Journal of Applied Biology & Biotechnology Vol. 11(3), pp. 245-249, May-Jun, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.111110

Total phenolic, flavonoid contents, and antioxidant activity of three selected *Portulaca grandiflora* mutants in MV8 generation as a result of recurrent irradiation technique

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ARTICLE INFO

Article history:
Received on: September 03, 2022
Accepted on: January 04, 2023
Available online: April 04, 2023

Key words:
Polyphenols,
Antioxidant activity,
Recurrent irradiation,
Ornamental crop.

ABSTRACT

Portulaca grandiflora belongs to the *Portulacaceae* family that is cultivated as an ornamental plant. Because of the sound pharmacological and curative effects of *P. grandiflora*, this plant can be used as a medicinal plant. Antioxidants are one of the important properties of *P. grandiflora.* Recurrent irradiation method using gamma-ray can modify the plant biochemical arrangement, and the resulting changes are heritable characters. The objective of the present experiment was to evaluate the phenolic content, flavonoids, and antioxidant activity of three *P. grandiflora* mutated genotypes in an advanced population of MV8 (eighth generation in *P. grandiflora* mutation breeding program). Before the extraction process, the three genotypes were measured fresh weight and dry weight. Extraction of the three test samples of *P. grandiflora* using the maceration method. Analysis of total phenolics was based on the Folin–Ciocalteu method and total flavonoids using the aluminum chloride solution method. The antioxidant capacity of the three *P. grandiflora* genotypes was examined using the free radical 1,1-diphenyl-2-picrylhydrazyl. Fresh weight and IC_{50} values of the three *P. grandiflora* genotypes were statistically significantly different. The antioxidant activity of *P. grandiflora* emanated from phenolic and flavonoid compounds. The recurrent irradiation method on *P. grandiflora* produced three novel genotypes that were tested to have potential antioxidant capacities that were useful for protecting the body from oxidative stress.

1. INTRODUCTION

Portulaca grandiflora is one of the potential ornamental plants centered on esthetic value and nutritional value. *P. grandiflora* is a species of the *Portulacaceae* family that has been shown to have various pharmacological effects [1]. Apart from highlighting the attractiveness of its morphology, *P. grandiflora* has various bioactive compounds that are helpful in folk medicine and maintain the health of the body's organs [2]. Ethnobotanical reviews noted that *P. grandiflora* has edible leaves [3]. Regarding its medicinal use, *P. grandiflora* functions as anti-inflammatory, antifungal, antiseptic, antidiabetic, and antispasmodic [4]. Furthermore, *P. grandiflora* is utilized to treat various mild-to-severe ailments [5].

As a medicinal and therapeutic plant, *P. grandiflora* has numerous phytonutrient compounds. *P. grandiflora* has a typical class of compounds named Portulacanones. Yan *et al.* [6] found that Portulacanones compounds have cytotoxic activity against human cancer cells. Besides phenolic compounds, the results of biochemical screening of *P. grandiflora* performed by Devi *et al.* [2] showed the presence of other compounds such as glycosides, phytosterols, saponins, and isoprenoids. Like most medicinal plants, *P. grandiflora* has a significant role in warding off free radicals. The capacity of plants to stave off free radicals is closely related to the presence of phenolic compounds [7].

Optimizing the potential of antioxidant activity in medicinal plants is necessary needed considering the number of emerging diseases nowadays. The antioxidant capacity of plants improves with the increase in the production of polyphenolic compounds [8,9]. Plant biochemical studies have reported considerable benefits of polyphenolic compounds on human health [10-12]. Different studies related to optimizing the plant growth environment contribute to the increase in plant secondary metabolites [13-17]. Moreover, improvement of the nutritional and biochemical properties of plants can be achieved through genetic improvement of plants. Mutation breeding, a combination of plant breeding programs and mutation techniques, is important in improving plant genetic potential [18]. One of the outcomes of the mutation breeding program is a new cultivar with more improved nutrition.

