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Germination, Physiochemical, and Morphology Changes of TR10 Rice Seeds Irradiated to Filtered and Unfiltered Neutron

Rosina Baadu[1,a], Khim Phin Chong[1,b], Jualang Azlan Gansau[1,c], Muhammad Rawi Mohamed Zin[2,d*] and Jedol Dayou[3,e*]

[1]Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

[2]Malaysia Nuclear Agency, 43000 Bangi, Selangor, Malaysia

[3]Energy, Vibration and Sound Research Group (e-VIBS), Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

[a]rosinabaadu@gmail.com, [b]chongkp@ums.edu.my, [c]azlanajg@ums.edu.my, [*d]muhammad_rawi@nuclearmalaysia.gov.my, [*e]jed@ums.edu.my

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Abstract. In 2020, Sabah's rice production was 117, 846 metric tonnes with a planted area of 40, 446 ha is relatively low compared to other states such as Selangor, which has 36,004 ha less planted area but can produce up to 159,535 metric tonnes more than Sabah. Low rice production in Sabah has contributed to the shortage of supply. Thus, new varieties could be needed that sufficiently produce yields that comparable to the efforts and cost of rice planting. One of the methods is producing new varieties that probably produce high yields using physical mutagen. From the literature available, gamma and neutrons were found to be the most common agents to increase rice production per unit planting area but used they were used separately. Theoretically, using fast neutron irradiation was found to produce a superior mutant. Thus, this paper aims to study the effect of rice seeds irradiated to both gamma and neutron simultaneous, and compare with neutron alone on the germination rates and physiochemical of rice seeds after the irradiations at different dosages. TR10 rice seeds were irradiated simultaneously to gamma and neutrons in the nuclear reactor core using Pneumatic Transfer System–PTS facility, while irradiation to neutrons alone was performed in a neutron chamber using Beam ports–BP facility at Nuclear Malaysia. The germination study shows a linear decrease as the irradiation doses increase in BP and fail to germinate in PTS. Yet, seeds irradiated in PTS showed no hazardous compounds and a remarkable increase in mineral content in seeds, particularly potassium and magnesium at 14 Gy. Similarly, to the shape of the starch granules in both facilities, the starch structure changes after being exposed to 14 Gy in PTS and 35 Gy in BP. As a result, seeds exposed to neutron alone induced considerably more inclusive effects and may raise growth performance and improve rice quality with optimal neutron dosage range from 7 to 14 Gy.

Introduction

In comparison to other grains, rice is one of the most significant food crops in Malaysian culture. Rice was consumed either as cooked rice or indirectly in the form of rice flour and the amount was increasing during festive seasons. According to Omar *et al.* [1], the annual average Malaysian consumption of rice is 80 kg per person (219.18 grams in daily rice consumption), with households in Sabah spending the most on rice at RM73/month, while households in Perlis spent the least at RM13/month, and the national average spending is approximately RM44/month. As Malaysia's rice output is still insufficient, the country needs to import from Thailand, Vietnam, and Pakistan. The need to enhance productivity, particularly for rice, has become crucial due to the crop's strategic importance. As a result, the introduction of high-quality rice seeds is one of the Ministry of Agriculture and Fisheries' initiatives aimed at increasing food production in Sabah and achieving the goal of 60% food production by 2030. High-quality rice seeds with improved qualities, drought tolerance, pest and disease resistance, and early maturity (may be planted three times a year), as a byproduct of induced mutation approaches, are being released to farmers for planting. One of the techniques currently used is a radiation-induced mutation which is a promising program in determining plant breeding.

In Malaysia, successful induced mutation breeding is not restricted to chemical mutagen; physical mutagens, such as gamma rays, ion beams, x-ray, and the most recent neutron were utilized [2]. Most of the mutation breeding programs were successfully done by using Malaysian Nuclear Agency (Nuclear Malaysia) facilities, especially gamma irradiator. Nuclear Malaysia is the leading research institute for mutation breeding since the 1980s, either alone or through collaboration with other agencies such as the Malaysian Agricultural Research and Development Institute (MARDI), National Landscape Department, United Plantation, International Atomic Energy Agency (IAEA), Japan Atomic Energy Agency (JAEA), and Higher Education Institution such as Universiti Putra Malaysia (UPM), Universiti Teknologi MARA (UiTM) and Universiti Malaysia Sabah (UMS) [3]. A pool research type reactor (TRIGA PUSPATI reactor) was used to facilitate neutron irradiation via the Rotary Specimen Rack, Pneumatic Transfer System (PTS), Central Thimble, Beam Ports, Neutron Radiography Facility (NuR2), and Small Angle Neutron Facility (SANS) instruments.

