World of Science: Journal on Modern Research Methodologies

Volume 2 Issue 6, Year 2023
ISSN: 2835-3072
https://univerpubl.com/index.php/woscience

Detection of the Effect of Microwave Radiation on the Phenotypic and Molecular Characteristics of *Radish Plant*

Najeeb Ibraheem Mohamed
Biology Department, Science of Collage, Kirkuk University, Iraq
Email : Najeeb.biotech@uokirkuk.edu.iq

Article Information

Received: Apr 23, 2023
Accepted: May 26, 2023
Published: Jun 15, 2023

Keywords: radiation, molecular properties, primers, DNA extraction

Abstract : The current study aimed to investigate the effect of microwave radiation on the molecular properties of radish. And detection of mutations resulting from exposure to microwave radiation on the genetic material of radish plant using RAPD-PCR technique. Samples were collected from the plants one month after the date of planting from all treatments, where (4-5) young leaves were taken from the growing apex and placed in special marked bags and transported directly to the laboratory to conduct the process of DNA isolation from them. DNA was extracted from the plant using the column tube method. The results of The RAPD indicators are characterized by their sensitivity to any change in the components of their interactions. Primer P1 molecular sizes ranged between 100bp-1500bp, and the number of produced bundles reached 31 bundles, ranging between 1-7 bundles per sample. Primer P3 molecular sizes ranged between 250 pb-1250, and the number of produced bundles reached 45 bundles, ranging between 1-9 bundles per sample. Primer P4 molecular sizes ranged between 200bp-1250bp, and the number of produced bundles reached 41 bundles, ranging between 1-8 bundles per sample. Primer P5 molecular sizes ranged between 150bp-1500bp, and the number of produced bundles reached 22, ranging between 1-7 bundles per sample. Primer P8 molecular sizes ranged between 150bp-1250bp, and the number of produced bundles reached 30 bundles, ranging from 1-6 bundles per sample. Primer P12 molecular sizes ranged between 250bp-1500bp, and the number of bundles produced reached 42 bundles, ranging between 1-12 bundles per sample. Primer P16 molecular sizes ranged between 200 bp - 1400 bp, and the number of produced bundles reached 16, whose molecular sizes ranged between 1-6 for one sample.

Introduction

The radish, Raphanus sativus L., is an annual herbaceous plant of the Brassicaceae family. The radish contains 18 chromosomes. It is a root vegetable of the cruciferous family [1]. It is cultivated for its edible root. The radish plant has a short stem Horizontal and circular leaves at ground level and oblong leaves with a length of 5-30 cm. The upper leaves of the plant are small. The root of

the plant is cylindrical or tapering and is usually red or white in color. The root skin color ranges from white to pink, red, purple, yellow, green to black, but The root is usually white, the roots get their color from anthocyanins, the red varieties get their color from anthocyanins, while the purple varieties get their color from cyanidins [2]. The main root is known to contain minerals, vitamins, and fiber, which are important for human health [3]. The glucosinolates of radish have been well identified from several studies, [4]. It contains an aliphatic form called glucoraphasatin, which is only available in radish [5]. Radish is a popular vegetable in Iraq and is used as a vegetable or salad, as both its leaves and roots are a good source of calcium, phosphorus, and ascorbic acid [6]. Radish provides a large amount of nutrients, especially rich in protein, fat, carbohydrates, calcium, sodium, phosphorus and potassium [7]. The main bioactive compounds produced in radish are glycosides, including glucosinolates such as chlorophanine, chlorocorosine, chlorofastin, and isothiocyanates such as sulforaphane and sulforaphane [8]. In addition, the reason for the sharpness of horseradish is due to the presence of some chemicals such as the enzyme Myrosinase and Glucosinolate isothiocyanate, which are produced by this plant [9]. Some have suggested that it has anti-cancer activities. In Germany in (2009) revealed an inverse relationship between dietary intake of glucosinolates and the risk of prostate cancer [10-11]. In addition, white radish extract inhibited abnormal proliferation of vascular smooth muscle cells [8]. The development of molecular indicators provided the necessary methods in terms of accuracy, speed, and shortening the effort and time to select the best through early recognition of the best molecular performance. These indicators use the nucleic acids (DNA and RNA) in the organism's body, and through them the researchers were able to overcome all the obstacles that faced the previous methods. Among these indicators is the RAPD - PCR (Randomly Amplified Polymorphic DNA), which was used to detect the relationship and genetic differences between radish varieties. There are many studies that used this technique, including a study in which 37 starters were used in the radish plant [12-13].

