# THE EFFECTS OF GLUCOSE AND GROWTH REGULATORS ON THE ORGANOGENESIS OF *Paeonia lactiflora* Pall. *IN VITRO*

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(Received November 4, 2010/Accepted November 29, 2010)

## A B S T R A C T

Herbaceous peony plants successfully propagated *in vitro* do not survive the transfer to the *ex vitro* environment. For other species, storage organ formation *in vitro* can limit the loss of plants during acclimatization. In the natural conditions, the renewal buds for the following year originate on the underground crown (metamorphosed underground shoot, rhizome) of herbaceous peony. A perennial crown and roots serve for the accumulation of the storage products and plant renewal.

The aim of the experiment was to investigate the influence of glucose (30, 60 90 g $l^{-1}$) and growth regulators (kinetin, IBA, $GA_3$) on the shoot, renewal bud, root growth and development of *Paeonia lactiflora* 'Jadwiga' *in vitro.* Excision of all leaves from isolated explants inhibited production of new shoots and leaves, and evidently induced formation of renewal buds. Increasing the glucose supply, especially in the absence of growth regulators, decreased production of shoots and outgrowth of leaves. The stronger inhibition of shoot growth by glucose was observed on the explants without leaves. By contrast, the beneficial effect of glucose on renewal bud formation was observed. A single supply of kinetin, IBA or $GA_3$ stimulated shoots and leaf growth and inhibited renewal bud formation, on the explants isolated with leaves. Interaction of kinetin, $GA_3$ and IBA (added together) and the highest glucose level enhanced the growth of shoots on the explants containing leaves, and increased the number of renewal buds, on the explants without leaves. Increasing glucose level enhanced the number of roots in the absence of growth regulators on the explants containing leaves. The supply of IBA in the medium containing 30 g $l^{-1}$ glucose, stimulated the root production on the explants without leaves. The addition of $GA_3$ or kinetin (singly or simultaneously with IBA) to the medium with different concentrations of glucose, strongly inhibited rooting.

The results presented here, show that a high level of glucose and exogenous growth regulators (kinetin, $GA_3$, IBA) together stimulate shoot and renewal bud for-

mation but the way of organogenesis depends on the presence or absence of leaves. The interaction between auxin (exogenous or endogenous) and glucose regulate root formation on the peony shoots but the final effect depends on the type of explants (with or without leaves). It is possible that leaves have a very important hormonal factors, which stimulate shoot growth or rooting and inhibit renewal bud formation.

**Key words**: *Paeonia lactiflora*, tissue culture, glucose, growth regulators, organogenesis, renewal bud formation

**Abbreviations**: $GA_3$ – gibberellic acid, IBA – indole-3-butyric acid, BAP – 6-benzylaminopurine, ABA – abscisic acid, MeJA – methyl jasmonate, TDZ – thidiazuron, MS – Murashige and Skoog medium

## INTRODUCTION

Peonies are perennial ornamental plants of the genus *Paeonia*, which belongs to the family *Paeoniaceae.* Herbaceous peonies are grown successfully in moderate, cold-winter climatic zones. The renewal buds for the following year originate on the underground crown, at the base of the annual stems. After the flowering of the aboveground annual stems (at the end of June), the new monocarpic shoot is initiated in the renewal bud. The renewal bud contains central and axillary (4-6) lateral shoots. It is protected by cover leaves (Barzilay et al., 2002; Czekalski and Jerzy, 2003). A perennial crown (metamorphosed underground shoot, rhizome) and roots serves for the accumulation of the storage products and plant renewal (Kapinos and Dubrov, 1993; Walton et al., 2007). The main storage carbohydrate is starch. The concentration of starch declines with resumption of the growth in spring (Walton et al., 2007).

