In Vitro Detection of Some Active Compounds in Stressed Callus of Mungbean (Vigna Radiata L.)

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Abstract

An experiment was conducted at the Ministry of Sciences and Technology/ Directorate of Agricultural Research, Genetic Engineering Department. In Vitro callus cultured on (MS) medium supplemented with different levels of NaCl, brassinolides and vitamin C. H.P.L.C technique was used to detect the phenolic compounds in different treatments of mungbean stressed callus. The results showed various responses for accumulation of phenolic compounds in different treatments of mungbean stressed callus for example, 15dS.m⁻¹NaCl+40mg.L⁻¹vitamin C+0.04mg.L⁻¹brassinolide) accumulated highest concentration of Hydroxybenzonic acid, Tannic acid, Gallic acid, Quercetin and Syrinigic acid (280.44, 135.16, 286.49, 778.93 and 778.93 µg.ml⁻¹) respectively, while highest accumulation of Chlorogenic acid, Gentisic acid, Sikimick acid and Coumaric acid were found in (6dS.m⁻¹NaCl+40mg.L⁻¹vitamin C+0.04mg.L⁻¹brassinolide) treatment reached (230.27, 208.21, 846.52 and 522.62 µg.ml⁻¹) respectively. Furthermore, 6dS.m⁻¹NaCl+0.04mg.L⁻¹brassinolide and 15dS.m⁻¹NaCl+0.04mg.L⁻¹brassinolide achieved highest accumulation (933.83 and 407.48 µg.ml⁻¹) for Ferulic acid and Protocatechuic acid respectively.

Key word: Vigna radiate, Callus, Salt stress, Secondary product, In vitro, Brassinolides, ASA.

1.Introduction

Mungbean (Vigna radiata L.) is one of the annual summer plants, which includes 170 species spread in the world [1]. For its nutritional value mungbean seeds are rich source of protein [2]. In addition to 4-6 g of carbohydrate and vitamin (A,B,C,D) which make it popular among vegetarians [3]. Furthermore, the sprouts of mung bean are consumed as a fresh salad in most of Asia and western countries[4,5], or used as forage [6]. Besides the nutrient value, mungbean contain many active compound which famous with their therapeutic effects, for example the lectin used as anticancer [7], the starch considered as suitable raw material for noodle making because it contains resistant starch that can escape digestion in the small intestine [8]. Plant products have been used as sources of nutrition, agrochemicals and pharmaceuticals and almost all of the world population depends on plant derived products [9]. Biotechnology applications of In Vitro technique offered many strategies for producing these compounds by means of large-scale in restricted area independent on climatic changes. Brassinolide (BRs) are steroidal phytohormones mediates various physiological processes which improves plant tolerance to abiotic stresses [10]. Exogenous application of brassinolides improved and alleviated plant growth under abiotic stress conditions [11]. Moreover, Ascorbic Acid (ASA) plays a vital role in related to the system of antioxidant compounds and modulating a number of fundamental functions in plants [12]. Exogenous application of (ASA) and brassinolides in vitro improved and alleviated plant growth beside increasing bioactive compounds under abiotic stress conditions. Therefore, the present study was designed with the aim of increasing phenols in stressed callus of mungbean via plant tissue culture technique.

2.Materials and Methods

This work was carried out at the Ministry of Sciences and Technology/ Directorate of Agricultural Research, Genetic Engineering Department during 2018-2019. In Vitro seeds of mungbean local verity were sterilized with ethyl alcohol (70%) for 30 seconds, followed by 2% commercial Clorox (6% sodium hypochlorite) for 15 , washed with sterile distilled water four times for few minutes to remove the excess of sterile substance. The sterilized seeds were germinated on basal salts of [13], medium supplemented with vitamins and sucrose (Table 1). Finally the culture incubated at 25 ± 2 °C under cool-white fluorescent light in culture room condition. Ten days later epicotyle were excised from the mungbean seedlings (Fig1a) and cultured on callus initiation media (Fig1b) supplemented with BA(0.5)+NAA(2) mg.L⁻¹ [14]. One month later a constant weight (100mg) of fresh callus exposed to different levels of NaCl media supplemented with different concentrations of Vitamin C and brassinolide described in Table (2)
Table 1. Seeds germination medium.

