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EXPERIMENTS AIMED AT THE INDUCTION OF SOMATIC EMBRYOGNESIS WITH TOMATO (*SOLANUM LYCOPERSICUM* L.*)* AND CAULIFLOWER (*BRASSICA OLERACEA* CONVAR. *BOTRYTIS* VAR. *BOTRYTIS* L.) GENOTYPES FOR THE PURPOSE OF ARTIFICIAL SEED PRODUCTION

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Table of Contents

1. Introduction and objectives

Year by year, the increasingly unpredictable weather conditions not only complicate food production but also challenge the foundational processes of seed production. As our agricultural system currently relies on hybrid seeds for yield potential and uniformity, the difficulties with controlled pollination, and the rising threat of economic and regulatory issues are becoming increasingly prevalent. The economic and social difficulties, extreme climate changes, and the emergence of new pathogens are directing attention toward the application of biotechnological methods.

Tomato (*Solanum Lycopersicum* L.) and cauliflower (*Brassica oleracea* convar. *botrytis* var. *botrytis* L.) both have significant nutritional and economic value. In addition, they are considered great model plants within their respective plant families, making them crucial for research in the field. Experiments involving these plants could be very relevant to the scientific community.

With the creation of artificial seeds, we could eliminate the need for the continuous maintenance of parental lines required for F1 hybrid seed production, as *in vitro* tissue culture methods would allow the direct production of plant clones. With a sustainably developed and optimized method, the process could be highly automated and synchronized in fermenters. In a controlled, artificial environment, a precisely planned quantity of propagative material could be available whenever needed, tailored to usage requirements. Furthermore, under sterile conditions, the propagated plant material is completely free of pathogens and viruses.

The ultimate goal of our research is to develop a methodology for generating propagules from plant *in vitro* cell cultures. This could be achieved through artificial seeds, or "synthetic seeds", which could contain meristematic cells (shoot or root derived) or somatic embryos developed through tissue culture techniques. To achieve this, multiple tomato and cauliflower varieties were used.

Our research aims to address the following objectives:

- to determine which plant-derived explant (hypocotyl, cotyledon, or first leaf) performs the best within a given genotype, in terms of callus induction, organogenesis, or somatic embryogenesis,
- to identify the most suitable combination of callus induction and somatic embryogenesis-inducing media,
- to assess which media combination results in successful somatic embryogenesis,

- to identify which medium combination induces organogenesis,
- to evaluate the regeneration potential of calli maintained in liquid culture.

2. Literature review

2.1. Introduction to the plant material

2.1.1. Tomato

The tomato (*Solanum Lycopersicum* L.) is an economically very significant species in the Solanaceae family. Its cultivation began in South America, with initial domestication presumed to have taken place in present-day Mexico. The species became widespread in Europe around the 16th century (Hodossi, 2009).

The plant belongs to the herbaceous plant group. During its life, it develops a robust taproot system and can form adventitious roots from the stem (Figure 1). Based on growth characteristics, two types are distinguished. In the case of determinate types, the apical meristem terminates in an inflorescence, and in indeterminate types, the main shoot remains active. The yellow flowers have anthers that form a tube around the stigma, allowing self-pollination to happen (Rashid and Singh, 2000).

Figure 1: *Solanum Lycopersicum* L. (Csapody and Priszter, 1961).

Its value in food production is prevalent, both as fresh and processed produce. The demand for the fresh, fruity vegetable is year-round, so the long-cycle greenhouse growing is gaining in importance in Hungary, too. The nutritional value is given by its high-water content and medium levels of vitamins B1, B2, B3, and C. Lycopene, the antioxidant responsible for the fruit's red colour, has been proven to neutralize carcinogenic free radicals in the human body (Hodossi, 2009).

Open-field, industrial tomato production has a long-standing tradition in Hungary, but year by year, the cultivation of fresh-market varieties intended for raw consumption is expanding. In recent years, the total cultivated area has declined; however, advancements in modern cultivation technologies have led to higher yields (Hungarian Central Statistical Office, 2023).

The plant has significant scientific value, in addition to its economic importance due to its use in a wide variety of biotechnological research. Numerous studies have focused on its *in vitro* regeneration potential in the past five decades (Bhatia et al., 2004). El-Farash et al. showed the influence of both genotype and explant type on successful regeneration and somatic embryogenesis.

2.1.2. Cauliflower

Cauliflower (*Brassica oleracea* convar. *botrytis* var. *botrytis* L.) belongs to the family Brassicaceae. It is an economically important crop, with cultivation and consumption mainly in Asia and Europe. The common ancestor of the cabbage group originated in the Mediterranean region, where continuous selection during domestication led to the development of modern cultivars known today (Swarup and Chatterjee, 1972).

Cauliflower is an annual species that develops a compact, thickened inflorescence meristem at the stem apex, known as the “curd” or “head”. This structure is the main marketable part of the plant, typically white, though purple, orange and green varieties are also cultivated (Figure 2). The species is generally self-incompatible. Its nutritional value is mainly due to its high levels of vitamin C, B1, and B2, as well as essential minerals (Nagy, 2009). In Hungary, cauliflower cultivation area declined notably in 2020, from 1100 hectares to 330 hectares, yet due to advances in cultivation technology, average yields have continued to increase year after year (Hungarian Central Statistical Office, 2023).

Tissue culture studies on cauliflower have primarily aimed at the production of artificial seeds. These experiments have employed root derived somatic embryos (Al Shamari, 2014) as well as secondary shoot apices regenerated from hypocotyl explants (Poon et al., 2012). Moreover, the large number of primary shoot meristems located within the inflorescence has been successfully utilized for cloning and regeneration experiments (Kieffer et al., 1995).

Figure 2: *Brassica oleracea* convar. *botrytis* var. *botrytis* L. (Csapody and Priszter, 1961)

2.2. Plant cell and tissue culture

Dénes Dudits and László Heszky (1990) defined plant cell and tissue culture as "...the *in vitro* cultivation of vegetative and generative cells, tissues, organs, and embryos on nutrient media under sterile, climatized, and controlled environmental conditions." According to their description, these techniques form the foundation of plant biotechnology. By maintaining sterility and providing an appropriate artificial environment, it becomes possible to preserve and propagate every part of a plant under laboratory conditions.

Micropropagation is a specific *in vitro* tissue culture method in which small plant segments (explants) are cultured in a sterile, artificial environment for the purpose of multiplication. The regeneration of tissues placed on nutrient media into complete plants can occur through either organogenesis or embryogenesis. In organogenesis, whole plants are regenerated from pre-existing primary meristematic tissues (from shoot or root apices) or from newly formed, *de novo* meristematic tissues. During embryogenesis, however, the embryo originates not from gametic cells or a zygote, but from somatic cells (Iliev et al., 2010). A schematic overview of the morphogenetic process is presented in Figure 3.

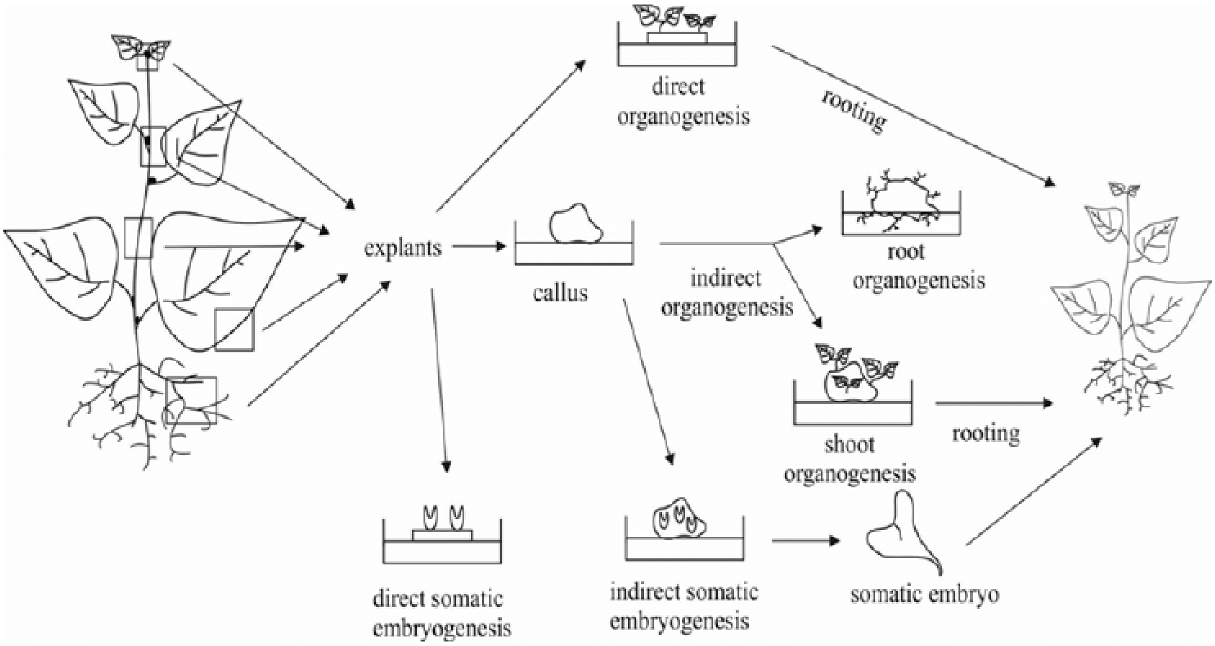

Figure 3: Routes of plant regeneration in tissue cultures *in vitro* from different explants (Adamczuk et al., 2012).

2.2.1. Callus culture

Upon wounding of a plant tissue, a physiological response occurs at the site of injury that induces the formation of callus cells. These undifferentiated cells possess the ability to divide and proliferate, developing a growing mass of tissue that covers the wound surface and serves as the basis for *in vitro* regeneration. This phenomenon was first described in 1902 by the Austrian botanist Gottlieb Haberlandt, who determined it as totipotency. Though his experiments already pointed towards tissue culture techniques, at that time, he could not yet prove that an entire, intact plant could be regenerated from a single cell. It was only by the late 1950s that this became possible, along with the establishment of liquid cell cultures that enabled the continuous maintenance and cultivation of individual cells (Dudits and Heszky, 1990). Skoog and Miller in 1957 demonstrated the effects of plant growth regulators on proliferating callus cells. During their experiments with tobacco plants, they found that auxins and cytokinins are essential for proliferation. They observed that the callus morphology depends on the relative concentration of the two hormones. Higher auxin concentration induces larger cells and adventitious root formation, while the increase in cytokinin content results in the formation of shoots and buds. With both growth regulators present in high concentration, only callus growth occurred, with no observable organ differentiation.