In herbaceous peony, the promoting effect of growth regulators on axillary and adventitious bud or root induction was demonstrated by a few authors (Hosoki et al., 1989; Albers and Kunneman, 1992; Tian, 2008; Gabryszewska, 2009). The plants successfully propagated *in vitro* do not survive the transfer to the *ex vitro* environment. The success of acclimatization has been reported by only a few authors (Hosoki et al., 1989; Albers and Kunneman, 1992; Gabryszewska, 2009; Gabryszewska and Kawa-Miszczak, 2010). However, after transferring to soil in the greenhouse, the peony microcuttings survive at a low percentage because of infection from pathogens (Albers and Kunneman, 1992; Tian, 2008; Gabryszewska, 2009; Gabryszewska and Kawa-Miszczak, 2010). Also, the growth of young plants is slow or stopped because of bud dormancy (Gabryszewska and Kawa-Miszczak, 2010).

Storage organ formation *in vitro* can limit the loss of plants during acclimatization. In the case of herbaceous peony, there are no studies concerning the *in vitro* formation of underground crown with renewal buds and/or roots. For other species, there are a number of factors that influence the induction and growth

of storage organs *in vitro*: environmental conditions (temperature, photoperiod, light quality), medium composition (type and concentration of growth regulators, carbohydrates, activated charcoal, gelling agents) and the genetic makeup of the plant (Ascough et al., 2008).

Plant growth regulators and their interaction with sugars play an important role in the storage organ induction of many plants propagated *in vitro*. Jasmonic acid and methyl jasmonate were capable of inducing *in vitro* tuberization of storage organ in many plant species. Jasmonic acid stimulated bulb formation of garlic (Ravnikar et al., 1993) and induction of tuber formation of potato (Koda et al., 1991) propagated in tissue culture. An increase in the level of jasmonates and a decrease in the level of gibberellins influenced the initiation of potato tuberization (Koda and Kikuta, 2001). Exogenous gibberellin added to the medium inhibited bulbing of *Allium cepa*. On the other hand, the inhibitors of gibberellin biosynthesis (ancymidol, flurprimidol, paclobutrazol) promoted bulb formation (Le Guen-Le Saos et al., 2002). The interaction between gibberellin and ABA and sucrose regulates tuber formation of potato (Xu et al., 1998). Sucrose plays an important role in the storage organ formation of many species propagated *in vitro*. The increase of the sucrose concentration in the medium stimulated the lily and onion bulb formation (Takayama and Misawa, 1980; Gerrits and De Klerk, 1992; Keller, 1993; Kästner et al., 2001), the corm induction of *Watsonia vanderspuyiae* (Ascough et al., 2008), the microtubers formation of *Solanum tuberosum* (Gopal et al., 2001), *Xanthosoma sagittifolium* (Omokolo et al., 2003), *Dioscorea cayenensis – D. rotundata* complex (Ovono et al., 2009) and the microrhizome production of *Zingiber officinale* (Zheng et al., 2008).

Sugars also act as signaling molecules whose transduction pathways influence developmental and metabolic processes. Some of the effects of sugars on plant growth and development suggest an interaction of sugar signals with hormonal regulation (Smeekens, 2000, Rolland et al., 2002, 2006). Glucose has emerged as a key regulator of many vital processes (Moore et al., 2003).

The influence of glucose and growth regulators on the *Paeonia lactiflora* renewal bud, shoot and root formation *in vitro* was investigated. In addition, the interactions between the glucose and growth regulators in the growth and development of different organs were studied.

## MATERIAL AND METHODS

The study was carried out on *Paeonia lactiflora* 'Jadwiga'. Plant material used for the experiment was propagated on the MS (Murashige and Skoog, 1962) medium containing: kinetin 1 mg $l^{-1}$ + 2iP 1 mg $l^{-1}$ + BAP 1 mg $l^{-1}$ + TDZ 0.01 mg $l^{-1}$ and sucrose 30 g $l^{-1}$. In the experiment two different peony explants were used: the pieces of crown with one shoot (with or without leaves). The influence of glucose (30, 60, 90 g $l^{-1}$)

