, and 1/1 mg/l GA3/IAA.

The 2:1 ratio (2 mg/l GA3 and 1 mg/l NAA) led to a statistically significant increase in the length of shoots and the number of internodes in comparison with the control (MS) in the work [22]. Upon this an increase in the ratio to 4:1 (4 mg/l GA3) led to an inhibition of the growth of microplantss.

In [23], GA3 levels of 4 and 5 mg/l in combination with NAA concentrations of 0.01 (400:1) and 0.02 (250:1) mg/l contributed to an increase in the length of stems by 20-90%, depending on the variety.

An increase in the length of stems by 70% was noted in [24] when using an MS medium with 3 mg/l GA3 and 1 mg/l IAA.

In turn, in [12], the best results for the length of stems and the number of internodes were obtained with the inverse ratio of GA3 and NAA (1:4), which concentrations were 0.25 mg/l and 1 mg/l, respectively. Nevertheless, the author did not use a hormone-free medium as a control in the study. In this connection, one can only talk about the superiority of this medium over monohormonal media with GA3, IAA, NAA and IBA with the same concentrations. The monohormonal medium with 1 mg/l of NAA also had the maximum values among the media with one type of hormone. In this connection, it can be assumed that the stimulating effect of the addition of GA3 for all auxins was on average the same.

The advantage of media with a combination of PGR over monohormonal media is shown in [25], where the best results concerning the length of stems and the number of internodes were obtained on the media with GA3 and IAA ration 3:1 and 1.5:1 (mg/l): the values were 1.8 times higher than on the medium without PGR. Cultivation of microplantss on the medium with 1 mg/l GA3 and 2 mg/l IAA increased the studied parameters to a lesser extent by 1.4 times. A slightly smaller increase in indicators was noted in the presence of the same IAA concentration, but, in the absence of GA3.

Thus, the presence of GA3 and auxins in the nutrient medium increases the growth rate of microplantss in comparison with any monohormonal medium [12]. At the same time, as in the case of a monohormonal medium with GA3, the acceleration of growth and development (formation of internodes) of microplantss does not always occur in parallel with an increase in the concentration of hormones.

At hormone levels below 1 mg/l, medium with 0.1-0.2 mg/l GA3 and 0.01-0.02 mg/l auxin (IAA or NAA) and the ratio of 10:1 is successfully used [17, 18]. A further increase in concentrations leads to a decrease in growth rates. Nevertheless, there is evidence that an increase in the ratio has an even greater stimulating effect, as does its decrease [19, 20]. At concentrations above 1 mg/l of phytohormones, an increase in the ratio of GA3 and auxins to 4:1 leads to growth inhibition [22]. The ratios 2:1, 3:1 are more effective in increasing stem length and number of internodes. Perhaps, at such levels, an increase in the GA3 concentration leads to a decrease in the concentration of endogenous gibberellins. At a certain ratio with auxins, this effect is leveled.

The enhancement of the auxin effect by GA3 introduction was also shown in [15]: the combination of GA3 with IAA increased the stem length by 91.5% compared to the control, while the increase of this trait on the monohormonal medium with IAA was up to 81.9%. In turn, the introduction of 5 mg/l BAP gave an increase in length of 46% (IAA + BAP) and 24.2% (IAA + GA3 + BAP).

A similar effect of applying BAP in combination with GA3 was noted in [23], where high GA3 concentrations (3, 4, and 5 mg/l) were involved. The BAP levels were, respectively,

1.5, 2, and 2.5 mg/l. On all three media, a decrease in the length of the stem and the number of internodes by 1.5-2 times was observed.

A decrease in the efficiency of potato propagation *in vitro* in the presence of BAP was also noted in [26], where the best results (length 5.8 cm, 15 internodes per plant) were obtained on a hormone-free MS medium, when compared with media with 0.25 mg/l and 0.5 mg/l BAP.

In [27], media with different ratios and concentrations of BAP and NAA were compared: 5:1 (0.5 mg/l BAP and 0.1 mg/l NAA), 10:1 (0.5 / 0.05 or 1 / 0.1 mg/l BAP/NAA), 15:1 (1.5 and 0.1 mg/L), 20:1 (1 / 0.05 or 2 /0.1 mg/l BAP/NAA), 40:1 (2 and 0.05 mg/l). In the absence of a hormone-free medium, one can only state the fact that the maximum length of internodes, as well as their number, are fixed on the medium with 1 mg/l of BAP and 0.1 mg/l of NAA. The values were 2-3 times higher than on the medium with BAP 0.1 mg/l, but with 2 times lower NAA concentration. The minimum values were obtained on the media with PGR concentrations of 0.5:0.1 and 0.5:0.05. In other cases, with an equal BAP level, a greater length of internodes and their number were observed on the medium with higher NAA concentration, which probably had a stimulating effect on the stem growth processes.

It can be said that the use of cytokinins (BAP) in combination with GA3 or auxins leads to a decrease in the stimulating effect of the latter. Cytokinins are widely used in the regeneration of potato shoots *in vitro* [2], and in the production of microtubers [28], but their use at the next stages during cutting is not justified. Gibberellins and cytokinins are antagonists in plant growth processes. At certain levels, they are able to suppress each other's signaling [29].

4 Conclusion

The available data on the effect of GA3 and auxins on the growth and formation of internodes of potato microplants *in vitro* are quite contradictory if we consider the concentrations and ratios of these phytohormones in nutrient media. Nevertheless, it can be stated that their joint introduction into the media increases the efficiency of plant propagation by 1.5-2 times. Of the described cultivation protocols, the best results have been obtained by the levels of GA3/IAA-NAA: 0,1-0,2/0,01 mg/l or 2-3/1 mg/l. The phenomenon of the nonlinearity of the effect of increasing GA3 concentrations in monohormonal media and in combination with auxins on the growth parameters of microplants requires additional research. The use of cytokinins in series cutting of potato plants *in vitro* is not relevant.

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