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EVOLUTION OF A METHOD FOR CALLUS INDUCTION OF STRAWBERRY (FRAGARIA ANANASSA DUCH)

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Abstract:

The aim of this study is to generate callus, while the aim of the experiment is to explore the influence of growth regulators on callus formation in young and mature strawberry leaves (Childers and Rosa Linda). The initiation of Callus from the meristematic cells of both Chandler and 'Li-Na' varieties was achieved. Various concentrations of NAA and Kin on Ms media, under dark and light conditions, were used to induction callus. The results showed that the greatest number of callus formation in Chandler and Rosa Linda occurred in the medium of multiple sclerosis that had 2 mg/l NAA with 0.3 mg/l Kin in dark conditions. Additionally, our protocol employs meristems as the source of detection for the induction of callus in both types of meristems. The protocol described in this study can be valuable in creating an in vitro system for strawberries, which holds great potential for genetic transformation and crop improvement.

Keywords: Strawberry, Growth Regulators, Callus Induction

1. INTRODUCTION

Tissue culture is considered to be aseptic culture of cells, tissues, organs or entire plants that are below the exact conditions of nutrition and habitat (Wijerathna-Yapa et al., 2023). that can be employed to create clones of plants. The resulting clones are genetically identical to the selection'sotype. The structured conditions often facilitate the culture of the environment in their progression and improvement. These factors have a full complement of nutrients, a pH that is medium, a temperature that is appropriate, and they have a full complement of gaseous and liquid conditions. The tissue culture method for plants can produce large quantities of plant tissue. Other than their function as a research tool, during the past few years, plant tissue culture methods have been utilized as an important industry context for the propagation of plants, the removal of disease, the development of plants and the production of secondary metabolites. Small pieces of tissue (termed explants) are able to produce multiple, successive plants using a nonstop process. One explant can be replicated into multiple thousand plants in a short period of time, regardless of the season or climate, on a year-round Basis (Kulak, 2022). Threatened, endangered and rare species have been cultivated and preserved by micropropagation as many developmental stages and smaller loads on a smaller number of plants in a preliminary space. Also, the tissue culture of plants was recognized as the most effective method for enhancing crops by creating both somaclonal and gametoclonal variants. The micropropagation method has a tremendous capacity to produce plants with a higher quality that are separate from the efficient variations of plants that have a well-disease-resistant and stress-management capabilities.(Chokheli and all. 2020). Which leads to the development of plants that are commercially significant? The production of plants via micropropagation has different advantages than the traditional method of propagation, which involves seeding, cutting, grafting and air-laying. It was considered to be a rapid procedure for the propagation of plants that lacked viruses (Hasnain, 2022). The culture of meristematic plants without the top part of a Banana has been accomplished (Lassois et al., 2013).

MATERIALS AND METHODS

To conduct the aforementioned experiment, we collected both young and mature leaves and internodes from Strawberry varieties 'Chandler' and 'Rosa Linda' at the Mashkhab Center for Scientific Research Laboratory. To ensure cleanliness, the explants were initially soaked in tap water for 30 minutes and then treated with a 1% concentration of Tween 20 for ten minutes. Afterward, they underwent multiple rinses with sterile distilled water. Further sterilization was carried out using aseptic techniques within a cabinet with a linear flow of air. The explants were sterilized with 50% (v/v) ethyl alcohol for 1 minute, followed by 0.1% (w/v) HgCl2 for 7 minutes. Finally, the explants were rinsed with sterilized water five times before being cut into appropriate sizes for cultivation on MS media (1.5 cm).

CALLUS INDUCTION:

Meristem cells from strawberries were cultivated under sterile conditions using a callus induction medium consisting of MS medium supplemented with vitamins, NAA (1, 2, 3 mg/L) and Kin (0, 0.3, 0.6 mg/ L) growth regulators,)3% sucrose(, and 3g/L gelrite per liter of medium. Prior to sterilization, the pH of the medium was adjusted to match the cultures, After that, the stock was split into two parts: one part was incubated in darkness or under a 16/8-hour light cycle at a temperature of 25±2°C for a duration of four weeks, with subculturing taking place every two weeks. The data resulting from this was described as the percentage of callus formation. Following the four-week period, the percentage of callus formation was recorded as a frequency.

The percentage of induced callus represents the ratio of the total number of plants that exhibited callus formation to the total number of plants that underwent cultivation. The rate of callus growth was quantified by measuring the increase in volume per day, while the density of the callus was determined by measuring the increase in density per day. Additionally, the improvement in overall quality was assessed by measuring the increase in quality per day.