and kinetin 1 mg $l^{-1}$, $GA_3$ 1 mg $l^{-1}$, IBA 1 mg $l^{-1}$ (added to the medium alone or together) on the shoot, renewal bud and root formation was investigated. Each treatment consisted of 3 jars with 5 explants. The experiment was repeated twice (2 series). Culture conditions were a 16 h photoperiod provided by cool-white fluorescent lamps (Philips TLD 36W/95) at 80 µmol $m^{-2}s^{-1}$ and a temperature of 25 °C.The observations and measurements were recorded after two months of culturing. The number of shoots, leaves, buds, and roots per explant were recorded. The statistical analysis of the treatments was tested using analysis of variance and means were compared by Duncan's multiple range test.

## RESULTS AND DISCUSSION

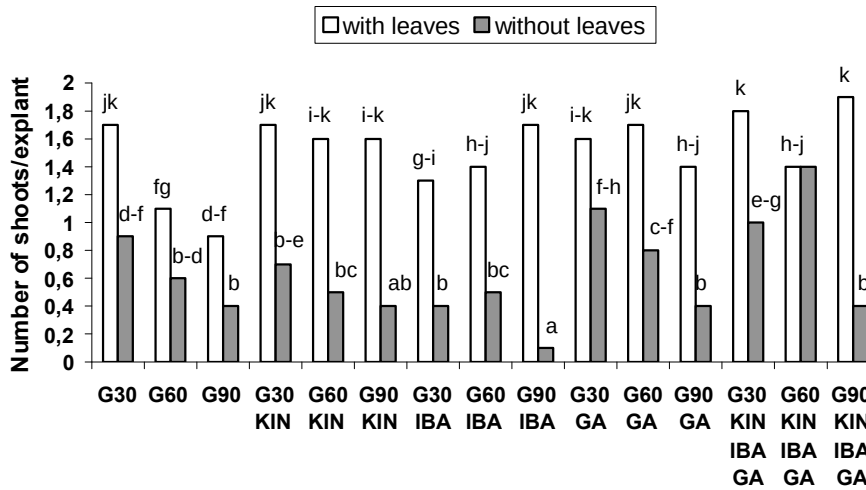
Plant growth regulators influence *in vitro* organogenesis in different species, in diverse ways. These variations are due to the fact that different species, organs or section of organs differ in their ability to produce endogenous growth substances. Also, the different species and organs show various abilities to recognize, take up, transport, metabolize and respond to the exogenous plant growth regulators (Cheesman et al., 2010). Studies *in vitro* seem to be a very useful tool for gaining a better understanding of the hormonal control of the development and growth of different organs.

After two months of culturing, the morphogenic response of *Paeonia lactiflora* 'Jadwiga' explants isolated with or without leaves were evaluated. Excision of all leaves strongly inhibited production of new shoots and leaves on the explants (Fig. 1 A, B; Fig. 3). Increasing the glucose (30-90 g $l^{-1}$) supply in the medium, especially in an absence of growth regulators, decreased production of shoots and outgrowth of leaves on the both kinds of explants. The stronger inhibition of shoot growth by glucose was observed on the explants without leaves. Presence of leaf and simultaneous supply of the exogenous growth regulators (kinetin, $GA_3$, IBA), added to the medium singly or together, had a stimulatory effect on the shoot and leaf growth of peony. It is possible, that exogenous growth regulators can overcome the sugar-derived inhibition and stimulate the shoot formation and growth. Some authors demonstrated the promoting effect of exogenous cytokinins on axillary or adventitious bud induction and multiplication of herbaceous peony (Hosoki et al., 1989; Albers and Kunneman, 1992; Tian, 2008; Gabryszewska, 2009). The axillary shoot formation was induced by BAP, TDZ or the addition of a few cytokinins together (Hosoki et al., 1989; Albers and Kunneman, 1992; Tian, 2008; Gabryszewska, 2009). The mixture of TDZ and cytokinins (BAP, 2iP, kinetin) in the medium was more effective compared to TDZ used alone (Gabryszewska, 1998). An increased level of TDZ (0.1-3 mg $l^{-1}$) in the medium strongly stimulated the adventitious shoot regeneration of herbaceous peony (Thian, 2008). The combination of

