Effect of Cysteine in Stimulating Some Active Compounds in Garlic Callus Under Different Light Spectra in Vitro

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Abstract

This study was conducted in tissue culture laboratories, Faculty of agriculture, University of Kufa in 2020. The experiment included studying the effect of two factors: type of light: (mixture spectra of red and blue LED R16 : B2), (Fluorescent : normal white light) in interaction with different concentrations of cysteine( 0, 50, and 100 mg.L⁻¹). A completely randomized design (C.R.D) were used with two factor and three replicates. The combined spectra (LED R16:B2) increased the active compounds Di-allyldisulfide and Vinyldithiin (131.33,121.41µg·g⁻¹ F.W. ) respectively. Cysteine (100 mg·L⁻¹) was significantly superior compare to other treatments in producing Di-allyldisulfide and Vinyldithiin content (158.87 ,144.35 µg·g⁻¹ F.W) respectively. Results also indicated that the interaction treatment between (LED R16:B2) and (Cysteine 100 mg.L⁻¹) gave significant increase in Di-allyldisulfide and Vinyldithiin content (184.28 , 146.07µg·g⁻¹ F.W) respectively. While the treatment (Fluorescent W) and (Cysteine 50 mg.L⁻¹) gave significant increase in Allicin content (195.38 µg·g⁻¹ F.W).

Keywords: LED, Cysteine, Allicin, Di-allyldisulfide, Vinyldithiin.

1. Introduction

Garlic (Allium sativum L.) has a medicinal value that may exceed the nutritional value, which makes it a plant that can be used in the treatment or resistance of many diseases; strengthens the body’s immunity, relieves dental pain, benefits patients with diabetes and high blood pressure through its effect in lowering blood sugar, reducing high blood pressure and preventing blood clotting, reduces the incidence of cancerous diseases because it contains antioxidants, a circulatory stimulant and is useful in reducing harmful low-density lipoproteins [1].

It is well known that (fluorescent lamps) light is an essential factor in most of cell culture in vitro. whereas, recently found that LED lamps (Light Emitting Diode) be more effective on cell cultures in vitro. Due to the amount of energy consumption is lower, its efficiency is high, and their temperature is low. Moreover higher life rate and low cost, and has given success in agricultural plant propagation laboratories successfully [2,3]. Light has significant effects on callus growth and morphology, induction of specific enzyme-linked activity in the production of secondary compounds which proved their efficiency in the medical field [4,5].

The sulfur-containing organic sulfur compounds in garlic had anti-bacterial properties. These properties are affected by increasing the number of sulfur atoms in these compounds when adding the precursor that contains sulfur in its composition and provides it organically to the plant. The sulfur amino acid (Cysteine) is characterized by containing sulfur in the side chain in its composition and being one of the basic components that enter into the synthesis of many proteins in the plant or the formation of the active site of many enzymes [6].

The research aims to increase some active substances in garlic using lighting and Cysteine precursor in vitro.

2. Materials and Methods

The experiment was conducted in tissue culture laboratory, Faculty of Agriculture, University of Kufa in 2020. This experiment used two type of light: (LED R16:B2 (L1) mixture of red and blue, Florescent (L2) white light ), three concentrations of Cysteine (T1 0, T2 50, and T3100 mg.L⁻¹) to stimulate producing active compounds. Allicin, Di-allyldisulfide and Vinyldithiin content in garlic callus in vitro. The local garlic used were obtained from local markets and the cloves of garlic were sterilized 20 minutes with 15% of NaOCl v/v (6 % active ingredient), then it washed by sterile distilled.
water three times. After sterilization the garlic cloves were placed in the test tube contain MS media (table 1) volume 10 ml. The explants were incubated to the growth room 25 ± 2 ° C. Callus initiation after 40 days was maintenance on the same callus media with different concentrations of Cysteine (0, 50 and 100 mg.L⁻¹). All explants were incubated under different type light: LEDR16:B2 and florescent (white light) for 16 h/d after four weeks of cultivation, Allicin, Di-allyldisulfide and Vinyldithiin content in callus were recorded using HPLC technique. Study was performed using the Completely Randomized Design (C.R.D) were used with three replicates and means were compared according to L.S.D. test at the level of 0.05.

Table 1. Components of used media in callus induction.

| Component     | Concentration (mg.L⁻¹) |
|---------------|------------------------|
| MS salts Full strength | 30000 |
| Sucrose       | 7000                  |
| Myo inositol  | 100                   |
| Agar          | 7000                  |
| BA            | 0.5                   |
| IAA           | 3                     |
| 2.4.D         | 1                     |

3. Results

3.1. Allicin content

Table (2) showed that fluorescent (L₂) significantly affected on callus for Allicin content (154.89 µg.g⁻¹ F.W) comparing to (107.26µg.g⁻¹ F.W for LED (L₁) treatment. Moreover, T2 and T3 showed the highest in Allicin content (169.74 and 160.65 µg.g⁻¹ F.W ), respectively compare with T1 (62.84 µg.g⁻¹ F.W). Interaction between type of light and different Cysteine concentration revealed that, combination of L₂T₂ gave the highest average 195.38 µg.g⁻¹ F.W compare with L₁T₁ gave 38.3 µg.g⁻¹ F.W.

