Influence of different concentrations and combinations of cytokinins and auxins on the proliferation of tomato (*Lycopersicon esculentum* Mill.) callus tissue

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***In vitro*, the effect of culture medium differing in the concentration and combination of plant growth regulators auxins and cytokinins on the callusogenesis and proliferation processes of two tomato cultivars H-2274 (Heinz) and Gardemarin belonging to the (*Lycopersicon esculentum* Mill.) species was studied. The best indicators of callus formation and proliferation of all three explants used in the H-2274 variety were observed when cultured in D variant (4 mg/l kinetin + 1 mg/l indolylacetic acid (IAA) for 8 weeks. Except for option F (BAP + NAA), there were no significant differences in the frequency of callus induction among the remaining 3 media tested. Callus induction and proliferation were lower in this variant than in other variants. Callus induction was poor in all variants tested in cultivar Gardemarin. The cultured callus tissue and nutrient medium in variant M (BAP-4mg/l + 2mg/l 2,4-D) started to darken in the second subcultivation period. Probably, callus tissue and darkening of the medium in variant M can be related to both hormonal content and genotype.**

***Keywords**: Tomato cultivars, in vitro, cytokinin, combination of phytohormones, darkening of the medium*

INTRODUCTION

Tomato belongs to the *Solanaceae* family due to its perennial nature, but it is cultivated commercially as an annual crop. As it contains a large amount of minerals and vitamins tomato is considered one of the most important vegetables in the human diet. Tomato (*Lycopersicon esculentum* Mill.) is rich in important substances such as vitamins A and C, flavonoids, zeaxanthin, lutein and lycopene. Studies show that eating tomatoes and tomato products is more effective than taking lycopene supplements in terms of heart health benefits. (Burton-Freeman et al., 2014) Other studies have shown that high blood lycopene levels are associated with lower mortality in people with metabolic syndrome, a group of risk factors that increase the risk of heart disease, diabetes and stroke. (Mordente et al., 2011) Lycopene in its composition protects cells and organs in the human body from harmful effects.

In recent years, scientists' interest in the tomato plant as a model plant has increased significantly due to the sequencing of its genome (The Tomato Genome Consortium, 2012). Tomato is a successful model for both basic and applied research programs. This is due to its ability to grow under different cultivation conditions, relatively short life cycle, relatively small genome (950 Mb), lack of gene duplication, nutritional value, as well as having several beneficial properties such as anticancer and antioxidant capacity (Khuong et al., 2013). The above characteristics show that tomato (*Lycopersicon esculentum* Mill.) has great potential for transgenic applications. The introduction of quality traits into commercial tomato cultivars is important to increase their nutritional value, yield and resistance to environmental factors (Gerszberg et al., 2015b). It is possible to obtain genetically improved plants for commercial purposes using biotechnological approaches. Creating simple and efficient regeneration systems is a fundamental premise for transformation. In vitro tomato culture has been successfully used for various biotechnological purposes, including clonal propagation of valuable commercial cultivars, virus-free plants, and genetic transformation. (Namitha and Negi, 2013; Li et al., 2011; Yarra et al., 2012; Namitha et al., 2013; Li et al., 2011). Exogenous phytohormones in the culture play an important role in the regulation of callus induction and organ differentiation or rooting.

Numerous researchers report that auxins - IAA, NAA (α-naphthaleneacetic acid), 2, 4-D (2,4-dichlorophenoxyacetic acid), and cytokinins - ZT and 6-BAP are are the hormones commonly used to improve callus induction and regeneration in tomato culture in vitro (Kantor et al., 2010;

Mamidala et al., 2011; Zhang et al., 2012).

Other researchers have tested KIN (kinetin), 2iP (6-(c,c-dimethylallyl amino purine), TDZ, and IBA (indole-3-butyric acid).. (Ashakiran et al., 2011). Rashid and Bal demonstrated that MS-medium enriched with kinetin 0.5 mg/l and BAP 0.5 mg/l was optimal for direct regeneration. (Rashid et al., 2010).