Malaysia has registered two rice mutant varieties, NMR151 and NMR 152, with improved characteristics such as drought tolerance, disease resistance (Blast disease), and higher seed yield [4] These strains were developed using MR219 as a parent strain, released by the Malaysia Agricultural Research and Development Institute in 2001 [5]. Mutant breeding has shown promising outcomes in increasing genetic diversity and producing superior varieties, addressing specific difficulties faced by rice growers.

For decades, plant breeders have utilized ionizing radiation to generate mutations. Gamma rays are commonly employed because it has high penetrating capability due to having shorter wavelengths. There are not just X-rays and gamma rays in our surroundings, but also neutrons. Thousands of low-energy neutrons exist at sea level [6]. Because neutrons have no electric charge, neutrons cannot directly ionize atoms or molecules. A neutron, on the other hand, can collide with a nucleus in an atom and produce secondary particles. These secondary particles have the ability to ionize atoms and molecules. In agriculture, neutron irradiation has been known for chromosomal damage and is best reserved for dry seeds. Furthermore, neutrons had a larger stimulating effect on many crops and were less affected by environmental factors compared to X-rays and gamma rays. Therefore, the purpose of this study was to evaluate the effect of irradiation treatment on TR10 rice seeds at different neutron irradiation facilities; Nuclear Reactor Core via PTS instrument and Neutron Irradiation Chamber at Beam Port #1 in TRIGA MARK II research of the Malaysian Nuclear Agency. Matured rice seeds were irradiated with different doses of neutron radiation.

Methodology

Plant Material Preparation. Rice seeds (TR10 rice variety) were collected from the Agriculture Department, Sabah. The mature and healthy seeds were exposed to different neutron irradiation facilities; Nuclear Reactor Core (PTS) and Neutron Irradiation Chamber at BP#1 at Malaysian Nuclear Agency, Selangor, Malaysia. The seedlings were subjected to neutron radiation over the period shown in Table 1.

Table 1. Time for exposed of seeds to neutron at Neutron Irradiation facilities

Dosage [Gy]	Neutron irradiation facilities	
	Nuclear Reactor Core (PTS)	Neutron Irradiation Chamber (BP#1)
0	0 sec	0 sec
7	13 sec	6 min 3 sec
14	27 sec	12 min 35 sec
21	40 sec	19 min 7 sec
28	54 sec	25 min 40 sec
35	67 sec	32 min 12 sec

The difference in exposure time for both irradiation facilities is to ensure the total dosage of thermal neutron are the same. However, the dosage of gamma is very large in the core compared to gamma dosage at BP#1. Thermal neutron and fast neutron flux in the core are around is in the order of 10^{13} n/cm^2/sec while thermal and fast neutron at BP#1 is around 10^6 n/cm^2/sec. The effect from fast neutron was maximized by filtering neutron in BP#1using cadmium (Cd) sheet with 1 mm thickness. Dosage of neutrons and gamma were measured from reading the TLD600 and TLD700 dosimeters.

Seed Germination. The irradiated seeds were disinfected with 5% sodium hypochlorite for 15 min and rinsed with distilled water three times prior to planting. The seeds were germinated on moist filter papers in the petri dishes under laboratory conditions with a temperature of 30±2 °C. The seed germination test was carried out by counting the emergence of the first shoot in each treatment including control on the 4th day after sowing and calculated using Eq. 1. 7 days after sowing, shoot length and root length were measured and seedling vigor was calculated using Eq. 2. The experiment was repeated three times.

$$Germination\ [\%] = \frac{Number\ of\ seed\ germinated}{Number\ of\ total\ seeds\ tested} \times 100\% \quad (1)$$

$$Vigor\ index = Germination\ \times Seedling\ length\ (shoot\ length + root\ length) \quad (2)$$

Fourier Transform Infrared (FTIR) Spectroscopy. The samples were dehusked and surface sterilized with 70% (v/v) ethanol. The samples were oven dried until the weight remained constant and ground into powdered form. About thirty (30) mg of each sample was placed onto the sample holder and measurements were carried out on a Perkin Elmer 2000 Series FTIR spectrometer. The measurement was performed at room temperature at 47% relative humidity. The spectrum resolution was set at 4 cm^{-1} (32 scans) and the scanning range was selected according to Qiu *et al.* [7] with the scanning range selected from 400 to 4000 cm^{-1}.

