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Variations of Stem Anatomy of Cabbage Seedlings (*Brassica oleracea* L.) Seedlings Produced from Heat-Shock Callus Tissue

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ABSTRACT

Background: The current study concentrated from an effect of heat- shock exposure of callus derived from stem segments of cabbage plants (*Brassica olerea* L.). **Objective:** The heat shock (20,25,30,35 and 40 Cº) for 10 and 20 minutes for each degree increased callus growth, size and easy to regenerate the callus to shoot and root. **Results:** Regeneration ratio reach up to 91.66%, especially in temperature 40 Cº for 10 minutes, and the numbers of shoots were 13, while in the same degree for 20 minutes the ratio of regeneration was 44.23% and the shoots were 2. On the other hand the anatomical characteristics are different in the shape, numbers of vascular bundles and the pith area. **Conclusion:** This study aimed to identify the anatomical variation in stem of seedlings produced from callus exposed to heat-shock.

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INTRODUCTION

Plant biotechnology its applications, are an important tool for breeding activity toward the enhancement of qualitative and quantitative traits of these important vegetable (Cristea *et al*.2012). Brassicaceae contain substantial quantities of bioactive compounds, which are good free radical scavenger and might have strong antitumor properties. (Caker, *et al.*, 2012). Climatic factors, such as extreme temperature (heat, cold and freezing), are major abiotic environmental stress that limit plant growth and development. Therefor agronomical yield and had a major role in determining the geographic distribution of plant species. (Krasenky & Jonak, 2012). The positive heat treatments in some systems of plants, stimulating metabolism ways in the cell presented in building up and accumulation of new proteins called (HSP) heat shock protein.(Lee *et al*., 2000). In addition the evidence due to that heat shock is inducting the oxidative operations and promote of synthesizing the enzymes antioxidant in number of plants. Such as the exposure of sunflower cell suspension culture to 40 Cº for 3hours.

As well as to recognize on the internal anatomical variations happens in tissue culture plants compared with the same plants produced from seeds clear differences in the anatomical features of *Cucuis milo* L. plants were produced from tissue culture including enlargement of diameter of stem and numbers of vascular bundles compared with seed plants. (Mavrona *et al*., 2000), and specific anatomical variation in epidermis and cortex in *Citrullus lanatus* plants and *Largenaria siceraria* (Yetisire *et al*., 2005).

This study aimed to identify the anatomical variation in stem of seedlings produced from callus exposed to heat-shock.

MATERIAL AND METHODS

Sterile seedling production:

Cabbage's seed (*Brassica oleracea* L.) were sterilized by soaking it in 6% sodium hypochloride solution (1:2 v/v) for 10 minutes, and washed the seed thoroughly by sterile water. Sterilized seeds were transported to the surface of 25ml MS0 (Murashige & Skoog,1962) medium contained in 100 ml(3 seeds/flask) , specimens were kept in culture room at condition of dark at 25 ± 2Cº, then the grown seedlings were transported to light and dark successive conditions 16/8 hours at light intensity 1500 lux.

Exposure of callus to heat shock:

One gram sample of stem derived callus 4 weeks old were each in sterile tube with cover of 25 ml capacity. Samples were exposed to each at (20, 25, 30, 35, and 40 Cº) for 10 and 20 minutes. Each exposed test tube was

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directly in water at roof temperature in waterbath, at the selected degree previously fixed (Irina *et al*., 2002). All heat- treated samples were placed on agar, solidified MS medium supplemented with 1.0 mg/L NAA+ 2.0 mg/L BA and incubated at the same conditions(Alradi, 2013).

Preparation of sections:

The basic steps of sample fixation, embedding in wax were followed as described (Al-haj, 1998) to prepare permanent various sections.

Results:

Effect of heat shock on callus stimulation from explants:

The results in table (1) indicate that all treatment enhanced callus stimulation.

Table 1: Effect of some treatment of heat- shock on callus production from stem explants of cabbage (*Brassica oleracea* L.).

