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RESEARCH ARTICLE

Modulation of axillary bud growth by phytohormones in surgically modified seedlings of chickpea

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Abstract: Growth is a unique biological attribute that normally follows a fairly identical pattern among similar organisms and their constituent organ systems. Young chickpea seedlings with intact cotyledons provide a unique model system to investigate a variety of growth correlative phenomena induced by selective organectomy and manifested through axillary bud outgrowth. The excision of shoot apex results in the development of a branch from the topmost leaf axillary bud whereas complete deshooting resulted in the development of a branch in the axil of each cotyledon. An additional removal of one cotyledon causes suppression of branch growth in the axil of the other intact cotyledon. This indicates that expression of the axillary branch growth is modified by persistent cotyledon. To understand the physiological mechanism of regulation of such multiorgan correlative growth responses, the seedling system was subjected to phytohormonal compounds like IAA, kinetin and Gibberellic Acid (GA). Some of the regulators brought about significant modulations of the axillary bud growth and resultant growth correlations. IAA and kinetin significantly inhibited growth of an intact seedling, and that of the non-suppressed branch in the deshooted seedling from which one cotyledon was excised. IAA also induced profuse rooting from the hypocotylar and cotyledonary nodal regions whereas kinetin induced multiple branches at the cotyledonary nodes. GA caused enhancement of growth of intact seedling and of the two axillary branches of unequal growth in the deshooted seedlings from which one cotyledon was also excised. The selected phytohormonal compounds tested were effective in modulating seedling growth, although they invoked diverse growth modulations in different cases.

Key Words: *Cicer arietinum,* growth correlations, seedling growth, selective organectomy, phytohormones

Introduction

A young seedling is a multiorgan composite with perfectly integrated structure and functions, with each organ having its own specialized physiology and influencing the growth and maintenance of other organs. Every organ participates in the realisation of its own growth and display of specific growth correlations in an integrated manner to produce a complete plant architecture. For instance, it is known that the growth of lateral buds into branches is modulated by the shoot apex (Cline 2000, Wilson 2000) or by shoot segments (Nagao and Rubinstein 1976), and by leaves (Berghage *et al.* 1989, McIntyre and Hsiao 1990). The basal branches inhibit axillary bud outgrowth in *Trifolium repens* (Thomas and Hay 2015) and in *Pisum sativum* (Thomas and Hay 2018). Similarly, the roots regulate the differentiation of aerial shoots (Prochazka *et al.* 1984). Likewise, the cotyledons play an important regulatory role influencing the early growth and establishment of seedling (Li *et al*. 1985) and shoot morphogenesis (Gambley and Dodd 1991). Such correlative growth responses are mainly regarded to be manifested through active and interactive participation of mobile factors which could be nutrients and/or hormonal in nature (Mason *et al*. 2014, Xu *et al*. 2020 and Kotov *et al*. 2021).

In order to demonstrate the involvement of growth regulatory substances, their exogenous application helps to understand the mechanism of behaviour for a particular growth response. Thus, the application of exogenous auxin generally results in the initiation and promotion of roots (Otiende *et al.* 2021) and initiation of lateral bud outgrowth (Li *et al.* 1995, Cline 1996); that of cytokinin stimulates lateral bud outgrowth in *Pisum sativum* (King and Van Staden 1988), apple *sp.* (Li *et al.* 2018) and multiple bud formation in strawberry (Waithaka *et al.* 1980) and *Glycine max*

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(Buising *et al.* 1994); and that of gibberellic acid promotes bud elongation after its release from apical dominance in strawberry (Waithaka *et al.* 1980), *Pisum sativum* (Prochazka *et al.* 1984).

Earlier investigation involving selective organectomy (removal of one or more organs) has proved immensely helpful to establish the physiological involvement of each organ (Saluja and Sawhney 2003, 2006). The excision of one cotyledon from deshooted seedlings causes an unequal growth of the two cotyledonary axillary buds with significant suppression of bud growth in the axil of intact cotyledon (Saluja and Sawhney 2003). The exogenous applications of phytohormones provide a powerful tool to simulate the endogenous hormonal situations leading to the realization or avoidance of a specific growth response. Here we employed selective organectomy and exogenous application of selected phytohormonal compounds like IAA, gibberellic acid and kinetin to establish their relative efficacy when dispensed through different organs in modulating seedling growth and growth correlations.