Table 2. Effect of light type and Cysteine concentrations on Allicin content (µg.g⁻¹ F.W.) in garlic callus.

| Type of light (L) | Cysteine concentration mg.L⁻¹ (T) | Mean of light |
|------------------|----------------------------------|---------------|
| T1 (0)           | 38.3                             | 107.26        |
| T2 (50)          | 144.1                            |               |
| T3 (100)         | 139.38                           |               |

3.2. Di-allyldisulfide content

The results presented in Table (3) shows a significant effect of LED (L₁) in Di-allyldisulfide content (131.33 µg.g⁻¹ F.W ) as compare with fluorescent (L₂) which gave (99.08 µg.g⁻¹ F.W). Also, there are a significantly affected between Cysteine concentrations, the treatment T3 achieved the highest average in Di-allyldisulfide content ( 158.87 µg.g⁻¹ F.W ) as compare with treatment T1, which it gave the lowest average reached (80.17 µg.g⁻¹ F.W ). The interaction between light type and Cysteine concentrations were also a significant effect in Di-allyldisulfide content, the treatment L₁T₃ gave the highest average reached (184.28 µg.g⁻¹ F.W), while the treatment L₂T₁ gave the lowest average only (74.70 µg.g⁻¹ F.W).

Table 3. Effect of light type and Cysteine in di-allyldisulfide content (µg.g⁻¹ F.W.) in callus of garlic.

| Type of light (L) | Cysteine concentration (mg.L⁻¹) (T) | Mean of light |
|------------------|-----------------------------------|---------------|
| T1 (0)           | 85.65                             | 131.33        |
| T2 (50)          | 89.07                             |               |
| T3 (100)         | 133.47                            |               |

3.3. Vinyldithiin content

Based on the results in Table (4), LED (L₁) significantly affected increase in Vinyldithiin content of garlic callus (121.41 µg.g⁻¹ F.W) when it compared with fluorescent (L₂) which gave (116.68 µg.g⁻¹ F.W). The results showed a significant increase between Cysteine concentrations in Vinyldithiin content, treatment (T₃) gave (144.35 µg.g⁻¹ F.W ) compared with
(T1) which gave value (93.81 µg.g⁻¹ F.W.). The combination of LED (L1) and Cysteine (T3) gave the highest average in Vinyldithiin content reached (146.07 µg.g⁻¹ F.W.), while the LED (L1) and Cysteine (T1) gave the lowest average by (86.81 µg.g⁻¹ F.W.).

Table 4. Effect of light type and Cysteine in Vinyldithiin content (µg.g⁻¹ F.W.) in callus of garlic.

| Type of light (L) | Cysteine concentration (mg.L⁻¹) (T) | Mean of light |
|------------------|------------------------------------|--------------|
| LED R18:B2 (L1)  | 86.81 (T1) 131.35 (T2) 146.07 (T3) | 121.41       |
| Fluorescent W (L2)| 100.82 (L1) 106.59 (L2) 142.62 (L3) | 116.68       |
| Mean of Cysteine | 93.81 (T1) 118.97 (T2) 144.35 (T3) |              |
| L.S.D. 0.05 L=1.112 T=1.362 LT=1.925 |

4. Discussion

The presence of red light in a high percentage in the LED lamps used in the study with wavelengths ranging from 610-700 nanometers, a region with maximum absorption, which positively affected on increasing growth. The formation of amino and organic acids needed for biological construction, Amino acids in plants are important for the synthesis of various natural products of secondary metabolism such as alkaloids. It also intervenes in increasing the plant's resistance to thermal and water stresses and participates in the synthesis of encouraging action of many enzymes and enzymatic chaperones [7-11]. As well as amino acid Cysteine is included in the synthesis of sulfur secondary compounds found in the callus cells of the garlic plant, it is considered one of the basic molecules that can be included in the secondary metabolism compounds through the food environment and increase or accelerate (or both) in the production of the desired compounds. In general, the addition of primers accelerates the production of secondary metabolic compounds [12,13].

Conclusion

From results this study, the treatment 100mg.L⁻¹ of Cysteine concentration under LED lighting type (R16-B2) condition is the best treatment and can be recommended to give highest accumulation of Di-allyldisulfide and Vinyldithiin content. Whereas, the treatment 50mg.L⁻¹ of Cysteine concentration under fluorescent lighting type condition is the best treatment may be recommended to give to give highest accumulation of Allicin.

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