Promoting lateral bud growth in Sweet potato (*Ipomoea batatas*)

– comparing the foliar application of synthetic phytohormone and coconut water

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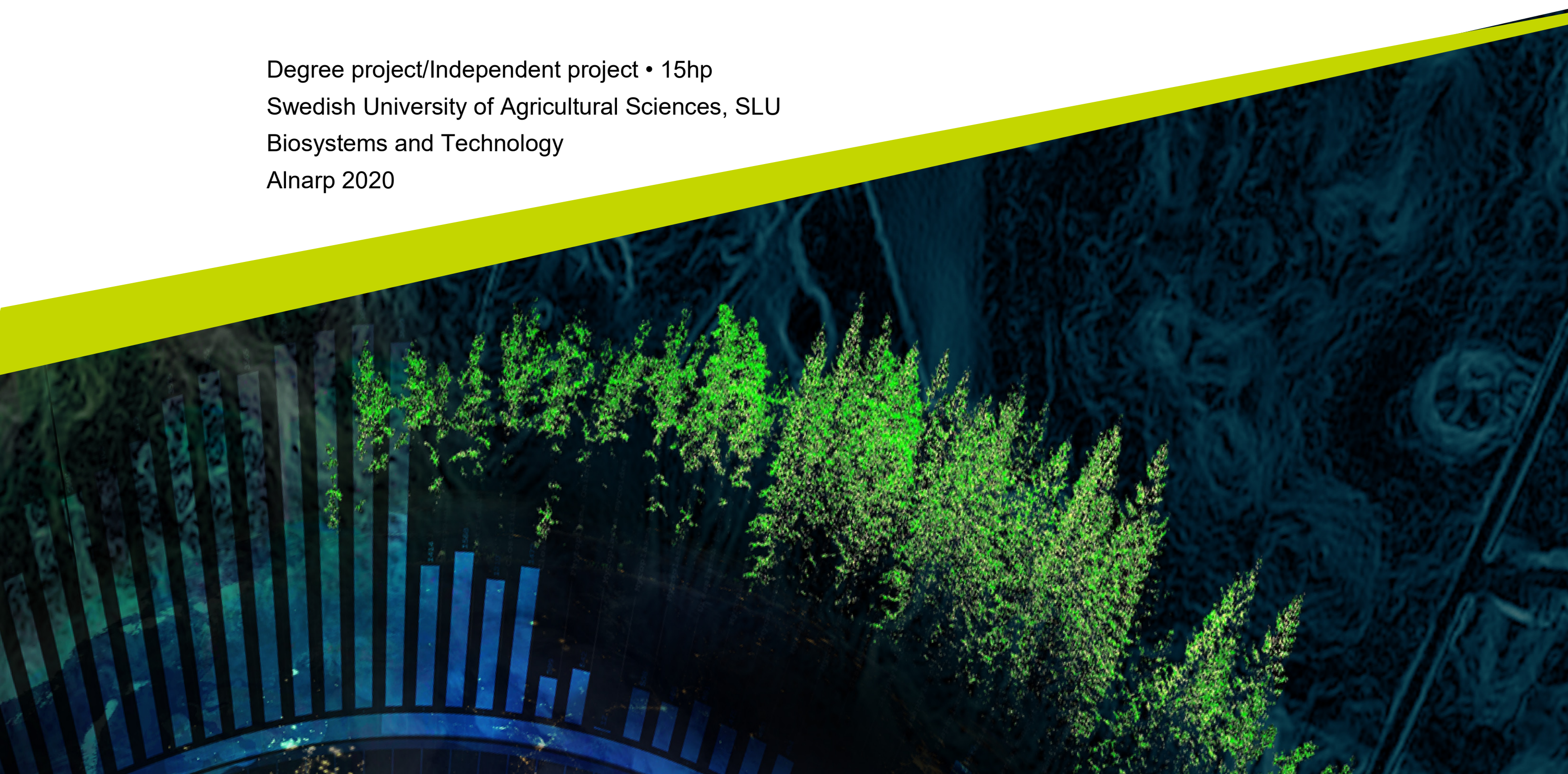

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Abstract

Sweet potato (*Ipomea batatas*) is an important crop grown worldwide with a rising interest in production in Europe and Sweden. Swedish producers are looking for ways to grow the crop but are facing problems because of low-quality slips from import which leads to a non-profitable production. This inadequate supply becomes a bottleneck for Swedish farmers. A Swedish propagation of slips would be beneficial to Swedish growers but in order to make such a production effective a solution to break the sweet potato's strong apical dominance is needed. Apical dominance is mainly controlled by auxin supply from the apical meristem. Cytokinin is a phytohormone that promotes lateral bud growth and can therefore also be used to aid the breaking of apical dominance. Although a lot is known about apical dominance there are a lot of unknown factors playing into how it is truly controlled. Coconut water (CW) is a natural, safe and organic biostimulant with enough phytohormones to affect other plants. An experiment was conducted where cuttings of sweet potato rooted in pumice stone and sprayed with a synthetic cytokinin (BAP) or a 20% dilution of CW in order to stimulate lateral bud growth. A control group without any treatment was also made. Every cutting in the experiment had its apex bud removed in order to cut off the main auxin supply. The results showed a 27% higher rate of stimulated lateral buds with BAP treatment and a 20% higher rate with the CW treatment compared to the cuttings without any treatment. The study is too small to prove a correlation but it’s enough to show an indication that these biostimulants might stimulate later bud growth. This indication can be used to motivate a study on a bigger scale.