These pioneering studies laid the groundwork for numerous subsequent research efforts and for industrially applied biotechnological methods that remain significant even today. When callus tissues form on solid media, or when explants are transferred into a liquid medium and kept under constant agitation, a cell suspension culture can be established. In such cultures, the newly formed cells occur either as small aggregates or as individual units. With regular subculturing, this system can be maintained over an extended period of time. The development of these techniques made it possible to cultivate single cells, forming the basis for large-scale micropropagation and for the production of plant-derived bioactive compounds via phytofermentation (Dudits and Heszky, 1990).

Numerous studies have been examining callus induction in tomato in recent years. As mentioned previously, the success rate of induction is strongly genotype- and explant-type-dependent; therefore, researchers have tested many cultivars and tissue sources, not just different growth regulators. Setiaji et al. (2020) worked with the hypocotyl explants of the ‘Permata’ variety and demonstrated the ideal concentrations of plant hormone for the induction of callus. They compared different concentrations of synthetic auxins (2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene acetic acid (NAA)) and the synthetic cytokinin 6-benzylaminopurine (BAP). The results showed that the 2 mg/l NAA and 0.2 mg/l BAP-supplied MS media (Murashige and Skoog, 1692) yielded the best results, with a 73,77% daily absolute callus growth rate. Jan and colleagues (2015), in the case of the cultivar ‘Peto-86’, identified the first true leaf and the stem as suitable explants. Their study showed that the combination of 4 mg/l 2,4-D and 0,5 mg/l BAP in MS medium produced the most desirable green, friable callus. Yaroshko and colleagues (2023) examined the cotyledons of genotypes ‘Micro-tom’ and ‘M82’. They found that the combination of 0,1 mg/l indole-3-acetic acid (IAA) and 1 mg/L zeatin added to Murashige and Skoog medium resulted in the highest callus yield.

Callus induction has also been intensively studied in cauliflower. In the study of Qamar et al. (2014), they used the hypocotyl explants of the cultivar ‘Chillout’. This resulted in the production of yellow, friable callus when cultured on MS medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l BAP. Similarly, Taha et al. (2011) examined both the hypocotyl and first true leaf explants’ ability to form callus with a non-disclosed cultivar. Their results showed that high-quality embryogenic callus could be obtained using low concentrations (0,05-0,1 mg/l) of 2,4-D in MS medium.

All in all, the literature shows that phenotype, explant source, auxin-cytokinin ratio, and the type of growth regulators used play a critical role in achieving optimal callus quality and quantity. The interaction of these factors not only governs the rate of callus formation but also influences cellular organization. Changes in the supplied hormone concentration in the media or in the physiological status of the donor plantlet can result in differences in callus morphology and embryogenic potential. The optimization of these specifications is crucial to establish a reproducible and efficient *in vitro* protocol which is suitable for both basic research and large-scale biotechnological applications.

2.2.1. Organogenesis and somatic embryogenesis

Morphogenetic plant regeneration can occur by two pathways: organogenesis or somatic embryogenesis. Both processes can proceed via either a direct or an indirect route. During regeneration via the direct pathway, embryos or meristematic cells grow *de novo* from tissues already differentiated, without an intermediate callus stage. This route typically results in faster, more genetically stable regeneration and minimizes the risk of somaclonal variation. On the contrary, the indirect pathway, undifferentiated callus cells first develop at the site of wounding and give rise to the embryos or secondary meristems. The dominant regeneration process is determined by the morphogenetic potential of the grown callus or cell suspension culture (Gamborg and Phillips, 1995). Somatic embryos are bipolar structures that contain the radicle and a shoot apex, similarly to classical zygotic embryos. The developmental progress is also characterized by the same stages (globular, heart-shaped, and torpedo forms) as described by Dudits and Heszky (1990). On the contrary, tissues formed through the organogenic pathway are unipolar; therefore, to achieve complete plant regeneration, a second induction step is required. One is needed for the formation of the shoot apex and another for the initiation of the root (Phillips, 2004). The callus tissue produced for plant regeneration can be morphologically distinguished as embryogenic or non-embryogenic, with only the former capable of forming somatic embryos. Embryogenic callus is typically yellowish in colour, has spherical cells, and a friable structure (Arnold, 2008). As with callus induction, the success of shoot regeneration and somatic embryogenesis is strongly dependent on genotype, explant type, and the combination and concentration of plant growth regulators in the culture medium.

In tomato, Yaroshko et al. (2023) successfully induced *de novo* shoots from cotyledon explants of the cultivars ’M82’ and ’Micro-Tom’. Danial and Ibrahim (2020) used the tomato genotypes ’Sandra’ and ’Rocky’ for their experiments. The explants used by them were hypocotyl-, cotyledon-, and first-leaf-derived. They achieved a 100% induction rate in their experiments using hypocotyl explants cultured on MS media supplemented with 1 mg/l BAP and 0,5 mg/l kinetin. For cotyledon explants, the combination of 1,5 mg/l BAP and 0,5 mg/l kinetin yielded the overall best results, though the number of shoots per explant was comparatively lower. Numerous studies have also addressed somatic embryo induction in tomato in recent years. Jan and colleagues (2015) achieved direct somatic embryogenesis from the first true leaves of the ‘Peto-86’ cultivar using MS media containing 4 mg/l 2,4-D and 0.5 mg/l BAP and observed the emergence of somatic embryos on day 13. Zahedi et al. (2023), while experimenting with the ‘Money Maker’ genotype, presented that 2 mg/l IAA and 5 mg/l BAP supplied media strongly promoted the formation of embryogenic callus and somatic embryos in the case of hypocotyl explants. Godishala et al. (2011) reported the highest embryogenesis frequency and the greatest number of somatic embryos directly formed on cotyledon explants when cultured on MS medium supplemented with 0,5 mg/l IAA, and 3,5 mg/l BAP.

In cauliflower, CV and Indulekha (2019) used young leaf explants cultured on MS medium containing 3 mg/l NAA and 0,5 g/l BAP, resulting in indirect shoot induction with an average of 3,8 shoots regenerated per explant. Qamar et al. (2014), as previously mentioned (in section 2.2.1. Callus Culture), were able to produce callus with strong regenerative potential, and after transferring it to MS vitamin medium supplemented with 2 mg/l kinetin, 6 mg/l GA_3, and 5 mg/l BAP, they confirmed that this protocol was effective for the organogenic regeneration of the ‘Chillout’ cultivar. A cell suspension culture was also established from callus tissue containing 0,5 mg/l BAP, in which microscopic observations revealed the presence of somatic embryos. Taha and colleagues (2011) achieved indirect embryogenesis from young leaf explants cultured on media containing 0,1 mg/l 2,4-D, successfully regenerating viable plants. In contrast, hypocotyl explants cultured on MS medium supplemented with 1 mg/l 2,4-D and 0,05-1,5 mg/l kinetin produced direct somatic embryos but were unable to develop beyond the globular stage. Al Shamari et al. (2015) observed somatic embryo formation on root and hypocotyl-derived callus of the ‘White Cloud’ genotype cultured on MS medium supplemented with 0,5 mg/l kinetin and 0,05mg/l IAA, with a higher embryo formation frequency in root-originating callus, compared to that from hypocotyls.

Overall, it can be concluded that the effectiveness of a somatic embryogenesis or an organogenic plant regeneration protocol is governed by multiple interacting factors, including the genotype, the explant source, and the type, ratio, and concentration of the applied growth regulators. The review of the literature available reveals that most studies have focused on widely available cultivars in Asia, while data on the available genotypes on the Hungarian market remain limited. This knowledge gap offers a great potential for further research to assess the morphogenetic capacity of locally available tomato and cauliflower varieties. Moreover, the reviewed literature shows that both somatic embryos and apical meristems can serve as viable sources for the development of synthetic seed technology. A comprehensive understanding of both morphogenic pathways may contribute to the establishment of efficient, reproducible, and genotype-specific regeneration systems and strengthen the technological foundation for future plant biotechnological innovations.

2.3. Artificial seeds

Propagation by seeds is the most widely used and natural method of plant reproduction. However, in certain species, this approach can be problematic due to limitations such as extremely small seed size, the absence of endosperm, or limited storability. In such cases, the use of artificial seeds provides an effective and innovative alternative.

A synthetic seed is an *in vitro*-produced structure designed to act similarly to the layered organization of a true seed. The outer coating usually built up of a protective gel matrix, commonly composed of sodium alginate, agarose, or sodium pectate, and it serves as a barrier against desiccation and environmental stress. Under this layer, an artificial endosperm can be found, supplied with essential nutrients, plant growth regulators, and, occasionally, antimicrobial or plant protection agents. At the core of this encapsulated system is the propagule, which, in the original concept proposed by Murashige (1977), could be exclusively a somatic embryo, but the term was later expanded to include any meristematic tissue derived from tissue culture, such as apical, axillary, or adventitious buds (Chaudhuri, 2020).

Artificial seeds can be classified into two major categories based on their water content: dehydrated and hydrated forms. In the case of dehydrated types, somatic embryos are embedded in a polyethylene glycol-based matrix and then dried in a controlled environment. This approach offers long-term storage and transportation, as dried seeds are compact, weigh less, are less sensitive to handling, and can be stored without refrigeration. However, it is applicable only to plant species whose embryos can withstand desiccation (Pond and Cameron, 2003). Hydrated artificial seed, in contrast, involves the encapsulation of the propagule in a hydrogel matrix. The most commonly employed encapsulation technique relies on an ion-exchange reaction between sodium alginate and calcium chloride ($CaCl_2$). During the process, explants are suspended in a sodium alginate solution and dispensed drop by drop into a $CaCl_2$ bath. In this process, the gel rapidly polymerizes into a spherical capsule (Reddy et al., 2012). An alternative approach reverses the order of these two solutions, resulting in "hollow" beads with a liquid core, which can improve oxygen diffusion and could reduce mechanical stress on the propagule (Winkelmann et al., 2004).

In tomato, several studies have explored the production of artificial seeds. Chirom et al. (2015) encapsulated *in vitro* grown shoot apices using a 2% sodium alginate and 100 mM $CaCl_2$ combination, which was identified as the most suitable for bead solidification. Porter (2008) also encapsulated apical buds using the "hollow capsule" technique and reported higher viability compared to solid, homogeneous gel structures. However, he observed that the biological activity of synthetic seeds declined significantly after only 15 days of storage, indicating a limited longevity after encapsulation.