| Component      | Concen. (mg·L⁻¹) |
|----------------|-------------------|
| MS             | 4400              |
| Glycine        | 2.0               |
| Myo-insitol    | 100               |
| Nicotinic acid | 0.5               |
| Thiamine-HCl   | 0.1               |
| Pyridoxine     | 0.5               |
| Sucrose        | 30000             |

Table 2. Codes of different treatments applied on mungbean callus

| Code | Treatments (mg·L⁻¹)                                      |
|------|--------------------------------------------------------|
| A1   | 6dS.m⁻¹NaCl                                           |
| A2   | 6dS.m⁻¹NaCl+40mg·L⁻¹vitamin C.                         |
| A3   | 6dS.m⁻¹NaCl+0.04mg·L⁻¹brassinolide                      |
| A4   | 6dS.m⁻¹NaCl+40mg·L⁻¹vitamin C+0.04mg·L⁻¹brassinolide    |
| A5   | 15dS.m⁻¹NaCl                                           |
| A6   | 15dS.m⁻¹NaCl+40mg·L⁻¹vitamin C                         |
| A7   | 15dS.m⁻¹NaCl+0.04mg·L⁻¹brassinolide                     |
| A8   | 15dS.m⁻¹NaCl+40mg·L⁻¹vitamin C+0.04mg·L⁻¹brassinolide   |

Figure 1. A Mungbean seedling of age 10 days. B callus initiated from epicotyls explants on media (BA(0.5)+NAA(2) mg·l⁻¹)

2.1 Extraction of phenolic compounds

Phenolic compounds were extracted from callus and separation condition estimating according to the procedure of [15]. Chromatographic Conditions: Phenols concentration determined using High-Performance Liquid Chromatography H.P.L.C. instrument (Shimadzu 2010 FLC). Column: (50cm×2.0mm, partial size 5μm) C-18 DB column, mobile phase consisted of acetonitrile: methanol: 0.1 phosphoric acid (6:3:1 v/v), detection UV set at 285nm, flow rate of 1.2 ml·min⁻¹ and temp. 25 °C.

Table 3. Retention time and area of standard compounds.

| Seq. | Compound                | Retention time | Area       |
|------|-------------------------|----------------|------------|
| 1    | Hydroxybenzonic acid    | 1.69           | 126907     |
| 2    | Tannic acid             | 2.67           | 126107     |
| 3    | Gallic acid             | 3.39           | 145941     |
| 4    | Quercetin               | 4.52           | 119001     |
| 5    | Syringic acid           | 4.90           | 169188     |
| 6    | Ferulic acid            | 6.11           | 209820     |
| 7    | Chlorogenic acid        | 7.49           | 321777     |
| 8    | Gentisic acid           | 8.66           | 221127     |
| 9    | Sikimick acid           | 9.80           | 117700     |
| 10   | Coumaric acid           | 10.50          | 179703     |
| 11   | Protocatechuric acid    | 11.39          | 179419     |
2.2 Statistical analysis

The experiment was designed in completely randomized (C.R.D) and treatments were triplicates. Means were compared at least significant differences (L.S.D) at P ≤ 0.05 level. Data analysis using Gen-Stat software.

3. Results and Dissection

Based on the data obtained from extracted callus exposed to salt stress and different concentration of vitamin C and the brassinolide, 11 active compounds were diagnosed by H.P.L.C depending on the availability of standard samples and chromatographic separation conditions shown in (Table3) namely Hydroxy-benzonic acid, Tannic acid, Gallic acid, Quercetin, Syrinigic acid, Ferulic acid, Chlorogenic acid, Gentisic acid, Sikimick acid, Coumaric acid and Protocatechuric acid. Hence Data in Table (4) showed A8 (15dS.m⁻¹NaCl+40mg.L⁻¹vitamin C+0.04mg.L⁻¹brassinolide) was superior to give highest concentration of Hydroxybenzonic acid, Tannic acid, Gallic acid, Quercetin and Syrinigic acid (280.44, 135516, 286549, 778593 and 778593 μg.ml⁻¹ respectively, while highest accumulation of Chlorogenic acid, Gentisic acid, Sikimick acid and Coumaric acid were found in A4 (6dS.m⁻¹NaCl+40mg.L⁻¹vitamin C+0.04mg.L⁻¹brassinolide) treatment reached (230.27, 208.21, 846.52 and 522.62 μg.ml⁻¹) respectively. Furthermore, A3(6dS.m⁻¹NaCl+0.04mg.L⁻¹brassinolide) and A7 (15dS.m⁻¹NaCl+0.04mg.L⁻¹brassinolide) achieved highest accumulation (933.83 and 407.48 μg.ml⁻¹) for Ferulic acid and Protocatechuric acid respectively.