Contents

Abbreviations

CW	Coconut water
Cytokinin	Phytohormone
BAP	Synthetic cytokinin (6-Benzylaminopurine)
SLU	Swedish University of Agricultural Sciences
Seed	The tuber of the sweet potato
Slips	20-30 cm long cutting from the sweet potato vine
MLE	Moringa leaf extract
Photo-assimilates	Biological compounds formed by assimilation using light-dependent reaction
Epidermis	The outer layer of the leaf

1. Introduction

1.1. Background

The sweet potato (*Ipomeas batatas*) is grown in many parts of the world, especially in Asia (66%), Africa (28%) and Latin America (4,6%). Sweet potato is also grown in Europe with a steady incline of production from 2010-2018 ("FAOSTAT," n.d.). Its rich in both starch and many minerals/antioxidants like β-carotene, Iron, Zinc, Calcium, Magnesium and can therefore add high-quality nutrition to the food supply if consumed regularly (Tumwegamire et al., 2011).

It may seem like the sweet potato is from the Solanaceae family based on its name but it's from the Convolvulaceae family and originates from either South or central America (*Geneflow 2009*, n.d.). Swedish producers have been looking for ways to be able to grow the crop in the Swedish climate and trials have been made in the southern part of Sweden. With good technique and the right variety, it's possible to cultivate a good harvest (Hansson 2020).

The sweet potato needs a stable climate to grow and it's usually propagated though a vegetative method by cutting 20-30 cm long cuttings called" slips" directly growing out of the tuber. Tubers are often referred to as seeds (Primomo & Pearson). Sweet potato like many other plants have different kinds of viruses that plague the plant and can cause a loss of yield from 25-40% and negatively impact the color of the skin or shape of the rhizome ("Sweet Potato Foundation Seed Production and the National Clean Plant Network Support," 2016). Therefore, it's very important to have vital and virus-free slips. It's also important to actively work to keep the cultivation healthy.

Sweden have a rising importation of Sweet potato, but a domestic production could be possible (*PxWeb - tabell*). According to Karlén *et al.* 2018 about 20-40 000 slips are required per hectare and that creates a high demand for Swedish farmers who want to cultivate the plant. Today the slips are produced mainly in the southern parts of Europe and are about five days old before they arrive to Sweden by truck (Hansson 2020). This is a problem because they often arrive damaged which affects the yield since slips-quality is very time sensitive because of its rapid growth (Karlén *et al.* 2018). If a local slips production could be established in Sweden it

would be possible to have a higher-quality supply of slips because of the short transportation chain which makes growing sweet potato more profitable for the farmer.
The propagation of Sweet potato slips is not without problems though. The plant only develops one terminal shoot from each vine, even if you remove the apex bud (Salunkhe and Kadam, 1998) and this becomes an issue when you need to produce many slips in a vegetative propagation. There are many ways to stimulate lateral bud growth and one way is by treating the sweet potato plant with phytohormones like cytokinins. Dormant lateral buds can be stimulated with cytokinin to grow and thereby increase the slips production (Dewir et al., 2020). Cytokines are available as synthetics, but they can be expensive and are hazardous towards personnel (PubChem). In order to appeal to a more organic production and decrease costs for the farmer, the option of using coconut water is worth exploring. Coconut water is rich in phytohormones like cytokinin, gibberellins and auxin (Yong et al., 2009) and can therefore be used to promote growth in sweet potato (Holwerda and Ekanayake, 1991).
This study will be focusing on why the lateral buds of sweet potato stay dormant and how its possible to break this dormancy by using synthetic cytokinin (BAP) or CW.

1.2. The sweet potato

The sweet potato is a vine that belongs to the family Convolvulaceae. The strategic growth pattern vines usually inhabit is to spread out as fast as possible and climb on top of other plants to access sunlight. To be able to expand in length the plant needs to focus its resources on growing its vine as long as possible and therefore the sweet potato have a strong apical dominance and rarely grows its lateral buds even if the apex bud is removed (Putz & Mooney 1991).
Unlike many other crops sweet potato is usually not propagated through seeds or tubers. The preferred propagation method is using slips which are a 20-30 cm long cuttings, taken from the stem. These slips are grown out of a sweet potato tuber. The tuber of the sweet potato is usually referred to as “seed”. The quality of these slips is very important because it dictates a big part of the yield produced in a growing season. To create a high-quality product of slips the producer must have a production free from disease with suitable production practices and harvest techniques (Primomo & Pearson).

