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Influences of gamma ray and polyethylene glycol to identified the drought-resistant in the rice (*Oryza sativa* L. cv. Riceberry) by plant tissue culture

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Abstract The drought-resistant on rice (*Oryza sativa* L. cv. Riceberry) was studied by mutation and tissue culture techniques. The dry seeds of rice were exposed gamma ray at 0, 20, 25, 30, 35 and 40 krad. Naked seeds were cultured on NB medium without growth hormone. The result indicated of lethal dose (LD_{50}) values of 28 krad that, could be highly related to mutagenic agent. The seeds were exposed to a dose at 28 krad of gamma rays and transferred onto NB medium supplemented with 0, 5, 10, 15 and 20% polyethylene glycol (PEG) compared with non-irradiation. The effect of irradiation and PEG was assessed on the shoot and root length, fresh and dry weight, total chlorophyll content and proline concentrations. The non-irradiation results appeared to the highest average of the shoot and fresh weight. On the contrary, the irradiated seeds produced the highest proline concentration and root length. The PEG decreased all the recored characters, except for the average of proline accumulation. The irradiation was the highest mean of root length, dry weight, chlorophyll, proline at the concentration of 15% PEG. This result gave some signal characteristics in drought tolerance against drought stress.

Keywords: Drought stress, Gamma radiation, LD_{50}, PEG, Rice (*Oryza sativa* L. cv. Riceberry)

Introduction

Drought is a one of natural disaster in the world and a major factor affecting the agricultural productivity because the increasing population of the world and the changing climate makes a lot of the crisis problem. In particular, rice is an important food crop and a large part of the world population consumes rice as the main food. Moreover, rice is the most significant cash crop in Thailand, exports 9.20 million tonnes of rice, worth about 119,215 million baht which is one of the main income earners in the country (Weerapad, 2012).

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At the present time, Riceberry rice (*Oryza sativa* L. cv. Riceberry) has been a popular brown rice for health conscious person. Riceberry is a native rice cultivar in the north and northeastern Thailand, the cross-breeding of rice, Jao Hom Nin and Khoa Dawk Mali 105 developed by Rice Research Center Kasetsart University for nutritional properties, and physical and cooking properties. In addition, rice bran and rice bran oil Riceberry also has antioxidant properties as well. The outcome is a deep purple whole grain rice contains anthocyanin occurs naturally, it is a pigment which are three primary colors following, red, purple, and blue pigments a natural coloring agent in food. Anthocyanin extract is a group of highly effective flavonoids or antioxidants. It is a nutraceutical could help slow down cell degeneration and reduce the risk of cancer and heart disease. Slow down the deterioration of the eyes. It inhibits pathogenic *Escherichia coli* in the gastrointestinal tract caused diarrhea and food poisoning. The medical system is used to make nutritional products.

However, rice is highly susceptible to drought stress about 50 percent of global production affected by drought. (Bouman *et al*., 2005). The water stress is the problem of global warming beginning to affect the ecology of rice such as reduced agricultural production, natural disasters, and epidemics, as well as economic problems and the variety of rice greatly reduced. Moreover, the area of rice production is limited. Therefore, Thailand should produce a lot of rice, there is enough rice for exports, which is a major source of income for the country because demand for world rice is likely to increase. The future of Thai rice is important to focus on high-quality production for export. Accordingly, it is important to study the drought tolerance of rice.

Techniques of plant tissue culture, genetic engineering, and molecular biology have developed and played a role in the adaptation or improvement of plant varieties. As well as increasing the yield and value of the crop, it has a high effect on trade and the economy. The technology is based on the basic principles of tissue culture, can be induced to complete the plant regeneration. Tissue culture techniques have a role in the study of drought tolerance of rice, it is a highly effective technique to study under sterile conditions and can be determined of drought stress to obtain the desired plant (Joshi *et al*., 2011).

The selection of drought-tolerant plants using tissue culture techniques can be cultured by culturing cells or groups of cells with synthetic media consist of osmoticum agent, the commonly used different concentration of Polyethylene Glycol (PEG), reducing the water potential and caused osmotic pressure in the culture (Muthuramu *et al*., 2008). The addition of osmoticum agent into the medium, that is imitated natural conditions for the crisis of drought. As a result, the plant is in a state of water stress, and death.

To improve breeding of rice plants, the use of irradiation. The gamma radiation is one of the most popular methods of inducing mutations, is penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It consists of photons in the highest observed range of photon energy. Gamma rays are used to induce mutations in organisms because it has high energy and can change with DNA. Normally, the genetic material of a living organism is responsible for regulating the characteristics of the organism. When genetically altered cells cause genetic changes.

The purpose of this study was to ascertain the drought tolerance of Riceberry rice by tissue culture, adding different concentrations of PEG6000 in culture media to simulate the drought condition. The study was based on observation of rice root growth, ability to germinate, effects of drought on rice seeds. The major plant response mechanism was the accumulation of proline. Proline accumulation was varies by species and plant varieties, tissue culture techniques are used in combination with gamma irradiation to improve rice varieties to study the ability to tolerate drought.

Materials and methods

Rice seeds cultivar Riceberry were received from Rice Research Center, Thailand. The dry seeds were separated into two group of the non-irradiation group used as a control and irradiation group, were compared. The naked seeds of each group were washed with water and sterilized with 70% alcohol (v/v) for 1 minute and soaking of 20% sodium hypochlorite (v/v) for 30 minutes at 230 rpm shaking speed. Then, the seeds were cleaned three times using sterile distilled water to remove sterilizing agents before blotted dry on a sterile coaster in a laminar airflow. After that, the sterilized seeds were transferred on NB medium enhanced with 30 g/L sucrose and 0, 5, 10, 15 and 20% of PEG6000 to simulate drought stress in tissue culture bottle and incubated at 25±2 ℃ under the light for 7 days. After culture, the seedling was recorded of the lengths of shoots and roots, plantlets fresh weights and plantlets dry weight 72 ℃ for 48 hours.