Table 4. Effect of the vitamin C and brassinolide in the concentration of phenols(μg.ml⁻¹) of mungbean callus exposed to salt stress.

| Phenols            | A1   | A2   | A3   | A4   | A5   | A6   | A7   | A8   | L.S.D |
|--------------------|------|------|------|------|------|------|------|------|-------|
| Hydroxybenzonic acid | 218.60 | 82.74 | 234.17 | 226.46 | 71.89 | 99.22 | 208.61 | 280.44 | 0.83  |
| Tannic acid        | 110.48 | 94.39 | 237.78 | 228.54 | 88.46 | 115.78 | 218.65 | 258.10 | 0.91  |
| Gallic acid        | 60.56  | 65.96 | 125.95 | 111.44 | 59.95 | 84.94  | 120.17 | 135.16 | 1.09  |
| Quercetin          | 93.53  | 193.05 | 276.77 | 184.72 | 125.21 | 180.80 | 236.51 | 286.49 | 0.86  |
| Syrinigic acid     | 342.37 | 727.07 | 769.57 | 575.95 | 456.26 | 560.22 | 733.88 | 778.93 | 0.90  |
| Ferulic acid       | 546.48 | 656.70 | 933.83 | 718.84 | 413.73 | 705.63 | 683.93 | 741.23 | 1.41  |
| Chlorogenic acid   | 7.64   | 155.27 | 203.95 | 230.27 | 115.69 | 123.39 | 173.55 | 191.32 | 0.90  |
| Gentisic acid      | 79.08  | 95.77  | 135.21 | 208.21 | 85.88  | 140.38 | 202.13 | 162.56 | 0.58  |
| Sikimick acid      | 351.56 | 632.25 | 817.99 | 846.52 | 528.43 | 560.59 | 807.55 | 699.39 | 1.07  |
| Coumaric acid      | 192.49 | 302.06 | 544.95 | 522.62 | 263.72 | 327.56 | 499.57 | 394.94 | 0.91  |
| Protocatechuric acid | 192.39 | 191.80 | 390.38 | 355.23 | 214.94 | 182.06 | 407.48 | 350.37 | 0.91  |
Production of natural products by In Vitro strategies is approach for utilizing optimal conditions (hormonal balance, vitamins and nutrient salts) among others for culture growth. Further advances by feeding culture with electors and stressors improved the productivity of active compounds. In the current experiment, the addition of NaCl salt, vitamin C and brassinolide to the medium had positive effects on the phenolic compound in callus derived from epicotyls of mungbean explants. However, many authors demonstrated the positive correlation for accumulation of medicinal product in stressed plants in vitro [16], found high accumulation of capsaisin alkaloid in callus derived from chilli pepper seedling explants, while [17], found high accumulation of Gallic acid in ruta plantlets subjected to salt stress. Vitamin C and brassinolide are stimulate the accumulation of phenols in plants cultured in stress media to reduce the negative damages of salinity, oxidation of ROS and neutralize toxic compounds by electron donation [18,19]. Phenolic compounds increase with the intensity of the different stresses to which the plant is subjected, including salt stress, as they act as scavengers of free radicals (Superoxide, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 3-Ethylbenz thiazoline-6-sulfonic acid(ABTS)), Also inhibit enzymes that work to generate free radicals (Xanthine oxidase, Lipoxygenase, Cyclooxygenase, Protein kinase and NADH Oxidase) [20-23], confirmed the importance of phenolic acids in plant tolerance to salinity, it has been observed that p-Coumaric acid has a role in reducing oxidative stress.

**Conclusion**

Our findings indicate that the production of phenolic compounds In Vitro in the presence of NaCl stress in combination with vitamin C and brassinolide proved a positive correlation when added to nutrient media in related to increase of phenolic compound.

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