1.2.1. Supply of slips in Sweden

Swedish farmers need to order slips from the southern part of Europe and because the slips from sweet potato are sensitive concerning both temperature and time, there have been difficulties in delivering high-quality slips because of the long distance. The slips will induce its tubers after 3 weeks from the time it is cut off from the mother plant which is why it's a very time-sensitive way of propagation. The problem of Swedish sweet potato production is getting the right number of cuttings in high-quality from an international supplier. The international suppliers don't have a variety that is suited for the Swedish climate and they have difficulties in delivering a big order of high-quality slips. Growing a hectare of sweet potato requires 20-40 000 slips. The potential of using established plants as planting material has been tried but the plants that get imported are either nutrient deficient or infected with disease or harmful insects. One perk of growing sweet potato in Sweden is the lack of established diseases like fungi, viruses and bacteria. This is because it's a new plant in Sweden that haven't had the time to develop these problems. Therefore, there is a lot less of a need to apply fungi- and pesticides. This is also why it's important to have domestic production because there is a risk of importing pathogens and pests when acquiring slips from countries where these are present. (Karlén et al. 2018).

A Swedish production is thereby possible and could give way for a more flexible and safe supply for the farmers.

1.2.2. Swedish cultivation practice

To adapt to the Swedish climate the sweet potato needs to be planted in raised beds with black plastic mulch. The purpose of this is to have adequate drainage and maintain a high enough temperature. It's important that the soil temperature doesn't go below +16°C so that the roots of the slips can get properly rooted. The plastic mulch also protects against unnecessary loss of water and inhibits growing weeds. If possible, it should be biodegradable. A homogenous sandy loam with proper drainage and a minimum amount of rocks will help to keep an even moisture and the right plant nutrition (Hansson 2020).

1.3. Sweet potato propagation

1.3.1. Seeds (tubers)

There are three stages of vegetative seed production: Breeder seed (G0), G1 root seed (foundation seed) and G2 root seed. Breeder seeds are cultivated by micropropagation in order to be free from diseases, especially viruses. The storage roots from the G0 seeds are planted to produce the tubers that will become G1 seeds.

The G1 seeds are multiplied to become the next generation, G2 from which the slips are produced that are sold to the market for commercial use.
In order to have cultivation free from pathogens, it's important to use certified slips. It's also possible for the farmers to propagate their own slips but not without any downsides (Primomo & Pearson). In a study made by Yoo & Lee 2013, they compared high quality and virus free slips to farmer's slips and there was quite a big difference in total yield, where the high-quality slips had a higher yield of 12-49%.

1.3.2. Slips

Slips are preferably taken from the shoot apex of a young plant but can also be taken from the middle or base of an older plant, although that will reduce the yield. The slips taken should have leaves left on the stem in order to promote good tuber production. Slips that are directly planted into the soil will produce a higher yield than those who have been stored. If the slips need to be stored, a 20-40cm long vine cutting is preferable. Such slips can be stored for 2-5 days (Ravi and Saravanan, n.d.).

1.3.3. Root sprouts

When harvesting roots for storage they are first cured and stored at 16 °C and 85% RH. Before the roots are planted, they experience a pre-sprouting period to induce sprouting. They are later buried in sand or soil beds and the sprouting will be induced by moisture and high temperature (Ravi and Saravanan, n.d.).

1.3.4. Root pieces

By cutting a storage root into smaller pieces with about a 2,5 cm thickness it's possible for the root piece to produce sprouts. A bigger cut produces larger sprouts, but it can't produce a sprout the same size as a complete storage root can. The shoot production from a root piece is more adventurous but the storage roots created are not as uniform as the ones produced from vine cuttings. Cut root pieces can be used as planting material but is not yet commercially available (Ravi and Saravanan, n.d.).

1.3.5. True seeds

The use of true seeds as a way of propagation is possible but it's not preferable because of the difficulties of its production. The flowering of sweet potato is unpredictable and when they do flower there is a problem of incompatibility and sterility. Compared to other methods true seed propagation does not look promising (Ravi and Saravanan, n.d.).

1.4. Claimed benefits of coconut water

Coconut is one of the few abundant agro-resources that are available cheap and with a complex chemical composition of phytohormones, although some challenges still exist where the CW (coconut water) needs to be processed in order to inhibit microbial growth. Since CW is a mix of different hormones another challenge is to have a homogenous or a specific subset of phytohormones, although these challenges are possible to solve through processing (Mamaril et al., n.d.). There is a lot of different products of coconut available for purchase like canned beverages of CW, coconut milk, dried coconut milk powder, coconut oil and young fresh coconuts. Many of these products are either a heavily processed mix of the entire endosperm of the coconut i.e. the CW and coconut meat or copra (Yong et al., 2009). In order to prolong the shelf life of CW today the process is mainly thermal treatment combined with chemical additives which may alter the original chemical composition of fresh CW (Prades et al., 2012).

The CW is a living endosperm and because of that, the content of the CW shifts in the different maturation stages. A study has been done by Mohd Lazim and Badruzaman, 2015 on two different varieties (Malayan Green Dwarf and Malayan Yellow Dwarf) of coconut *(Cocos nucifera L.)* to examine how the concentration of the main cytokinin in CW (trans-zeatin) changes within the shifting maturation stages and it was found that it was highest at the immature and mature stage, respectively. Since the phytohormones in the CW are at their most natural state before any processing is done, the trial will use CW from young fresh coconuts.

