Callus cell proliferation from broccoli leaf slice using IBA and BAP in vitro culture: Its biochemical and antioxidant properties

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Abstract

Plant tissue or cell culture keeps a significant role in micropropagation in the plant production industry. Combination of 6-Benzylaminopurine (BAP) and other plant growth regulators like 1-Naphthaleneacetic acid (NAA) or Indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) was used in the most of the research in tissue culture. The study was carried out to investigate the optimization of the concentration of IBA and BAP combination (0, 0.25, 0.50, 1.0, 1.50, 2.0, 2.5, 3.0 and 3.5 mg/l) for the root, callus and leaf proliferation from the leaf cutting slice. The highest number (6.75) of root proliferation was observed in the concentration of 2.0 mg/l IBA + 0.25 mg/l BAP combination. The callus initiation was found in the concentration of IBA 1.0–3.5 mg/l + BAP 1.0–2.0 mg/l. However, the highest callus weight was observed at the concentration of IBA 1.5 mg/l + BAP 1.0 mg/l combination than other combination of concentrations. Positively leaf initiation and formation was better in the concentration of IBA 1–3.5 mg/l + BAP 1.0–2.0 mg/l combination. In addition, the 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential was higher (70.1%) in leaves extract than in callus extracts (46.3%) at the concentration of 10 mg/ml though both extracts had lower DPPH free radical scavenging activity compared to the positive control, vitamin C and BHT. The results conclude that the optimum concentration was IBA 1.5 mg/l + BAP 1.0 mg/l combination to produce callus cell

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proliferation and concentration of 2.0 mg/l IBA + 0.25 mg/l BAP combination was the optimum for root proliferation of broccoli in vitro.

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Specialization Table

| Subject area                  | Biology                        |
|-------------------------------|-------------------------------|
| More specific subject area    | Plant cell and tissue culture Biotechnology |
| Type of data                  | Table and Figure              |
| How data was acquired         | Culture in Growth chamber, antioxidant activity, DPPH free radical measured by spectrophotometer. |
| Data format                   | Raw data collection and analyzed. |
| Experimental factors          | Single factor                 |
| Experimental features         | 10 replicates were used as CRD design |
| Data source location          | Hail city, Saudi Arabia and Kuala Lumpur, Malaysia |
| Data accessibility            | Data are presented in this article. |

Value of the data

1. The data provides the information of the effect of different concentrations of IBA (auxin) and BAP (cytokinin) on the leaf, root and callus proliferation from broccoli leaves slice in vitro culture.
2. This data would be valuable for further studies of physiological, biochemical and antioxidant activity in broccoli explants in vitro culture.

1. Data

In the data, the effects of IBA and BAP on the callus, roots and leaf formation have been shown (Table 1.2). In Table 1.3, effects of different combinations of hormones on the fresh weight of callus produced have been mentioned. Furthermore, measurement of the OD reading of control, positive control (vitamin C and BHT) and other samples have been taken at 515 nm using spectrometer

| Table 1.1  |
|------------|
| Standard procedures for MS media preparation. |
| Component | Unit |
|-----------|------|
| MS powder form with vitamin | 4.4 g |
| Sucrose    | 30 g |
| Phyta gel  | 2.8 g |
| pH         | 5.8  |
2. Materials and methods

2.1. Preparation of Murashige and Skoog (MS) basal media

The MS basal media [1] were used as control and seed germination was prepared following the standard procedures for MS powder form preparation (Table 1.1). MS powder form was added in a beaker with 800 ml distilled water followed by 30 g of sucrose and 2.8 phyta gels and adjusted the pH to 5.8 so that the final volume of the medium was 1000 ml.