STATISTICAL ANALYSIS:

Using a completely randomized design known as a Kurd, the experiment employed a factorial design. The factors included callus induction under two different photoperiod conditions: a dark photoperiod or 16/8 hours of light/dark. Additionally, two factors were manipulated at three different levels: NAA (1, 2, 3 mg/L) and relatives (0, 0.3, 0.6 mg/ L). To ensure reliability, ten replicas, each comprising three samples, were created for this study. Prior to conducting the variance analysis, the normality of the data was assessed using SAS software . To determine significant differences between treatment means, the Duncan multiband test (destroyed) was utilized, with a level of $\alpha = 0.05$ serving as the threshold for a significant difference.

RESULTS AND ANALYSIS

Callus induction under dark conditions.

The regeneration of plants and the formation of callus in two different varieties of Strawberry, namely 'Chandler' and 'Rosa Linda', were the subjects of experimentation. To induce callus, the scarified region of the Strawberry's meristem was utilized. The explant used for callus formation was the meristem, and the medium employed was MS media (Murashige and Skoog, 1962), which consisted of varying concentrations (1,2, and 3 mg/L) of NAA and (0, 0.3, and 0.6 mg/L) of Kin. The experiments were conducted both in darkness and under a 16/8 hour photoperiod. Interestingly, the percentage of callus formation was higher in dark conditions compared to the 16/8 hours of light. The results presented in Fig 1,2 revealed that Rosa Linda' exhibited the highest frequency of callus induction in dark conditions , while Chandler had the lowest . Furthermore, the expansion rate of callus was evaluated in both darkness and under a 16/8 hour photoperiod, using different concentrations.

Table (1) displays the effects of NAA and Kin on callus development in various strawberry varieties. Interestingly, two of the varieties exhibited exceptional rates of callus development when cultured in a medium containing 2 mg/L NAA and 0.3 mg/L Kin in the absence of light. This specific mixture of 2 mg/L NAA and 0.3 mg/l relatives was most effective in promoting the growth of callus in the 'Chandler' and 'Rosa Linda'varieties. As a result, to promote the growth of callus, the medium was increased by 2 mg/L NAA and 0.3mg/L of relatives in the dark.

Callus induction under 16/8 hour photoperiod.

Callus induction was performed using mature embryos such as implantation and a photoperiod of 16/8 hours. To achieve this, Ms media with varying concentrations of NAA and Kin were used. Fig 1,2 contains the results of callus induction for the strawberry varieties 'Chandler' and 'Rosa Linda'. It was noted that the inclusion of NAA and Kin in the center of Ms had a positive effect on callus induction for both types. Under a 16/8-hour light period, the use of NAA 2 mg/l + Ken 0.3 mg/L resulted in the highest callus induction percentages for the Chandler variety, achieving 66.7% and 72.20% respectively. Meanwhile, for the Rosa Linda variety, the percentages of callus induction were 92.2.00% and 82.00% when using NAA2 mg/l + Ken 0.3 mg/L . Statistical analysis revealed significant effects of different varieties and interactions between varieties and plant growth regulators. Among the varieties, 'Chandler' exhibited the highest rate of callus induction at 86.00%, while 'Rosa Linda' had the lowest rate at 73.90% . Furthermore, noticeable variations were observed in the size of callus development.

Fig 2 reveals notable variations in both the different varieties tested and the concentrations of NAA and Kin under a 16/8 hour photoperiod. To evaluate the impact on callus growth rate, different concentrations of NAA and Kin were employed. The treatments exhibited significant differences in growth. The data unequivocally show that a concentration of 2 µg/L of NAA alone leads to increased callus growth in both the 'Chandler' and 'Rosa Linda' strawberry varieties, resulting in the highest growth rate.

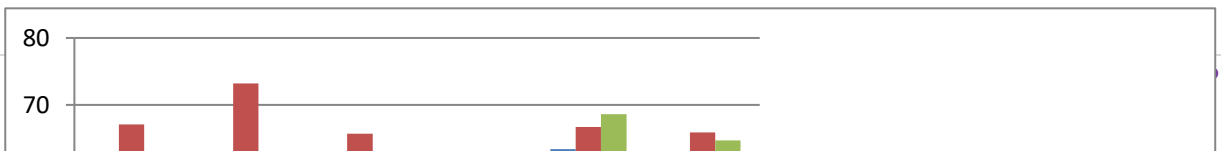


Figure 1: The impact of varying concentrations of NAA and Kin, as well as different lighting conditions (darkness or a 16/8 hour photoperiod), on the percentage of callus induction in Strawberry var. Chandler was examined.