Determination of Mineral Content in Rice Seeds. Approximately 1 g dried powder of rice seed TR10 was digested in 10 ml of 65% nitric acid solution and left for 24 hours for digestion. After 24 h, the solution was heated on a hot plate at 70 °C until the solution turned from clear to pale yellow. Hydrogen peroxide was added for accelerating the process of sample oxidation. After cooling, the solution was filtered through a Whatman membrane filter paper (0.45 µm) and a sufficient amount of deionized water was added to make the final volume up to 50 mL, stored in a centrifuge tube, and kept at 4 °C in a refrigerator until it was analyzed with Inductively coupled plasma-optical emission spectrometry (ICP–OES). The determination of minerals includes Phosphorus (P), Potassium (K), Magnesium (Mg), Sodium (Na), Manganese (Mn), Zinc (Zn), and Iron (Fe).

Microstructure by Scanning Electron Microscopy (SEM). Random seeds samples were broken transversely at the mid-region using a razor blade and the morphology of samples was observed by using an SEM machine (Model Hitachi S3400). The accelerating voltage used in this study was 15kV at magnifications of 500x and 2000x. Cross-sections of the samples were manually snapped, mounted on an aluminum stud using adhesive tape, and sputter-coated with a thin layer of gold to reduce the charging effect during the imaging process. The size (diameter - µm) of 100 particles was measured randomly using ImageJ software (http://rsbweb.nih.gov/ij (accessed on 25 July 2022)).

Data Analysis. All the experiments were replicated three times. All data were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) test was performed followed by Tukey's HSD multiple comparison tests to determine significant differences at significance level $P< 0.05$ with IBM SPSS Statistics (Version 26).

Results and Discussion

This study was undertaken to determine the effect of varying doses of neutron radiation by using different neutron irradiation facilities for effective mutation breeding in rice (TR10). Understanding the effect of neutron irradiation on crop plants will help in its utilization for increasing crop diversity.

Seed Germination. This research discovered all seeds irradiated in a nuclear reactor core (PTS) showing failed to germinate, therefore no data for germination percentage and vigour index could be obtained from the PTS facility. In contrast with PTS, seeds irradiated in BP#1 with varied doses of neutron were presented in Table 2.

Based on the analysis, seed germination percentage irradiated in BP#1 does not show significant differences between treatments. The germination percentage shows a linear decrease as the irradiation doses increase over control. TR10 irradiated with 7 and 14 Gy shows 95% percentage germination compared to control (100%), while the lowest germination percentage, 85% is detected in seeds irradiated with 21 and 35 Gy. In addition, varied dosages of irradiation treatment show different results in shoot, root, and plant length. The highest percentage increase in root length (32.25%) and plant length (13.67%) over control was seeds treated with 7 Gy. However, the lowest increase in a shoot, root, and plant length is seed treated with 28 Gy with -14.10%, -13.84%, and -13.98% respectively. Overall, seeds treated with 7 Gy are the most vigorous and while seeds irradiated with 28 Gy are the least vigorous with seed vigour index at 8,710.55 and 6,070.75 respectively.

According to [8, 9], the variation in germination remains quite complex to evaluate because several environmental factors such as temperature, water, light, pH, and soil moisture influence each stage of the germination process in different ways. Each factor determines the percentage of germinated seeds and the rate of germination [10]. In general, the use of neutron affects the seeds germination. [11] found that *Ocimum basilicum* irradiated with 60 Gy fast neutron inhibited plant development after the germination, with the stopping of progress in cotyledons and no complete plant development occurs. While 50 Gy also damaged the plant development, it was still able to grow some mutant plants with phenotypic variations such as leaf mutation, spearmint/spiky leaves, and split leaves, suggesting that regardless of the dosage, fast neutron irradiation is suitable for the new genetic development of *Ocimum basilicum*.

Table 2. Effect of neutron radiation by using neutron irradiation chamber (BP#1) on germination and growth parameters of seedlings of TR10 variety of rice.