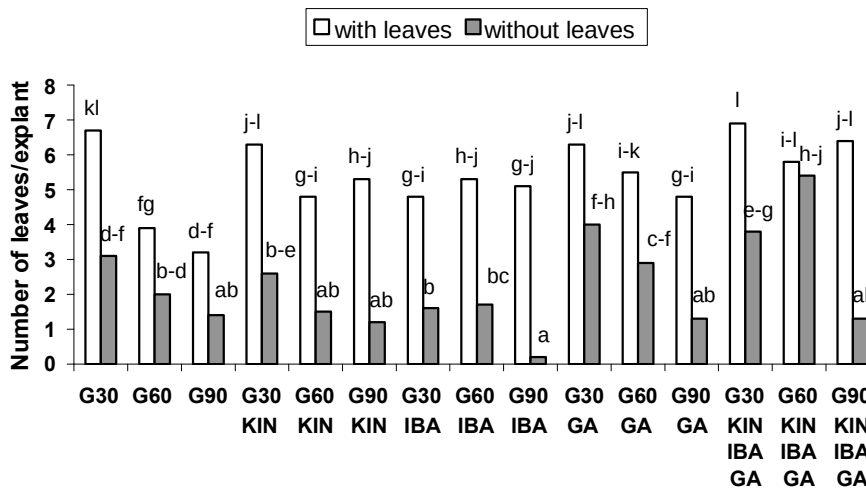
cytokinins with a high level of $GA_3$ (10 mg $l^{-1}$) and sucrose (60-90 g $l^{-1}$) significantly increased multiplication rate (8 shoots/explant) of *Paeonia lactiflora* 'Jadwiga' by axillary branching (Gabryszewska, 2009). A model has been proposed in which sugar inhibits the peony axillary bud growth by negative regulation of the production and/or signal transduction of gibberellin. Exogenous gibberellins can overcome the sugar-derived inhibition and stimulate the axillary shoot growth in the presence of cytokinins. However, the addition of exogenous auxin, to the above mentioned medium, inhibited the shoot growth and influenced the progression of shoots from a juvenile to adult phase. Application of gibberellin does not overcome the auxin-derived inhibition (Gabry-szewska, 2009).

At present, there is no information on the mechanism of *in vitro* formation of renewal buds on underground crown (rhizome) of *Paeonia lactiflora*. The data indicate that excision of all leaves promoted the renewal bud formation in the absence of exogenous growth regulators as well as in their presence in the medium (Fig. 2 A; Fig. 3, 4). The addition of 1 mg $l^{-1}$ kinetin, IBA or $GA_3$ (singly or together) inhibited renewal bud formation on the explants isolated with leaves. Similarly, in tissue culture of *Dioscorea* spp., cytokinin inhibited tuberization. But a high level of sugar in the medium can overcome the cytokinin-derived inhibition and stimulate the tuber formation (Lauzer et al., 1995). On the other hand, the highest tuberization of *Xanthosoma sagittifolium* was obtained on medium with BAP and a high concentration of sucrose (80 $gl^{-1}$) (Omokolo et al., 2003). The renewal bud formation of peony 'Jadwiga' was induced by glucose on a growth regulator-free medium (Fig. 2 A). It is known that soluble sugars could affect the formation of adult structures, such as leaves and tubers (Gibson, 2005). Interaction of all growth substances (kinetin, $GA_3$, IBA added together) and high glucose level induced renewal bud formation (about 4.5/explants), on the peony explants isolated without leaves (Fig. 2 A). On the same kind of explants, the single addition of $GA_3$ to the medium with glucose, resulted in a similar growth of buds as that on the medium which had an absence of growth regulators. Recently, it was reported that interaction of gibberellin and sugars affect storage organ formation in various species of plants *in vitro* (Xu et al., 1998; Ascough et al., 2008; Zheng et al., 2008). In the case of *Watsonia vanderspuyiae*, interaction between $GA_3$, sucrose and reduced temperature, promoted corm formation (Ascough et al., 2008). Also, the addition of $GA_3$ together with a high level of sucrose (80 $gl^{-1}$) markedly increased rhizome production of *Zingiber officinale* (Zheng et al., 2008). For this species, the effect of gibberellin on rhizome induction was larger than that kinetin or NAA. The dominant role of GA in the potato tuber formation was demonstrated by Xu et al. (1998). In addition, sucrose regulates