CW contains a long list of phytohormones like auxin, cytokinin, gibberellins, abscisic acid and salicylic acid with the concentration of cytokinin being strong enough to support cell division (Yong et al., 2009). The trail of this thesis will compare the effects of BAP and CW. Although it’s good to take into consideration that CW contains other hormones like auxin, gibberellin and even some unknown chemical components that may interact together with the cytokinin and have a synergetic effect (Yong et al., 2009). To get the effects of CW there are different ways to apply it to a plant. Studies have proven a notable effect by adding CW *in vitro* to a MS [Murashige and Skoog 1962] (Akhiriana et al., 2019) but in order to appeal to farmers, a foliar application method is worth exploring.

1.5. Apical dominance and its regulators in plants

Within the plant, there are many ways to control and affect how and what will happen. One of the most common ways to do this is by adding exogenic phytohormones and other regulators. There are a lot of different kinds of hormones in the plant-like gibberellin, ethylene, brassinosteroids, abscisic acid. The two most commonly occurring phytohormones within plants are auxins and cytokinins. These are the main groups of phytohormones involved in apical dominance (Taiz and Zeiger, 2014).

1.5.1. Auxins

Auxin is essential to how the plant grows and is used in almost every part of it. Indole-3-acetic acid (IAA) is the primary organic plant auxin as well as other kinds of auxins like phenylacetic and 4-chloro-IAA who are used less often in the plant. The main production of auxin is found in the apical meristems, young leaves and young fruits. Although almost every living cell of the plant appears to be able to produce small amounts of auxin. Auxin has polar transportation from the shoot to the roots via PIN and ABCB proteins. Since the main production of auxin occurs in the top shoot meristem and is transported by a polar one-way system, it's possible to cut off the main source of auxin in the plant by removing the top meristem. Auxin is a simple molecule and because of that it's easy to synthesize and therefore there is a lot of different synthetic compounds with auxin activity (Taiz and Zeiger, 2014).

1.5.2. Cytokinins

Cytokinins are phytohormones and their main function is to stimulate plant cells to divide and handle stresses like drought and salinity. Cytokinins are also part of many functions within the plant like apical dominance, leaf senescence, vascular development, promotion of sink activity and breaking of bud dormancy. Cytokinins are mainly synthesized in the roots into different types like kinetin and zeatin. Many synthetic cytokinins have been created including phenylurea-type N, N′-diphenylurea, thidiazuron, adenine-type kinetin, and 6-BA (BAP) (Feng et al., 2017).

1.5.3. Apical dominance

There is somewhat of an antagonistic relationship between auxin and cytokinin where they have contrasting activities. In physiological studies, it has been noted that applying a higher concentration of auxin than cytokinin to plant tissue will promote root formation, while the opposite will promote shoot formation. Their relationship is important in the regulation of apical dominance, where the lateral buds only grow depending on how strong the apical dominance is. A weaker apical dominance will allow lateral buds to grow at the same time as the apical meristem. In order to break apical dominance, the apical meristem that synthesizes auxin may be removed and therefore stop the biggest supply of auxin. The cytokinin located in the lateral buds will then promote them to grow (Taiz et al. 2015). The strength of the apical dominance varies depending on the species. Plants with a weak apical dominance like Arabidopsis or greenhouse-grown Coleus can induce growth in their lateral buds without the removal of the apical meristem. Weak apical dominance resembles the developing behavior of plants with sylleptic growth where there is no dormancy period between bud formation and outgrowth. Partial inhibition of lateral bud outgrowth can be termed as “intermediate” and functions in a way that makes it possible for lateral buds to break dormancy and grow without removing the apical meristem. This intermediate apical dominance is present in plants like bean or petunia. A strong or complete apical dominance that is present in Tradescantia, Helianthus, or indoor-grown Ipomoea requires the removal of the apical meristem in order to break lateral bud dormancy (Cline 1997).
Although the theory that auxin is the only factor that inhibits bud outgrowth has been questioned in a study by Mason et al., 2014 that claims that breaking apical dominance is dependent on the sugar redistribution by removing the high sugar-demanding apical meristem. This makes the sugars relocate to the lateral buds and initiates growth before any change in the amount of auxin at the lateral bud has been made. The study also claims that by artificially increasing sucrose levels at the bud, the gene that is responsible for maintaining bud dormancy BRANCHED1 is repressed and the bud can break dormancy. The trials of this study were performed on pea (Pisum sativum).
Another interesting study was made by Hosokawa et al. 1990 on *Ipomea nil*. In the study, they removed not only the apex bud but undeveloped foliage as well. The resulting effect of removing all buds and foliage at all nodes above the 4th was a strong promotive effect on lateral bud outgrowth. In the study, they discuss that it appears that within Ipomea the 0-5cm stem apex has little effect on the lower lateral bud outgrowth and that the entire stem growth region or the young expanding leaves. Concluding the study, they found out that the more the apical stem-growth region was removed, the more the lateral bud outgrowth increased. The study found that the 13cm apex from the stem tip was the part of the vine was the most influential on lateral bud outgrowth and that the basal part was less so and that

removal of small leaves (1-5 cm in length) had a lot more effect than leaves lager than 5cm. These smaller leaves had more effect on apical dominance than the 0.5 cm stem apex. It was also demonstrated that AgNO3 (inhibitor of apical dominance) was only effective when the whole shoot was treated and not just the apex. Which suggests that the whole stem is involved in apical dominance and not just the apex.