Dose [Gy]	Germination [%]	Shoot length [mm]	Root length [mm]	Plant length [mm]	Vigor Index
0 (control)	100.00[a]	40.13[ab]	40.53[ab]	80.66[ab]	8,066.00
7	95.00[a]	38.09[abc]	53.60[b]	91.69[a]	8,710.55
14	95.00[a]	41.15[b]	47.68[ab]	88.83[a]	8,438.85
21	85.00[a]	36.15[ac]	48.56[ab]	84.71[ab]	7,200.35
28	87.50[a]	34.47[c]	34.92[a]	69.38[b]	6,070.75
35	85.00[a]	35.40[c]	44.36[ab]	79.76[ab]	6,779.60

Different letters mean that there are significant differences between the measured characteristics at the level of $\alpha = 0.05$ based on the Tukey test.

FTIR Spectral Analysis. The FTIR spectrum of the neutron irradiate rice seed (TR10) using different facilities and six levels of neutron dosage are given in Figure 1. FTIR spectroscopy provides a rapid, non-destructive, operator-friendly, reliable, and precise method that allows the identification of the presence of organic and inorganic compounds in the sample. According to [12], the peak positions of organic compounds for the presence of lipids can be viewed in the spectral range of 3000 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} for proteins, and 1200 – 800 cm^{-1} for carbohydrates. In all doses of neutron radiation, seeds irradiated in BP facility show no significant difference compared to the control. Meanwhile, the spectra of seeds irradiated in PTS are found to be different in all dosages of neutron radiation. Furthermore, the spectra did not change, indicating that no new compounds are formed. The small peaks at 921 cm^{-1}, 925 cm^{-1}, 923 cm^{-1}, 934 cm^{-1}, and 931 cm^{-1} are related to the presence of starch and lipid which includes C-O stretching / C-C stretching / C-O-C stretching / C-O-H stretching (1200 – 900 cm^{-1}) [12, 13]. The increase of band intensities at 991 cm^{-1} and 997 cm^{-1} by neutron irradiation confirm the decrease in the ordered structure of the treated starches. The major peaks at 1649 cm^{-1}, 1646 cm^{-1}, 1638 cm^{-1}, and 1640 cm^{-1}, and small peaks at 1530 cm^{-1}, 1536 cm^{-1}, and 1540 cm^{-1} are ascribed to amide I peak, representing C=O stretch in proteins and the amide II peak, which represents N-H bending in proteins, respectively. The band at 1640 cm^{-1} is also attributed to adsorbed water, H-O-H bending vibrations that indicate intermolecular and intramolecular hydrogen bonding. The slight alteration in protein between PTS and control may be caused by pressure stress during irradiation as well as dehydration. Heat-induced and pressure-induced structural changes in soybean protein, according to [14]. All the FTIR spectra of organic compounds (lipid, proteins, and carbohydrate) show higher intensity at seeds treated in PTS neutron irradiation facilities as compared to BP#1 and control.

