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Determination of vitamin C levels in callus, regenerated shoots and tap roots from carrot (*Daucus carota* L.)

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Abstract

One of the essential root vegetables that is abundant in bioactive substances like vitamin C is the carrot. There is a great deal of concern and research about the variation in vitamin C content of fresh *Daucus carota*. With an emphasis on tissue culture especially, this study seeks to advance existing approaches in the field of plant biotechnology. callus induction utilizing MS media supplemented with various concentrations of NNA and BA from 2cm pieces of hypocotyls. through using MS media supplemented with 1 mg/L NAA + 4 mg/L TDZ under light luminescence, shoots were regenerated from calluses. Vitamin C levels were measured in the mother plant's tap roots, regenerated shoots, and callus tissue by using UV spectrophotometry method. The findings showed that vitamin C concentration in callus tissue and newly grown shoots was superior to that in tap roots and mother plants, and that the level of vitamin C increased in newly grown shoots of plants.

Keywords: *Daucus carota,* Vitamin C levels, Shoot regeneration.

Introduction

Fruits and vegetables are high in minerals and phytochemicals, which are known for their nutraceutical and health advantages (Tiwari & Cummins, 2013). The plant generated about 3000 chemical compounds that are extensively utilized in a variety of applications such as medications, food coloring, and colors (Núñez et al., 2006). The cultivated carrot *D. carota* L. is one of the most well-known vegetable plants in the world due to its great production capacity and usage as a fresh or processed product (Dawid et al., 2015). Because of their high nutritional value and exceptional preservation properties, they serve an important role in human nutrition (Leja et al., 2013).

D. carota L. consider a good source for carbohydrate, mineral and vitamin include vitamin A and vitamin C (Surbhi et al., 2018). Vitamin C plays an important role in the body's defense against bacterial and viral infection, iron absorption, wound and tissue healing, collagen formation, and reducing the body-harmful effects of free radicals (Igwemmar et al., 2013).

Carrots have more vitamin C content than plums, pears, and grapes, among other fruits and vegetables. The overall vitamin C concentration in plant tissues increasing throughout summer due to the increased strength of light. Vitamin C concentrations have also been seen to be influenced by the horticulture crops' maturity at harvest time, with reduced concentrations as carrot age at harvest time rose (Leong & Oey, 2012). It seems acceptable to employ tissue culture methods for its production given the influence of environmental and genetic variables on the level of carotene and vitamin C present in fruits, seeds, and leaves. Since then, several papers have been published on the effect of various growth factors on callus initiation (Khashan & Muhsin, 2015).

Materials and Methods

Callus Induction and Shoots Regeneration: Sterile seedlings production: For seed culture, *D. carota* seeds were used. Healthy seeds were cleansed with detergent water and kept in running water for one hour. After being submerged in a solution of sodium hypochlorite (2%) for 15 minutes, 70% ethanol for one minute, and finally 3-5 times in sterile water, the seeds were surface sterilized. (Awika, 2004).

Callus Induction: Seeds were cultured on free MS medium. After two weeks of cultivation resulted in

the germination of seeds with typical roots and shoots. Cotyledons from the in vitro grown seedlings (two weeks old) of *D. carota* were used as source of explants and cultured on MS medium (Murashige and Skoog, 1962) the MS medium supplement with different concentration of NNA and BA (Table 1). The media placed in the dark at with temperature of 25±1°C. Callus initiated after 4 weeks grown in culture media (Pant & Manandhar, 2007).

Shoots Regeneration: One gram each, of green and healthy callus were transferred to 30 ml of the selected regeneration of agar-solidified medium (MS + 1 mg/L NAA + 4 mg/L TDZ) in 100 ml volume glass jars, then maintained in this medium for about a 4 weeks and incubated under light luminescent 3000 Lu×24/day at 24±1 C to regenerate shoots from callus (A Mohammed & K Al-Mallah, 2013).

Vitamin C determination: According to (Khan et al., 2006), the vitamin C content was calculated. The amount of vitamin C is stated as mg/100 g of fresh weight.