1.5.4. Natural cytokinin

Natural cytokinin has been shown to be an effective biostimulant. Leaf extract from the plant Moringa containing a cocktail of different phytohormones has even been shown to be more effective than BAP. In a study by Yasmeen et al., 2014 they compared the two biostimulants as foliar applications on tomato (*Solanum lycopersicumL.*). The MLE (Moringa leaf extract) produced a higher number of flowering branches, a number of flowers, and the highest bio-mas of fruits per plant compared to the BAP-treatment. MLE has also been shown to ameliorate the water deficit stress on two wheat cultivars (AARI-11 and Millat-11) thanks to its content of antioxidants that protect against oxidative damage (Nawaz et al., 2016). A natural biostimulant like MLE has in this case been shown to have multiple functions by not only adding phytohormones like cytokinin but also having other perks like antioxidants that protect from damage.
Seaweed Extract-Based cytokinin containing trans-zeatin has also been shown to be an effective biostimulant (Zhang and Ervin, 2008). CW has been used to enhance In vitro propagation of Kiwifruit (*Actinidia deliciosa*) together with BAP, with the goal to make it a more efficient and reliable (Nasib et al., n.d.). The combination of BAP and CW has also been used to replace zeatin in (*Olea europaea L.*) micropropagation (Peixe et al., 2007). Unfortunately, not much is known about the CW application *ex vitro* because of the small number of studies made on the subject. In an email conversation with Jean W.H Yong Professor of Horticulture, at SLU Swedish University of Agricultural Sciences he claims:
“Based on what we did for many years, there is no known formal publication (including foreign languages) using CW as a foliar spray, despite the global practice. This is an ironic and interesting fact about coconut water. As I often shared, many effective practises in Australia and Asia are never published.” (Yong 2020).

1.6. Exogenous variables

The sweet potato (*Ipomea batatas*) is a tropical plant that likes a warm and temperate climate and is commonly cultivated as an annual crop. Depending on the conditions the plant's growing period is between 12-35 weeks. Factors that determine axillary shoot production/growth are both genetic and environmental. The stem branching of different varieties of sweet potato can be put in three different categories: erect bushy, spreading, or intermediate. The sweet potato plant normally produces a primary, secondary, and tertiary branch depending on which period of growth it's in. The amount of branching the stem does is dependent on the application of N, irrigation, the photoperiod (promoting branching at 8 hours), soil moisture and spacing (Ravi and Saravanan, n.d.).

The different ways of the plant to be able to adjust to abiotic stresses are considered in the experiment of this thesis by removing the apical meristem and adding exogenic phytohormones in CW or BAP to promote axillary bud growth.

1.6.1. Foliage application

There are mainly two different ways to apply biostimulants to a plant, by the foliage or by the roots. In the case of applying phytohormones to the roots, it's possible to do it in vitro by supplementing an MS (Murashige and Skoog) medium with phytohormones (Akhiriana et al., 2019). Even though this way of applying CW or BAP is more controlled and precise it is an expensive way of application and not very likely to be profitable for farmers to propagate their slips. The perks of foliage application are how it's cheap and easy. Because of the nutrients in CW, an application to the soil is not preferable because the CW can be contaminated with microbial growth (Yong et al., 2009) (Mamaril et al., n.d.).

When a leaf is sprayed with a solution of phytohormones they enter the plant via the open stomata. There are often more stomata openings on the underside of the leaves and therefore it's important that the foliage application reaches the underside (Franke, 1967). The amount of solution absorbed can vary a lot depending on the different characteristics of the leaf. Some leaves are more hydrophobic because of the complex biopolymer cuticle that integrates with lipids i.e. waxes and if the solution is water-based that can affect the amount of the application that is absorbed (Koch and Ensikat, 2008). Once the solution has entered via the stomata it is absorbed through the membrane of the stomata and then taken up into the cytoplasm by active transport (Park et al., 2017).

In a study made by Liu et al. 2020, IAA (auxin), GA3 (gibberellin) and abscisic Acid (ABA) was applied to two varieties of sweet potato's foliage to investigate the exogenous hormone's role in reducing the loss of plant growth when under stress from K+ deficiency. The study showed a successful effect of foliage application of phytohormones in sweet potato.

1.6.2. Paste application

Orchids are propagated asexually as well, and this is done by inducing dormant buds. Orchids have a meristem hidden under the protective epidermis of the bud and by removing it and applying a so-called “Keiki paste”, it’s possible to artificially induce the orchid to create a new offshoot from the mother plant. “Keiki paste” is a commercial product and plant growth regulator mixture usually containing an auxin like α-naphthaleneacetic acid (Lee & Yeung 2018).

1.7. Source and sink

In a plant, there are sink and source tissues. The source tissues are those who can produce enough carbohydrates to supply the sink tissues. Sink tissues are often the parts that are under development in the plant and can’t supply itself with enough carbohydrates like the rots, the stem, tubers, growing shoots, flowers, tubers, fruits, developing seeds, developing leaves and growing shoots. The carbohydrates are unloaded and leave the sieve elements (cells containing sap rich in sugars and other organic molecules) and transported from source to sink through the phloem via short-distance-transport. This transport can occur in many ways depending on the plant and what kind of source it is. Some sources supply only some sinks and the specific transportation through the phloem depends a lot on variables like distance, vascular connections, development, and modification of different translocation pathways (Taiz et al. 2015). The relationship between different sinks and sources is also depending on sink strength.

