

Determination quercetin content, antioxidant and antimicrobial activity of genotype mutant Samosir shallots irradiated by gamma rays

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Abstract. The aim of the research was to study the variation in antioxidant and antimicrobial activity as well as the total quercetin content of the fifth generation genotypes mutant Samosir shallot irradiated by gamma rays. The studies conducted included the assessment of quercetin content, antioxidant and antimicrobial activity in shallot bulbs after long- term storage (6 months in the room temperature). Quercetin content of 20 selected genotype mutants of irradiated shallot bulbs along with untreated populations were calculated using quercetin (QU) as a standard. Antioxidant activities of 8 genotype mutant were determined using DPPH. Antimicrobial activity of bulb extracts were tested against six bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* and one yeast *Candida albicans*. The results showed that population of genotype mutants irradiated with dosage 2Gy, 4 Gy, 5 Gy and 6 Gy have higher quercetin content than control samples. None of the genotype mutants exhibited antibacterial inhibitory against all microorganism tested except for the sample number 2 and 6 (bulbs generated from the plants irradiated by gamma rays with dosage at 2 Gy and 6 Gy). There was also none of the genotypes observed exhibited significant antioxidant efficacy.

1. Introduction

Shallot (*Allium cepa* L. var. *ascalonicum* Backer) belongs to the Liliaceae family, is a common food spice, used widely in many parts of the world. For many centuries various species of genus *Allium* have been used as flavoring vegetable and as medicines for curing various diseases.

Shallot is abundant with biologically active compounds of antioxidant properties. These include, among others, flavonoids and phenolic acids [1-5]. Flavonol levels in the edible portion of *Allium* vegetables including leeks, shallots, green onions, garlic, and onions range from less than 0.03 to 1 g/Kg of vegetables [6]. Analysis of shallot extracts has confirmed the presence of flavone and polyphenolic derivatives such as quercetin, quercetin 4 β -glucoside, quercetin 7,4 β -diglucoside, quercetin 3,4 β -diglucoside, and quercetin mono-D-glucose, suggesting that it also may have antioxidant properties [7]. It is well known that quercetin has a pronounced effect to allergies, asthma, arthritis, cancer, diabetic complications, goat, neurodegenerative disorder and osteoporosis [8]. *Allium* species are a rich source of phytonutrients, useful for the treatment or prevention of a number of diseases,



including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) [9].

In in-vitro experiments, onion showed antibacterial and antifungal activity against both gram-positive and gram-negative bacteria (including enteropathogens), pathogenic yeast (*Candida* spp.) and some skin-pathogenic fungi. Alliums were important in warding off plague and other microbial infections over the centuries [10]. Allium crops are well known for their biological activity resulting from the presence sulphur compound in the bulbs, however other compounds, namely flavonoids and other polyphenols, are also in the area of interest. Flavonoid compounds have received considerable attention because of their potential health-promoting properties for human consumers. Flavonoids show wide range of biological properties, mainly connected with beneficial effect on cardiovascular system and with their antioxidant activity [11]. Allium vegetables are rich in flavonoles, primarily quercetin, and among the onion varieties the most abundant in quercetin are shallots and red onion [12]. It contains sulphur compound like allyl propyl disulphide, chromium and vitamin B6 known as compound that could reduce homocysteine, a factor cause heart attack and stroke. *Allyl propyl disulphide* in shallots could also increase insuline content and decrease glucose content in the blood. Chromium could decrease triglyceride and cholesterol in the blood. It also contains high flavonoids like quercetin that can reduce the risk of cancer [13].

Flavonoids and phenolic acids are the most important groups of secondary metabolism in plants that consider as good sources of natural antioxidant in human diets. In the last decade, gamma irradiation has been drawn the attention as a new and rapid method to improve the qualitative and quantitative characters of many crops. Irradiation with gamma rays is currently used as a tool in mutation breeding technology for enhancing the production of plant secondary metabolites like alkaloids or to increase biomass production in medicinally valuable plants [14]. Gamma rays were reported to be the most efficient ionizing radiation of creating mutants in plants as they can induce high mutation numbers in plants. It could also modify physiological characteristics to create new mutants with improved properties that can produce higher amounts of commercially essential metabolites, developing varieties that are agriculturally and economically significant, and contain high productivity potential [15-16].