Artificial mutation technique using gamma-ray irradiation, ionizing radiation, is frequently utilized to increase plants' morphological

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diversity and phytochemical content. Hong *et al.* [19] reported an increase in the activity of antioxidant enzymes and the phenolic content of wheat due to short and long exposure to gamma irradiation. El-Beltagi *et al.* [20] conveyed that the 5 kGy gamma-ray irradiation dose increased the glucose, fructose, flavonoid, and phenolic content of date fruits. Due to gamma-ray irradiation, the proline and polyphenols content improved significantly in *Terminalia arjuna* [21]. The recurrent irradiation method is one of the four methods of applying artificial mutations that plant mutation breeders prefer to maximize plant genetic diversity [22]. Several studies and reviews have reported increased morphological variations caused by gamma-ray mutagenesis using the recurrent method. Nevertheless, changes in biochemical composition due to recurrent irradiation treatment are still severely limited.

A plethora of comprehensive research on exploring and improving the biochemical compounds of *P. grandiflora*, particularly the polyphenol content, has been conducted [1-5]. However, to the best of our knowledge, no studies report the alteration of polyphenolic contents and the antioxidant capacity of *P. grandiflora* obtained from the gamma-ray mutation. This research aims to obtain information on the phenolic and flavonoid contents along with the antioxidant activity of three selected mutants of *P. grandiflora* in an advanced population (MV8 population), which is obtained through recurrent induced mutation using gamma-ray irradiation.

2. MATERIALS AND METHODS

2.1. Study Region

The process of harvesting three test genotypes of *P. grandiflora* was carried out in the experimental field of Sabisa Farm, IPB University, Sindang Barang, Bogor, West Java, and the biochemical analysis of *P. grandiflora* was situated at Tropical Biopharmaca Research Center, IPB University, Bogor, West Java.

2.2. Plant Material Preparation and Extraction Method

In this study, three *P. grandiflora* genotypes that arose out from the recurrent irradiation method were selected to measure the phenolic, flavonoid, and antioxidant activity. The underlying reason for selecting the three potential genotypes of *P. grandiflora* was based on the emergence of new morphological characters after eight generations of mutation breeding using the gamma-rays recurrent method [Table 1]. The whole plant part of the *P. grandiflora* was extracted as the test sample solution. The sample extraction was using the maceration method with slight modifications [23]. Beginning with weighing the fresh weight, then the three selected genotypes of *P. grandiflora* were dried using an oven until the water content reached less than 10%. Soon after, the dry weight is weighed, and the dry sample is formed into a powder. The powder samples of *P. grandiflora* were immersed in aqueous ethanol (70% v/v) for 48 h. The ratio between extract and solvent is 1:10. The filtrates were evaporated to obtain the sample in the form of a paste.

2.3. Total Phenolic Content (TPC) Analysis

The phenolic content of *P. grandiflora* is estimated using the Folin–Ciocalteu method with modifications [24]. Folin–Ciocalteu solution consisting of 160 ml of purified water, 10 ml of Folin–Ciocalteu reagent (10%), and 20 ml of Na_2CO_3 (10%) was mixed with *P. grandiflora* extract and incubated for 30 min. The absorbance of the sample was measured at a wavelength of 750 nm using a microplate reader (Epoch BioTek, USA). The TPC was defined as mg gallic acid equivalent per gram of extract (mg GAE g^{-1}).

2.4. Total Flavonoid Content (TFC) Analysis

The flavonoid assay was carried out using the aluminum chloride ($AlCl_3$) solution method with a slight change [25]. The test solution consisted of a mixture of 10 ml of *P. grandiflora* sample, 60 ml of methanol, 10 ml of $AlCl_3$ (10%), 10 ml of potassium acetate (CH_3COOK) with a concentration of 1 molar, and 120 ml of distilled water. The sample mixture was stored in the dark for 30 min. The sample wavelength was measured at 415 nm. Total flavonoids in the sample were expressed in mg of quercetin equivalent per gram of extract (mg QE g^{-1}).