The current study focused on the study of the effect of nutrient media differing in the concentration and combination composition of plant growth regulators on the callusogenesis and morphogenic response of two tomato cultivars belonging to the species (*Lycopersicon esculentum* Mill.) *in vitro*.

MATERIALS AND METHODS

Two varieties of tomato plant (*Lycopersicon esculentum* Mill.) H-2274 (Heinz) and Gardemarin were used as research objects. H-2274 is a quick-ripening, thick-skinned, oval-shaped fruit, with an average fruit weight of 140-160 g. The Gardemarin variety is a medium-ripening variety, growing up to 70 cm in height, smooth, bright red, and highly productive. The seeds were first kept in 70% ethyl alcohol for 10 seconds and then washed with autoclaved distilled water. After that, the seeds were kept in -5% NaOCl solution for 20 minutes and surface sterilized. After sterilization, the seeds were washed repeatedly with distilled water until the chemical solution was removed. Once the seeds were ready for germination, they were planted in test flasks in a pre-prepared ½-thickness Murashige and Skoog (MS) (Murashige et al., 1962) nutrient medium. Macro elements, microelements, $CaCl_2$, chelate and agar were added to the composition of MS nutrient medium. All manipulations were carried out under aseptic conditions under laminar boxing. The seeds were first stored in the dark at a temperature of 25ºC. Seedlings were observed after about 2-3 days and then switched to 16 hours of light and 8 hours of darkness regime. Hypocotyl, cotyledon, epicotyl developed in 12-14-day-old plants. In order to transfer the obtained tomato explants to culture, the cotyledon, hypocotyl and epicotyl parts were placed in standard MS medium. The leaves were cut into approximately 8 mm segments and the adaxial surface lowered on the medium, the hypocotyl was divided into approximately 5 mm segments horizontally, and the epicotyl was placed vertically and cultured. 0.5 mg/l nicotinic acid, 0.1 mg/l thiamine, 0.5 mg/l pyridoxine, 2 mg/l glycine, and 100 mg/l inositol were added to the standard MS nutrient medium. The pH of the medium was measured between 5.5-5.6. As an inducer of callus formation, auxin and cytokinins were used in different concentrations and combinations. Phytohormones were added to the environment for primary callus induction according to the following scheme (Table 1).

Table1. Composition of culture media used for callus induction and proliferation of tomato

	Medium code	Composition
1	MS1	without phytohormones (A)
2	MS2	BAP-4mq/l+ 0.1mq/l IAA (C)
3	MS3	Kinetin-5mq/l +1mq/l IAA (D)
4	MS4	BAP-3mq/l + 0.5 mq/l NAA (F)
5	MS5	BAP-4mq/l+2mq/l 2,4-D (M)

20 explants were taken for each variant. Experiments were carried out in 2 repetitions.

RESULTS AND DISCUSSION

The seeds of both varieties of S. *esculentum* tomato plant were germinated in ½ MS medium (without hormones). After 7 days, H-2274 (Heinz) had a germination rate of 98%, while Gardemarin had a very low germination percentage. Only 12 of the planted seeds germinated. 10 days after germination, the sprouts reached a height of 3-4 cm.

Explants were selected from 3 different parts of 10-12-day-old seedlings and placed in a callus-inducing medium. Callus formation started after about 12 days. In all variants of both cultivars swelling and callus cells were observed on the edges of the explants, except for variant A (without phytohormones) (Fig. 1).

Morphologically, the callus was fragile and characterized by a pale yellow color.

The use of cytokinins alone or in combination with auxins improves callus induction in many tomato cultivars and their necessity in the nutrient medium has already been proven for many varieties (Nasher et al., 2010), Among the cytokinins, BAP and its role in (*Lycopersicon esculentum*) tissue culture have been well studied. However, information about the role of kinetin in callus induction, morphogenesis and morphogenesis pathway determination is insufficient. In this experiment, the effect of kinetinin on the induction and proliferation of callus in 2 tomato cultivars was evaluated.