A lot of work has been carried out on the beneficial effects of gamma irradiation in improving the crop, however for my knowledge, there is no report informing about impact of gamma irradiation on enhancement antioxidant and antimicrobial activity of shallot mutant.

The aim of the research was to investigate the variation in antioxidant and antimicrobial activity as well as the total quercetin content of the fifth generation genotype mutant Samosir shallot irradiated by gamma rays.

2. Materials and Methods

2.1. Preparing plant materials

Uniform and healthy dry bulbs 2,5 months after harvest collected from Samosir farm, selected with the weight ranging from 1,3 g to 1,7 g, by doses of irradiated along with non-irradiated local Samosir shallot were packaged in 0.1 mm thick paper bags of 10 cm x 22 cm dimension and sealed. The bags were subjected to gamma rays irradiation in radiator Chamber 4000 A using ^{60}Co gamma source at Patir Batan, Jakarta, by exposing them to gamma irradiation with doses ranging from 1 to 9 Gy. Subsequently, the irradiated bulbs along with unirradiated bulbs (control) were planted in experimental field for generation advance. Bulbs of the M_1V_1 generation were planted in 2014 for generation advance in an experimental field. Bulbs harvested individually gave rise to the M_1V_2 population. In the following year, M_1V_2 were planted again in experimental field, aiming at the generation advance. Each plant was harvested individually, giving rise to M_1V_3 genotype. Selected mutants by dosage of irradiation M_1V_3 were planted giving rise to M_1V_4 and M_1V_5 . Six months after harvesting, populations of M_1V_5 were used as the samples to be analysed in antioxidant and antimicrobial activity and five months shallot bulbs stored in the content of quercetin.

2.2. Assay of Quercetin content

The methanol extracts (250 ml each) of 19 genotype mutant of Samosir shallot along with control plant bulbs (after storage for 5 months in the room temperature) were mixed with 1.25 ml of distilled H₂O and 75 ml of a 5% NaNO₂ solution. After 5 minute, 150 ml of a 10% AlCl₃ H₂O solution was added and filtered for 6 minute. About 500 ml of 1 M NaOH and 275 ml of distilled H₂O were added to the mixture, mixed well and the intensity of pink color was measured at 510 nm. *The level of total Quercetin concentration was calculated using quercetin (QU) as a standard* [17]. The results were expressed as mg of quercetin /g fresh weight of bulb tissues.

2.3. Evaluation antimicrobial and antioxidant activity of M_1V_5 treated and untreated bulb

Preparation of plant extracts

Plant materials were ground using an electric mill (GM100 Retsch, Germany). Each sample of 8 (eight) genotype mutant along with control plant bulbs (5 g) was macerated with 150 ml of 80 % ethanol and placed on a shaker (200 rpm) (GFL3005, Germany) for 24 h. All procedures, stated above, were carried out at room temperature. Extracts from each specimen were subsequently filtered and concentrated (evaporated to dryness) using a rotary vacuum evaporator (R-200 Buchi, Switzerland) at 40 °C. Dried residues were dissolved in 100 % dimethyl sulfoxide (DMSO) to obtain a stock concentration of 51.2 mg/ml, which was kept at -20 °C until used.

2.3.1. Assay of antimicrobial activity

In this study, four bacterial strains and one yeast were tested. The following American Type Culture Collection (ATCC) standard strains were purchased from Oxoid (United Kingdom) for analysis: *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. Six clinical isolates of antibiotic-sensitive as well as antibiotic-resistant *S. aureus* strains (SA1, SA2, SA3, SA4, SA5, SA6, SA7, SA8, SA9, SA10) were provided by University Hospital in Motol (Prague, Czech Republic). Microorganism cultures were stored in Mueller-Hinton broth (MHB) (Oxoid, United Kingdom) at 4 °C until used. Prior to antimicrobial tests, microorganisms were re-cultured at 37 °C for 24 h (48 h for *C. albinos*).