Several studies also focused on encapsulating different propagules in cauliflower. Qamar and colleagues (2014) investigated somatic embryos as the encapsulated material, mixing them in a 1:4 ratio of sodium alginate and $CaCl_2$. The synthetic seeds they produced successfully regenerated plants *in vitro*, and the transplanting to soil was also successful. Similarly, Poon et al. (2012) encapsulated secondary shoots from hypocotyl explants into an MS-based medium with the addition of 0,3 mg/l NAA and 3 mg/l BAP. The achieved germination rates of the experiment reached 70% after seven days of storage, and 63% after 30 days, with viable plantlet regeneration confirmed in both cases.

The artificial seed system therefore offers a promising alternative to conventional propagation, especially when traditional seed sowing is constrained by technical or biological limitations. Encapsulation of the propagules produced *in vitro* in hydrogel matrices. The long-term preservation of valuable plant germplasm and the maintenance of elite genotypes under controlled conditions are enabled. Nonetheless, several challenges must be addressed before this technology can be adopted for commercial-scale application. Large-scale, cost-effective, and reproducible production of viable somatic embryos or meristematic tissues is still technically demanding. In the meantime, future research must prioritize the optimization of tissue culture systems, the enhancement of encapsulation materials, and the improvement of long-term storage to ensure sustained viability. In summary, artificial seed technology holds substantial potential as a bridge between *in vitro* culture systems and field-level applications. To achieve its full economic and practical impact, further advancement in the underlying biotechnological processes is required to support its integration into modern production systems.

3. Material and method

3.1. Plant material

To establish the experimental plant population, seeds from four tomato and four cauliflower genotypes were germinated under *in vitro* conditions. The tomato cultivars used were ‘Bobcat F1’ (determinate growth habit, medium-early maturity), ‘Dominet’ (greenhouse cultivar, extra-early type), ‘Hapynet’ (open-field variety with determinate growth), and ‘Sagatan’ (early-maturing, open-field cultivar). The cauliflower genotypes included: ‘Andromeda’ (fresh market, open-field type with white curds), ‘Amidala’ (white curded, fresh market cultivar), ‘Clarina’ (suitable for both fresh consumption and processing, white curds), and ‘Clementine’ (orange-curded, fresh market variety). The varieties’ seeds were provided by Syngenta Hungary Ltd.

3.2. Plant raising and callus induction

Prior to sowing, the seeds were soaked in distilled, autoclaved water to promote germination. During soaking, the sterilizing solution was prepared by dissolving 10 g of calcium hypochlorite in 90 mL of distilled water. After filtering the solid undissolved material, one drop of Tween20 was added to the solution to enhance surface adherence. For disinfection, 5 ml of the calcium hypochlorite solution was used for every 8-10 seeds. The seeds, together with the solution, were transferred into a new sterile flask under a laminar airflow cabinet (BL-1200 F, Defi). The sealed flasks were then placed on a rotary shaker (Multi-Shaker PSU 20, Biosan) for 20 minutes. Following the sterilization, the seeds were rinsed five times with sterile distilled water to remove any residual disinfectant. Between each rinse, the flasks were returned to the shaker for 10.15 minutes to ensure thorough washing. Finally, the sterilized seeds were

Figure 4: The *in vitro* grown tomato (left) and cauliflower (right) plantlets an the 14th growing day. The genotypes from left to right are: ‘Bobcat F1’, ‘Dominet’, ‘Hapynet’, ‘Sagatan’, ‘Andromeda’, ‘Amidala’, ‘Clarina’ and ‘Clementine’.

placed onto hormone-free MS medium and incubated in a growth chamber under controlled environmental conditions at 22 °C, with a 16/8-hour light/dark photoperiod (Figure 4).

When the tomato and cauliflower seedlings reached 21 days of age, three types of explants (hypocotyl, cotyledon, and first true leaf) were excised and transferred onto callus induction media. In the case of both plant species, all four genotypes were cultured using each of the three explant types on two different media compositions containing plant growth regulators, as well as on MS control. The media formulations were the following: MS + 2 mg/l 2,4-D + 0,5 mg/l BAP (Qamar et al., 2014); MS + 4 mg/l 2,4-D + 0,5 mg/l BAP (Jan et al., 2015). The pH of the media was adjusted to 5,7-5,8, and Plant Agar (Duchefa, The Netherlands) was added at a concentration of 8 g/l. The sterilized media were poured into Petri dishes (10 cm diameter), after which the explant samples were carefully arranged and grouped according to genotype and explant type.

3.3. Somatic embryogenesis

After four weeks of cultivation, the calli developed on the explants were transferred to two different somatic embryogenesis induction media. Based on the literature, for cauliflower, the somatic embryogenesis medium described by Rihan and Fuller (2018) was selected with the following composition: MS + 0,05 mg/l IAA + 0,5 mg/l kinetin + 2% sucrose. For tomato, the media formulation proposed by Godishala et al. (2011) as applied, consisting of MS + 3,5 mg/l + 0.5 mg/l IAA + 3% sucrose. In both cases, the pH was adjusted to 5,7-5,8, and Plant Agar was added at a concentration of 8 g/l. Approximately 25 ml of each sterilized medium was poured into 10 cm diameter Petri dishes. For traceability, both the original explant type and the callus induction medium were clearly labelled on each culture.

3.4. Callus suspension culture

From the ‘Dominet’ and ‘Sagatan’ tomato and the ‘Clarina’ and ‘Clementine’ cauliflower genotypes, the developed calli on the previously described induction media were transferred into the liquid (agar-free) versions of the same callus induction media. Approximately 25-30 ml of the sterilized liquid medium was dispensed into 50 ml Erlenmeyer flasks. These were maintained in darkness on a rotary shaker, and the suspension was subcultured every two weeks following sufficient cell proliferation. Starting from the second subculture, 1 ml of the cell suspension was pipetted onto both solid callus induction and solid somatic embryogenesis induction media to promote further tissue development and to observe the embryogenic potential of the cultured cells.

3.5. Artificial seed encapsulation

Shoot apices developed indirectly from the explants of the ‘Clarina’ cauliflower genotype were encapsulated with both a root regeneration medium and a growth regulator-free medium. The MS-based solutions contained macronutrients at half strength and, in the case of the former, were supplemented with 2 mg/l BAP, 1 mg/l thidiazuron (TDZ), 1 mg/l meta-topolin (TOP), 0,1 mg/l indole-3-butyric acid (IBA), 2 ml/l WUXAL® Super (Kwizda Agro Hungary Ltd.), and 15 g/l sodium alginate. Following autoclave sterilization, 500 mg/l 2-(N-morpholino)ethanesulfonic acid (MES) was added, which was filtered through a 0.2 µm membrane filter. For the solidification of the artificial seeds, a 50 mM $CaCl_2$ solution was prepared.

Under sterile conditions, the shoot apices formed on the explants were carefully excised and transferred into the encapsulation media. Each shoot apex was drawn individually into a cut (4 mm diameter) pipette tip, then released near the surface of the $CaCl_2$ solution while gently agitating the liquid to ensure even bead formation. Then the $CaCl_2$ solution with the formed capsules was placed on a rotary shaker for 30 minutes to obtain adequate structural integrity of the gel spheres (Figure 5)

Figure 5: Encapsulated cauliflower shoot apices in $CaCl_2$ solution.

Then, the only MS-based beads were put on solidified MS media while the root-inducing ones were put in Jiffy® peat pellets (33 mm diameter), which were sterilized under UV light and hydrated with sterile water (3 pellets per 1 l glass jar). Immediately after encapsulation, three artificial seeds were sown directly into the hydrated peat. The remaining capsules were stored at 4 °C and planted after 5, 10, and 20 days to assess the effect of storage.

3.6. Effect of NAA

To assess the effect of an auxin-analogous growth regulator, the media composition described by Fári et al. (1995) was modified. The explants were collected from 20-day-old seedlings, at a developmental stage when the cotyledons were fully expanded and the first leaves had only emerged. The halved cotyledons and the upper 2/3 of the hypocotyl were then placed on media containing MS macronutrients and micronutrients, B5 vitamins, and 4 mg/l NAA, and, in the case of tomato genotypes, 6% sucrose, and, in the case of cauliflower, 2%. From the calli obtained from 'Dominet', 'Sagatan', 'Clarina', and 'Clementine' varieties, liquid cultures were also started. The liquid medium composition was identical to the solid version, except for its higher NAA concentration (10 mg/l). For suspension culture, 100 ml Erlenmeyer flasks were used, each containing 10 ml of liquid medium.

3.7. Data evaluation

The growth rate of calli cultured on both callus-induction and somatic-embryogenesis-induction media was quantified using ImageJ. A total of 2070 data points were recorded for the callus induction phase and 1570 for the somatic embryogenesis stage across both species. Statistical analyses were performed using the R statistical environment (R Development Core Team, 2015), while data visualization and figure generation were carried out with Microsoft Excel (Microsoft 365 version). Differences were considered statistically significant at $p < 0{,}05$ and distinct letters were used to denote significantly different groups.

4. Results and their evaluation

4.1. Results of callus induction media

For the sake of traceability, the genotypes were assigned the codes presented in Table 1. The letter "P" stands for tomato, and "K" for cauliflower.

Table 1: The genotypes used in the experiments and their codes.