**A**



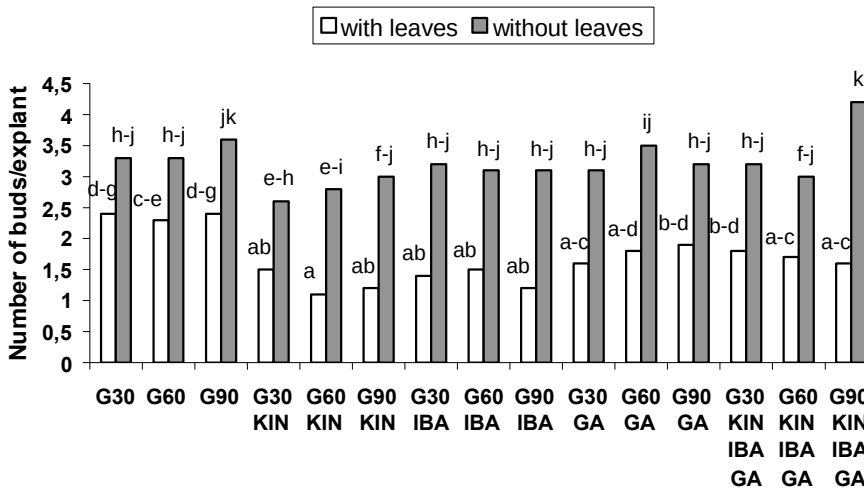

**B**




Explanations: G – glucose, GA – gibberellic acid, KIN- kinetin
Means marked with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test

**Figure 1**. The influence of glucose (30, 60, 90 g $l^{-1}$) and growth regulators (kinetin, $GA_3$, IBA – 1 mg $l^{-1}$) on the number of shoots (A) and leaves (B) of *Paeonia lactiflora* 'Jadwiga' *in vitro*

**A**

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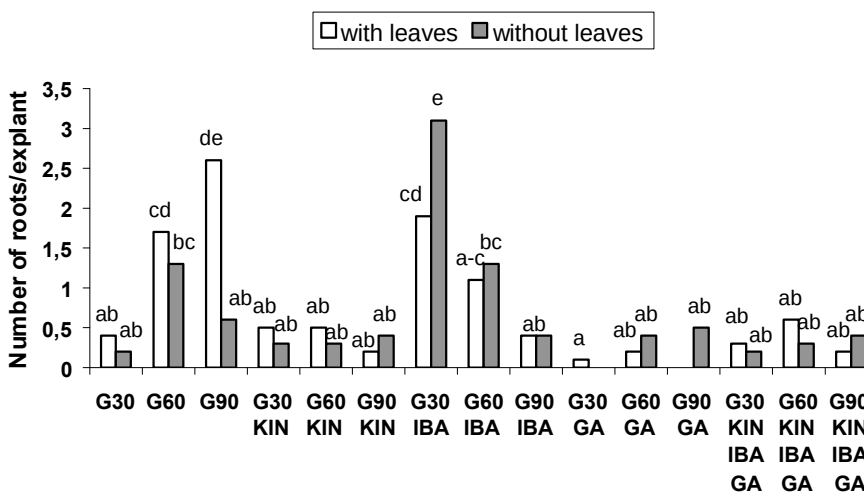


Explanations: G – glucose, GA – gibberellic acid, KIN- kinetin
Means marked with the same letter are not significantly different at $p \leq 0.05$, according to Duncan's multiple range test

**Figure 2**. The influence of glucose (30, 60, 90 g $l^{-1}$) and growth regulators (kinetin, $GA_3$, IBA – 1 mg $l^{-1}$) on the number of renewal buds (A) and roots (B) of *Paeonia lactiflora* 'Jadwiga' *in vitro*

tuberization of potato by influencing the endogenous gibberellin level. Other plant growth regulators are involved in the regulation of tuber formation but their effects seem to depend on the final GA content in the tissue.