Preparation of solutions:

5% Metaphosphoric acid -10% acetic acid: 15 g of solid metaphosphoric acid were dissolved in a combination that also contained 450 ml of deionized water, 40 ml of glicial acetic acid, and 500 ml of volumetric flask. The mixture was then filtered to remove the particles, and the liquid was collected.

85% Sulphuric acid Added 85 ml of sulphuric acid to volumetric flask and complete the volume to 100 ml by deionized water

The following items were purchased: 10% Thiourea solution (Loba Chemie) 2,4-Dinitrophenyl-hydrazine solution (sigma).

Standard vitamin C solution: To create a 500ppm stock solution, 0.1 g of standard crystalline ascorbic acid was dissolved in 1 L of distilled water then create the calibration solution (1-20 ppm).

Sample preparation: After homogenizing a 1 g sample with around 5 ml of a 5% metaphosphoric acid-10% acetic acid solution, a homogeneous dispersion was produced. After that, the sample was quantitatively transferred into a 10 ml volumetric flask and obtained with a gentle shake. Then, to adjust the concentration, the 5% metaphosphoric aceid-10% acetic acid solution was added. In order to determine the sample's vitamin C level, the solution was then filtered, and the clear filtrate was kept.

Estimation of the Amount of Vitamin C: The filtered sample solution was treated with a few drops of bromine water until the color changed. The surplus bromine was then removed by adding a few drops of thiourea to create a clean solution. Following that, the normal and oxidized ascorbic acids were fully incorporated into the 2,4-Dinitrophenyl-hydrazine solution. Using a spectrophotometer and the 2,4-Dinitrophenyl-hydrazine dye coupling process to measure total vitamin C.

Result and Discussion

Calibration Curve: Ascorbic acid stock solution with a 500 ppm concentration was used to produce a series of solutions for the calibration curve. The ascorbic acid concentration in each sample was calculated by graphing the concentration range (1–20 ppm) vs the matching absorbance.

Seed germination: After three days of inoculation, the D. carota seeds that were grown on MS medium began to germinate. All of the plantlets grew healthy roots and shoots. 81% of the seeds overall grew after being planted.

Callus production: Table 1 showed result of callus induction by different concentration of hormone. The highest callus frequency was observed on MS medium supplemented with 1.0 NAA+ 1.0 BA. The results demonstrated that NAA is essential for the continuity of carrot callus production in certain concentration and induction of callus for short time when compensation with BA at equal concentration. (Hutchinson et al., 1994) report the presence of BA besides NAA is important to enhance steadily production of callus. According to a similar publication, an MS medium containing 1.0 mg/L of both NAA and BA resulted in the callus of up to 83.3% of carrots (A Mohammed & K Al-Mallah, 2013).

Shoot regeneration: The findings showed that the placement of stem callus on MS medium consist of .0 mg/L NAA and 4.0 mg/L TDZ resulted in a significant capacity for shoot regeneration Table (2). After transferring calluses onto the regeneration media for 4 weeks, the shoots started to regenerate. In these trials, adding TDZ to NAA in MS media resulted in excellent shoot development. According to (Murthy et al., 1998), a TDZ is a powerful regulator of cotton ball in vitro plant morphogenesis. This could be because TDZ, which is thought to as a cytokinin that stimulates shooting, may really operate as a modifier of endogenous cytokinin metabolism and auxin due to its high concentration (Taha et al., 2009).

Table (1): The period of callus induction by using different type of media

Induction media (mg/L)	N.O induction	Callus initiation %	Period of initiation
MS+ 0.5 NAA	15	42.9	4 weeks
MS+ 1 NAA	27	77.1	4 weeks
MS+0.5 BA	4	11.4	6 weeks
MS + 1 BA	8	22.9	7 weeks
MS+0.5 NAA+ 0.5 BA	12	34.3	6 weeks
MS+0.5 NAA+ 1.0 BA	12	34.3	6 weeks
MS+1.0 NAA+ 0.5 BA	18	51.4	5 weeks
MS+1.0 NAA+ 1.0 BA	30	85.7	3 weeks

Table (2): Effects of adding 1.0 mg/L NAA and 4.0 mg/L TDZ to MS media on carrot (*Daucus carota* L.) shoot regeneration

Regeneration medium	Callus segment	Shoots formation	Period of shooting
MS+1NAA+4 TDZ	126	79.4 %	4 weeks

Vitamin C determination: Results, shown in the figure-1 illustrates the differences in vitamin C content affected with the concentrations of plant hormone in callus culture media. Observed the highest of vitamin C content in callus grown on nutrient medium supplemented by equal amount of NAA and BA 1mg/L. Shoot regeneration from callus tissue culture significantly differ in their vitamin C content characteristics from tap roots of donor plants. The finding of investigation indicates increasing of plant hormone in media for callus induction lead to increase of vitamin C level.