Sink strength can also be called sink dominance and is based on which sink attracts the most photo-assimilates, creating a sink priority. The theory of how sink dominance is regulated is still not completely clear but since it’s related to apical dominance the two phenomena have a lot in common (Lemoine et al., 2013). Sink dominance can be explained by how the sink containing the least amount of photo-assimilates will attract the most photo-assimilates because of the lower hydrostatic pressure gradient in the pressure-flow mechanism for translocation in the phloem. What would in this case make a sink higher priority is the speed of carbohydrate metabolism. A way to affect the sink priority can be by removing higher prioritized sinks and therefore let the secondary sink accumulate more photo-assimilates (Taiz et al. 2015). Sucrose synthase is an enzyme that breaks down sucrose in many tissues of the plant and thereby creates lower hydrostatic pressure, which regulates sink strength. Sucrose synthase activity has been found to be lower in secondary sinks and higher in primary sinks and has, therefore been a way to dedicate sink strength (Sung et al., 1989). In a study of tomato, they created mutant plants with repressed Sucrose synthase genes. The plants showed a higher expression of PIN1 proteins in developing leaves which might suggest an alteration in auxin signaling.

The results of the study suggest a possible role of sucrose synthase in the leaf's development and its ability to alter the auxin-signaling pathway (Goren et al., 2017). A minireview suggests the hypothesis that the fruit's development is regulated by the export of auxin (IAA) of the earlier developed fruits which inhibits the export of later developed fruits. This regulation happens at the junction of different auxin streams and the lower auxin-export of the secondary sink/fruit acts as the signal of prioritization (Bangerth, 1989).

1.8. Objective, research questions and hypothesis

Objective

The objective of this study is to understand why the lateral buds of sweet potato stay dormant and if it's possible to break this dormancy by using synthetic cytokinin (BAP) or CW.

Research questions

- How does a plant regulate and control apical dominance?
- In what ways is it possible to stimulate lateral bud growth?
- What options are there in biostimulators and which are the most sustainable?
- What kind of biostimulants does CW contain and are they effective?

Hypothesis

A foliar application of CW and BAP on sweet potato cuttings will stimulate lateral bud growth and break the apical dominance.

Limitations

The number of cuttings and treatments are limited and won't make up a statically concrete result. The settings of the greenhouse were also limited because it had to be adjusted to other experiments conducted at the same time. This experiment was conducted under limited time and may have been extended if possible.

2. Materials and methods

2.1. Experiment

2.1.1. Material and methods

The experiment was performed April-May 2020 in the greenhouse of the SLU, *Swedish University of Agricultural Sciences, Alnarp*. Four varieties of sweet potato (*Ipomoea batatas)* were used. The four different varieties of sweet potato were labeled either 1, 2, 3, or 4, see *Table 1*. Six cuttings of each variety per treatment were prepared, from the shoot apex, each cutting was cut after 3 mature leaves. Please see *Table 2* for each individual treatment. The base leaf was later removed from the stem to make room for root formation. The apical meristem of every cutting was removed. 5cm of the basal part of the cutting was placed into a 1-liter pot of 2-8mm pumice stone and the pot was marked with an individual number based on future treatment. This was done on all the 72 cuttings and they were placed in gutters (see *Figure 2*) inside a greenhouse on the 23rd of April 2020. The settings of the greenhouse were DT 20°C, NT 18°C with shadow fabric on radiation higher than 500W/sqm with no added illumination.
The cuttings were left to grow roots for 12 days inside the greenhouse with a plastic film covering all the cuttings, being provided with manual irrigation and fertilization. After 12 days on the 5th of May 24 cutting's leaves were sprayed on the upper and lower epidermis with a 1g/l BAP solution till the dripping point. 24 cuttings were sprayed with a 20% dilution of CW from young coconut and deionized water till the dripping point. The foliar application was done in the middle of the day at 12:00. The cuttings were left to absorb the biostimulants for two weeks without any plastic film covering them. 14 days after application every cutting was analyzed by observing if the dormant bud had been stimulated. Lateral buds were categorized in 3 ways. A: dormant, B: partly stimulated with new leaf development and C: fully stimulated bud with an emerging shoot, see *Figure 1*. The leaves and number of flowers were also counted.

Treatments

- Application method: Foliar application
- Six cuttings of each variety were treated with CW deluded to a 20% concentration with deionized water and categorized into C and F
- Six cuttings of each variety were treated with 1mg/L BAP concentration and categorized into B and E
- Six cuttings of each variety did not undergo any treatment and were included in the control-category and categorized into A and D.
- For example, 2EB is New Orleans, BAP-treatment and number two of the three cuttings.

A = Dormant B = Partly developed C = Bud fully developed

Figure 1.