The present results indicate, that the leaves as the source of endogenous growth regulators, also influenced root formation on the peony shoots. Explants isolated with leaves produced roots on the growth regulators free-medium but with the presence of glucose (Fig. 2 B, Fig. 3). Increasing glucose level strongly enhanced the number of roots (from 0.4 to 2.6 roots/explant) in the absence of growth regulators, on the explants containing leaves. But, the addition of $GA_3$ or kinetin to the medium with different concentrations of glucose strongly inhibited rhizogenesis. Also, the presence of $GA_3$ and kinetin (added together) in the IBA-containing medium significantly suppressed the rooting on the both kinds of explants. The excision of leaves and supply of IBA (1 mg $l^{-1}$) promoted rhizogenesis (3 roots/explant) on the shoots growing at the lowest concentration of glucose (30 g $l^{-1}$). Thus, it seems that the interaction between auxin (exogenous or endogenous) and glucose, regulate root formation on the peony explants *in vitro*. In a previous study, it was found that exogenous IBA added alone had no effect on IAA production but added together with a high level of sucrose stimulated IAA production in peony 'Jadwiga' shoots *in vitro* (Gabryszewska, 2009). In the *Zingiber officinale* rhizome culture, the influence of sucrose concentration on the endogenous level of IAA was also very little. In contrast, the production of gibberelins and ABA changed sharply when the sucrose level was increased (Zheng et al., 2008). The promoting effect of the increasing sucrose concentration on adventitious root formation was demonstrated for apple microcuttings (Calamar and De Klerk, 2002). In addition, there was an interaction between sucrose and auxin. For many others plant species it was stated that soluble sugars could affect the formation of adventitious roots and can act as signaling molecules in this process (Gibson, 2005).

Among all the plant growth substances, jasmonates play an important role in various morphogenic events in plants including tuberization, tuberous root or bulb formation (Tampe et al., 2001). In tissue culture of the peony 'Jadwiga', a simultaneous supply of MeJA with IBA inhibited root formation on the shoots growing at high temperatures. In contrast, the addition of indomethacin (inhibitor of jasmonic acid biosynthesis) to the medium with IBA stimulated rooting and leaf formation (Gabryszewska, 2009). Previously, MeJA had been shown to induce bulb (lily, garlic) and tuber (potato) formation or to inhibit adventitious shoot (radiata pine) regeneration (Koda et al., 1991; Ravnikar et al., 1993; Shimasaki and Fukumoto, 2000, Tampe et al. 2001). The role of jasmonates in the organogenesis of herbaceous peony should be studied in detail.


**Figure 3**. The influence of glucose at different concentrations and growth regulators (IBA, $GA_3$, kinetin) on the shoot, renewal bud and root formation of *Paeonia lactiflora* 'Jadwiga' *in vitro*

**Figure 4**. The renewal buds (*arrows*) formed on the crown of *Paeonia lactiflora* 'Jadwiga' (explant without leaves) grown on the MS medium containing glucose 90 g $l^{-1}$ and IBA, $GA_3$, kinetin (1 mg $l^{-1}$) added to the medium together

In conclusion, the results presented here show that a high level of glucose and exogenous growth regulators (kinetin, $GA_3$, IBA) together, stimulate shoot and renewal bud formation but the way of organogenesis depends on the presence or absence of leaves. The interaction between auxin (exogenous or endogenous) and glucose regulate root formation on the peony shoots but the final effect depends on the type of explants (with or without leaves). It is possible that leaves have very important hormonal factors, which stimulate shoot growth or rooting and inhibit renewal bud formation.