2.2. Media in the autoclave

MS basal media with auxin was prepared by adjusting the pH to 5.8 by using 1 N HCl and 1 M NaOH. Then, the media was fractional in 30 ml and was added into jam jars (7 × 4.5 cm²) and autoclaved at 15 psi and 121 °C for 20 min. After that, the sterilized media were cooled and kept in culture room under dark condition. Preparation of media was completed a week before use to reduce water condensation in jam jars and the media was sterilized completely.
Table 1.2
Effects of IBA and BAP on the roots, leaf and callus formation from broccoli leaf slice.

| IBA  | BAP  | No. of root formation | Observation of callus | Leaf proliferation |
|------|------|-----------------------|-----------------------|--------------------|
| 0    | 0    | 0                     | –                     | –                  |
| 0    | 0.25 | 0.25 ± 0.25           | –                     | –                  |
| 0    | 0.5  | 0                     | –                     | –                  |
| 0    | 1.0  | 0                     | –                     | –                  |
| 0    | 1.5  | 0                     | –                     | –                  |
| 0    | 2.0  | 0                     | –                     | –                  |
| 0    | 2.5  | 0                     | –                     | –                  |
| 0    | 3.0  | 0                     | –                     | –                  |
| 0    | 3.0  | 0                     | –                     | –                  |
| 0.25 | 0.25 | 1.0 ± 0.41            | –                     | –                  |
| 0.25 | 0.5  | 1.75 ± 0.25           | –                     | –                  |
| 0.25 | 1.0  | 2.0 ± 0.41            | –                     | –                  |
| 0.25 | 1.5  | 3.5 ± 0.65            | –                     | –                  |
| 0.25 | 2.0  | 2.5 ± 0.29            | –                     | –                  |
| 0.25 | 2.5  | 2.0 ± 0.41            | –                     | –                  |
| 0.25 | 3.0  | 2.25 ± 0.25           | –                     | –                  |
| 0.25 | 3.5  | 2.25 ± 0.23           | –                     | –                  |
| 0.5  | 0.25 | 1.5 ± 0.29            | –                     | –                  |
| 0.5  | 0.5  | 1.25 ± 0.25           | –                     | –                  |
| 0.5  | 1.0  | 1.5 ± 0.65            | –                     | –                  |
| 0.5  | 1.5  | 1.75 ± 0.48           | –                     | –                  |
| 0.5  | 2.0  | 2.0 ± 0.71            | –                     | –                  |
| 0.5  | 2.5  | 1.75 ± 0.48           | –                     | –                  |
| 0.5  | 3.0  | 1.5 ± 0.29            | –                     | –                  |
| 1.0  | 0.25 | 1.75 ± 0.49           | –                     | –                  |
| 1.0  | 0.5  | 1.25 ± 0.29           | –                     | –                  |
| 1.0  | 1.0  | 1.5 ± 0.29            | Callus initiated       | +                  |
| 1.0  | 1.5  | 1.5 ± 0.29            | Green and whitish callus | +            |
| 1.0  | 2.0  | 1.75 ± 0.48           | Compact and globular callus | +         |
| 1.0  | 2.5  | 1.5 ± 0.29            | –                     | –                  |
| 1.0  | 3.0  | 2.0 ± 0.41            | –                     | –                  |
| 1.0  | 3.5  | 3.25 ± 0.75           | –                     | –                  |
| 1.5  | 0.25 | 2.75 ± 0.48           | –                     | –                  |
| 1.5  | 0.5  | 3.5 ± 0.65            | Callus initiated       | –                  |
| 1.5  | 1.0  | 2.5 ± 0.65            | Green and whitish callus | +            |
| 1.5  | 1.5  | 2.75 ± 0.48           | Compact and globular callus | +         |
| 1.5  | 2.0  | 1.5 ± 0.65            | –                     | –                  |
| 1.5  | 2.5  | 2.25 ± 0.48           | –                     | –                  |
| 1.5  | 3.0  | 2.25 ± 0.25           | –                     | –                  |
| 1.5  | 3.5  | 2.0 ± 0.41            | –                     | –                  |
| 2.0  | 0.25 | 6.75 ± 0.49           | –                     | –                  |
| 2.0  | 0.5  | 3.5 ± 0.65            | –                     | –                  |
| 2.0  | 1.0  | 2.5 ± 0.29            | Callus initiated       | +                  |
| 2.0  | 1.5  | 3.5 ± 0.65            | Green and whitish callus | +            |
| 2.0  | 2.0  | 2.5 ± 0.29            | Compact and globular callus | +         |
| 2.0  | 2.5  | 3.0 ± 0.41            | –                     | –                  |
| 2.0  | 3.0  | 2.5 ± 0.29            | –                     | –                  |
| 2.0  | 3.5  | 2.75 ± 0.25           | –                     | –                  |
| 2.5  | 0.25 | 2.5 ± 0.65            | –                     | –                  |
| 2.5  | 0.5  | 2.25 ± 0.25           | –                     | –                  |
| 2.5  | 1.0  | 2.5 ± 0.29            | Callus initiated       | +                  |
| 2.5  | 1.5  | 4.0 ± 0.41            | Green and whitish callus | +            |
| 2.5  | 2.0  | 2.0 ± 0.41            | Compact and globular callus | +         |
| 2.5  | 2.5  | 3.0 ± 0.71            | –                     | –                  |
### Table 1.2 (continued)