Treatments Cº/min.		No. of stem cultured	Explants responded	Callus %
20	10	60	42	**70**.00
	20	66	48	**72**.72
25	10	62	48	**77**.41
	20	64	50	78.12
30	10	58	46	79.31
	20	62	53	85.48
35	10	60	52	86.66
	20	60	54	90.00
40	10	50	48	96.00
	20	62	40	64.51

In addition the images in figure.1 showed of the beginning callus stimulated is derived from stem egments of cabbage plants (*Brassica oleracea* L.) in solid MS media is provided with 1.0 mg/L NAA + 2.0 mg/L BA (figure1-A). In the other hand stimulated callus which exposure to heat shock in optimum temperature at 40Cº for 10 minutes to show increasing size of callus (figure 1-B), and the shoot is in initiating from callus (figure 1-C).

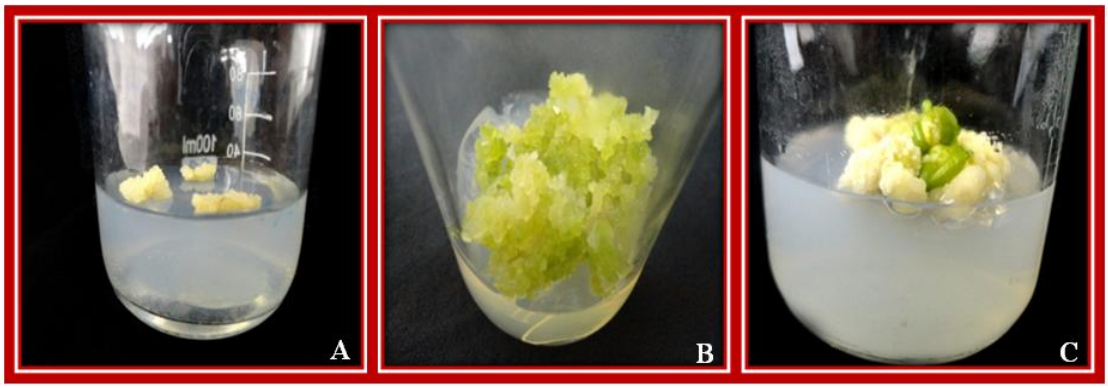

Fig. 1: A-Callus initiation from cabbage's stem, B-Increasing callus in size after exposure to heat-shock, C-Start shoots from callus.

Table(2) show the effects of treatments heat-shock on callus regeneration and the number of shoots, it was the maximum at 40Cº for10 minutes to arrival the segments regenerated 22, the number of shoots 13, and the ratio of regeneration was 91.66.

Table 2: Effect of some treatments of heat-shock on regeneration of callus of cabbage *Brassica oleracea*.

Treatments Cº/min.		No. of callus exposed	Segment regenerated	No. of shoots	Regeneration (%)
20	10	21	4	3	17
	20	25	6	3	20.8
25	10	28	8	3	28.57
	20	25	7	5	26.92
30	10	27	10	5	37.03
	20	26	14	7	53.84
35	10	25	17	8	68.00
	20	23	18	11	78.00
40	10	24	22	13	91.66
	20	26	12	2	46.15

In the figure-2 images indicate number of shoots (figure2-A), and complete regeneration to hole plant under tissue culture conditions (figure2-B). The plant transported to the soil after 30 day from callus is begging regeneration (figure2-C).

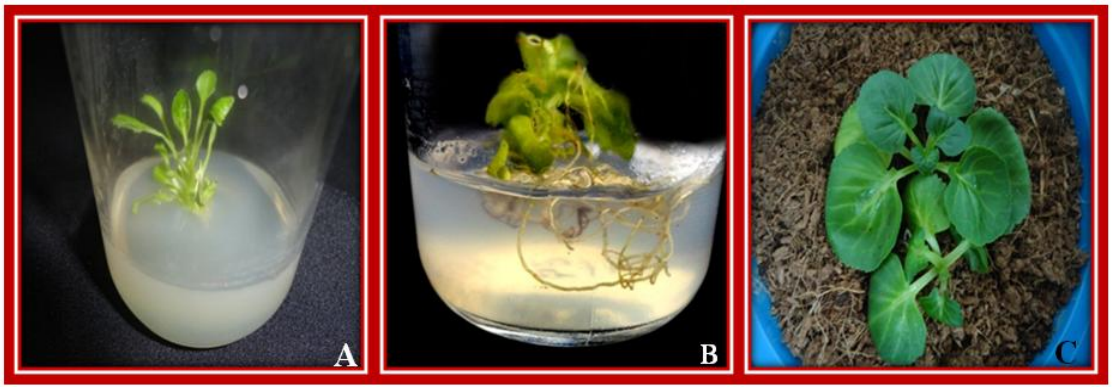

Fig. 2: A-Number shoots regenerated of callus, B- Complete regeneration to shoots and roots, D- Adaptation the plant by transported to the soil.