Materials and methods

Culture and organectomy

Seeds of *Cicer arietinum* L. were surface sterilized with 0.1% mercuric chloride and 70% ethanol, rinsed and pre-soaked in distilled water (DW) for 4 h, planted in Petri dishes lined with moist Whatman paper (No 1) discs and maintained at 25 ± 2ºC, under continuous illumination. On the 7th day, the testa was removed from the cotyledons and the seedlings were subjected to surgical treatments involving excision of selected plant organs following the procedure as reported earlier (Saluja and Sawhney 2003). The following seedling system types were prepared (Fig. 1): A) intact unmodified seedling (control); B) seedling with shoot excised up to 0.5 cm (complete deshooting), root up to 3.0 cm (partial derooting) and both cotyledons intact; C) seedling with shoot excised up to 0.5 cm (complete deshooting), root up to 3.0 cm (partial derooting) and one cotyledon excised. The seedlings were cultured in half strength Hoagland's nutrients solidified with 1% agar.

Phytohormonal treatment

The modified seedlings were treated with exogenous application of 10^{-5}M and 10^{-3}M concentrations of IAA, GA and kinetin dispensed through the cotyledonary axillary bud(s) or cotyledon(s) as a micro drop using a sterile glass syringe, by its placement in the axil(s) of the cotyledon(s) and on the inner flat surface(s) of the cotyledon(s) for the respective seedling organ. Each micro drop contained 7.6 µl of the phytohormonal solution of the requisite concentration. The treatments were given after 0, 1, 2 and 3 days of the surgical modifications of the 7-day old seedlings. For continuous application to the root system, the phytohormone in the requisite

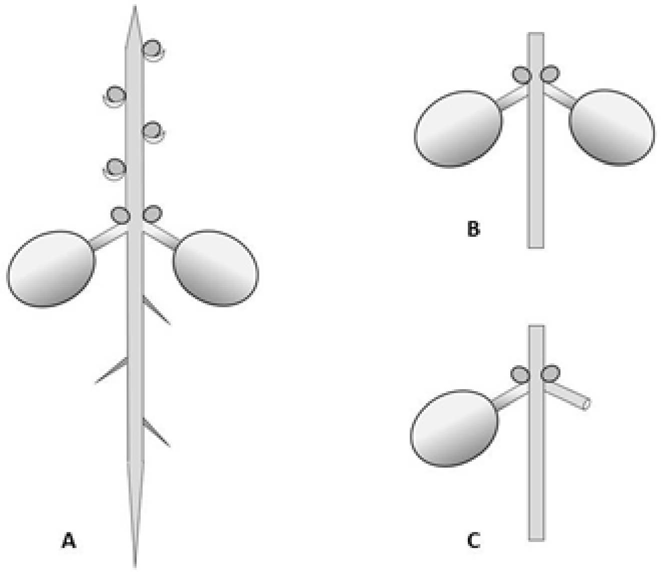

Figure 1. Diagrammatic representation of unmodified and surgically modified seedlings. **A** Unmodified intact seedling. **B** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with both cotyledons intact. **C** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with one cotyledons intact (Adapted from Saluja and Sawhney, 2003).

concentration was supplemented in the culture medium (agar) that supported the seedlings. The entire experiment was conducted in a growth chamber maintained at 25 ± 2ºC under continuous illumination for 21 days.

Observations and statistics

The cumulative length of the seedling main axis/cotyledonary axillary branch(es) was recorded after 14 days of giving surgical/ phytohormonal treatment. The data was statistically analysed for ± SE of the mean values.