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# WPŁYW GLUKOZY I REGULATORÓW WZROSTU NA ORGANOGENEZĘ PIWONII CHIŃSKIEJ (*Paeonia lactiflora* Pall.) *IN VITRO*

Eleonora Gabryszewska

S T R E S Z C Z E N I E

Sadzonki piwonii chińskiej pochodzące z rozmnażania *in vitro* bardzo słabo aklimatyzują się w szklarni i nie podejmują dalszego wzrostu. U innych gatunków roślin formowanie *in vitro* organów spoczynkowych, zawierających materiały zapasowe, wpływa korzystnie na aklimatyzację roślin rozmnażanych *in vitro*. W warunkach naturalnych piwonia chińska wytwarza na kłączu pąki odnawiające, z których w następnym sezonie rozwijają się jednoroczne pędy nadziemne. Kłącze i zgrubiałe korzenie gromadzą substancje zapasowe. Przeprowadzone badania miały na celu określenie wpływu glukozy i regulatorów wzrostu (kinetyna, IBA, $GA_3$) oraz ich współdziałania w procesie formowania oraz wzrostu pędów, pąków odnawiających i korzeni u piwonii chińskiej odmiany 'Jadwiga' rosnącej *in vitro*. Doświadczenie przeprowadzono na fragmentach kłącza pochodzących z rozmnażania *in vitro*.

Odcięcie liści z izolowanych eksplantatów wpływało hamująco na powstawanie i wzrost pędów, natomiast stymulowało formowanie pąków odnawiających. Wzrost stężenia glukozy w pożywce ograniczał formowanie pędów i wzrost liści, szczególnie u eksplantatów rosnących na pożywce bez regulatorów wzrostu. Glukoza silniej hamowała wzrost pędów u eksplantatów pozbawionych liści. Stymulujące działanie tego cukru obserwowano w procesie formowania pąków odnawiających. Pojedyncze zastosowanie regulatorów wzrostu (kinetyna, IBA, $GA_3$) sprzyjało powstawaniu pędów oraz hamowało formowanie pąków odnawiających na eksplantatach zawierających liście. Łączne zastosowanie wszystkich regulatorów wzrostu (kinetyna, IBA, $GA_3$) i glukozy w najwyższym stężeniu (90 g $l^{-1}$) stymulowało powstawanie i wzrost pędów na eksplantatach izolowanych z liśćmi, a pąków odnawiających na eksplantatach bez liści. Wzrost stężenia glukozy w pożywce zwiększał liczbę powstających korzeni na eksplantatach zawierających liście, rosnących na pożywce bez regulatorów wzrostu. Dodanie IBA do pożywki z najniższym stężeniem glukozy (30 g $l^{-1}$) najsilniej stymulowało ukorzenianie eksplantatów z odciętymi liśćmi. Egzogenna giberelina i kinetyna, zastosowane łącznie lub odzielnie z IBA, silnie hamowały powstawanie korzeni.

Uzyskane wyniki wskazują na współdziałanie glukozy w wysokim stężeniu i łącznego zastosowania egzogennych regulatorów wzrostu (kinetyna, IBA, $GA_3$) w procesie formowania zarówno pędów, jak i pąków odnawiających, jednakże kierunek organogenezy uzależniony jest od obecności lub braku liści na izolowanych eksplantatach. Współdziałanie auksyn (egzogennych, endogennych) z glukozą reguluje powstawanie korzeni, ale ostateczny wynik ukorzeniania zależy również od zachowania lub odcięcia liści. Można przypuszczać, że liście zawierają istotny hormonalny czynnik, który stymuluje rozwój pędów i korzeni oraz hamuje powstawanie pąków odnawiających.

**Słowa kluczowe**: *Paeonia lactiflora*, kultury tkankowe, glukoza, regulatory wzrostu, organogeneza, powstawanie pąków odnawiających