Cauliflower		Tomato	
Andromeda	K1	Bobcat F1	P1
Amidala	K2	Dominet	P2
Clarina	K3	Hapynet	P3
Clementine	K4	Sagatan	P4

In the callus induction experiment, the effects of three different media were evaluated on the plant species and explant types. These included: a growth regulator-free MS basal medium, a medium supplemented with 2 mg/l 2,4-D and 0,5 mg/l BAP, and another containing 4 mg/l 2,4-D and 0,5 mg/l BAP. For clarity and consistency in the figures, the plant hormone-containing media were designated "2" and "4", respectively, according to their 2,4-D

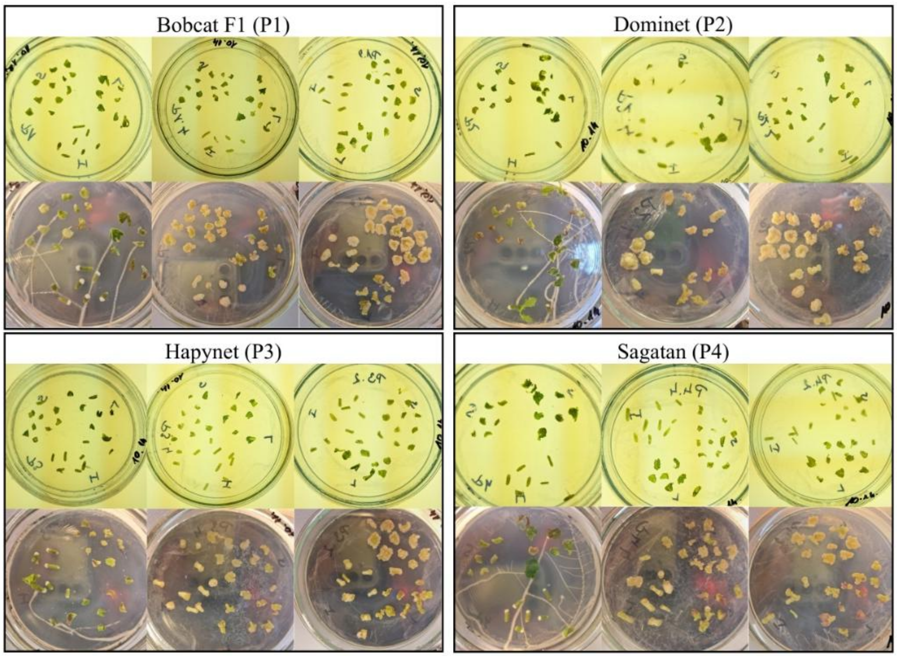

Figure 6: Callus growth of tomato genotypes from left to right on MS, "4", and "2" media. The upper row shows the explants immediately after placement, while the lower row depicts their condition 16 days later.

concentrations. Figures 6 and 7 illustrate the callus induction and the extent of callus growth, observed in the tomato and cauliflower genotypes after 16 days of culture.

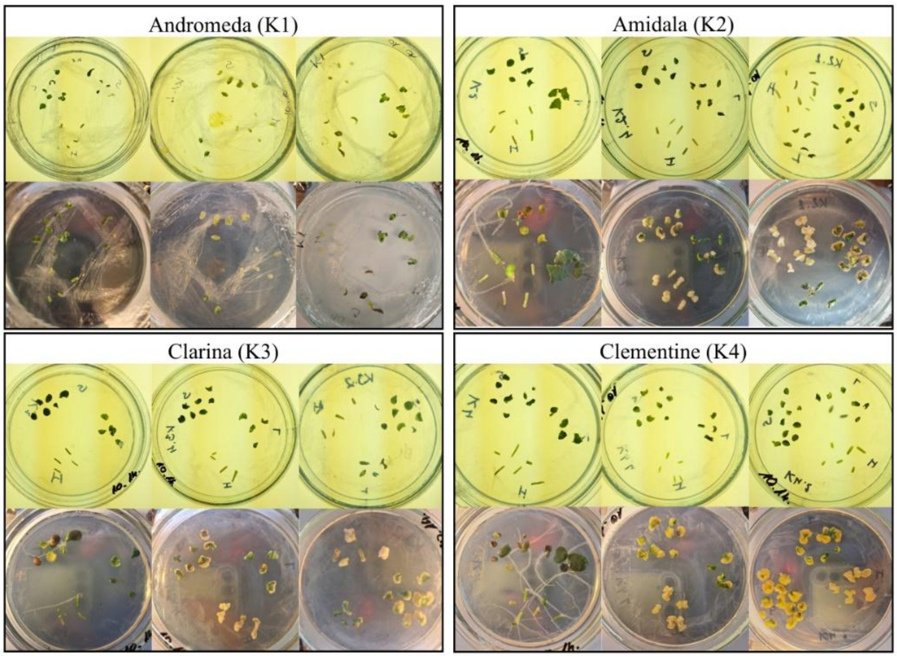

Figure 7: Callus growth of cauliflower genotypes from left to right on MS, “4”, and “2” media. The upper row shows the explants immediately after placement, while the lower row depicts their condition 16 days later.

Figure 8 presents the average daily callus growth rate of the tomato genotypes. Statistical evaluation was performed using Multivariate ANOVA. As shown in the figure, the addition of auxin (2,4-D) enhanced average callus growth compared to the MS control medium. In all genotypes, cotyledon explants cultured on media supplemented with growth regulators showed a significantly higher daily callus growth rate than those cultured on hormone-free media. Although the growth rate of cotyledon explants was consistently higher on the media containing 2 mg/l 2,4-D than on the media containing 4 mg/l 2,4-D, a statistically significant difference was only observed for the ‘Bobcat F1’ (P2) genotype. In the case of hypocotyl explants, a significant difference was only observable between the media “2” and the MS control. The only exception was the variety ‘Dominet’ (P2), for which both hormone-containing media induced a statistically higher growth rate. No significant difference was found between the two hormone treatments for this genotype, although the “2” medium produced a slightly higher mean growth rate. The addition of 2 mg/l to the MS media significantly enhanced the growth rates across all varieties in the case of the first true leaf explants. An exception to this was the ‘Bobcat F1’ (P1), for which no statistical difference was detected between the treatments. The first leaf explants of the genotype ‘Dominet’ (P2) were the only ones where the average daily growth rate was higher on the media “2” than on the media “4”, though both values remained considerably higher than those on MS. The significantly increased callus growth was only demonstrated on the 2 mg/l 2,4-D medium compared with the regulator-free control in the case of the leaf explants of ‘Hapynet’ (P3) and ‘Sagatan’ (P4).

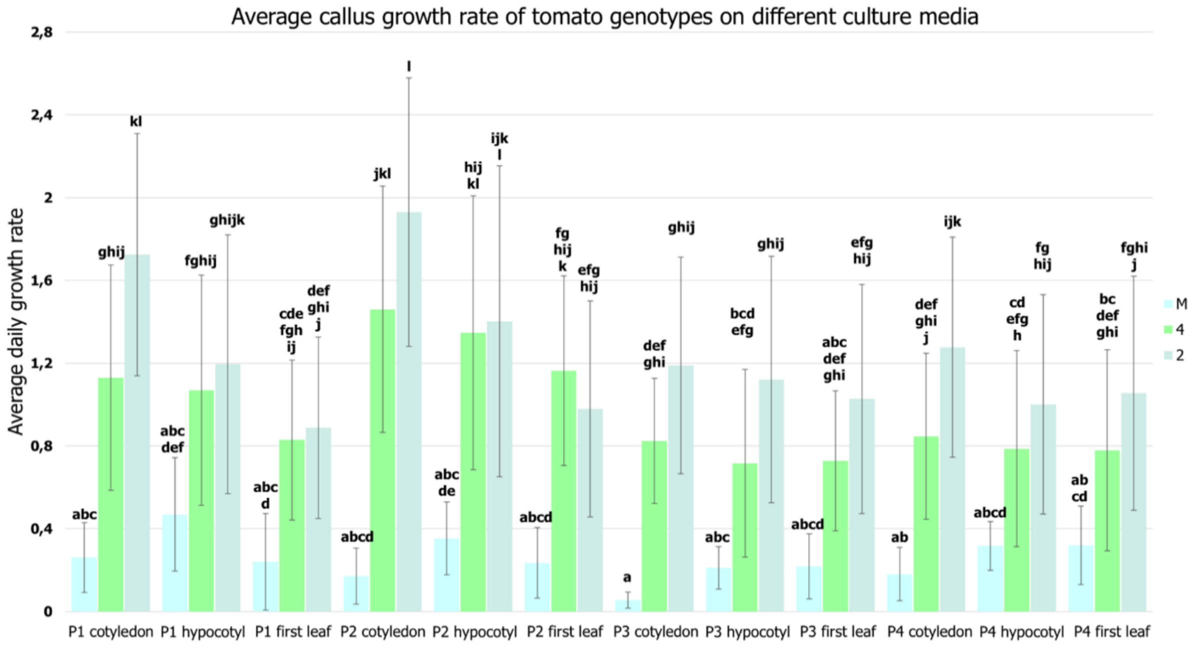

Figure 8: Effect of growth regulator free MS and the two plant hormone containing media on the callus growth rate of three explants from four tomato genotypes.

In the case of the genotype ‘Bobcat F1’ (P1), the cotyledon explants reached the highest growth rate on the media “2”. It is only statistically comparable to the hypocotyl results obtained on the same medium, which showed the second-highest values overall. Although ‘Dominet’ (P2) exhibited the largest callus growth rate across the entire experiment (on the “2” medium) in the case of the cotyledon explant, significantly, it was only larger than the results of the explants cultured on the MS control and the first leaf within the variety. No statistical differences were observed between the two growth regulator concentrations within the varieties ‘Hapynet’ (P3) and ‘Sagatan’ (P4). The results from medium “2” were statistically distinct from those on MS medium in all cases, while medium “4” showed significant differences only for cotyledon explants compared to MS.

Across all genotypes and explant types, there was no observable statistical variation in the daily average growth rate on MS media. In the case of the media “4”, a significant difference was only shown between the cotyledon explant of the variety ‘Dominet’ (P2) and all the explants of the varieties ‘Hapynet’ (P3) and ‘Sagatan’ (P4). The 2 mg/l 2,4-D treatment caused significantly higher callus induction in the cotyledon and hypocotyl explants of “Bobcat F1’

(P1) and 'Dominet' (P2) compared with all other genotypes and explant types. Based on these findings, tomato genotypes generally performed better on the media "2", despite earlier studies reporting enhanced callus induction at higher auxin concentrations (Jan et al., 2015).

Figure 9 shows the average daily callus growth rate of the cauliflower genotypes. Similarly, to the analysis of the tomato, a Multivariate ANOVA was conducted. Due to poor germination of the 'Andromeda' (K1) genotype, insufficient replication prevented its inclusion in the statistical analysis. As observed in tomato, cauliflower explants also show an increase in callus growth under the influence of hormone-containing media. For all genotypes, the cotyledon explants present significantly greater average callus proliferation on the hormone-supplemented media than the MS control. Though no statistical difference could be seen between the two growth regulator-supplemented media. The same trend was seen for the hypocotyl explants, except in the case of the 'Clarina' (K3) genotype, where no significant difference was found between the hormone-free and the media having 2 mg/l 2,4-D. Among the first leaf explants, only the 'Clementine' (K4) showed a statistically significant difference between the control and growth regulator-supplied media.