Results and discussion

The intact seedling showed normal growth of the terminal bud, and none of the axillary buds differentiated to produce a branch till the termination of the experiment after 21 days, pointing to a perfect apical dominance. The modification of the seedling by complete deshooting and partial derooting with both cotyledons intact, resulted in the emergence of two cotyledonary axillary branches from the axil of each cotyledon (Fig. 2). This correlative growth of cotyledonary axillary bud is due to loss of apical dominance, brought into play by the removal of the shoot apex, and is considered to be realized through either direct or indirect involvement of phytohormones or nutrient diversion towards the active meristem or a composite of both factors (Cline 2000, Wilson 2000, Kotov *et al.* 2021). The transcriptome and molecular analysis involving specific genes for axillary bud outgrowth have been proposed (Ahmad *et al.* 2020). However, the removal of one of the two cotyledons from such deshooted seedlings resulted in the display of unusual growth correlations, with complete suppression of branch growth in the axil of the intact cotyledon whereas its own axillary bud developed into a normal branch (Saluja and Sawhney 2003).

IAA is the chief auxin in higher plants. The involvement of auxins in regulation of lateral bud has been already well established by studies in which decapitation resulted in the loss of apical dominance and a subsequent auxin application to the cut stump effectively restored it (Cline 2000 and Balla *et al.* 2016). The efficacy of IAA in modulating seedling growth in *C. arietinum* appeared to be organ specific. Thus, 10^{-3}M IAA did not influence seedling growth in any appreciable

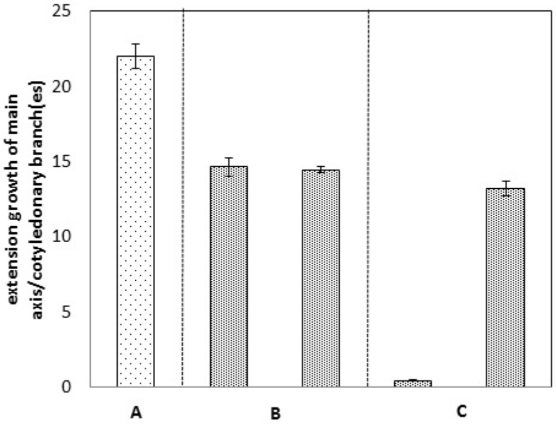

Figure 2: Extension growth of 21-days old unmodified and surgically modified chickpea seedlings. **A** Unmodified intact seedling. **B** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with both cotyledons intact. **C** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with one cotyledon removed.

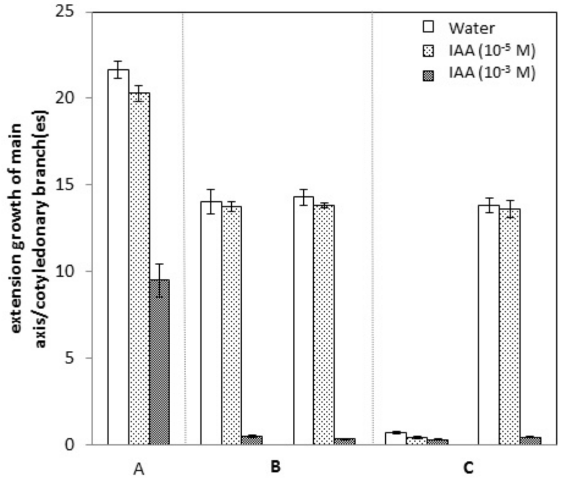

Figure 3: Effect of IAA on extension growth of 21-days old unmodified and surgically modified chickpea seedlings. **A** Unmodified intact seedling. **B** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with both cotyledons intact. **C** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with one cotyledon removed.

manner when dispensed through cotyledons or cotyledonary axillary buds. IAA up to 10^{-3}M when dispensed through roots significantly inhibited growth of the intact seedlings and of the normal branches in the modified seedlings whereas the suppressed branch remained unaffected (Fig. 3). IAA has been earlier shown to promote growth of *Citrullus lanatus* hypocotyls (Carrington and Esnard 1988), *Pisum sativum* internodes (Yang *et al.* 1993) and *Zea mays* coleoptiles (Iino 1995). On the contrary in the present investigation with *Cicer arietinum* IAA invoked a negative response. The inhibition of the seedling may not be taken as a supraoptimal response since the lower concentration (10^{-5}M) under identical conditions was completely ineffective. The known mediation of auxin induced promotory effects through their influence on cell division and enlargement activities (Cleland 1995), may still hold good for the inhibitory effects, as seen in the present case. This appears to be so because IAA is seen to influence primarily the fresh growth of intact seedlings (equivalent to net growth) as well as the formation of branches of normal growth in deshooted seedlings.