Among the four media combinations tested in cultivar H-2274 (Heinz), variant D showed effective callus formation and after subcultivation intensive proliferation. In this variant, kinetin was used instead of BAP. At this time, active callus induction was observed in all three types of explants. This indicates that kinetin is more efficient than BAP.

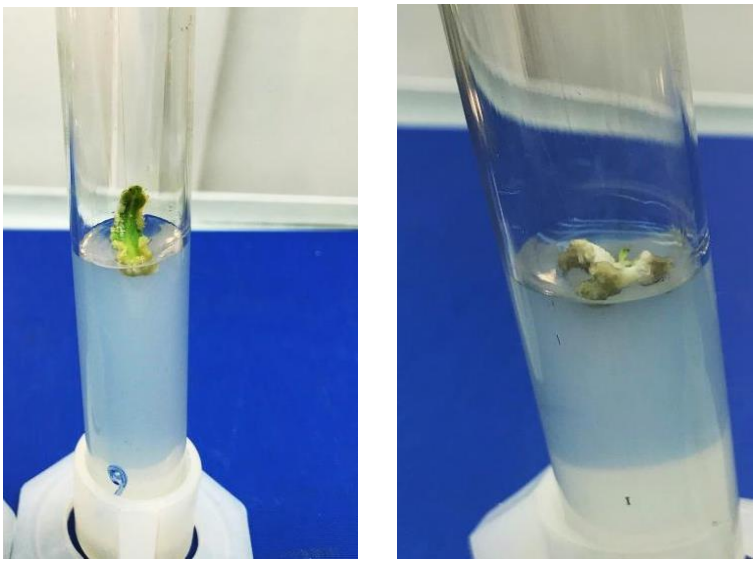

Fig. 1. Induction of callus cells on day 12 of cultivation in variant C in tomato cultivar H-2274 (Heinz)

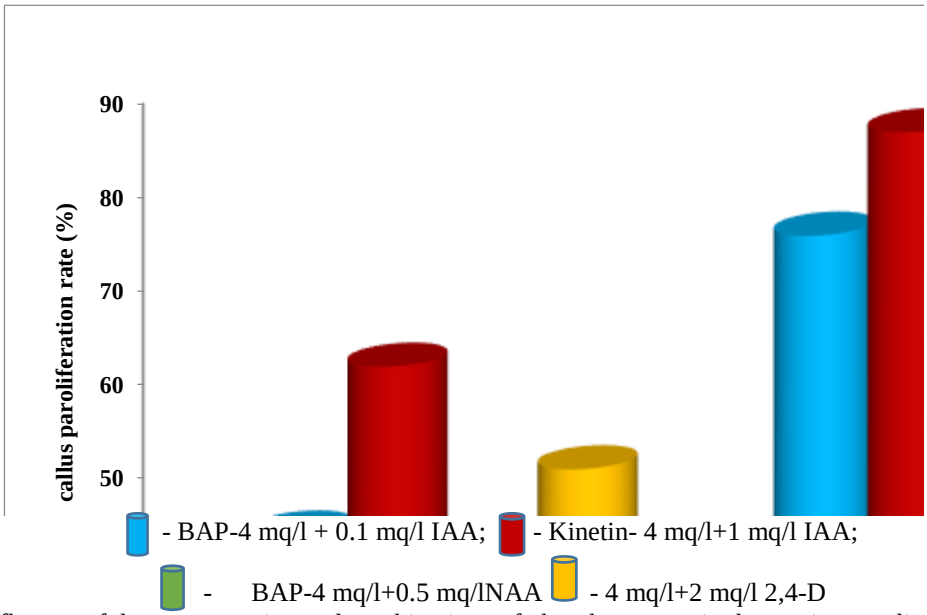

Fig. 2. Influence of the concentration and combinations of phytohormones in the nutrient medium on the proliferation of tomato callus in variety H-2274