2.5. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

Antioxidant capacity analysis using the stable free radical DPPH referring to Nurcholis *et al*. [26] with modification. A 200 ml of extract consisting of each of the six concentration levels (0. 31.25, 62.5, 125, 250, 500, 1000, and 2000 ppm) was combined with 2 ml of DPPH (0.1 mM) and 0.8 ml of methanol. The test solution was homogenized for 1 h. Absorbance is measured using a microplate reader (Epoch BioTek, USA) at a wavelength of 517 nm.

2.6. Data Analysis

The fresh weight and dry weight data of three *P. grandiflora* genotypes are presented in a bar chart processed with Microsoft Excel 2010. The mean biochemical properties of *P. grandiflora* were tested statistically by analysis of variance (ANOVA) and followed by Tukey's test at a 5% significance level to express a significant difference. Correlation analysis was performed by R studio software (corrplot package).

3. RESULTS AND DISCUSSION

3.1. Fresh and Dry Weight

Figure 1 provides information on the fresh and dry weights of the three selected genotypes of *P. grandiflora*. It is readily apparent that PA was recorded with the least fresh and dry weight, while PC showed the highest weight among the three genotypes. After the drying process, the three genotypes of *P. grandiflora* experienced an extreme reduction in weight. The highest dry weight was recorded in the PC genotype with 84 gr, and the lowest weight was recorded in the PA genotype with 69 g. Engagingly, PC genotype, plants with pink flowers, showed the lowest plant length and flower diameter compared to the other two genotypes (PA and PB) but had the highest weight among the two different genotypes.

Although exogenous factors influence plants' fresh and dry weight, genetic factors cannot be separated from their contribution to plant mass. Vanani *et al.* [27] have explored the variety of fresh and dry

Table 1: Phenotypic descriptions of three selected *P. grandiflora* mutants.

Genotypes	Sample code	Flower color	Stem color	Leaves color	Plant length (cm)	Flower diameter (cm)
PGA50+35 3.1.23	PA	Pink-purple	Orange-brown	Green	29.51–32.15	3.0–3.2
PGB50+45 11.1.8	PB	Pink-purple	Orange-brown	Green	27.43–31.37	2.8–3.2
PGC75+55 8.1.22	PC	Pink	Orange-brown	Green	21.13–25.04	2.5–3.0

weight in *Lolium perenne* genotypes. Sujatha *et al.* [28] reported their findings that the weight measurement of the genotypes of Gerbera, one of the well-known ornamental plants, proved to be significantly different. Chaimala *et al*. [29] found the genetic basis of the six test genotypes of Jerusalem artichoke to affect plant growth characteristics such as total biomass and shoot and tuber dry weight. In this study, the genotype diversity in fresh and dry mass was derived from the induced artificial mutation using gamma-ray irradiation, even though external factors also play a role in plant conditions.

3.2. Phenolics, Flavonoids, and Antioxidant Activity

The antioxidant, total phenolic, and total flavonoid activities of selected genotypes of *P. grandiflora* are summarized in Table 2. In this study, we measured the antioxidant effect of *P. grandiflora* extract through the reaction capacity with the stable free radical DPPH. Dzoyem *et al.* [30] explained that the smaller the IC_{50} value of an extract, the better the antioxidant potential. The IC_{50} value of the ethanolic extract of three genotypes of *P. grandiflora* showed that the PB sample had the highest antioxidant activity (270.56 g mL^{-1}), followed by the PA sample (301.15 g mL^{-1}), and last, the PC sample (327.59 g mL^{-1}). Bi *et al.* [31] classifying IC_{50} values based on the efficacy of antioxidant activity: Weak (IC_{50} >500 g mL^{-1}), medium (IC_{50},100–500 g mL^{-1}), and strong (IC_{50} >500 g mL^{-1}). Referring to the former category, the antioxidant activity of the three selected genotypes of *P. grandiflora* was moderate. However, the IC_{50} of ascorbic acid, one of the positive controls frequently used for *in vitro* assays, far greater compared to the IC_{50} value of the three *P. grandiflora* genotypes (3.77 µgmg^{-1}). The antioxidant capacity of the three potential genotypes of *P. grandiflora* was higher than *Apium graveolens* and *Sonchus arvensis* that had been analyzed by Sukweenadhi *et al.* [32]; three genotypes of *Curcuma aeruginosa* reported by Nurcholis *et al.* [26]; and four extracts of medicinal plants evaluated by Laryea [33]. Nevertheless, the methanol extract of *Caesalpinia volkensii*, *Vernonia lasiopus*, and *Acacia hockii* had more promising antioxidant activity than the three examined extracts of *P. grandiflora* [34].