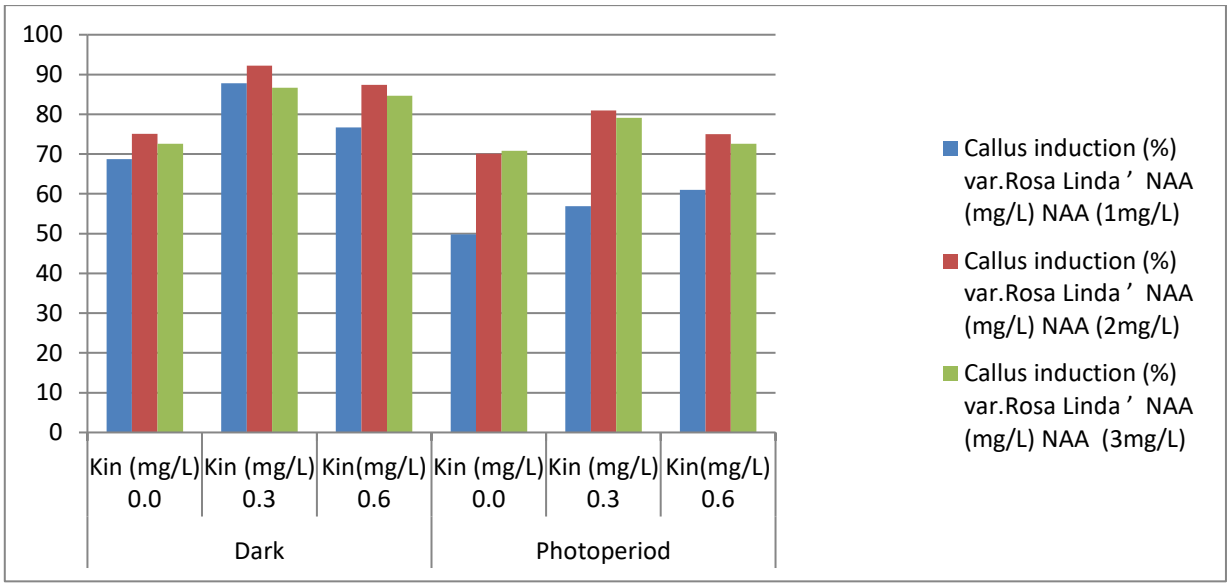


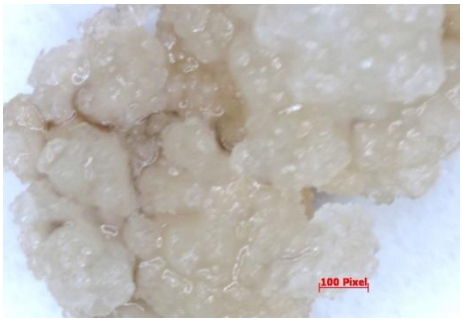
Figure 2: The percentage of callus induction for Strawberry var. Rosa Linda was examined under various conditions, including different concentrations of NAA and Kin, as well as different illumination settings (dark or a 16/8 hours photoperiod).

Table (1) The growth of callus in Strawberry var. Chandler' and 'Rosa Linda' was studied under various concentrations of NAA and Kin, as well as different lighting conditions (16/8 hours photoperiod or dark).

		Calli incitement (%) var Chandler		Calli incitement (%) var. Rosa 'Linda	
NAA (mg/L)	Kin (mg/L)	Dark	16 hours light	Dark	16 hours light
1	0	+	++	++	+
	0.3	+	++	++	+++
	0.6	+	++	++	+++
2	0	++	++++	++	+++
	0.3	++++	++	++++	++
	0.6	+++	++	+++	+++
3	0	++	++	+++	+++

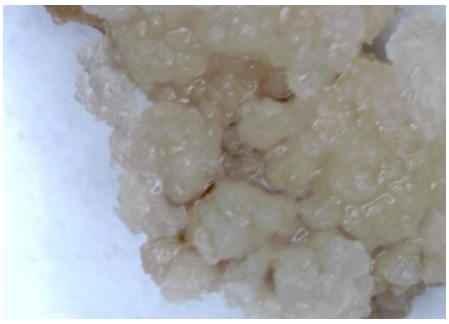
	0.3	++	++	+++	+++
	0.6	+++	++	+++	+++

C (+), D (++), B (+++) A (++++) C .very poor D poor B good A very good



A

C



B

D

A: Callus induction after 4 weeks on MS medium supplemented with 2.0 mg/l NAA and 0.3 mg/l Kin (Dark) var Rosa Linda

B: Callus induction after 4 weeks on MS medium supplemented with 2.0 mg/l NAA and 0.3 mg/l Kin (Dark) var Chandler

C: Callus induction after 4 weeks on MS medium supplemented with 2.0 mg/l NAA and 0.3 mg/l Kin (light) var RosaLinda

D: Callus induction after 4 weeks on MS medium supplemented with 2.0 mg/l NAA and 0.3 mg/l Kin (light) var Chandler.