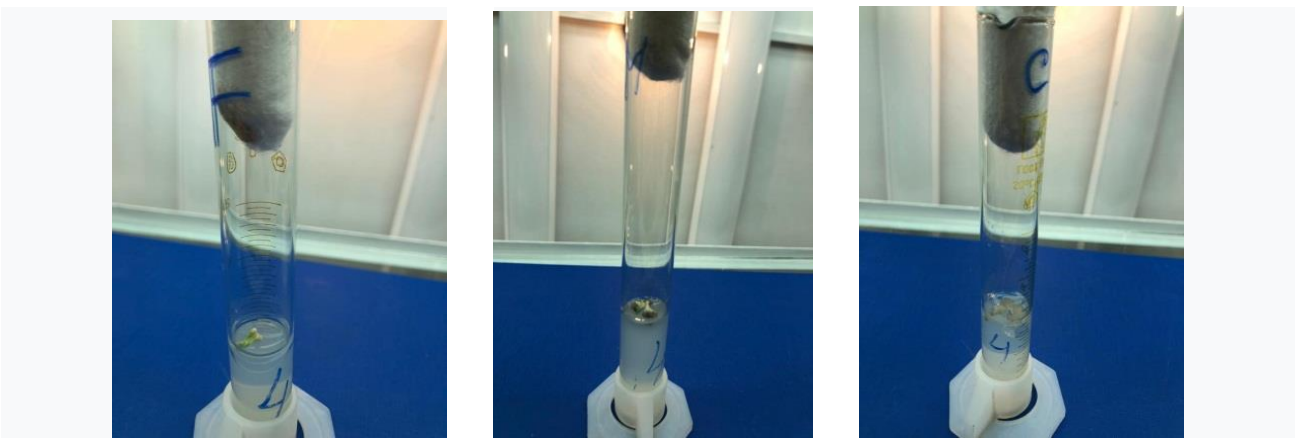

Fig. 3. Induction of callus cells on day 12 of cultivation in variant C in tomato cultivar Gardemarin

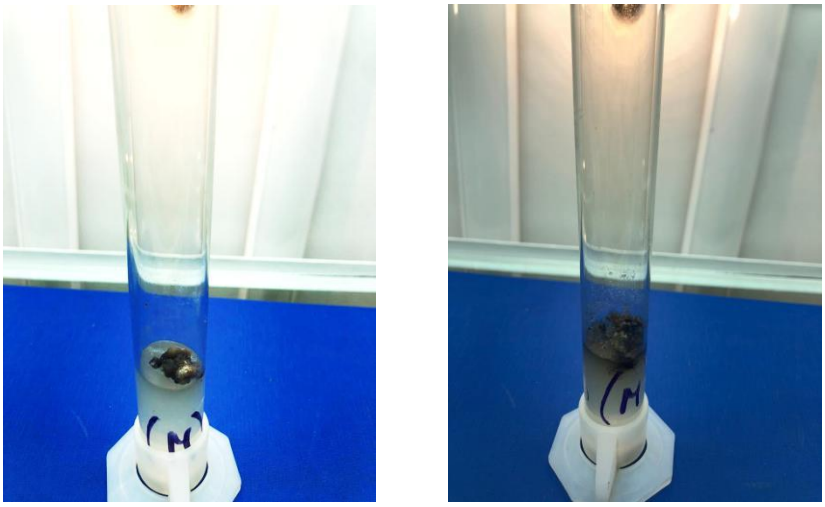

Fig. 4. Darkening of the nutrient medium and callus tissue in the M variant (BAP-4 mq/l + 2 mq/l 2.4-D)

Except for variant F (BAP + NAA), there were no significant differences in the frequency of callus induction among the remaining 3 media tested. Callus induction and proliferation were lower in this variant than in other variants. In general, the best result in the formation of callus was shown by the joint explant - epicotyl. Different results of this explant in terms of callus formation were observed in variant M. In this variant, the hypocotyl reacted very weakly to this combination of phytohormones (Fig. 2).