IAA at 10^{-3}M induced profuse rooting from the hypocotylar and cotyledonary nodal regions of all types of seedlings, which is in accordance with the known role of IAA in the promotion of rooting (Otiende *et al.* 2021).

The efficacy of kinetin in modulating seedling growth in *C. arietinum* appeared to be organ-specific. Thus, 10^{-3}M kinetin did not influence seedling growth in any appreciable manner when dispensed through either cotyledons or cotyledonary axillary buds. However, when dispensed through roots, 10^{-3}M kinetin effectively inhibited the extension growth of intact as well as the emergence of normal branches upon complete deshooting (Fig. 4). The suppressed branch remained unaffected. Foliar sprays of kinetin are known to reduce internodal elongation with fewer leaves differentiation in *Vigna ungiculata* (Argall and Stewart 1984).

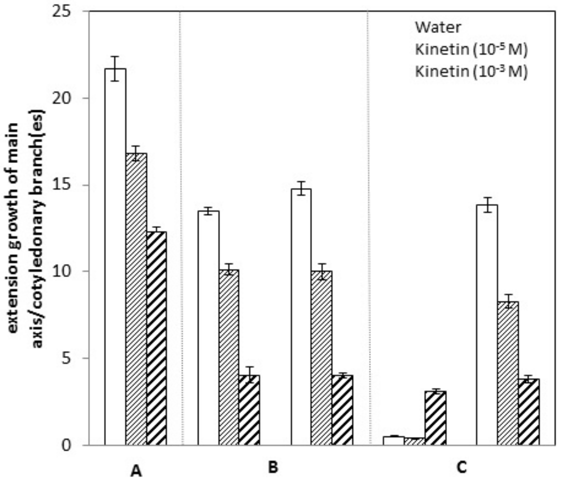

Figure 4. Effect of kinetin on extension growth of 21-days old unmodified and surgically modified chickpea seedlings. **A** Unmodified intact seedling. **B** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with both cotyledons intact. **C** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with one cotyledon removed.

The root treatment of 10^{-3}M kinetin also appeared to interfere with the persistent apical dominance in the unmodified seedlings by activating a few basal axillary buds to form branches. The cytokinin-caused loss of apical dominance and promotion of lateral bud outgrowth and its molecular mechanism has been reported in apple (Li *et al.* 2018). This loss of apical dominance induced by kinetin appeared to be physiologically different from the one induced by decapitation in terms of polarity and magnitude of response. As in the former case these were the lowermost four nodes that produced branches in comparison to the lone uppermost node in the latter case. Such a differential response to decapitation and cytokinin application has also been reported in *Hygrophila* sp. (Cutter and Chiu 1975). The root treatment of 10^{-3}M kinetin appeared to have moved upwards causing branching of intact seedlings in an acropetal order. That kinetin-induced loss of apical dominance was also stronger than that caused by complete deshooting, as it caused multiple bud formation by the differentiation of additional branches from the axil of cotyledons in all seedlings. Multiple bud formation on the germinating embryonic axis and shoot development have also been reported in *Glycine max* (Buising *et al.* 1994) and *Macrotyloma uniflorum* cotyledonary node explants (Mohamed *et al.* 1998). However, without quantitative determinations it would be difficult to deduce that external kinetin applications to root actually augmented their endogenous cytokinin levels at the

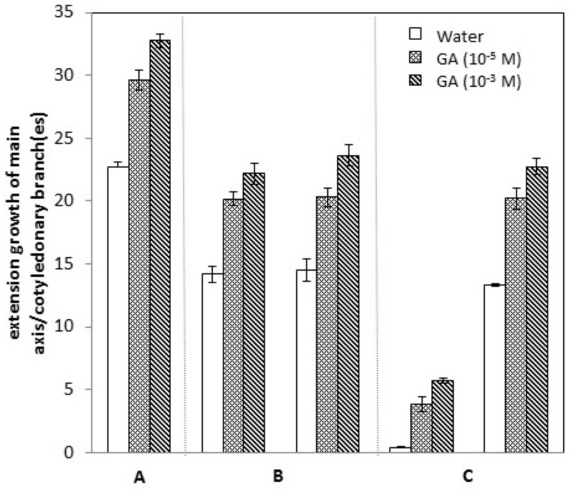

Figure 5. Effect of GA on extension growth of 21-days old unmodified and surgically modified chickpea seedlings. **A** Unmodified intact seedling. **B** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with both cotyledons intact. **C** Seedling with shoot removed upto 0.5 cm (complete deshooting) and root upto 3.0 cm (partial derooting) with one cotyledon removed.

target sites to enhance branching.