In vitro antimicrobial activity ethanol extracts derived from bulb of 8 genotype mutant along with control plant bulbs were determined by broth micro dilution method using 96-well microtiter plates as described by the Clinical and Laboratory Standards Institute and Cos *et al* (2006) in [18]. Minimum inhibitory concentration (MIC) is expressed as the lowest concentrations that inhibited bacterial growth by ≥80% compared with that of the agent-free growth control. Antibiotics ciprofloxacin, oxacillin, tetracycline and tioconazole were used as positive control. Oxacillin and tetracycline were used as markers for methicillin and tetracycline resistance.

2.3.2. DPPH radical-scavenging assay (assay of antioxidant activity)

Slightly modified method previously described by [19] was used. Initially, two-fold serial dilutions of each sample were prepared in methanol (175 L) in 96-well microtiter plates. Subsequently, 25L of freshly prepared 1mM DPPH (2, 2-Diphenyl-1-picrylhydrazyl) in methanol solution was mixed with the sample in each well plates creating a range of concentrations from 2 to 5120 g/mL (final volume of 200 L) to start the radical-antioxidant reaction. The mixture was kept in the dark at room temperature. After a 30 min incubation period, the absorbance was read against blank at 517 nm using Infinite 200 reader (Tecan, Switzerland). Results were expressed as half maximal inhibitory concentration (g/mL IC₅₀).

3. Result and discussions

In this study, 19 genotype mutants along with control bulbs (20 weeks after harvested) for the total of quercetin content and 8 genotype mutant (24 weeks after harvested) of irradiated shallots by gamma

rays along with control bulbs (unirradiated) , were assayed for in vitro anti-microbial and antioxidant activity.

3.1. Total Quercetin content

Biochemical differentiation based on quercetin content revealed that shallot bulbs irradiated at 6 Gy, 5Gy, 4 Gy and 2 Gy with the plant code number 6(3-4-4) and 6(2-5-6), 5(4-5-1), 4 (6-8-5) and 2 (6-7-9) exhibited quercetin content (QC) of 1.7 mg/g, 1.6 mg/g and 1.6 mg/g, 1,6 mg/g and 1.6 mg/g respectively, higher than unirradiated plant (control) (Table 1). However, the study also revealed that among genotypes themselves which were irradiated at the same dosage, exhibited different QC. For example between 5(4-5-1) and 5(5-1-2) that irradiated with 5 Gy exhibited QC 1.6 g/mg and 1.2 g/mg and among different dosage 4(6-8-5), 4(7-2-4), 3(13-2-3), 3(11-4-6) and 2(19-6-3) exhibited QC 1.6, 1.2, 1.2, 1.3, and 0.9mg/g respectively.

Table 1. The total quercetin concentration of Samosir Shallot irradiated by gamma Rays 5 months after harvest

No	Plant code number	Dosage of irradiation	Total Quercetin concentration (mg/g)
1	1(4-2-4)	1 Gy	1.3
2	1(5-4-2)	1 Gy	1.0
3	2(6-7-9)	2 Gy	1.6
4	2(9-7-5)	2 Gy	1.0
5	2(13-6-4)	2Gy	1.2
6	2(9-7-5)	2Gy	1.2
7	2(19-6-3)	2Gy	0,9
8	3(11-4-6)	3Gy	1.3
9	3(13-2-3)	3Gy	1.2
10	4(6-8-1)	4Gy	1.2
11	4(6-8-5)	4Gy	1,6
12	4(7-2-4)	4Gy	1.2
13	4(7-8-4)	4Gy	0.7
14	5(4-5-1)	5Gy	1.6
15	5(5-1-2)	5Gy	1.2
16	6(2-5-6)	6Gy	1.6
17	6(3-4-4),	6Gy	1.7
18	8(1-3-3)	8Gy	0.6
19	9(1-3-2)	9Gy	0.9
20	Control	Unirradiated	1.1

Gamma irradiation was reported to induce oxidative stress with over production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism [20]. This indirect effect of irradiation is important in vegetative cells, the cytoplasm of which contains about 80% water. These free radicals create alterations in plastid ultra-structure and mitochondria [21] and fragmentation of the endoplasmic reticulum, golgi apparatus, DNA and proteins by breaking chemical bonds [22]. These radicals can induce damage in cellular membranes [23] and damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose [24]. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes,

alteration in photosynthesis, modulation of the anti-oxidative system, and accumulation of phenolic compounds [25].