Table 1. Labeling of varieties

1	Beni Azuma
2	Orleans
3	Errato Deep Orange
4	Radiance

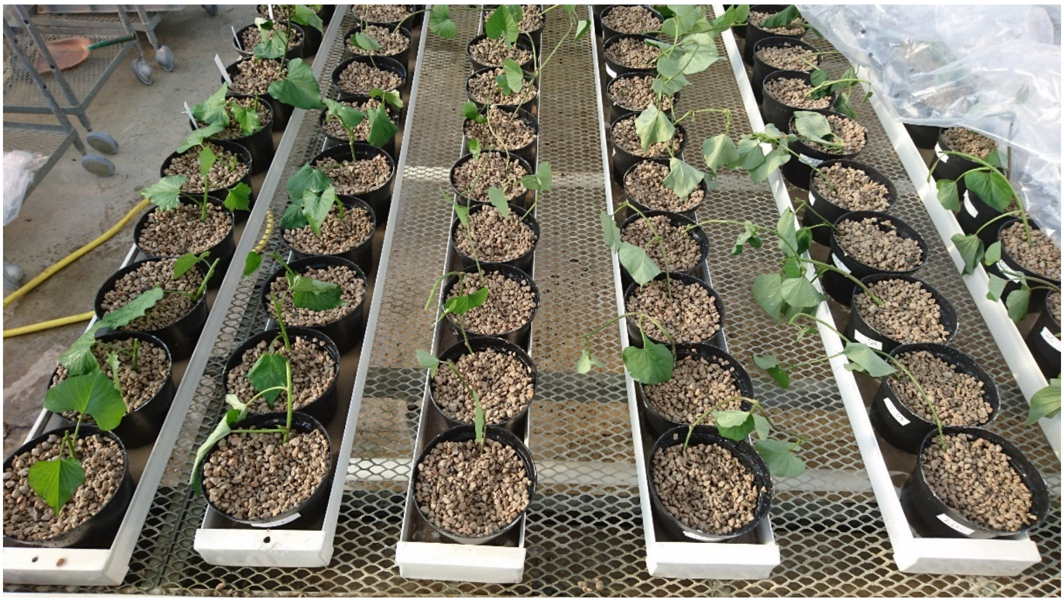

Figure 2. Cuttings planted in pots with pumice stone placed in gutters

Table 2. Design of experiment, (number and treatment of individual cuttings)

Control				Control			
1A:	1AA	1AB	1AC	**1D:**	1DA	1DB	1DC
2A:	2AA	2AB	2AC	**2D:**	2DA	2DB	2DC
3A:	3AA	3AB	3AC	**3D:**	3DA	3DB	3DC
4A:	4AA	4AB	4AC	**4D:**	4DA	4DB	4DC
BAP				**BAP**			
1B:	1BA	1BB	1BC	**1E:**	1EA	1EB	1EC
2B:	2BA	2BB	2BC	**2E:**	2EA	2EB	2EC
3B:	3BA	3BB	3BC	**3E:**	3EA	3EB	3EC
4B:	4BA	4BB	4BC	**4E:**	4EA	4EB	4EC
CW				**CW**			
1C:	1CA	1CB	1CC	**1F:**	1FA	1FB	1FC
2C:	2CA	2CB	2CC	**2F:**	2FA	2FB	2FC
3C:	3CA	3CB	3CC	**3F:**	3FA	3FB	3FC
4C:	4CA	4CB	4CC	**4F:**	4FA	4FB	4FC

3. Results

3.1. Indication of an effect

The total difference of stimulated buds compared to the control group with the BAP treatment was a 27% increase and the cuttings treated with CW had a 20% increase. The different varieties reacted differently to the treatments with Beni Azuma having a 75% increase comparing the BAP to the control. The Orleans variety showed very little effect from the CW or BAP treatments with no increase and even a decrease from the CW treatment. Erato Deep Orange showed a small increase and Radiance had no increase with BAP but a 100% increase with CW. The small amount of plants in each variety makes it difficult to see a concrete pattern of behavior but collectively its possible to see an indication of effect.

Please view the excel attachment for a complete display of the results.

Table 3. Total number of stimulated buds (Sum of B and C)

	Beni Azuma	**Orleans**	**Errato Deep Orange**	**Radiance**	**SUM**
Control	8	8	5	3	24
BAP	14	8	8	3	33
CW	10	7	5	6	28

4. Discussion

4.1. Future studies

The results for all cuttings treated with BAP showed an increase of 27% of stimulated buds and for CW 20% compared to the untreated plants. The conclusion is that a leaf application with either BAP or CW might stimulate lateral buds to start growing.

The Radiance (4) developed flowers during the experiment and so did the mother plant the cuttings was taken from. This is interesting considering that if a cutting is taken from a mother plant that is about to bloom, the cutting will bloom as well. This could be a good thing to take into consideration when propagating slips because it most likely affected the sink priority and therefore there were fewer resources and space to develop lateral buds. The lower percentage of stimulated buds in the CW-treatments might be because of the lower cytokinin content compared to the 1g/L BAP concentration.