Materials & methods

Design of experiment

All (5) treatments (FC, F1, F2, F3, F4) were cultivated. Where we divided the seeds into five groups in order for the seeds to be placed in water, which coincides with Sunday 10/30/2021 by placing them in containers and keeping them in a dark place for 48 hours, then we exposed the samples to different times of microwave waves, which coincides with Tuesday 11/1/ 2021, and it was worked on in the Molecular Biology Laboratory - University of Tikrit - College of Science - Department of Life Sciences, and then the samples were cultivated on the same day in the farms of Al-Alam district of Salah Al-Din Governorate.

Sample collection

Samples were collected from the plants one month after the date of planting from all treatments, where (4-5) young leaves were taken from the growing apex and placed in special marked bags and transported directly to the laboratory to conduct the process of DNA isolation from them.

DNA extraction

DNA was extracted from the plant using the column tube method.

Method of extracting DNA

- The leaves of the plant are washed with distilled water and left to dry. Then we take 5-10 leaves of the plant and put them in a ceramic mortar and add Liqiuid nitrogen to it and we grind the leaves well until they turn into a powder.
- transfer the mixture to a 2 ml Pendrof tube
- Add 800 microliters of preheated Lysis buffer-1 and then incubate the samples in a water bath for 30 minutes at 60C, stirring every five minutes.
- Add 200 microliters of protein precipitation buffer to the sample, then vortex the sample, then freeze the samples for 5 minutes.
- mix the samples with a vortex, then put the samples in a centrifuge at a speed of 14,000 for 5 minutes (to precipitate the insoluble particles).
- The clearant is withdrawn by a fine pipette, where we withdraw 500, 600, or 700 microliters into a new Pendrov tube.
- A similar volume of Binding buffer is added to the test tube containing the sample and mixed well by inversion and then by vortex for 10 seconds.
- Prepare a Column tube. Then, 600 microliters of the sample are transferred to a Column tube.
- put the samples in a centrifuge for 1 minute at a speed of 10,000 revolutions per minute.
- keep the filter and discard the sediment (note that if the sample does not pass through the filter, we repeat the centrifugation process at a speed of 14000 for a minute.
- Add 500 microliters of washing buffer-1 (washing buffer-1), then put the samples in a centrifuge for one minute at 10,000 rpm.
- Keep the filter and get rid of the sediment.
- Add 500 microliters of washing buffer-2 (washing buffer-2), then put the samples in a centrifuge for 1 minute at 10,000 rpm.
- Keep the filter and get rid of the sediment.
- Drying without adding anything in a centrifuge for 2 minutes at 13000 speed to remove residual ethanol.
- Transfer the filter to a new Pendrov tube.
- Add 100-150 microliters of preheated Elution buffer at a temperature (60C) to R.T and incubate for five minutes (or leave for five minutes)
- The samples are placed in a centrifuge for 2 minutes at 10,000 rpm at room temperature.
- Disposing of the colum filter and keeping the pendentroof tube containing the extracted DNA and stored at -20C.

Electrophoresis technology

The electrophoresis process is performed to detect the presence of DNA, and the electrophoresis process takes place in two stages, and each stage has a specific time and electric current, and the time, electric current, and voltage vary according to the size of the basin used in the electrophoresis process.

Primers

In the current study, table (1) showed the primers that used to diagnosis some genes in Raphanus sativus radish.

Table (1): showed the primers that used in this study

No	Primer	Sequence 5′➞➞➞ 3′
P1	OP A-01	CAGGCCCTTC
P3	OP B-12	CCTTGACGCA
P4	OP C-08	TGGACCGGTG
P5	OP H-16	TCTCAGCTGG
P8	OP B-20	GGACCCTTAC
P12	OP D10	GGTCTACACC
P16	OP D-18	GAGAGCAAC

RAPD_PCR

An amount of 2 microliters of DNA extracted from the plant was taken and placed in special tubes for PCR using a micropipette, 2 microliters of previously prepared primer enzymes were added, and 14 microliters of distilled water were added to special tubes for PCR Pre-mix form Bioneer (pcr) and the mixture was mixed properly.