| IBA  | BAP  | No. of root formation | Observation of callus              | Leaf proliferation |
|------|------|-----------------------|------------------------------------|--------------------|
| 2.5  | 3.0  | 2.5 ± 0.29            | –                                  | –                  |
| 2.5  | 3.5  | 2.5 ± 0.29            | –                                  | –                  |
| 3.0  | 0.25 | 2.0 ± 0.41            | –                                  | –                  |
| 3.0  | 0.5  | 4.0 ± 0.41            | –                                  | –                  |
| 3.0  | 1.0  | 2.5 ± 0.29            | Callus initiated                   | +                  |
| 3.0  | 1.5  | 2.75 ± 0.25           | G Green and whitish callus         | +                  |
| 3.0  | 2.0  | 3.5 ± 0.65            | Compact and globular callus        | +                  |
| 3.0  | 2.5  | 3.0 ± 0.41            | –                                  | –                  |
| 3.0  | 3.0  | 2.5 ± 0.29            | –                                  | –                  |
| 3.0  | 3.5  | 2.75 ± 0.63           | –                                  | –                  |
| 3.5  | 0.25 | 2.75 ± 0.48           | –                                  | –                  |
| 3.5  | 0.5  | 4.0 ± 0.41            | –                                  | –                  |
| 3.5  | 1.0  | 3.25 ± 0.48           | Callus initiated                   | +                  |
| 3.5  | 1.5  | 2.5 ± 0.29            | Green and whitish callus           | +                  |
| 3.5  | 2.0  | 2.75 ± 0.48           | Compact and globular callus        | +                  |
| 3.5  | 2.5  | 2.5 ± 0.29            | –                                  | –                  |
| 3.5  | 3.0  | 3.0 ± 0.41            | –                                  | –                  |
| 3.5  | 3.5  | 2.5 ± 0.29            | –                                  | –                  |

Mean ± SE (n=10). + = organ (leaf) formation was indicated. – = no-indication of organ formation.

### Table 1.3

Effects of different combination of hormone on the fresh weight of callus produced from leaves.

| BAP  | IBA  | Callus weight |
|------|------|---------------|
| 1.0  | 0.25 | 0.6 ± 0.15    |
|      | 0.5  | 1.02 ± 0.06   |
|      | 1.0  | 1.4 ± 0.09    |
|      | 1.5  | 3.9 ± 0.15    |
|      | 2.0  | 2.35 ± 0.23   |
|      | 2.5  | 1.8 ± 0.11    |
|      | 3.0  | 2.15 ± 0.07   |
| 1.5  | 3.5  | 2.18 ± 0.4    |
|      | 0.25 | 2.7 ± 0.15    |
|      | 0.5  | 2.4 ± 0.04    |
|      | 1.0  | 2.37 ± 0.12   |
|      | 1.5  | 2.45 ± 0.10   |
|      | 2.0  | 2.3 ± 0.09    |
|      | 2.5  | 2.35 ± 0.06   |
|      | 3.0  | 2.0 ± 0.17    |
| 2.0  | 3.5  | 2.22 ± 0.10   |
|      | 0.25 | 2.47 ± 0.19   |
|      | 0.5  | 1.4 ± 0.15    |
|      | 1.0  | 1.85 ± 0.04   |
|      | 1.5  | 2.15 ± 0.06   |
|      | 2.0  | 2.15 ± 0.06   |
|      | 2.5  | 2.35 ± 0.12   |
|      | 3.0  | 2.3 ± 0.04    |