Since this experiment only has a small amount of plants treated it's not possible to claim any proven effect. Although what would be possible is to continue examining how it's possible to affect the apical dominance in sweet potato. Based on the research presented in this thesis there are different ways it could be possible to continue. A new experiment on a bigger scale could be a way to confirm the effect of BAP or CW has with foliar application. During the experiment, the BAP and CW were applied mid-day but the optimal time for foliar absorption varies from plant to plant and what conditions it is exposed to. A new trial might explore what the preferred time and conditions are for optimal foliar absorption.

A treatment of combining BAP and CW can be tested as it showed to have a good result in vitro by Nasib et al., n.d. In the study by Mamaril et al. they successfully extracted phytohormones from CW which could be a way of increasing CW's potency in future experiments. Also, the way Hosokawa et al. 1990 removed leaves and parts of the vine to induce later bud growth. An artificial increase of sucrose levels as done in the study by Mason et al., 2014 may also be a way to induce lateral buds' growth but it may not be practical on a large scale.

The time in-between treatment and reading of results as well as the number of foliar treatments may be extended but, in this experiment, most of the lateral buds had been stimulated and a longer time period or more treatments may have been superfluous.
The mechanisms of apical dominance are not yet fully understood today. There is a lot of research done to prove the different actors involved in inducing or breaking apical dominance but it's still unclear exactly in what way plants have a high apical dominance. What's also unclear is in what way the plant decides how it's going to allocate its different resources. For example, we know how sugar is allocated from source to sink because of the difference of the lower hydrostatic pressure gradient in the pressure-flow mechanism for translocation in the phloem and that sucrose synthase breaks down carbohydrates but not how the plant chooses which sink that is going to be prioritized. If it would be possible to truly know how to control apical dominance in plants, it could be possible to further develop more effective propagation techniques.

4.2. Environment and sustainability

When developing new techniques, it's important to consider how sustainable the new method is and how it affects the environment. Knowing which concentration of BAP or CW that is the most effective is important, to not overuse the biostimulant and be able to use enough to have the best effect. One of the problems of using BAP is that it is hazardous to both the environment and human health (PubChem). Therefore, it may not be a suitable option in large scale production and that is why using a natural biostimulant like CW is preferred but CW also carries some problems of its own. Coconuts are not grown locally in Scandinavia and the majority are grown in Asia (FAOSTAT), so the CW needs to be transported a long way before it can be used in Sweden. Because of this long distance from production to consumption, it's preferable to use a local resource in Sweden. Ascophyllum nodosum is a seaweed that can be used for its cytokinin content by producing a seaweed extract and it may be a more sustainable option to CW because of its local origin in Scandinavia (Ascophyllum nodosum (Linnaeus) Le Jolis 1863 :: Algaebase). The effectiveness of the seaweed extract is to be further explored. Compost tea may also be a local biostimulant that can be used but more research is needed (Arancon et al.). Natural biostimulants in general are a pretty new concept and researchers are doing more and more research on their possibilities. Some studies in the west have been done on their effects and possibilities but the research done in eastern countries like China is not fully understood because of the language barrier. It's the same when talking about the sweet potato's growth pattern and plant physiology. Most research that is being done is on the tuber which is usually the yielding part of the plant but since it's possible to use the foliage as a food resource as well (Bovell-Benjamin 2007) more studies should be conducted on its behavior. A collaboration between western and eastern universities may help to bridge this gap. The potential and possibility of different combinations of these biostimulators should be explored to create a safe and sustainable way of plant manipulation.

4.3. Swedish sweet potato production

Creating a Swedish supply of slips is a way of supplying local producers with enough high-quality slips to make sweet potato production profitable. The perks of this local production are not only high-quality but also the way a production may be more adjustable to the wants and needs of the grower when factors like weather complicate the growing season. Domestic production will also prevent the potential import of disease and pests that may come with importing. Keeping pests out of Sweden will also keep the organic farming of sweet potato possible for longer. It is also more environmentally friendly because of the shorter transportation-chain.

To have the financial incentive to establish this production there needs to be enough farmers interested in growing sweet potato. A way of creating more demand is by supplying not only Swedish farmers but farmers from countries like Denmark, Norway, and Finland as well. This production would have to be from micro-propagation to G2 slips because the few producers in Europe can't supply G0 seeds. Swedish farmers can be inspired by the way Canadian farmers are developing methods to grow and propagate sweet potato in a similar climate to Sweden. The reason why it's difficult to buy any seeds in Europe is that the market is very small, and the knowledge of sweet potato propagation is lacking ("Storage capacity of sweet potatoes in Europe challenge for year round supply"). Domestic production will also aid the food security of Sweden as the country won't need to rely on international suppliers.

The fact that sweet potato is the 6th biggest crop (*Sweetpotato Facts and Figures*) in the world makes it quite surprising how little research that been done on the subject and because of the rising demand of the product in Europe more research is needed to supply farmers with useful information.

4.4. Conclusion

The sweet potato is an important crop and there is a possibility to have a Swedish production but the bottleneck preventing that is the low quality of slips from southern Europe.

To sum up this thesis, apical dominance is a subject where there’s been a lot of research done but, we need to do more research on how it works to understand it completely and control it in plants.

CW is a safe biostimulant containing different phytohormones with a lot of versatility and uses but so far there is a lack of knowledge and available research results in English to assess the potential use for biostimulants in the future. CW could be combined with other organic biostimulants or a synthetic one like BAP.

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