The efficacy of GA application in modulating seedling growth of *C. arietinum* also appeared to be organ specific. 10^{-3}M GA did not significantly influence the seedling growth when dispensed through cotyledons or cotyledonary axillary buds. However, when given through roots 10^{-3}M GA or even lower concentration 10^{-5}M significantly promoted the growth of intact seedling as well as that of branches of both normal and suppressed growth that emerged upon complete deshooting (Fig. 5). GA has been shown to promote shoot elongation in *Jatropha curcas* (Ni *et al.* 2015) and specific GAs promote branching in hybrids of *Populus* (Katyayini *et al.* 2020).

Conclusion

The present investigation emphasises the role played by different organs in an interactive manner in the expression of overall seedling growth and growth correlations. The selected phytohormonal compounds invoked diverse growth modulations in different cases. All phytohormonal compounds were effective in modulating seedling growth when dispensed through roots in comparison to their almost complete inertness even at reasonable high concentration levels when applied to the cotyledons or cotyledonary axillary buds. The organ-specific efficacy could be due to the differential sensitivity of the treated organ towards the applied regulator, their concentration as well as differential rate and extent of uptake or the greater availability in terms of quantum and duration.

References

Ahmad S, Yuan C, Yang Q, Yang Y, Cheng T, Wang J, Pan H & Zhang Q 2020 Morpho-physiological integrators, transcriptome and coexpression network analysis signify the novel molecular signatures associated with axillary bud in chrysanthemum. *BMC Plant Biology* **20** 145

Argall J F and Stewart K A 1984 Effects of decapitation and benzyladenine on growth and yield of cowpea (*Vigna ungiculata* (L.) Walp). *Ann Bot* **54** 439-444.

Balla J, Medvedova Z, Kalousek P, Matijescukova N, Friml J, Reinohl V and Prochazka S 2016 Auxin flow-mediated competition between axillary buds to restore apical dominance. *Scientific Reports* **6**:35955

Berghage R D, Heins R D, Erwin M J H and Carlson W 1989 Pinching technique influences lateral shoot development in poinsettia. *J Am Hort Sci* **114** 909-914.

Buising C M, Shoemaker R C and Benbow R N 1994 Early events of multiple bud formation and shoot development in soyabean embryonic axis treated with the cytokine, 6-benzylaminopurine. *Am J Bot* **81** 1435-1448.

Carrington C M S and Esnard J 1988 The elongation response of watermelon hypocotyls to indole-3-acetic acid: A comparative study of excised and intact plants. *J Exp Bot* **39** 441-450.

Cleland R E 1995 Auxin and cell elongation. In: Plant Hormones: Physiology Biochemistry and Molecular biology, ed. Davies P J, Kluwer Academic Publishers, Dordrecht, Pp 214-227.

Cline M G 1996 Exogenous effects on lateral bud outgrowth in decapitated shoots. *Ann Bot* **78** 255-266.

Cline M G 2000 Execution of the apical replacement apical dominance experiment in temperate woody species. *Am J Bot* **87** 182-190.

Cutter E G and Chiu 1975 Differential responses of buds along the shoot to factors involved in apical dominance. *J Exp Bot* **26** 828-839.

Gambley R L and Dodd W A 1991 The influence of cotyledons in axillary bud adventitious shoot production from cotyledonary nodes of *Cucumis sativus* L. (cucumber). *J Exp Bot* **42** 1131-1135.

Iino M 1995 Gravitropism and phototropism of maize coleoptiles: Elevation of the Cholondy-Went theory through effects of auxin applications and decapitation. *Plant Cell Physiol* **36** 361-367.