For 'Amidala' (K2), the cotyledon explants cultured on the media "2" displayed the highest callus growth rate within the genotype, which was significantly higher than any value on MS medium, as well as for hypocotyls on the medium "4" and first leaves on the medium "2". Apart from the growth rates measured in the case of cotyledon explants on hormone-

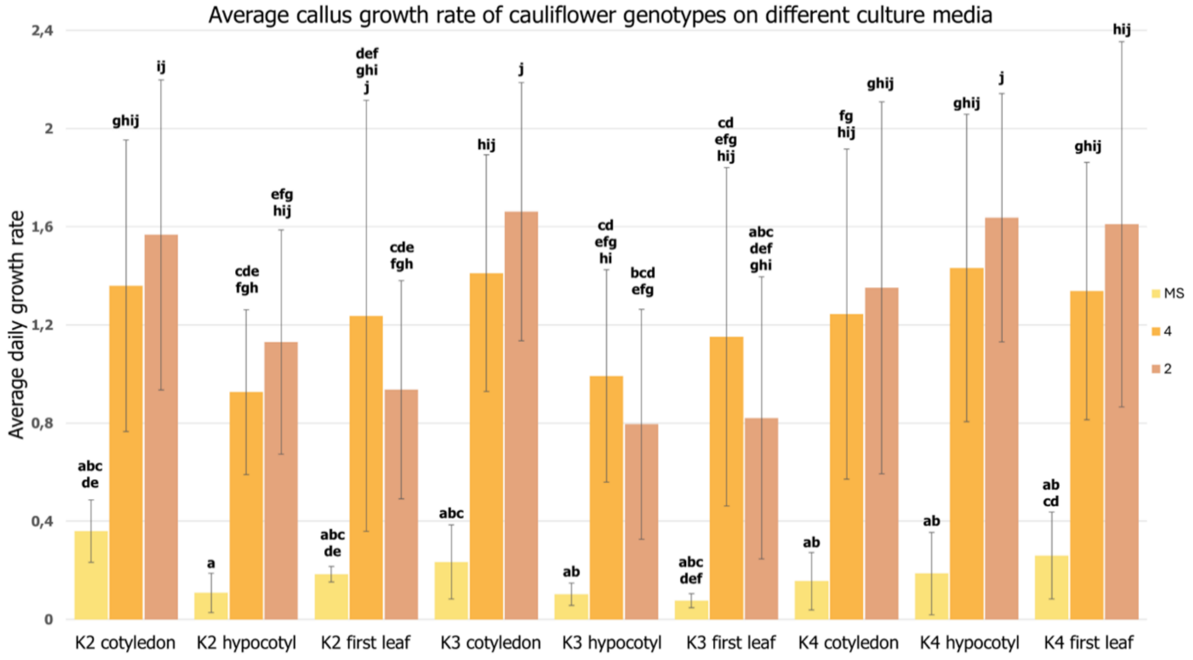

Figure 9: Effect of growth regulator free MS and the two plant hormone containing media on the callus growth rate of three explants from three cauliflower genotypes.

supplied media, no notable differences were detected between the MS-grown calli on cotyledon explants and the true leaf explants, or among the explants cultivated on media containing growth regulators. In the case of ‘Clarina’ (K3) genotype, the cotyledon explants on the media “2” produced the overall highest callus induction rate. This showed a significant difference from the hypocotyl explants on both growth regulator-containing medium, from the first leaf explant on the 2 mg/l 2,4-D media, and from the explants on MS. The average growth of its hypocotyl and true leaf explants was lower on the “2” medium compared to the medium “4”, although no statistically significant difference was seen between them. The cotyledon explants of ‘Clementine’ (K4) displayed slightly lower mean growth rates than its other explants, but these differences were not statistically significant. The effect of hormone-supplemented media was clearly distinguishable from that of the MS control.

No significant differences were observed among genotypes and explants on the hormone-free or 4 mg/L 2,4-D media. The highest callus induction rates were achieved by the cotyledon explants of ‘Clarina’ (K3) and the hypocotyl explants of ‘Clementine’ (K4) on the “2” medium, both of which showed significantly higher values than the true leaves of ‘Amidala’ (K2) and the hypocotyls of ‘Clarina’ (K3). The analysis of cauliflower callus growth rates clearly reflects the genotype and explant-dependent variability in tissue culture performance, as described previously in the literature (Gubis et al., 2004).

On the plant growth regulator free MS media, the growth of roots then shoots were observable across all genotypes, in the case of cotyledon and true leaf explants (Figure 10).

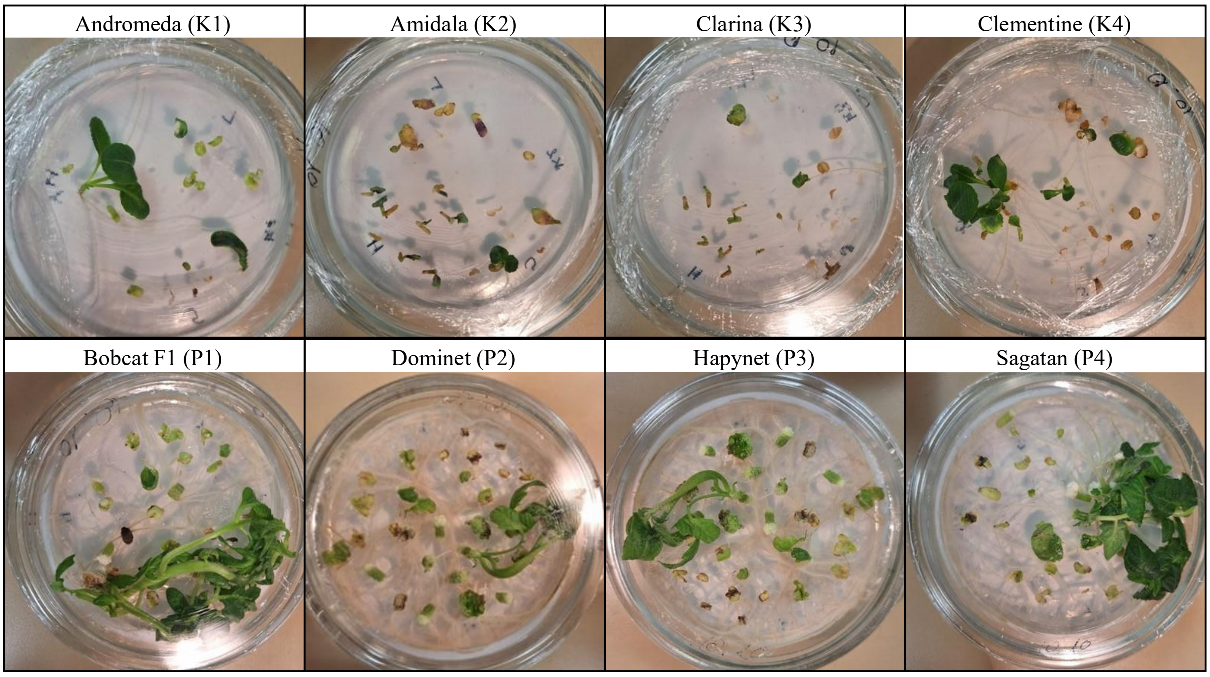

Figure 10: Tomato (bottom row) and cauliflower (top row) genotypes on MS media after 60 days of culturing.

4.2. Results of somatic embryogenesis induction media

Figures 11 and 12 illustrate the response of tomato and cauliflower genotypes cultured on somatic embryogenesis induction media. During the experiment, the calli were consistently distinguished by the type of explant and callus-induction media from which they originated. In the following graphs, the media combinations are shown in the order of the cultivation sequence. The first is the specific callus-induction medium, followed by transfer to the somatic embryogenesis-induction medium. Each combination was assigned a letter code as follows:

- A: “2”: MS + 2 mg/l 2,4-D + 0,5 mg/L BAP, followed by cauliflower somatic embryogenesis medium: MS + 0,05 mg/l IAA + 0,5 mg/l kinetin + 2% sucrose
- B: “4”: MS + 4 mg/l 2,4-D + 0,5 mg/l BAP, followed by cauliflower somatic embryogenesis medium: MS + 0,05 mg/l IAA + 0,5 mg/l kinetin + 2% sucrose
- C: “2”: MS + 2 mg/l 2,4-D + 0,5 mg/L BAP, followed by tomato somatic embryogenesis medium: MS + 3,5 mg/l BAP + 0.5 mg/l IAA + 3% sucrose
- D: “4”: MS + 4 mg/l 2,4-D + 0,5 mg/l BAP, followed by tomato somatic embryogenesis medium: MS + 3,5 mg/l BAP + 0.5 mg/l IAA + 3% sucrose.

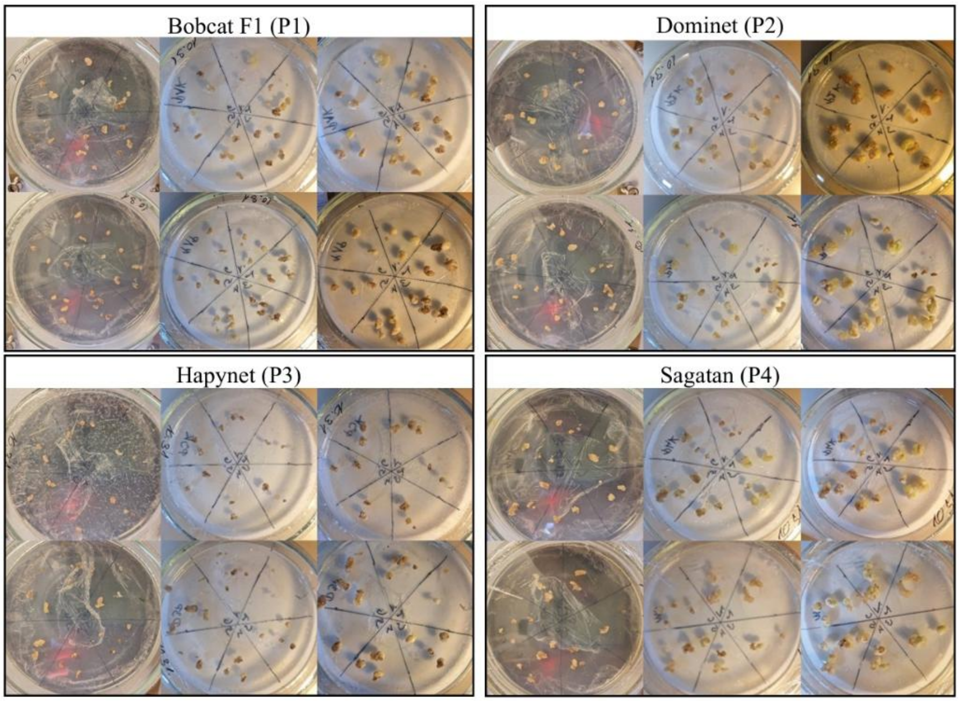

Figure 11: Tomato genotypes on the somatic embryogenesis inducing medias. The upper row represents the medium composition optimized for cauliflower according to literature data, while the lower row shows the medium developed for tomato. From left to right, the images illustrate the progression of growth over 7 day intervals.