Results & Discussion

DNA extraction

The study included the extraction of DNA from the radish plant, which was treated with different times of microwave radiation, and after the isolation of the DNA, the purity and the appropriate concentration for conducting PCR reactions were confirmed, and all samples were transferred using electrophoresis to detect the genomic DNA on an agarose gel at a concentration of 1%, and after the migration process, the gel was photographed using Gel Documentation system to see the DNA bundles and estimate their size as in the figure (1).

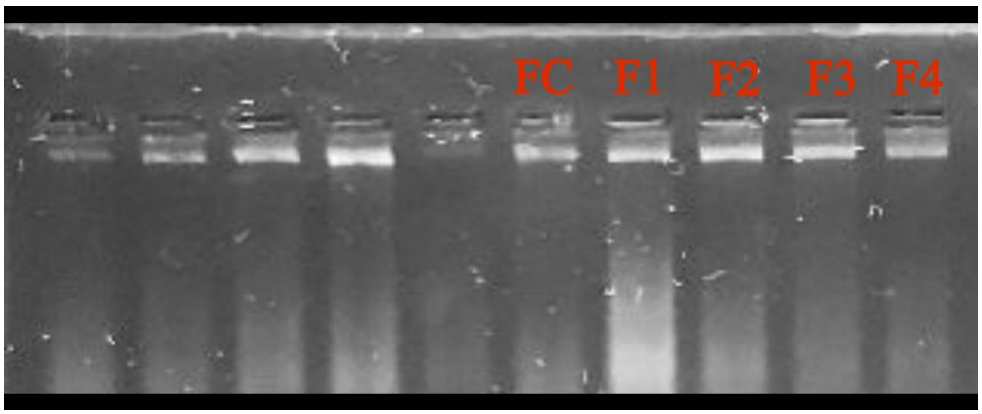

Figure (1): Results of genome electrophoresis on an agarose gel

The results of the RAPD-PCR indicators

The RAPD indicators are characterized by their sensitivity to any change in the components of their interactions. Therefore, they are considered among the reactions that are difficult to repeat and obtain the same result [14-15]. It was shown through the results obtained in this study that the concentration of the template DNA mainly affects the results, especially in the intensity of the band that appears on the Agros Gel. It leads to the dispersion of the outputs in the early stages of the program, which leads to not obtaining clear results and prominent bands, as well as leads to the appearance of a smear smear between the bands. It was shown through the results that the best concentration of the DNA template is 50 ng for the reaction sample. Sample dilutions were adjusted to 50 ng / microliter per liter using a Nano drop device. The difference in band density when DNA concentrations are controlled is a discriminatory feature that can be used in diagnosis [16].

Primers result

The results of the primers used in this study are the primers produced, which amounted to 7. The following is a detailed explanation of the prefixes produced:

Table (2): results of primers used in RAPD reactions

Primer	The number of sites produced	number of public sites	number of variation sites	Total number of bands	number of generic bands	Number of variation bands	Number of unique packages	Number of missing bands	Variation %
P1	11	2	9	31	10	21	4	1	81
P3	9	5	4	45	20	25	-	3	44
P4	8	6	2	41	30	11	-	2	25
P5	7	3	4	22	15	7	3	1	57
P8	8	6	2	30	20	10	1	1	25
P12	9	-	9	42	-	42	-	3	100
P16	6	-	6	16	-	16	1	2	100
Total	58	22	36	227	95	132	9	13	62

Primer P1 (OP A-01)

Primer P1 showed eleven sites for the bundles, and all of these bundles were differentiated except for only two sites, which are general sites in this primer, their molecular sizes ranged between 100bp-1500bp, and the number of produced bundles reached 31 bundles, ranging between 1-7 bundles per sample. This Primer in the F1 sample is characterized by only one absent bundle, where Its molecular size was 900 bp, and the F2 sample was characterized by only three unique bands, as its molecular size was 1250 bp-1500 bp, respectively, 600 bp. The F3 sample was characterized by one unique band and an absent band. The molecular size of the unique band was 800 bp. As for the molecular size of the absent band, it was 500 bp as ahown in table (2) and figure (2).