(Ho et al., 2021) indicate the NAA and BA concentration effected on bioactive content in root cultures (Singh, 2019) reported increase of NAA markedly increased the Vitamin C content of the fruits. Also (Khashan & Muhsin, 2015) reported the vitamin C, Carbohydrate and β-carotene content of carrot affected with the concentrations of plant hormone in callus culture media.

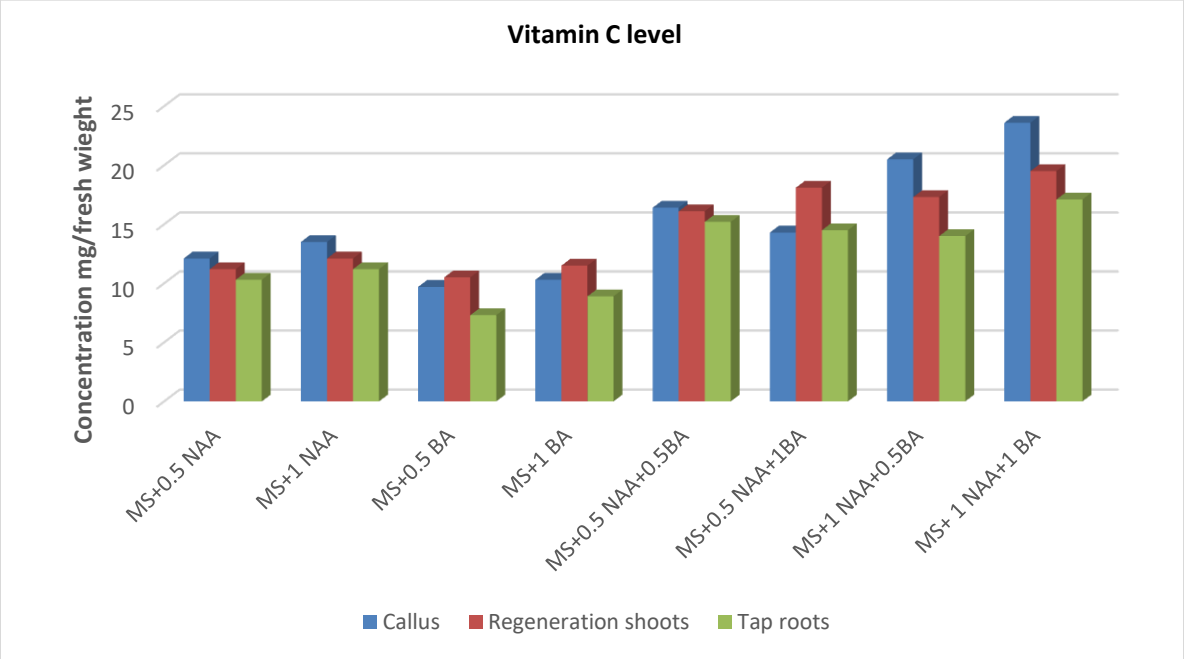

Figure (1): The effect of different types of media in vitamin C level in callus, regeneration shoots, and tap roots.

Conclusion

The growth and development of *D. carota* can be significantly influenced by the concentrations of plant hormones, particularly NAA and BA. MS medium supplemented with 1.0 NAA and 1.0 BA showed the highest callus frequency and resulted in callus induction for a short period. Additionally, adding TDZ to NAA in MS media resulted in excellent shoot development. Moreover, increasing the concentration of plant hormones in the media for callus induction led to an increase in the vitamin C level. Therefore, the use of plant hormones can be a valuable tool in enhancing the growth and development of *D. carota*, especially in callus culture and shoot regeneration, which can have implications for biotechnology and plant breeding.

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