Callus produced per leaves explant. Mean ± SE (n=10).
2.3. Seed sterilization and germination in the MS media

Seeds of broccoli were obtained from the nursery. A total of 100 seeds were used on MS [1] basal medium. The 20 jam jars were used to culture the seeds and five seeds were germinated on every jam jars. The seeds were washed in 70% ethanol for about 5 min, and then rinsed in 15% chlorox for 15 min. The seeds were brought into the laminar flow hood and further rinsed with sterile DH20 for a few seconds. Then, the sterilized seeds were germinated on MS basal media for 7 days. This process was carried out under aseptic condition in the laminar flow. The seeds were exposed to light from cool white fluorescent tubes for a photoperiod of 16 h in the incubation room at 25–28 °C.

2.4. MS basal media with IAA and IBA and BAP (2nd time media preparation)

The MS media with IBA and BAP were used as rooting media, MS powder form was added in a beaker filled with 800 ml distilled water and 30 g of sucrose was added. Then, the hormones with specific concentration were added. The pH was similarly adjusted and 2.8 g phyta gel was added, so that 1000 ml of medium was prepared. The media with hormones were prepared for 10 replicates of each hormone concentration. The BAP (as cytokinin) and IBA (as auxin) concentrations were 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3 and 3.5 mg/l.

2.5. Leaf cutting slice culture on MS supplemented with IBA and BAP

After one week of germination, 7 days seedlings were selected as a source of explants. The explants leaves were cut and transferred into the media with different concentrations of IBA and BAP 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 mg/l. Each treatment was consisted as five replications. Broccoli leaves were sliced in the clean bench. After that, in vitro culture on MS basal was performed. The leaf slice cultures were put in the growth chamber in the incubation room at 25–28 °C. Randomized complete block designed (RCBD) was used during sampling setting.

2.6. Antioxidant activity of broccoli

The antioxidant was evaluated based on the scavenging activity on the stable 2,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical measured by spectrophotometer [2]. DPPH was useful reagent for investigating the free radical activities of compounds. A freshly prepared DPPH solution was exhibited a deep purple color with maximum absorption at 515 nm. The DPPH observation was a non-enzymatic method currently used to provide basic information on the ability of extracts to scavenge free radical. The OD reading of control, positive control (vitamin C and BHT [3]) and all samples were taken at 515 nm using spectrometer. The use of the percentage of free radical scavenging activity was calculated by the following formula for vitamin C. Vitamin C = [(A control – A sample)/A control]x100 [3].

| Samples                          | OD reading (515 nm) |
|----------------------------------|---------------------|
| Control (ethanol 95%)            | 0.058               |
| Vitamin C                        | 0.005               |
| BHT                              | 0.008               |
| Leaves extracts (10 mg/ml)       | 0.017               |
| Callus extracts (10 mg/ml)       | 0.031               |
| Leaves extracts (20 mg/ml)       | 0.027               |
| Callus extracts (20 mg/ml)       | 0.036               |

Table 1.4 Measurement of the OD reading of control, positive control (vitamin C and BHT) and other samples taken at 515nm using spectrometer.
2.7. Data collection

Root and callus formation and leaf proliferation were observed and data were collected after one month of treatment setting. The 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical was measured.

2.8. Design and statistical analysis

Randomized block design was used during sampling setting. Standard deviation and then standard error was made to compare the replicates. Least Significant Difference (LSD) test was used for data analysis.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.11.061.

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