DISCUSSION

Callus refers to a mass of unorganized parenchymatous cells derived from plant tissue. The tissues used to initiate callus formation depend on plant tissues and growth hormone used. Generally, a higher auxins concentration in growth medium induces callus formation. The quality and quantity of callus mass depends on various factors like explants, plant growth regulators and light/dark incubation etc. Among different growth regulators Kin and 2, 4-D were found to be most suitable for callus induction in strawberry. For the successful implementation of a highly effective method of Strawberry transformation, it is imperative to generate a callus from a meristem. Numerous tries have remained showed on suitable explants in Strawberry to improve the proliferation of meristem cells in culture. In this study, a significant increase in callus induction was observed when incorporating NAA Indole-3-acetic acid and Kin into the fertilizer. However, this combination proved to be crucial for callus production. Throughout the history of callus culture, plant growth regulators have played a significant role, then impact of different Plant hormones on plant redevelopment in Strawberry callus cultures has been extensively studied (Maiman et al., 2013 and Sahida et al., 2022). Typically, the media used for the induction and propagation of strawberry plants requires a strong auxin, NAA Indole-3-acetic acid is the most commonly used. Plant regeneration media, on the contrary, lack a growth regulator or consist of a weak auxin with a cytokine. On the contrary, exposing callus cultures to high auxin concentrations with cytokines led to the formation of radicals (Dar et al., 2021). The interaction between cytokine-containing auxins and the plant's ability to perceive and respond to growth regulators is said to have multiple important effects, as documented by emenecker et al.(2020). Our results indicate that the administration of NAA Indole-3-acetic acid and its relatives in most cases has a positive effect on callus induction and plant growth. Similar conclusions were made in strawberries by Abbas et al.(2013). These collective findings will have a significant impact on increasing the frequency of callus induction, and this is necessary for the development of crops and the rapid spread of plants through biotechnology, according to Villalobos-Ishimbiz et al.(2022). In addition, the type of implantation genotype can have a significant impact on callus induction. To circumvent this, we tried different lighting configurations and concentrations of NAA and relatives to increase the

percentage of callus formation and growth (Mahood et al., 2022). Light has a significant role in the induction of calluses, the growth of cells, and the production of plants, as documented by Cavallaro et al.(2022). Gago et al. (2014) also documented the significant impact of light in the in vitro procedure. The effect on the progression of organized tissues is documented in detail. Distinguishing the light conditions that are most beneficial to growth involves multiple factors, including light quality, intensity, and daily duration (Mehbub et al., 2022). The response to light is different for different cell types and the Strawberry plant's genotype (Roosta et al., 2024). Interestingly, Strawberries that were grown in darkness had a higher cell mass than those that were grown with a light-dark cycle of 16 hours of light and 8 hours of darkness (Chung et al., 2022). Dark conditions are beneficial for creating beautiful Strawberry plants that promote cell growth (Zhao et al., 2023). While the increase in callus typically promotes embryogenesis in both dark and light conditions, dark conditions specifically lead to an increase in the number of plants/seedlings and a higher percentage of successful plantings (Pathi et al., 2013). It's important to note that light is not necessary for the growth of callus, and many investigations will form callus in the absence of light (Cavallaro et al., 2022). However, some research suggests that light can be employed to promote callus growth and maintain its potency (Adil et al., 2019). The potential for superior cald quality and increased cell growth is apparent in the strawberry cald grown in darkness compared to the 16/8 hour light/dark cycle, as mentioned by Zhao et al.(2023). The induction of a highly active mitotic state, facilitated by DNA hypermethylation caused by NAA, promotes the creation of embryos, as demonstrated by Ji et al.(2019). Pires (2020) described the procedure of producing somatic embryos from the stem cell population of strawberries in the dark,

CONCLUSION

The optimal concentrations of media for callus formation were determined based on the significant impact they have on the formation of callus. In our research, the greatest amount of callus formation in Chandler was greater than that in Rosa Linda, the increase in the latter was achieved by culturing the stem cells on media that was supplemented with 2 mg/LNAA and 0.3 mg/L Kin in the dark. However, NAA with Kin has an effect on the generation of callus.

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