The anatomical changes in cross sections of stems:

Cross section of stem derived from callus exposure to heat- shock (figure.3), is bigger than cross section of stem in seed plants (figure.4), in size and its more folded or curvatures. The stomatal system is looking wide (figure.3-A), compared with in (figure.4-A). In the cells cortex showed as corner cholenchyma (figure.3-b), but is absence in another (figure.3-B). The numbers of vascular bundles in cross section of stem of tissue culture 9 bundle/section. (figure.3-C), while being 7 bundle/section in cross section of stem in seed plants. (figure.4-C), as well as the pith zone is wide (figure.3-D), compared with another (figure.4-D).

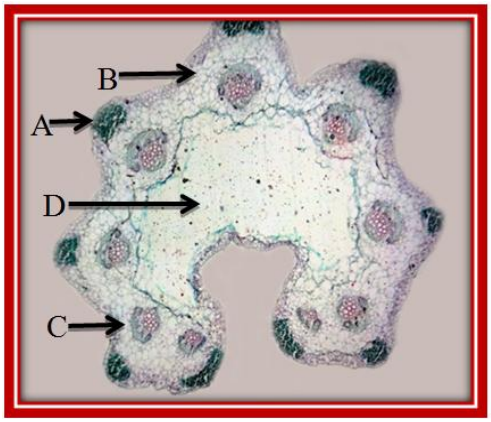

Fig. 3: C.S. cabbage's stem derived from callus exposure to heat- sho.

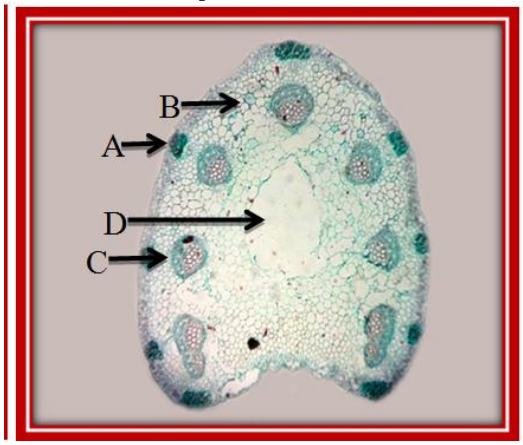

Fig. 4: C.S. cabbage's stem derived from seed plants.

The more studies had proven on role of using the heat shock in promoting some biotic activities in the cell. (Sholpan *et al*., 2001). Callus increase in size which is exposure to heat treatments, maybe is attributable to enhancement of protein and chlorophyll in the cells of callus, because of the heat shock. (Al-Mallah & Al-Nema, 2007). The positive role in obtaining to increase permeability of cellular membranes, which is to attract Ca^{+2} and some nutrients. (Mejia *et al*., 1995). A study found that exposure the cell suspension for heat shock lead to augment cells divisions in rice plants. (Thompson *et al*., 1989), as well as that lead to early cells divisions and overmuch of the numbers in callus permodia is initiating from cell suspension to sunflower plants. (Rasheed & Kasim, 2006). The effect of promoting to this treatment is representing to remove all the barriers in the cell membrane through formatting temporary pores. (Joersbo & Brunstedt, 1991). In high temperature lead to decrease of regenerating for callus to shoot and root, that perhaps return to obtain damage in proteins of the cells. (Bukner *et al*., 1998), or is crashing the responsible enzymes of it building. (Soloman *et al*., 1999), that is consequent cells death. (Loschiavo *et al*., 2000). This study focused on the anatomical characteristics, because of the value to recognize the changes as reason to culture the plants under *in vitro* conditions, environmental conditions effected on the anatomical features to enhance are desirable features to produce plant is able in resistance the worse conditions. (Edwin *et al*., 2008). In the other hand a study found the numbers of vascular bundles increase compared with the same plant is culturing in the soil. (Alradi, 2013).

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