On the photographs, the labelling can be observed as follows: each Petri dish is divided into six parts; within each group of six, the explants are arranged in pairs from left to right as hypocotyl, cotyledon, and true leaf, meaning that 2/6 of the division is one type of explant. According to the letter codes (for each genotype), the upper rows show the cauliflower somatic embryogenesis media, each 1/6 alternating A" and "B", while the lower rows correspond to the tomato somatic embryogenesis media, each 1/6 alternating "C" and "D".

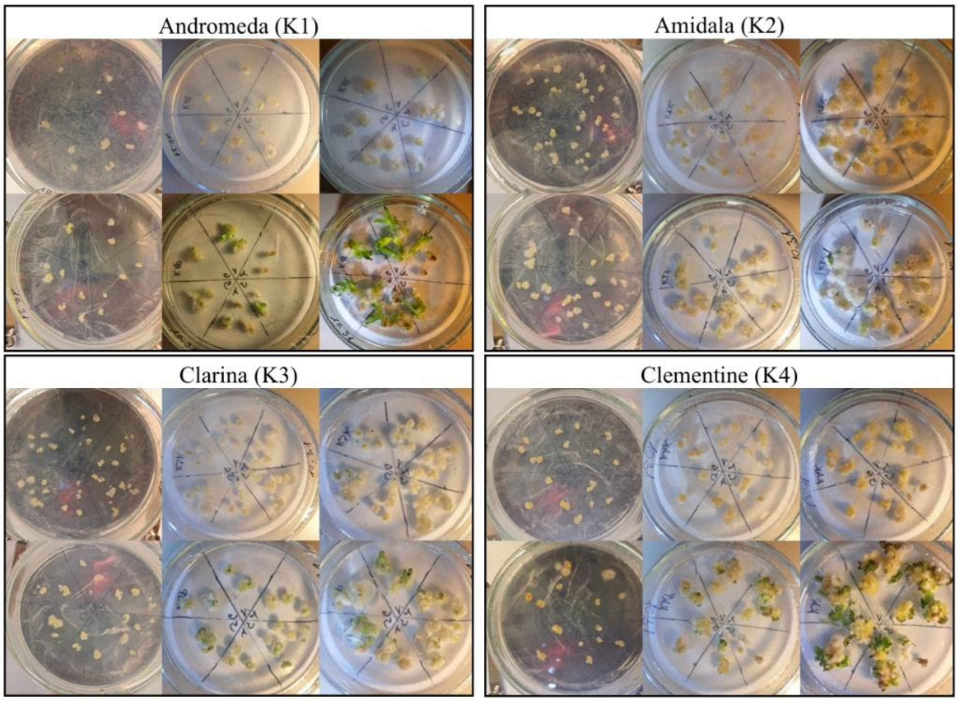

Figure 12: Cauliflower genotypes on the somatic embryogenesis inducing medias. The upper row represents the medium composition optimized for cauliflower according to literature data, while the lower row shows the medium developed for tomato. From left to right, the images illustrate the progression of growth over 7 day intervals.

During our examination, we couldn't detect the formation of somatic embryos on any of the somatic embryogenesis-inducing media, genotypes, or explants. In the case of tomato, no shoot formation was observed on any of the tested media. In contrast, all of the cauliflower genotypes exhibited indirect shoot formation. For each medium combination ("A", "B", "C", and "D"), the number of regenerated shoots emerging from the calli was evaluated, as shown in Figure 13. Only media combinations "C" and "D," which were the most notable in genotypes 'Clarina' (K3) and 'Clementine' (K4), and lesser but still present in 'Andromeda' (K2). According to the literature, both media used in combination "D" (for callus induction and somatic embryogenesis) were originally developed for tomato (Godishala et al., 2011; Jan et al., 2015). In contrast, in combination "C", only the somatic embryogenesis medium was tomato-specific. Shoot formation was also observed in the 'Amidala' (K1) genotype.

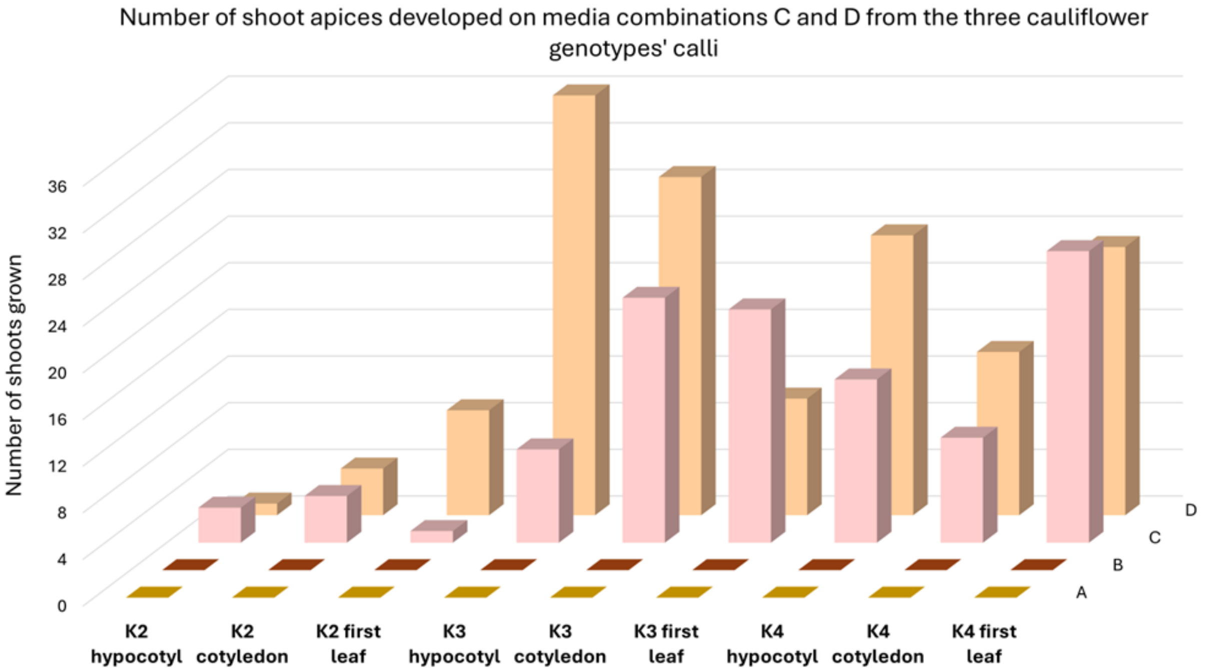

Figure 13: Number of shoots developed from calli of three cauliflower genotypes across three explant types cultured on four different medium combinations. The Y-axis represents the number of shoots formed.

4.3. Callus suspension culture

Following the transfer of calli grown on solid media to liquid callus induction medium, a gradual increase in cell growth was observed. Even after periodic subculturing into fresh media, cell proliferation remained slow. During the subculturing process, approximately 50 Petri dishes were subsequently inoculated with cells from the liquid culture using both solid callus-induction and somatic embryogenesis-induction media. During our experiments, no notable callus growth was observed on either medium. Only the cauliflower genotype ‘Clarina’ (K3) produced an organogenic cell mass, which later developed both roots and shoots when transferred to the “2” medium (Figure 14).

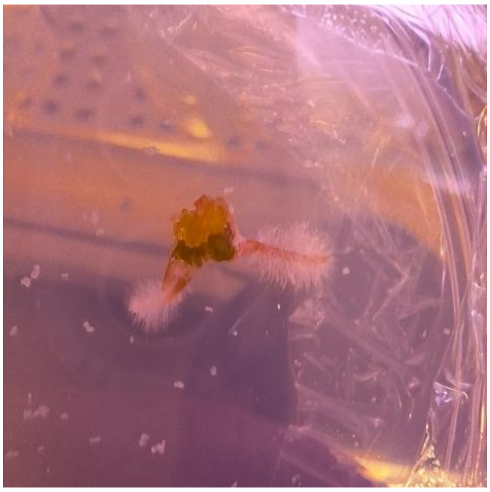

Figure 14: Organogenic callus derived from liquid culture of the genotype ‘Clarina’ (K3).

4.4. Artificial seed encapsulation

The encapsulated shoot apices of the ‘Clarina’ (K3) genotype were maintained in sterile conditions. Shoot growth started with both the plant hormone-free and the root regenerating capsules, but neither could initiate root growth (Figure 15). We could not observe differences between any of the stored capsules. Shoot initiation was observed on individuals within all storage groups, but the contiuation of growth falled short.

Figure 15: The encapsulated shoot apices of the genotype ‘Clarina’ (K3)under sterile conditions on MS (left) and on hydrated Jiffy peat pellets (right).

4.5. Effect of NAA

The culturing of cotyledon and hypocotyl explants of the three cauliflower genotypes on NAA-containing media resulted in callus induction and organogenesis (Figure 16). Cotyledonary explants rapidly developed numerous roots, while hypocotyl explants produced roots only after several weeks. Beginning on the eighth week, shoot formation was also observed, typically fewer in cotyledonary explants (~3 shoots) and more abundant in hypocotyls (~10 shoots). Nearly all hypocotyl explants produced shoots, while the cotyledon explants showed that shoot formation was concentrated in a single sample.