Phenolic content (TPC) in the three selected genotypes of *P. grandiflora* ranged from 3.70 to 4.72 mg EAG 100 g^{-1}, PC genotype had the lowest TPC content, while PA genotype was recorded with the highest TPC. Likewise, with regard to TFC, The PC genotype had the lowest flavonoid content (1.04 mg EK 100 g^{-1}), while in contrast, the PB genotype possessed the highest flavonoid content (1.16 EK 100 g^{-1}). Interestingly, in terms of morphology, the genotype of *P. grandiflora* with pink petals tended to have lower polyphenol content and antioxidant properties than the genotype with pink-purple petals. Interestingly, in terms of morphology, the genotype of *P. grandiflora* with pink petals tended to have lower polyphenol content and antioxidant properties than the genotype with pink-purple petals. Our findings agree with Gonçalves *et al.* [35] who found that the pink petal orchid had low phenolic content. Nonetheless, in particular cases, the phytochemical content in pink petal plants is greater than in colorless petals. Hallman [36] studied the polyphenol content in pink and white *Robinia* flowers and found that pink flowers contain more phytochemicals than white flowers. Another study conducted by Yang and Lu [37] revealed that white petal *Macadamia* flowers contain higher polyphenols and antioxidants than pink petal flowers. These unique phenomena occur based on the availability of different active secondary metabolites in each species. It is necessary to conduct further analysis, metabolite profiling, to compare the widespread secondary metabolites contained between pink petal and pink-purple petal of *P. grandiflora*.

3.3. Correlation Analysis

Figure 2 illustrates the relationship among the observed biochemical constituents of *P. grandiflora*. TPC and TFC have a solid positive correlation value of 0.84. This result aligns with the fact that flavonoid is one of the compounds deriving from phenolic compounds. These results correspond to the findings of Calvindi *et al.* [24] who informed a positive correlation between TPC and TFC in winged wings, but in contrast with Wairata *et al.* [38] who discovered a negative correlation between TPC and TFC in *Garcinia forbesii*. Moreover, regarding the antioxidant

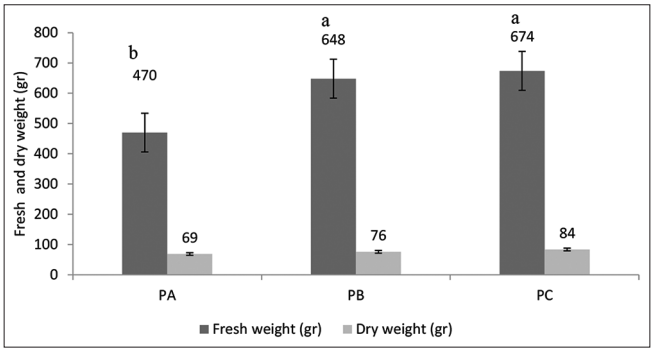

Figure 1: Comparison of fresh and dry weights among three *P. grandiflora* genotypes. Different letters display significant differences based on the Tukey *post hoc* test at the 5% level.