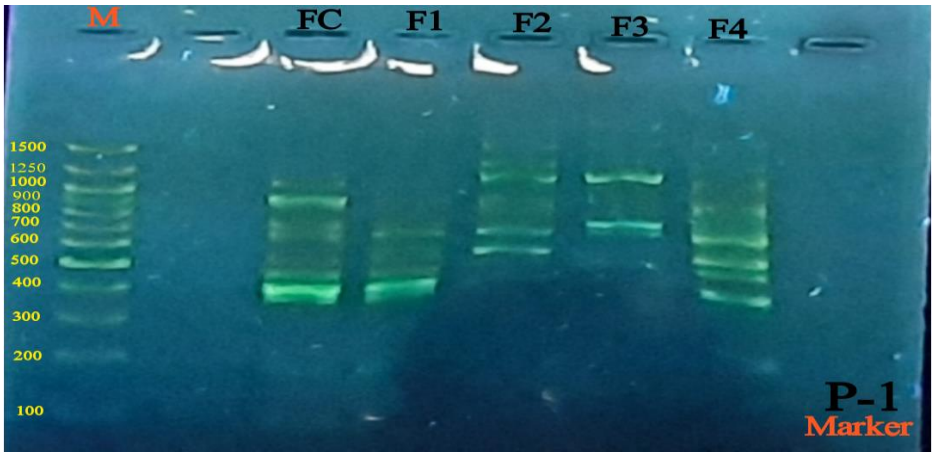

Figure (2): results of the Primer (opA-01) P1 stage on agarose gel with a concentration of 1.5%

Primer P3 (OP B-12)

The primer P3 showed nine sites for the bundles, and all of these bundles were general except for four sites, which are different sites in this primer, their molecular sizes ranged between 250 pb-1250, and the number of produced bundles reached 45 bundles, ranging between 1-9 bundles per sample. respectively between bp250-bp1250, and the F3 sample was characterized by only one absent band, with a molecular size of 700 bp, as shown in figure (3).

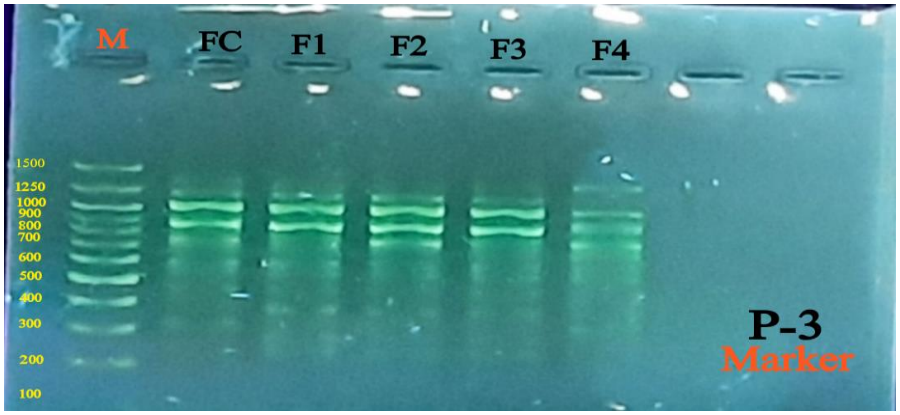

Figure (3): results of the Primer (**OP B-12**) P1 stage on agarose gel with a concentration of 1.5%

Primer P4 (OP C-08)

The Primer P4 showed 8 sites for the bundles, and all of these bundles were general except for two sites, which are different sites in this Primer, their molecular sizes ranged between 200bp-1250bp, and the number of produced bundles reached 41 bundles, ranging between 1-8 bundles per sample. The F1 sample, with only an absent band, had a size between 200 bp, as shown in Figure (4).