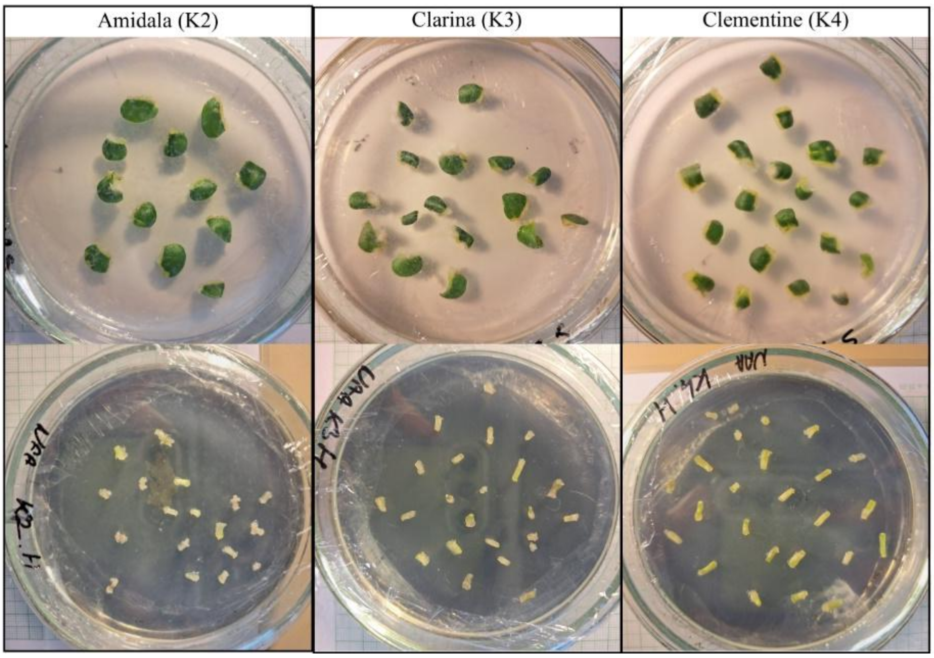

Figure 16: The cotyledon (top row) and hypocotyl (bottom row) explantums of the three cauliflower genotype on media conatining 4 mg/l NAA after two weeks of cultivation.

For the four tomato genotypes, NAA-supplemented media mostly induced organogenesis, and nearly no callus formation was observable. Adventitious roots appeared within a short time on cotyledonary explants, accompanied by minimal callus formation. Root development in hypocotyl explants occurred later, with only limited callus proliferation (Figure 17).

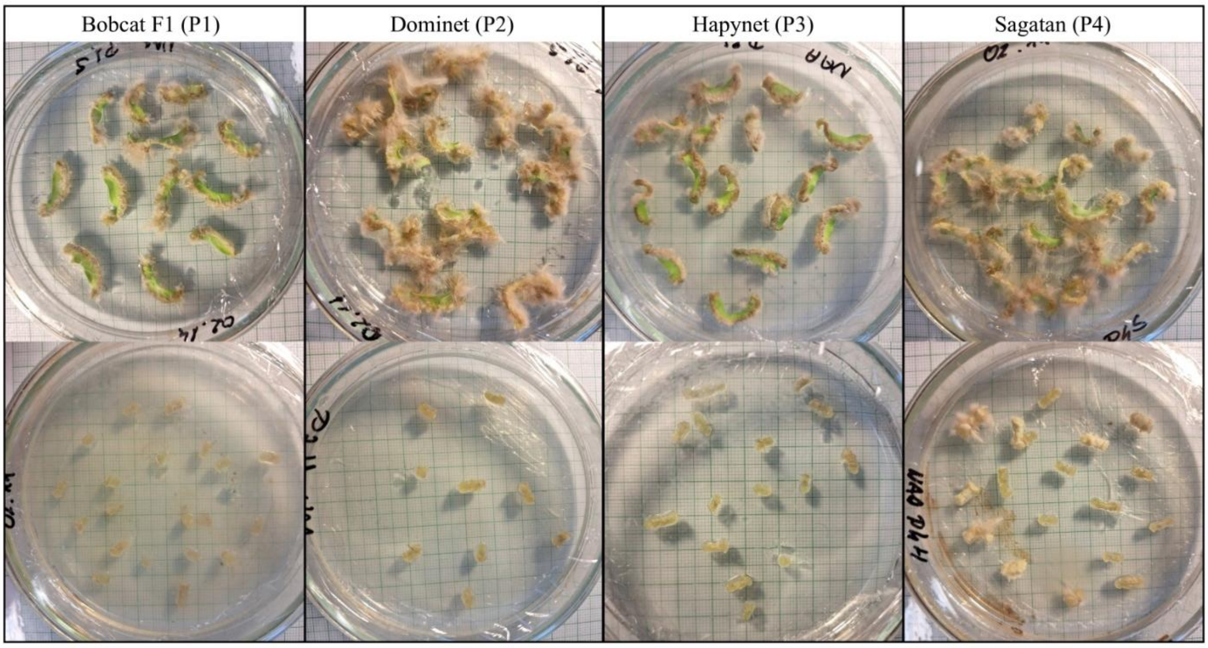

Figure 17: The cotyledon (top row) and hypocotyl (bottom row) explantums of the four tomato genotype on media containing 4 mg/l NAA after two weeks of cultivation.

Saeed et al. (2019) reported the formation of an embryogenic structure called a "Rhizoid Tuber" on the root tips of tomato explants cultured on NAA containing MS media. Similar structures could be seen during our experiments. These were excised and subsequently transferred to an embryo maturation medium (MS + 5 mg/l BAP + 2% sucrose). These Rhizoid Tuber-like formations were maintained separately according to genotype and explant type (Figure 18). From the third week onward, greenish tissue proliferation was observed, characteristic of the early stages of embryogenic tissue development, but final embryogenic maturation couldn't be achieved.

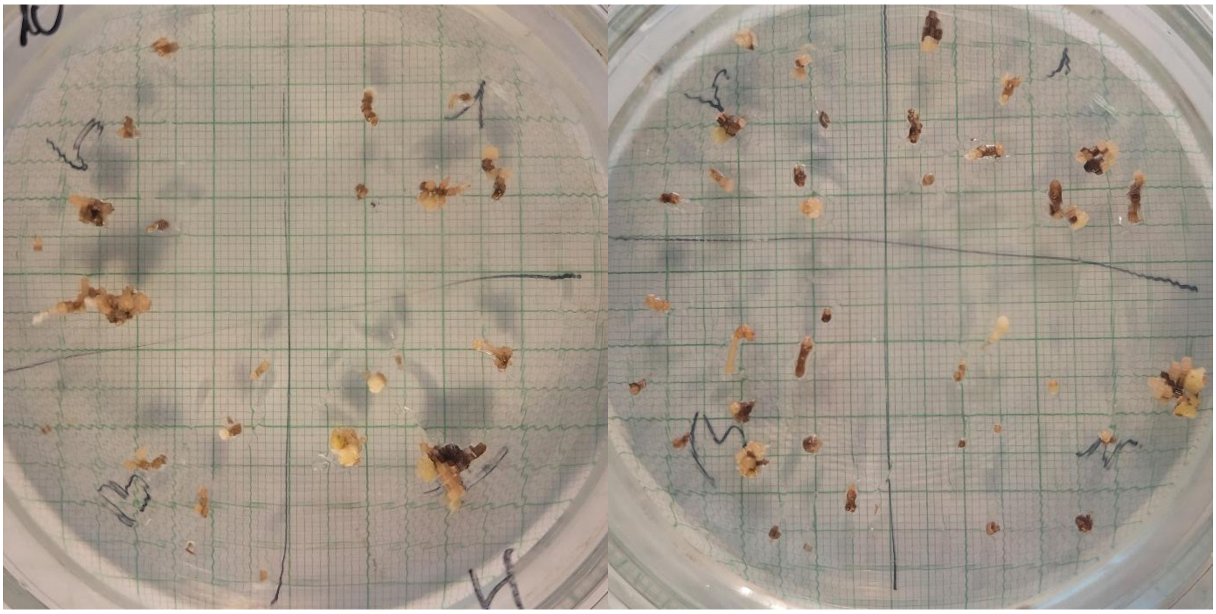

Figure 18: Tomato hypocotily (left) and cotyledod (rigth) derived "Rhizoid Tubers" on the maturation media containing 5 mg/l BAP.

In callus cell suspension cultures initiated from the 'Clarina' (K3) and 'Clementine' (K4) genotypes from the NAA-containing media, the calli derived from 'Clarina' (K3) cotyledon and hypocotyl explants showed a high capacity for callus and root formation, with visible shoot primordia too. The 'Clementine' (K4) calli showed lower proliferation and produced only a few roots. By contrast, the tomato genotypes 'Dominet' (P2) and 'Sagatan' (P4) did not generate viable calli in the liquid medium containing 10 mg/L NAA (Figure 19).

Figure 19: The cotyledon expant derived organogenic cell mass on the genotype ‘Clarina’ (K3) (left) and the non viable calli observed from the tomato genotypes.

5. Conclusions and proposals

Based on the results of our callus induction experiments in tomato and cauliflower, it can be concluded that, on average, cotyledon explants were the most suitable for callus proliferation across all tomato genotypes and in most cauliflower genotypes, except for ‘Clementine’ (K4). These results, in the case of cauliflower, indicate a greater effect of the genotype and explant than of the supplied growth regulator. According to the findings of Jan et al. (2014), in the tomato genotype ‘Peto-86’, higher 2,4-D concentrations promoted callus induction when using leaf and stem explants. In contrast, our results showed that callus growth favoured lower auxin concentrations in the genotypes examined. For cauliflower, no consistent trend could be established, as the callus growth rate varied more and showed genotype and explant effects over the applied growth regulator.

Contrary to previous studies, our experiments aimed at inducing somatic embryogenesis did not result in the formation of observable somatic embryos in either tomato or cauliflower genotypes. This could be attributed to an interaction between the auxin analogue growth regulator and the genotype (Saeed et al., 2019). Interestingly, the embryogenesis-inducing medium described by Godishala et al. (2011) for tomato induced organogenic callus formation in the cauliflower genotypes tested in our study.

The NAA-containing media successfully induced organogenic callus formation, followed by root development within a few weeks. Notably, root growth was also detected in liquid culture in the ‘Clarina’ (K3) cauliflower genotype. Previous studies on both tomato and cauliflower have reported successful shoot regeneration from root explants (Pavlović et al., 2010; Peres et al., 2001). This approach could also serve as a promising direction for further research and may provide suitable starting material for artificial seed encapsulation from liquid culture-grown propagation material.

6. Summary

The production of hybrid seed used in modern crop cultivation has become increasingly challenging due to difficulties in controlled pollination, extreme fluctuations in climatic conditions, and various technical, economic, and regulatory hardships. With advancements in biotechnology, opportunities are arising to improve and alleviate or overcome these limitations. Tomato (*Solanum lycopersicum* L.) and cauliflower (*Brassica oleracea* convar. *botrytis* var. *botrytis* L.) are both economically important vegetable crops that have been widely studied in the field of plant tissue culture. These techniques allow the *in vitro* maintenance, regeneration, and propagation of plant cells and tissues.