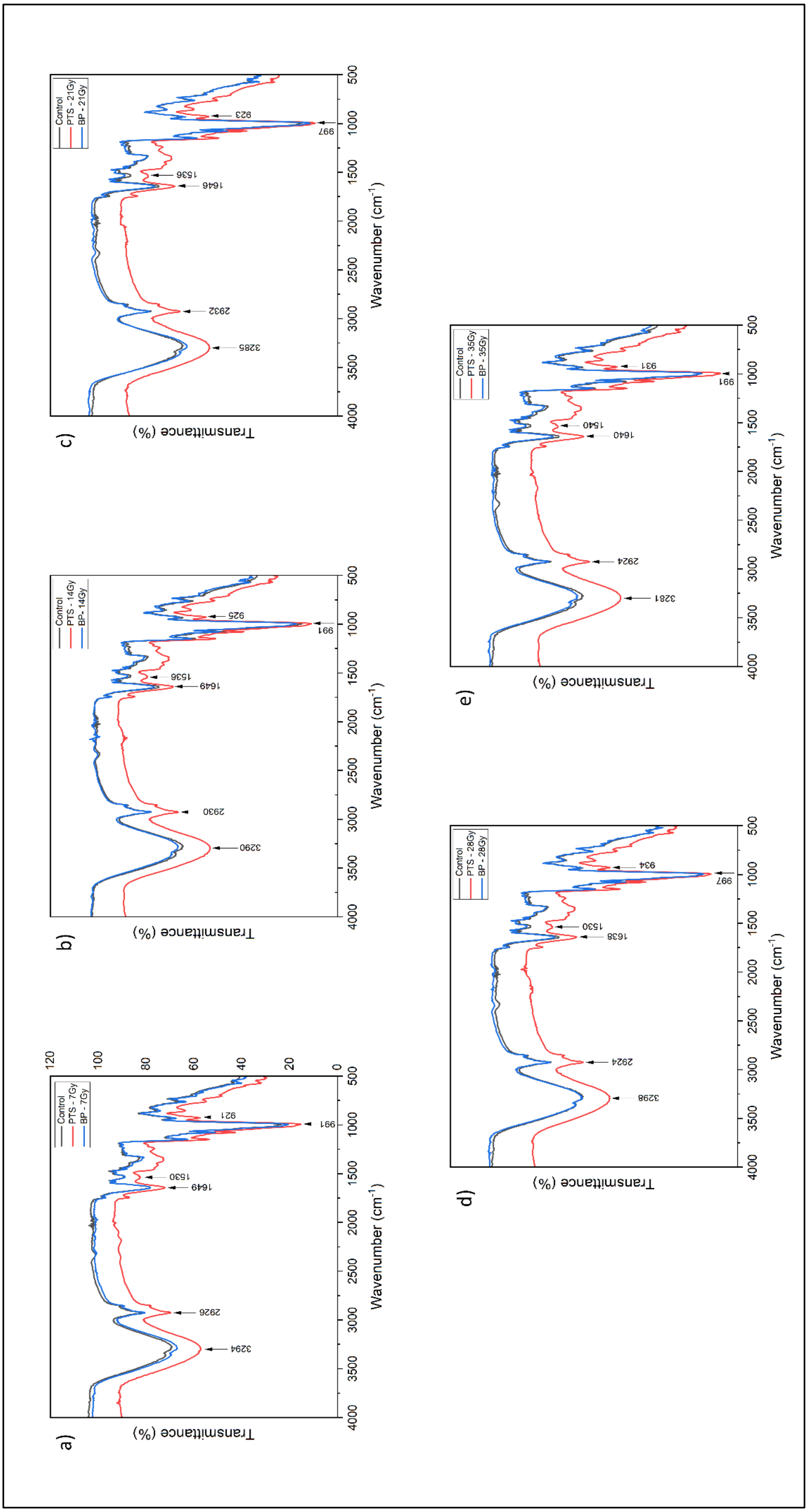

Fig. 1. Fourier transform infrared (FTIR) spectra for TR10 rice seeds irradiate with different facilities of neutron radiation at doses a (7Gy), b (14Gy), c (21Gy), d (28Gy), and e (35Gy).

Mineral Content. In this research, the analysis of mineral contents in TR10 rice varieties was done by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES) in order to determine the effect of neutron radiation on the mineral content using different facilities.

Minerals are one of the essential components needed in the normal diet for the human body to function properly. The mineral composition in TR10 rice varieties results from the current study is shown in Table *3*. There are significant differences in the phosphorus, potassium, and magnesium content among all the treatments at $p<0.05$. The primary minerals identified in irradiated rice in this study for P range from 0.94±0.00 to 2.48±0.03 mg/g, K from 507.07±9.57 to 1434.13±26.83 mg/g, and Mg from 30.53±0.52 to 83.63±1.03 mg/g. According to [15] data, the average amount of P, K, and Mg in flour, rice, white and unenriched was 94 mg/100g, 75 mg/100g, and 22.9 mg/100g, respectively, indicating that rice irradiated with neutron has greater amount in K and Mg, but lower in P. The highest percentage increase in P (69.86%), K (56.25%), and Mg (4.81%) composition over control was from seeds treated with 14 Gy and bombarded in the nuclear reactor core (PTS).

According to [16], rice is enriched with minerals such as Fe and Zn to minimize disease related to mineral inadequacies. The current study shows that TR10 irradiated in PTS has the highest increase in mineral content compared to the control at 72.85%. Meanwhile, the unirradiated seed (control) has the maximum mineral concentration in both Fe (9.95 mg/g) and Mn (18.30 mg/g) as compared to all irradiated seeds in both facilities. The loss of Mn and Fe in all treatments could be due to neutron irradiation damage, which causes transmutation, collision displacement, and ionization effects in materials, resulting in changes in the microstructure and material properties [17].

Table 3. The mineral content of TR10 rice varieties treated with different neutron irradiation facilities.