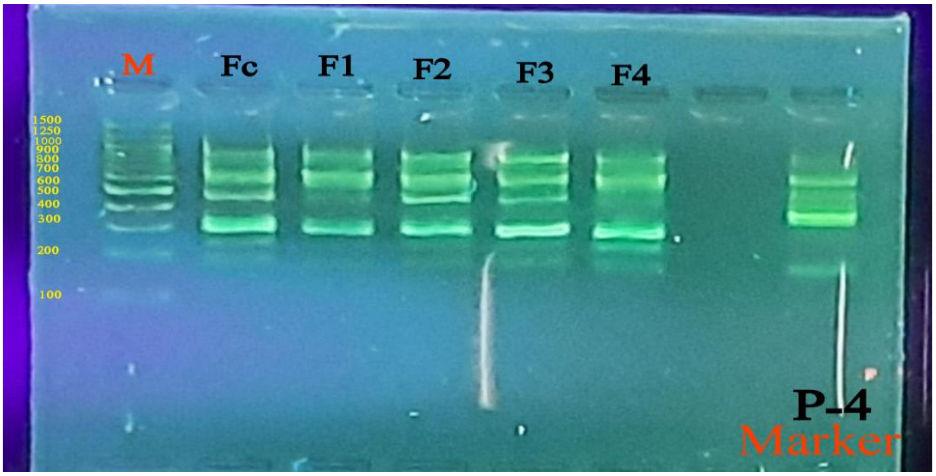

Figure (4): results of the Primer (OP C-08) P1 stage on agarose gel with a concentration of 1.5%

Primer P5(OP H-16)

The primer P5 showed seven sites for the bundles, and all of these bundles were different except for three sites only, which are general sites in this primer, their molecular sizes ranged between 150bp-1500bp, and the number of produced bundles reached 22, ranging between 1-7 bundles per sample. This primer in the FC sample is characterized by only one absent bundle, where Its molecular size was 800 bp, and the F1 sample was characterized by only one unique band, with a molecular size of 150 bp, and the F4 sample was characterized by only two unique bands, with a molecular size of 250-bp1250, respectively, as shown in figure (5).

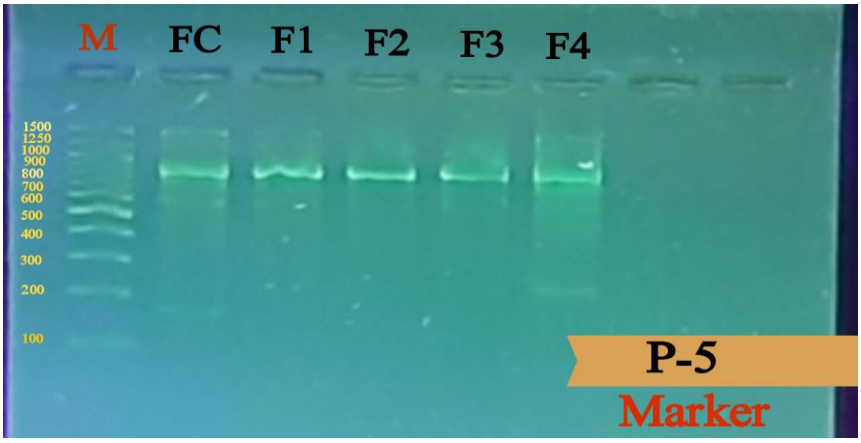

Figure (5): results of the Primer (OP H-16) P1 stage on agarose gel with a concentration of 1.5%

Primer P8 (OP B-20)

The primer P8 showed seven sites for the bundles, and all of these bundles were general except for three sites, which are different sites in this primer. Their molecular sizes ranged between 150bp-1250bp, and the number of produced bundles reached 30 bundles, ranging from 1-6 bundles per sample. This primer in the F2 sample is characterized by only one unique bundle. Where its molecular size reached 700 bp, and the F4 sample was distinguished by an absent band only, its molecular size was 1250 bp, shown in the figure (5).

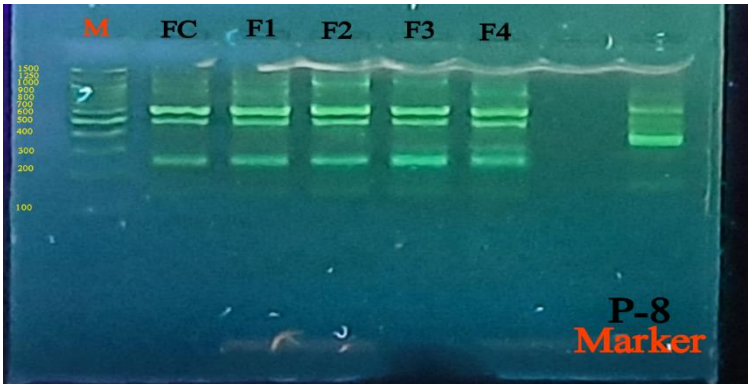

Figure (6): results of the Primer (**OP B-20**) P1 stage on agarose gel with a concentration of 1.5%