The aim of our research was to develop an efficient methodology using these tissue culture techniques to induce callus and produce propagation material suitable for potential artificial seed production. During our investigation, we focused on finding answers to the following questions:

- which plant-derived explant (hypocotyl, cotyledon, or first leaf) performs the best within a given genotype, in terms of callus induction, organogenesis, or somatic embryogenesis,
- to identify the most suitable combination of callus induction and somatic embryogenesis-inducing media,
- which media combination results in successful somatic embryogenesis,
- which medium combination induces organogenesis,
- what regenerative capacity is showed by calli maintained in liquid culture?

The plant material used in this study was, in the case of tomato: ‘Bobcat F1’ (P1), ‘Dominet’ (P2), ‘Hapynet’ (P3), and ‘Sagatan’ (P4). And in the case of cauliflower: ‘Amidala’ (K1), ‘Andromeda’ (K2), ‘Clarina’ (K3), and ‘Clementine’ (K4).

To achieve our objectives, two plant growth regulators were tested in callus-induction media. All three explant types for each genotype were cultured on three media variants: the “2” medium containing 2 mg/l 2,4-D and 0,5 mg/l BAP; the “4” medium containing 4 mg/l 2,4-D and 0,5 mg/l BAP; and an MS control medium without growth regulators. The results clearly demonstrated genotype- and explant-dependent differences in callus growth rate, with cotyledon explants generally showing a higher proliferation rate than other tissues. Tomato genotypes typically showed greater callus growth rate on the medium “2” than on the medium with the higher auxin concentration (4 mg/l), while a similar trend could not be observed for the cauliflower genotypes, which could only be assessed genotype by genotype.

In the next step, calli were transferred to somatic embryogenesis-inducing media. The composition of these were the following: MS + 0,05 mg/l IAA + 0,5 mg/l kinetin + 2% sucrose and MS + 3,5 mg/l BAP + 0.5 mg/l IAA + 3% sucrose. Although the formation of somatic embryos was not observed on any of the media combinations, organogenic tissue induction occurred in all cauliflower genotypes on the latter embryogenesis-inducing media. The highest number of shoots was recorded in the 'Clarina' (K3) genotype, which was then selected for our artificial seed encapsulation experiments.

The grown shoots of the 'Clarina' (K3) genotype were encapsulated in both a growth regulator-free MS and a root-inducing medium based on MS salts containing half-strength macronutrients, supplemented with 2 mg/l BAP, 1 mg/l TDZ, 1 mg/l TOP, 0,1 mg/l IBA, 2 ml/l WUXAL® Super, and 500 mg/l MES. For gel formation, 15 g/l sodium alginate and 50 mM $CaCl_2$ were used. Then the MS-based beads were put on solidified MS media, and three of the root-inducing ones were immediately planted into sterile peat pellets, while the remaining capsules were stored at 4 °C for 5, 10, and 20 days before planting. The encapsulated shoot apices were maintained under sterile conditions, and although shoot growth began in both types of capsules, root growth was not observed in either. We could not observe differences between the groups of the stored capsules.

The tomato and cauliflower genotypes were also cultured on media containing 4 mg/l NAA, which induced callus formation (only in the cauliflower genotypes) and organogenesis. Root development was observed on both cotyledon and hypocotyl explants across all genotypes, while shoot formation occurred only in cauliflower. On cotyledon explants, fewer shoots (~3) developed compared to hypocotyls (~10). In tomato genotypes, "Rhizoid Tuber" like structures could be observed, previously described as embryogenic formations by Saeed et al. (2019). These structures were transferred to an embryo maturation medium (MS + 5 mg/L BAP + 2% sucrose), where greenish tissue growth, characteristic of early embryogenic development, appeared by the third week, but maturation was not reached.

The initial callus suspension cultures, established in liquid versions of the callus induction media, showed slow proliferation. Even after periodic subculturing, no significant callus growth or embryogenic induction was observed when the cells were reintroduced onto solid media. One exception was noted in the cauliflower genotype 'Clarina' (K3), where organogenic callus developed, forming both roots and shoots. Calli derived from the 4 mg/l NAA-containing solid MS B5 media of the ‘Dominet’ (P2), ‘Sagatan’ (P4), ‘Clarina’ (K3), and ‘Clementine’ (K4) genotypes were transferred to liquid culture containing 10 mg/L NAA. The ‘Clarina’ (K3) calli originating from cotyledon and hypocotyl tissues showed a visibly high proliferating response and root formation, with the shoot apex also emerging. A similar but less pronounced response was observed for the ‘Clementine’ (K4) genotype. In contrast, tomato genotypes did not produce viable callus tissues in liquid culture.

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MATE Organizational and Operational Regulations
III. Requirements for Students
III.1. Study and Examination Regulations
Appendix 6.13: The MATE Uniform Thesis /thesis / final thesis / portfolio guidelines
Annex 4.2: Declaration of public access and authenticity of the thesis/thesis/dissertation/portfolio

DECLARATION

the public access and authenticity of the thesis

Student's name: Vasas Dominika

Student's Neptun code: AB96UZ

Title of thesis: EXPERIMENTS AIMED AT THE INDUCTION OF SOMATIC EMBRYOGNESIS WITH TOMATO (SOLANUM LYCOPERSICUM L.) AND CAULIFLOWER (BRASSICA OLERACEA CONVAR. BOTRYTIS VAR. BOTRYTIS L.) GENOTYPES FOR THE PURPOSE OF ARTIFICIAL SEED PRODUCTION

Year of publication: 2025

Name of the consultant's institute: Institute of Genetics and Biotechnology

Name of consultant's deparment: Department of Genetics and Genomics

I declare that the final thesis[1] submitted by me is an individual, original work of my own intellectual creation. I have clearly indicated the parts of my thesis or dissertation which I have taken from other authors' work and have included them in the bibliography. Furthermore, I declare that the artificial intelligence tools (e.g. text generation, linguistic correction, translation, data analysis) used during the preparation of the thesis did not substitute my own research and creative work; their use was indicated either in the list of sources or in the methodology section, and I acted in accordance with professional and ethical expectations.

If the above statement is untrue, I understand that I will be disqualified from the final examination by the final examination board and that I will have to take the final examination after writing a new thesis.

I do not allow editing of the submitted thesis, but I allow the viewing and printing, which is a PDF document.

I acknowledge that the use and exploitation of my thesis as an intellectual work is governed by the intellectual property management regulations of the Hungarian University of Agricultural and Life Sciences.

I acknowledge that the electronic version of my thesis will be uploaded to the library repository of the Hungarian University of Agricultural and Life Sciences. I acknowledge that the defended and

- not confidential thesis after the defence
- confidential thesis 5 years after the submission

will be available publicly and can be searched in the repository system of the University.

Date: 2025 year october month 31 day

Student's signature

DECLARATION

Vasas Dominika (name) (student Neptun code: AB96UZ)
as a consultant, I declare that I have reviewed the final thesis/[✓ thesis]/dissertation/portfolio[1] and that I have informed the student of the requirements, legal and ethical rules for the correct handling of literary sources.

[✓ I recommend] / do not recommend[2]

the final thesis / dissertation / portfolio to be defended in the final examination.

The thesis contains a state or official secret: yes [✓ no]*[3]

Date: 2025 year october month 31 day

insider consultant

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[1] The other types should be deleted while retaining the corresponding thesis type.

[2] The appropriate one should be underlined.

[3] The appropriate one should be underlined.

MATE Organizational and Operational Regulations
III. Requirements for Students
III.1. Study and Examination Regulations
Appendix 6.13: The MATE Uniform Thesis /thesis / final thesis / portfolio guidelines
Annex 7: Declaration of independent supervisor for Institute of Technology

DECLARATION OF INDEPENDENT SUPERVISOR

As independent supervisor of

Vasas Dominika (name of student) (Neptun code of student: AB96UZ)

I declare, that the student regularly attended the pre-scheduled consultations.

Date: 2025 year october month 31 day

independent supervisor

Declaration of Students and Doctoral Candidates on the Use of Artificial Intelligence (AI)"

1. general information:

Name of the student:	**Vasas Dominika**
Neptun ID:	**AB96UZ**
Level of program (mark with X):	☐ BSc/BA ☒ MSc/MA ☐ Doctoral School (PhD) ☐ Other: ....
Name and code of the subject*:	
Title of the work:	**EXPERIMENTS AIMED AT THE INDUCTION OF SOMATIC EMBRYOGNESIS WITH TOMATO (SOLANUM LYCOPERSICUM L.) AND CAULIFLOWER (BRASSICA OLERACEA CONVAR. BOTRYTIS VAR. BOTRYTIS L.) GENOTYPES FOR THE PURPOSE OF ARTIFICIAL SEED PRODUCTION**

* Not required to be completed in the case of a doctoral dissertation.

2. Declaration on the Use of AI

I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

(Please choose one of the options below!)

☐ A) I have not used any artificial intelligence system or service.

(If you selected this option, completing the subsequent tables is not required.)

☒ B) I have used an artificial intelligence system or service.

(Please fill in the relevant tables!)

3. Details of Artificial Intelligence Usage

TABLE I: Assistant or Minor Usage (e.g., translation, language proofreading, brainstorming, etc.)

(For these uses, attaching the specific prompts and responses is not required.)

Purpose of Use	**Name and Version of the AI Tool Used**	**Affected Section (if not applicable to the entire text)**
Translation	Grammarly, web-based version in October 2025	

TABLE II: Significant Content Contribution (e.g., generating an entire figure or a longer text section)

(In these cases, documenting the key prompts used and the raw responses provided by the AI, and attaching them as an appendix to the work, is required.)

Purpose of Use	Name, Version, and Access Information of the AI Tool Used	Exact Number of the Affected Chapter / Figure / Table	Entry Number of the Appendix Containing the Prompt Log

3/A. Additional Rules Prescribed by the Lecturer (if any)

If the instructor or supervisor of the course has established specific rules or expectations regarding the use of AI tools, please summarize them in the field below:

For example: prohibition of AI use for certain types of tasks; only specific tools are permitted; different citation requirements; documentation format, etc.

Rules Prescribed by the Lecturer or Supervisor

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4. Declaration Applicable to All Students:

I declare that I have critically reviewed, edited, and incorporated any content potentially generated by AI in all cases. I take full responsibility for every element of the submitted work, including its originality and scientific validity. I acknowledge that the Hungarian University of Agriculture and Life Sciences may check the submitted work with an artificial intelligence detector and may initiate proceedings if my declaration is found to be false or incomplete.

Place and Date: Gödöllő, 2025. October month 31 day

………………………………………………………… ……………………………………………………

Signature of the Student **Signature of the Advisor/Supervisor**