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Effect of Induced Mutation on Antioxidant Activity in *Ocimum basilicum* Linn

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Abstract

Five batches from the same stock of seeds of *Ocimum basilicum* were irradiated at 5, 10, 15, 20 and 25 Gy, respectively using ^{60}C source. Methanolic leaf extracts of these samples and a control were evaluated for their antioxidant activity by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method using M_2 plants. All the methanolic extracts showed antioxidant activity. The IC_{50} of the methanolic extracts of the six different treatments, control, 5 Gy, 10 Gy, 15 Gy, 20 Gy and 25 Gy, showed antioxidant activity with IC_{50} values of 100, 90, 86, 61, 71 and 70 µg/ml, respectively. Three individual mutants, M-15-5, M-20-6 and M-15-4, had IC_{50} values of 26, 30 and 40 µg/ml, respectively. These mutants were from the 15 Gy and 20 Gy treatments. From the results, it is confirmed that induced mutation can be employed to create variation in the levels of free radical scavenging activity in *O. basilicum* and can therefore serve as a tool for breeding for high levels of antioxidant activity in *O. basilicum*.

Keywords

***Ocimum basilicum*, Nonirradiated, Irradiated, Antioxidant Activity, Mutant, 1,1-Diphenyl-2-picryl-hydrazyl**

1. Introduction

The use of natural antioxidants by consumers and the scientific community is on the increase since epidemiological studies have shown that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer [1] [2] Natural antioxidants may be used as reducing agents, free radical scavengers, complexes of pro-oxidant metals and quenchers of reactive oxygen species. Antioxidant activity is

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mostly due to flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins [3]. Currently, there is considerable interest in new natural antioxidants to replace the synthetic ones that are used in foods and cosmetics.

Ocimum basilicum belongs to the plant family Lamiaceae (syn. Labiatae). Species belonging to this family have been reported to contain high levels of dietary antioxidants [4]-[6], and the *in vitro* antioxidant potency of these species has been revealed in numerous studies [7].

In Ghana, *O. basilicum* is cultivated as homegarden herb and it is used in traditional medicine, but it is mainly known for its culinary properties as seasoning. The search for varieties of this plant species with high levels of antioxidant would require a breeding exercise to increase its genetic base. Induced mutation is a breeding technique employed to increase the genetic base of plant and animal species after which individuals with high levels of the desirable traits are then selected. The experiment aims at using induced mutation to create genetic variation in *O. basilicum* and then select for plant individuals with high levels of antioxidant activities. In Ghana, medicinal plant breeding has received little attention and the current work seeks to set the pace for breeding medicinal plants.

2. Materials and Methods

DPPH was obtained from Sigma Aldrich Co. (St. Louis, USA). All other chemicals used were of analytical grade.

2.1. Seed Multiplication

Seeds from accessions of *Ocimum basilicum* were collected from homegardens in Accra and Aburi in Ghana. Seeds were nursed and one hundred and five (105) seedlings were transplanted in the field. Seeds were harvested from these plants and bulked. Six batches of seeds were prepared and five of them were subjected to five different treatments of irradiation. The remaining batch served as a control.

2.2. Irradiation of Seeds

The five batches of seeds were sealed in polyethylene bags (ca 80 µm thick) and placed in ice-cooler boxes prior to irradiation and after irradiation. Samples were irradiated at 5, 10, 15, 20 and 25 Gy, respectively at the Radiation Technology Centre of the Ghana Atomic Energy Commission using ^{60}C source. Non-irradiated (control) and irradiated samples were stored in a deep freezer at −4˚C until needed for use.

2.3. M_1 Generation

The irradiated and non-irradiated seeds were nursed in wooden trays. Seedlings were transplanted into polyethylene bags. Seeds obtained from M_1 seeds were subsequently sown in M_2 generation. Leaves from the M_2 and non-irradiated plants were harvested and air-dried for four days and pulverized.

2.4. Preparation of Crude Plant Extract

The crude extracts were obtained by dissolving a known amount of the pulverised leaves in 98% methanol to obtain a stock solution of 5 mg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (3, 4, 6, 8, 10, 15, 25, 40, 50, 75, 100 µg/ml).

2.5. Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract

The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 100 µg/ml. An amount of 0.002% DPPH was prepared in methanol. One millilitre of this solution was mixed with 1 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using a spectrophotometer against methanol [8]. One (1) ml of methanol with 1 ml of DPPH solution (0.002%) was used as the blank. The optical density was recorded and percent of inhibition was calculated using the formula given below:

Percent inhibition of DPPH activity = $\frac{(A-B)}{A}\times 100$, where A is optical density of the blank and B is optical

Table 1. *In vitro* antioxidant activity of the M_2 generation methanolic extracts.

Test compound (methanolic extract)	IC_{50} (µg/ml) (Mean ± SD)
Control	100 ± 0.1
T-5 Gy	90 ± 1.2
T-10 Gy	86 ± 1.0
T-15 Gy	61 ± 1.5
T-20 Gy	71 ± 1.1
T-25 Gy	70 ± 1.3
Ascorbic acid (aq.)	3.1

Table 2. *In vitro* antioxidant activity of the methanolic extracts of the three best M_2 generation mutants.

Test compound (methanolic extract)	IC_{50} (µg/ml) (Mean ± SD)
M-15-5	26 ± 0.1
M-15-4	40 ± 1.1
M-20-6	30 ± 0.12
Ascorbic acid (aq.)	3.1

density of the sample.

2.6. Statistics and IC_{50}

Decolorization was plotted against the sample extract concentration and a linear regression curve was established to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the DPPH free radical by 50%. All the analyses were carried out in triplicate and the results expressed as mean ± SD. Statistical analyses were performed using SAS computer software.

3. Results and Discussion

The crude methanolic extracts of the treatments: control, 5 Gy, 10 Gy, 15 Gy, 20 Gy and 25 Gy showed antioxidant activity with IC_{50} mean values of 100.0 ± 0.1, 90.0 ± 1.2, 86.0 ± 1.0, 61.0 ± 1.5, 71.0 ± 1.1, and 70.0 ± 1.3 µg/ml, respectively. The IC_{50} mean value for ascorbic acid was 3.1 ± 0.8 µg/ml. The results indicate that the antioxidant activity of the crude extracts of the irradiated plants is higher than that of the control but less than that of ascorbic acid (**Table 1**). **Table 2** shows three individual mutants which have relatively low IC_{50} values. Mutants M-15-5, M-20-6 and M-15-4 had IC_{50} values of 26.0 ± 0.1, 30.0 ± 0.12 and 40.0 ± 1.1 µg/ml respectively. These mutants were from the 15 Gy and 20 Gy treatments.

In earlier studies, seven phenolic compounds namely gallic, vanillic, syringic, caffeic, 2,5-dihydroxybenzoic, rosmarinic and p-coumaric acids were identified in methanolic extracts of three different nonirradiated varieties of *O. basilicum* where DPPH radical scavenging activities of 63%, 53% and 52% were observed [9]. The observed IC_{50} values for the methanolic extracts can therefore be attributed to phenolic compounds among other phytochemical constituents such as tannins, reducing sugars and proteins.

In the present study, the free radical scavenging activity of the methanolic extract was confirmed. It is also confirmed that induced mutation can be used to create variation in the levels of free radical scavenging activity in *O. basilicum* and can serve as a tool for breeding for high levels of antioxidant activity in *O. basilicum*.

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