Primer P12 (OP D10)

The primer p12 showed nine sites for the bundles, and all of these bundles were different in this primer, their molecular sizes ranged between 250bp-1500bp, and the number of bundles produced reached 42 bundles, ranging between 1-12 bundles per sample. Only one was absent, with a molecular size of 400 bp, and the F3 sample was distinguished by only one absent band, with a molecular size of 800 bp, as shown in the picture (5-7).

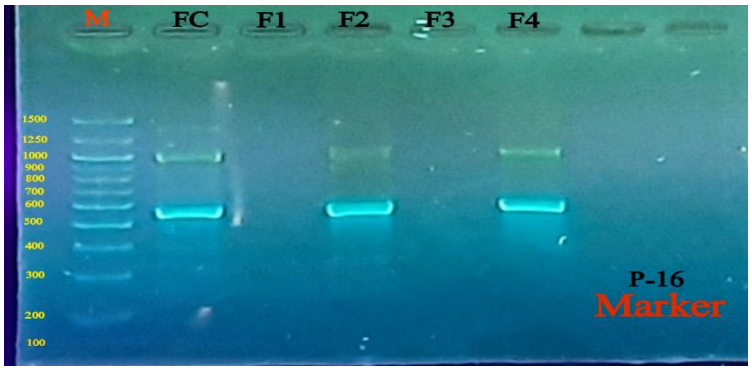

Figure (7): results of the Primer (OP D10) P1 stage on agarose gel with a concentration of 1.5%

Primer P16 (OP D-18)

The primer P16 showed eight sites for the bundles, and all these bundles were different in this primer, their molecular sizes ranged between 200 bp - 1400 bp, and the number of produced bundles reached 16, whose molecular sizes ranged between 1-6 for one sample. The molecular size reached 300 bp, and the F4 sample was characterized by only one absent band, with a molecular size of 1400 bp, as shown in the figure (8).

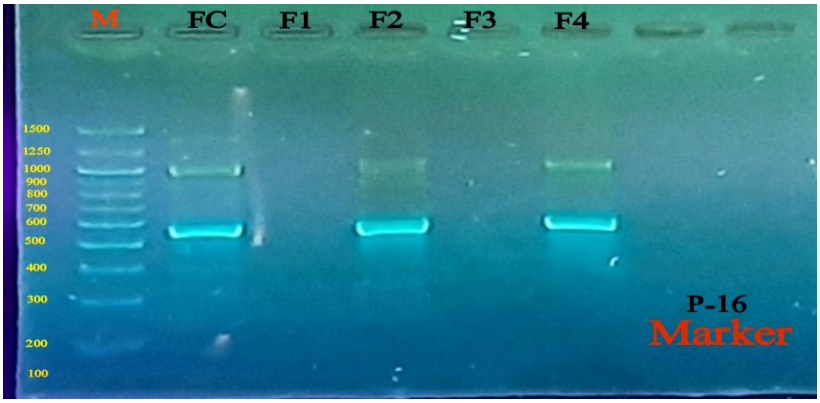

Figure (8): results of the Primer (OP D-18) P1 stage on agarose gel with a concentration of 1.5%

Conclusions

The longer the duration of exposure to microwave radiation, the greater the effect on phenotypic and molecular characteristics. Microwave radiation has positive effects, as some phenotypical traits of radish were improved. There is a clear effect on the genome of the DNA sequences resulting from the effect of microwave radiation.