Dose [Gy]	Irradiation Facilities	Mineral Contents [mg/g]						
		P	K	Mg	Na	Mn	Zn	Fe
0	Control	1.46±0.02	917.83±23.07	79.79±1.36	54.00±3.06	18.30±0.10	20.00±1.65	9.95±0.05
7	PTS	2.28±0.00	1288.83±49.09	73.65±1.85	82.23±3.74	13.17±0.21	14.37±0.50	0.74±0.01
	BP#1	1.47±0.01	888.30±18.70	68.95±1.04	28.43±0.64	14.87±0.12	11.87±0.31	6.07±0.02
14	PTS	2.48±0.03	1434.13±26.83	83.63±1.03	80.23±1.44	17.30±0.26	28.10±0.46	0.72±0.00
	BP#1	1.08±0.01	629.93±8.83	50.94±0.27	32.17±0.12	10.77±0.06	15.77±0.46	4.49±0.02
21	PTS	0.94±0.00	507.07±9.57	30.53±0.52	197.00±8.02	0.17±0.06	6.47±1.39	0.35±0.01
	BP#1	1.16±0.01	683.93±10.33	55.38±0.62	37.10±0.69	13.73±0.15	12.87±0.35	5.78±0.05
28	PTS	2.00±0.02	1206.10±35.71	68.83±1.53	92.80±2.10	9.80±0.10	29.53±1.29	0.31±0.00
	BP#1	1.48±0.00	881.00±13.60	68.57±1.02	33.83±1.06	16.00±0.10	10.60±0.36	6.20±0.05
35	PTS	2.05±0.03	1348.17±20.84	75.61±0.09	136.77±1.42	14.37±0.15	34.57±1.18	1.28±0.00
	BP#1	1.29±0.01	789.43±18.28	70.09±1.69	39.50±1.25	17.77±0.06	11.47±0.25	8.92±0.16

*Values are expressed as a mean of three replicates

SEM Analysis of The Endosperm Structure. The cross-sectional appearances of the rice kernels were evaluated and exhibited in Figure *2* to study the structural changes in the endosperms TR10 rice variety influenced by neutron irradiation. According to [18], the diameter of granules in plants ranges from less than 1 µm to more than 100 µm and can be angular, oval, round spherical, or irregular in form. Further, rice starch compound granules have diameters of up to 150 µm, are polyhedral in form, are fine solid, and comprise multiple (20-60) individual granules.

Irradiation appears to have generated minor modifications in the morphology of the granules. The starch shape changes after neutron irradiation with 14 Gy in PTS and 35Gy in BP, becoming more spherical, shrinking and showing partial aggregation. [19] discovered that the number of small-size granules increased in both inner and outer layer endosperm of rice grain treated with 2 KGy of gamma irradiation. The alterations in the starch granular structure were dose-dependent, with a high dosage of energetic and penetrating radiation capable of damaging large starch molecules causing some starch granules to fracture along the cleaved molecules, resulting in the creation of small size granules [20, 21].