Katyayini N U, Rinne P L H, Tarkowska D, Strnad M and van der Schoot C 2020 Dual role of gibberellin in perennial shoot branching: Inhibition and activation, *Front Plant Sci* **11** 736

King R A and Van Staden J 1988 Differential responses of buds along the shoot of *Pisum sativum* to isopentyladenine and zeatin application. *Plant Physiol Biochem* **20** 237-246.

Kotov A A, Kotova L M and Romanov G A 2021 Signaling network regulating plant branching: Recent advances and new challenges. *Plant Science* **307** 110880.

Li C J, Guevara E, Herrera J and Bangerth F 1995 Effect of apex and replacement by 1-napthylacetic acid or cytokinin concentration and apical dominance in pea plants. *Physiol Plant* **94** 465-469.

Li G, Tan M, Cheng F, Liu X, Qi S, Chen H, Zhang D, Zhao C, Han M and Ma J 2018 Molecular role of cytokinin in bud activation and outgrowth in apple branching based on transcriptome analysis. *Plant Molecular Biology* **98** 261-274.

Li J R, Proctor J T A and Murr D P 1985 Effects of cotyledon removal on apple seedling growth and distribution of ^{14}carbon-labelled photosynthates. *Can J Bot* **63** 1736-1739.

Mason M G, Ross J J, Babst B A, Wienclaw B N and Beveridge C A 2014 Sugar demand, not auxin, is the initial regulator of apical dominance. *PNAS* **111** 60926097.

McIntyre G I and Hsaio A I 1990 The role of expanded leaves in the correlative inhibition of axillary buds in milkweed (*Asclepias syriaca*). *Can J Bot* **68** 1280-1285.

Mohamed S V, Jawahar M and Jayanalan N 1998 Establishment of efficient method for plant regeneration from cotyledonary node expants of *Macrotyloma uniflorum* (Lam.) Verdc. *Physiol Mol Biol Plants* **4** 139-142.

Nagao M A and Rubinstein B 1976 Early events associated with lateral bud growth of *Pisun sativum* L. *Bot Gaz* **137** 39-44.

Ni J, Gao C, Chen M, Pan B, Ye K and Xu Z 2015 Gibberellin promotes shoot branching in the perennial woody plant *Jatropha curcas*. *Plant and Cell physiol* **56** 1655-1666.

Otiende M A, Fricke K, Nyabundi J O, Ngamau K, Hajirezaei M R and Druege U 2021 Involvement of the auxin-cytokinin homeostasis in adventitious root formation of rose cuttings as affected by their nodal position in the stock plant. *Planta* **254** 65.

Prochazka S, Navratilova D and Blazkova J 1984 Transport of benzyl-8-^{14}C-adenine from roots into the shoots of pea (*Pisum sativum* L.) seedlings with outgrowth of lateral buds. *Beitz Biol Pflanz* **58** 421-426.

Saluja S and Sawhney S 2003 Interorgan influences in seedling growth of chickpea revealed through selective organectomy. *Ind J Plant Physio*l **8(3 N.S.)** 205-213.

Saluja S and Sawhney S 2006 Deshooting-activated cotyledonary axillary bud growth and its correlation with nodal vasculature in chickpea seedlings. *Phytomorphology* **56(1&2)** 1-6.

Thomas R G and Hay M J M 2015 Regulation of correlative inhibition of axillary bud outgrowth by basal branches varies with growth stage in *Trifolium repens*. *J Exp Bot* **66** 3803.

Thomas R G and Hay M J M 2018 Mechanisms by which basal branches suppress axillary bud outgrowth in pea (*Pisum sativum*). *Botany* **96.**

Waithaka K, Hildebrandt A C and Dana M N 1980 Hormonal control of strawberry axillary bud development *in vitro*. *J Am Soc Hort Sci* **105** 428-430.

Wilson B F 2000 Apical control of branch growth and angle in woody plants. *Am J Bot* **87** 601-607.

Xu J, Li Q, Yang L, Li X, Wang Z and Zhang Y 2020 Changes in carbohydrate metabolism and endogenous hormone regulation during bulbet initiation and development in *Lycoris radiate*. *BMC Plant Biology* **20** 180.

Yang T, Law D M and Davies P J 1993 Magnitude kinetics of stem elongation induced by exogenous indole-3-acetic acid in intact light grown pea seedlings. *Plant Physiol* **102** 717-724.