References

1. Ishida M, Nagata M, Ohara T, Kakizaki T, Hatakeyama K, Nishio T. (2012). Small variation of glucosinolate composition in Japanese cultivars of radish (Raphanus sativus L.) requires simple quantitative analysis for breeding of glucosinolate component. Breed Sci 62: 63–70.
2. Brickell, Christopher, et,al. (1992). The Royal Horticultural Society Encyclopedia of Gardening (Print). London: Dorling Kindersley. pp. 356-357.
3. Sun, Y.; Wang, J.; Qiu, Y.; Liu, T.; Song, J.; Li, X. (2018). Identification of 'Xinlimei' radish candidate genes associated with anthocyanin biosynthesis based on a transcriptome analysis. Gene, 657, 81-91.
4. Liang, X.; Lee, H.W.; Li, Z.; Lu, Y.; Zou, L.; Ong, C.N. (2018). Simultaneous Quantification of 22 Glucosinolates in 12 Brassicaceae Vegetables by Hydrophilic Interaction Chromatography-Tandem Mass Spectrometry. ACS Omega, 3, 15546–15553.
5. Yi, G.; Lim, S.; Chae, W.B.; Park, J.E.; Park, H.R.; Lee, E.J.; Huh, J.H. (2016). Root Glucosinolate Profiles for Screening of Radish (Raphanus sativus L.) Genetic Resources. J. Agric. Food Chem, 64, 61-70.
6. Kang, So Youn Won, Mi- Suk Seo , Jeongyeo Lee, Si Myung Lee, Soo-jin Kwon and Jung Sun Kim. (2020). Induction of Glucoraphasatin Biosynthesis Genes by MYB29 in Radish (Raphanus sativus L.) Roots, Genomic Division, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 54874, Korea Int. J. Mol. Sci., 21, 5721.
7. Yuyun Sugiharti, Mukhamad Su'udi, Sooyeon Lim, Jongkee Kim. (2014). Isolation and expression of FMOgs-ox1 from Korean Radish Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea ScienceAsia, (40) :278–284.
8. Baenas, N.; Piegholdt, S.; Schloesser, A.; Moreno, D.A.; Garcia-Viguera, C.; Rimbach, G.; Wagner, A.E. (2016). Metabolic activity of radish sprouts derived isothiocyanates in drosophila melanogaster. Int. J. Mol. Sci., 17, 251.
9. Nakamura, Y.; Nakamura, K.; Asai, Y.; Wada, T.; Tanaka, K.; Matsuo, T.; Okamoto, S.; Meijer, J.; Kitamura, Y.; Nishikawa, A. (2008). Comparison of the Glucosinolate-Myrosinase Systems among Daikon (Raphanus sativus, Japanese White Radish) Varieties. J. Agric. Food Chem., 56, 2702-2707.
10. Steinbrecher, A.; Nimptsch, K.; Husing, A.; Rohrmann, S.; Linseisen, J.(2009). Dietary glucosinolate intake and risk of prostate cancer in the EPIC-heidelberg cohort study. Int. J. Cancer, 125, 2179-2186.
11. Wang, N.; Wang, W.; Huo, P.; Liu, C.Q.; Jin, J.C.; Shen, L.Q. (2014). Mitochondria-mediated apoptosis in human lung cancer a549 cells by 4- methylsulfinyl-3-butenyl isothiocyanate from radish seeds. Asian Pac. J. Cancer Prev. 15, 2133-2139.

12. LIU, L. et al. (2007). Genetic diversity analysis of radish germplasm with RAPD, AFLP and SRAP markers. Acta Horticulturae, v. 1, n. 760, p. 125-130.
13. Kong QS, Li XX, Xiang CP, Wang HP, Song JP, Zhi HY (2011). Genetic diversity of radish (Raphanussativus L.) germplasm resources revealed by AFLP and RAPD markers. Plant Mol. Biol. Rep. 29(1):217-223.
14. Al-Jubouri, Zuhair Ahmed Muhammad (2013). A study of quantitative, molecular and physiological traits as indicators for drought analysis and adopting them as selective guides in evaluating genotypes of soft wheat (Triticum) (SSR, aestivum L.), Master Thesis, College of Science - University of Tikrit.
15. Saleh, Ghada Saad. 2020. Gene expression of Sippo and SiNST1 genes and its relation to quantitative and qualitative traits of sesame seed oil.Sesame in dicum L. Master Thesis - College of Science - University of Tikrit.
16. Becerril F, Calantzis C, Turnau K, Caussanel JP, Belimov AA, Gianinazzi S, Gianinazzi-Pearson V (2002) Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three Pisum sativum L. genotypes. J Exp Bot 53:1177-1185.