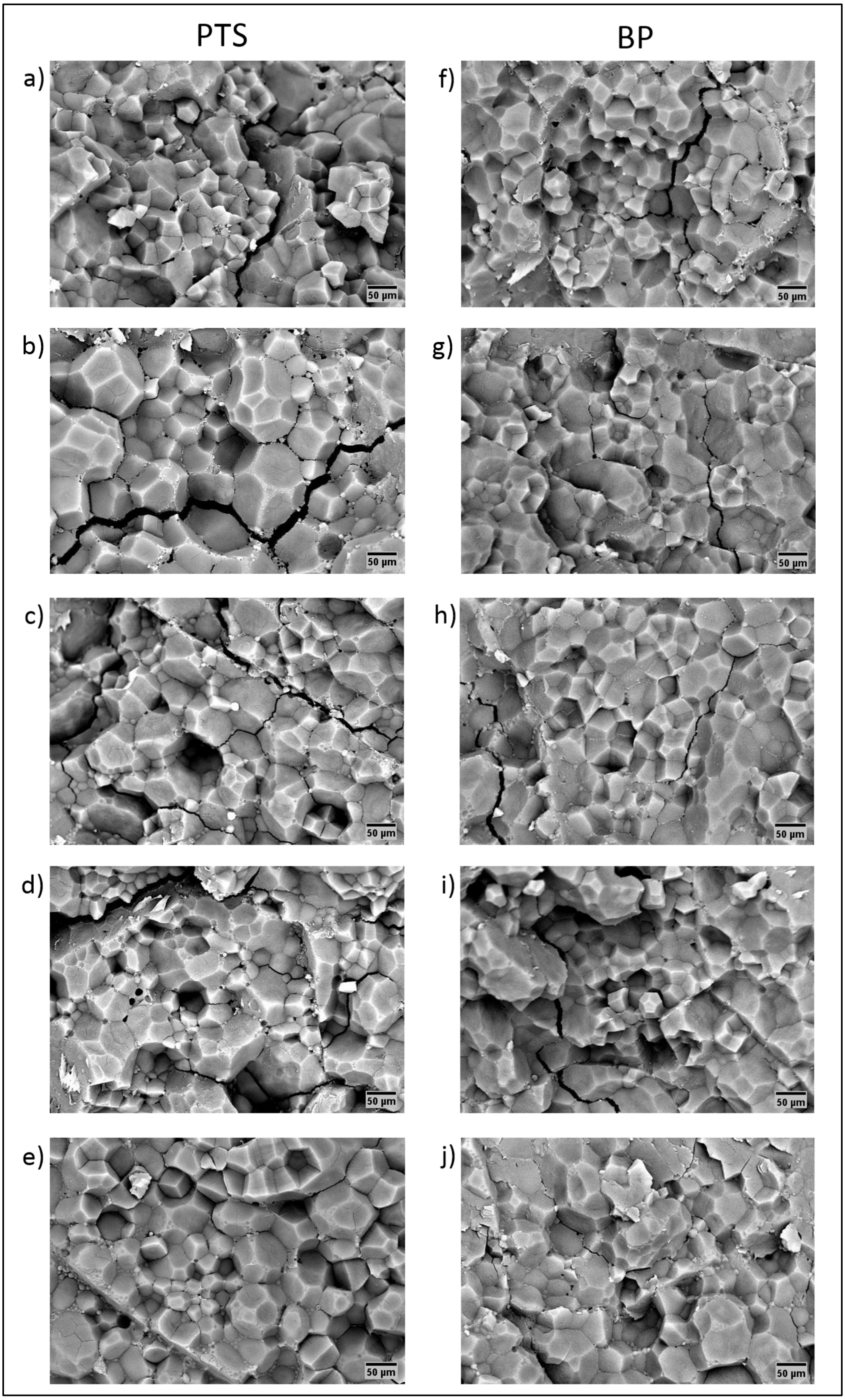

Fig. 2. Cross-sectional appearances of TR10 rice seeds irradiated in PTS (a-e) and seed irradiated in BP (f-j).

Conclusion

This article has provided a quick overview on the effect of using filtered (in neutron irradiation chamber, BP#1) and unfiltered (in nuclear reactor core using pneumatic transfer system or PTS) neutron sources at Malaysian Nuclear Agency's nuclear reactor, to the germination, physiochemical and morphology of TR10 Rice Seeds. The major goal is to improve the TR10 rice traits while also being concerned about changes in nutritional components caused by neutron irradiation, therefore these results may give some relevant information for crop breeders in mutant breeding programs.

It was found that seeds irradiated to unfiltered neutron source inside the nuclear reactor core failed to germinate. On the other hand, the seed germination decreased with the increase of neutron dosage for the seeds irradiated to filtered neutron in neutron irradiation chamber, BP#1. Similar trend was found on the seed vigour index. On the microstructure observation, the starch granules exhibit the appearance of small starch granules when irradiated with a neutron, although this did not have a significant effect when compared to seed treated with a high dosage of 14 Gy in PTS and 35 Gy in BP#1. In terms of physiochemical properties, all irradiated rice seeds in all neutron dosages shows no new harmful compounds are produced. For mineral composition, generally rice seeds irradiated to unfiltered neutrons turned to be higher compared to irradiation to filtered neutrons except Fe. In addition, P, K, Na and Zn contents found to be higher in seeds irradiated to unfiltered neutrons whereas all mineral content in the seeds irradiated to filtered neutrons are lower, even when compared to the control seeds. As a whole, irradiation to filtered neutrons may successfully boost the growth performance while also improving rice quality with optimum neutron dosages ranging from 7 to 14 Gy.

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Conflicts of Interest. The authors declare no conflict of interest.

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