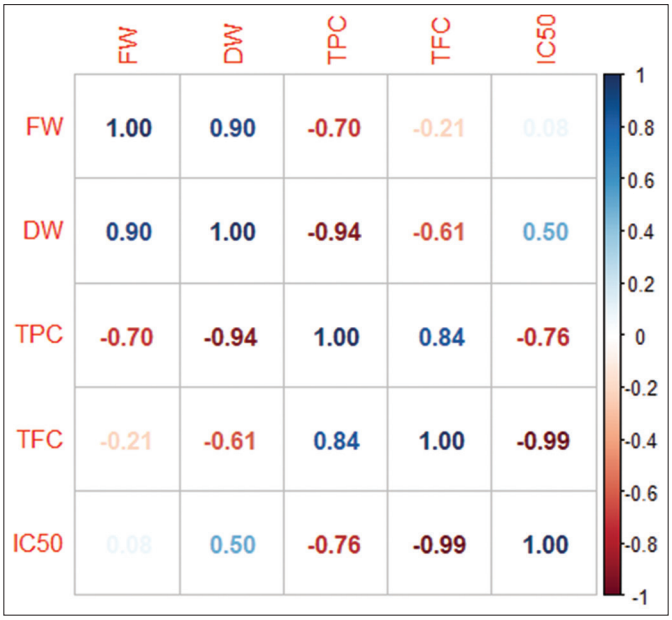

Figure 2: Correlation matrix on the biochemical properties of *P. grandiflora.*

Table 2: The mean values of phenolic, flavonoid, and antioxidant capacity of three selected genotypes of *P. grandiflora.*

Sample	Genotypes	IC_{50} (g mL^{-1})	TPC (mg EAG 100 g^{-1})	TFC (mg EK 100 g^{-1})
PA	PGA50+35 3.1.23	301.15[a]	4.72	1.11
PB	PGB50+45 11.1.8	270.56[a]	4.57	1.16
PC	PGC75+55 8.1.22	327.59[a]	3.70	1.04
A	Ascorbic acid	3.77[b]	-	-

Different letters display significant differences based on the Tukey *post hoc* test at the 5% level.

potential of *P. grandiflora*, TPC and TFC have strong negative correlation values with IC_{50} of –0.76 and −0.99, respectively. The negative correlation values, particularly between phenolic, flavonoid, and IC_{50}, imply that the higher the phenolic and flavonoid content of *P. grandiflora*, the lower the IC_{50} value. Potent antioxidant expressed with a low IC_{50} value. Essence, TPC, and TFC are markedly responsible for the strength of the antioxidant properties of *P. grandiflora*.

4. CONCLUSION

The three mutated genotypes of *P. grandiflora* resulting from artificial irradiation using gamma-rays had antioxidant capacity derived from phenolic and flavonoid compounds. These favorable genotypes have the prospect of being new multifunctional cultivars.

5. ACKNOWLEDGMENTS

The present research is fully supported by PDUPT research program (186/E5/PG.02.00.PT/2022) from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

6. AUTHORS' CONTRIBUTIONS

Waras Nurcholis conducted the research design, conducted research, analyzing the data, wrote manuscript draft, and improved the manuscript. Syarifah I. Aisyah conducted research designs and wrote drafts, Regina A. M. Saraswati conducted the research, analyzing the data, and wrote manuscript draft, and Yoshua S. Yudha wrote draft, improved the manuscript, and analyzing the data.

7. FUNDING

The Ministry of Research, Technology and Higher Education of the Republic of Indonesia through PDUPT research program with number of 186/E5/PG.02.00.PT/2022.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Nurcholis W, Aisyah SL, Saraswati RA, Yudha YS. Total phenolic, flavonoid contents, and antioxidant activity of three selected *Portulaca grandiflora* mutants in MV8 generation as a result of recurrent irradiation technique. J App Biol Biotech. 2023;11(3):245-249. DOI: 10.7324/JABB.2023.111110