To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments [26]. This defense was brought about by alteration in the pattern of gene expression. This led modulation of certain metabolic and defensive pathways. The results of this study revealed that variation in quercetin content was observed in irradiated plants. Table 1 showed that gamma rays irradiation caused the variation in QC of bulbs, however the total QC of the sample was not determined by dosage of gamma irradiation, since genotype mutants irradiated with the same dosage also exhibited the different in total QC.

3.2. Antimicrobial activity

Antimicrobial activity of nine (9) genotype mutants along with control plants had been evaluated in vitro against four bacterial species and one yeast. Complete results for antimicrobial activity of tested bulbs extracts are summarized in Table 2. It can be seen that none of the genotype mutants and control plant exhibited antibacterial inhibitory against all microorganism tested except for the sample number 2 and 6 (bulb generated from the plants irradiated by gamma rays with dosage at 2 and 6 Gy) that could only exhibited *Staphylococcus aureus* at high concentration. This results contrast with the statement mentioned before. This probably caused by the bulbs of all genotypes mutant along with the control plant had been kept too long (6 months) in improperly condition (at room temperature) which can affect their quality and biological characteristics. Prefously, the extract of *A. cepa* has been described to possess antimicrobial effect against *Vibrio cholera* [27] and *S. aureus* [28]. The essential oil of this plant also exhibited strong antimicrobial activity against *Salmonella enteridis*, *Aspergillus Niger* and *Penicillium cyclopium* [29], complementing the results found in this study.

Table 2. Minimum inhibitory concentration (MIC) of the fresh shallot extract with different dosage of gamma ray irradiation

Treatment Dosage of irradiation	MIC ($\mu\text{g/mL}$)				
	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
	ATCC 25923	ATCC 29212	ATCC 25922	ATCC 27853	ATCC 10231
Control	>512	>512	>512	>512	>512
1	>512	>512	>512	>512	>512
2	512	>512	>512	>512	>512
3	>512	>512	>512	>512	>512
4	>512	>512	>512	>512	>512
5	>512	>512	>512	>512	>512
6	512	>512	>512	>512	>512
8	>512	>512	>512	>512	>512
9	>512	>512	>512	>512	>512
ATB	1	16	1	16	0.5

ATB Antibiotics used as positive control

3.3. Antioxidant activity

Results for antioxidant activity of tested plants extracts are summarized in Table 3. It can be seen that none of these genotypes exhibited significant antioxidant efficacy. As mention before, this might be caused by the bulbs of all genotypes mutant along with the control plant had been kept too long (6 months) in improperly condition (at room temperature) which can affect their quality and biological characteristics.

Table 3. Antioxidant activity of fresh shallot extracts with Different irradiation dosage.

Sample (Gy)	Antioxidant activity (IC ₅₀ µg/mL)
Control	> 537.375
1	> 537.375
2	> 537.375
3	> 537.375
4	> 537.375
5	> 537.375
6	> 537.375
8	> 537.375
9	> 537.375
Trolox (Vit.E)	52.59

4. Conclusions

Population of genotype mutants irradiated with dosage 2Gy, 4 Gy, 5 Gy and 6 Gy have more benefit such as higher quercetin content, compared to control samples. None of the genotype mutants exhibited antibacterial inhibitory against all microorganism tested except for the sample number 2 and 6 (bulb generated from the plants irradiated by gamma rays with dosage at 2 and 6 Gy) that could only exhibited *Staphylococcus aureus* at high concentration. In addition, there was also none of the genotypes observed exhibited significant antioxidant efficacy.

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