All variants of the 12 plants that germinated in the Gardemarin variety were planted. In contrast to H-2274 variety, callus induction was weak in all variants tested. In variant F, callus cells were observed only in the cotyledon. In variant C, the callus was transparent and watery, amorphous. In all variants, the increase in proliferation and biomass of callus cells after subcultivation was not visually observed (Fig. 3).

It is an interesting fact that the cultured callus tissue and nutrient medium in variant M (BAP-4mg/l + 2mg/l 2,4-D) started to darken during the second subcultivation period (Fig. 4).

When plant tissues and cells are introduced into in vitro culture, there are often problems with darkening of the tissues and nutrient medium, which has an extremely negative effect on the progress of the experiment. This usually results in the death of plants. This darkening is associated with the oxidation of phenolic compounds, which are widely represented in plants (Shimelis, 2015). During the oxidation of phenolic compounds, highly reactive chemical compounds o-quinones are formed, which interact with each other and with other cell compounds such as amino acids or proteins to form high molecular weight compounds - melanins. Melanins paint damaged surfaces darkly (Baimukhametova, 2020).

Darkening of callus tissue and culture medium can be influenced by environmental factors. The presence of light and high temperature increases the rate of darkening by increasing enzyme activity. Probably, callus tissue and darkening of the medium in variant M can be related to both hormonal content and genotype. Genotype effects play an important role in callus formation, and consequently, great importance should be placed on cultivar selection to establish regeneration protocols through organogenesis. High concentration levels of cytokinins always improved callus induction with low concentration of auxins. Low and high kinetin concentrations produce less callus (Capote et al., 2000).

Thus, the results obtained using medium concentrations in our experiments indicate that kinetin concentrations close to and equal to other cytokinins can be a potential callus inducer in tomato regeneration.

Considering the morphogenesis data available to date, there is still a need to improve the tomato tissue culture protocol for in vitro mass propagation of various commercially important cultivars.

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Sitokin və auksinlərin müxtəlif qatılıq və kombinasiyalarının tomat (*Lycopersicon esculentum* Mill) bitkisinin kallus toxumalarının proliferasiyasına təsiri

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In vitro şəraitdə (*Lycopersicon esculentum* Mill.) növünə aid H-2274 (Heinz) və Qardemarin iki tomat sortunun kallusogenez və proliferasiya proseslərinə bitki böyümə tənzimləyiciləri auksinlər və sitokininlərin konsentrasiya və kombinasiya tərkibi ilə fərqlənən qida mühitlərinin təsiri öyrənilmişdir. H-2274 sortunda istifadə olunan hər üç eksplant kallusun əmələ gəlməsi və proliferasiyasında ən yaxşı göstəriciləri qida mühitinin D variantında (4 mq/l kinetin + 1 mq/l indolilsirkə turşusu (İAA) 8 həftə ərzində kultivasiya edildikdə müşahidə olunmuşdur. F variantı (BAP + NAA) istisna olmaqla, sınaqdan keçirilmiş qalan 3 mühit arasında kallus induksiyasının tezliyində əhəmiyyətli fərqlər yox idi. Bu variantda kallusun induksiyası və proliferasiyası digər variantlarla müqayisədə aşağı olmuşdur. Qardemarin sortunda sınaqdan keçirilən bütün variantlarda kallusun induksiyası zəif olmuşdur. M variantında (BAP-4 mq/l + 2 mq/l 2,4-D) becərilən kallus toxuması və qida mühiti ikinci subkultivasiya müddətində qaralmağa başlamışlar. Ehtimal ki, M variantında kallus toxuması və mühitin qaralması həm hormonal tərkib, həm də genotiplə əlaqəli ola bilər.

Açar sözlər: *Tomat sortları, in vitro, sitokininlər, fitohormonların kombinasiyaları, mühitin qaralması*