The effect of gamma ray on rice seeds

The seeds of ricberry rice were irradiated with acute gamma irradiation (Caesium-137) at 0, 20, 25, 30, 35 and 40 krad in the Gamma Irradiation Service and the Nuclear Technology Research Center (GISC). Manually dehusked seeds were externally sterilized as the above method. The seeds were placed on NB medium supplemented with 30 gL^{-1} sucrose and 2.6 gL^{-1}

phytagel an incubated at 25±2℃ at the fluorescence light. The seed germination and LD_{50} values were recorded after 28 days of culture.

The evaluation of total chlorophyll content

The test total chlorophyll was a modified method of Hiscox (1979). Leaf tissue with 100 mg fresh weight was cut around 1 mm. The cut leaves were immersed with 10 ml Dimethyl sulfoxide reagent and incubated at 65℃ for 4 hours. The liquid extract was determined at 645 nm and 663 through the use of a spectrophotometer. The data were calculated for the total chlorophyll content according to the formula of Anon (1994).

The determination of proline accumulation

The test proline accumulation of the samples was carried out according to Bates *et al*. (1973). The rice leaves with 5 mg fresh weight were homogenized with 3% sulfosalicylic acid. The extracted was mixed with 2 mL of glacial acetic acid and ninhydrin reagent incubated at 100℃ for 30 min. The reaction was stopped in the ice bath. Then add 4 mL toluene and shaking for 15-20 seconds. The liquid extract was assessed at 520 nm by the spectrophotometer.

The statistical analysis

The experiment was designed as two factors factorial in Completely Randomized Design (CRD) with three replications; 10 experimental unti per replication. The factors included 2 levels of the non-irradiation group and irradiation group and 5 levels of PEG 0, 5, 10, 15 and 20%. Data were analyzed by using IBM SPSS Statistics 23.0 programme. All treatments were compouted by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$ level.

Results

Effect of gamma ray in rice seeds

The various radiation doses showed the survival rate of 57.50 to 28.00 percent (Fig.1), The percentage survival rate at 50 percent (LD_{50}) was 28 krad, as shown in Fig. 2. The characteristics of rice plants are presented in Fig. 3A-3E appeared that the rice plant was green shoots. The rootlets were plumped and branched off from the seminal roots. At 40 krad, the morphology of some rice plants was changed color from green to brown. The length of the shoot and root was decreased. The survival rate was at least 28 percent, as shown in Fig. 3F

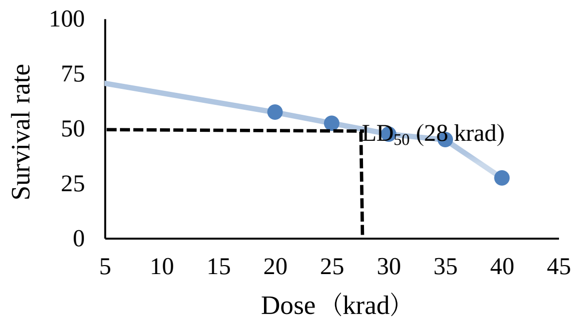

Figure 1. Lethal dose 50 of riceberry rice seeds were cultured for 4 weeks. After exposed to acute gamma irradiation (Caesium-137) at 0, 20, 25, 30, 35 and 40 krad, respectively

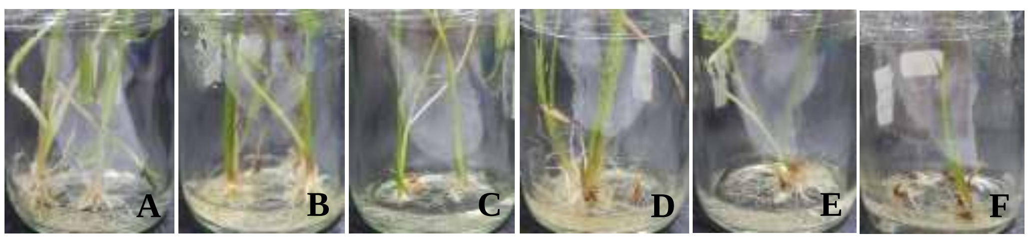

Figure 2. The morphology of Riceberry rice seeds on NB medium without plant growth regulator was cultured for 4 weeks. (A) Non-irradiated rice seedling as a Control and (B-F) irradiated rice seedling at 20, 25, 30, 35 and 40, respectively

Shoots length of seedlings

The non-irradiated seedling performed the mean value of shoot length increased compared with the irradiated ones was demonstrated. The application of PEG during seedling growth was associated with decreased the shoot length irrespective of gamma ray application (Fig.3). The interaction between irradiation and PEG% appeared that the largest shoot length of 24.15 mm was recorded for non-irradiation treatment with 0% PEG compared with irradiation treatment, which reached 17.07 mm. The non-irradiated treated sample produced the largest shoot length in concentrations of 15% PEG which reached 5.66 mm compared with the irradiated samples, which reached 4.56 mm. The morphology of non-irradiated seedling and irradiated seedling after culture on NB medium enhanced with various concentration of PEG6000) Fig.%).

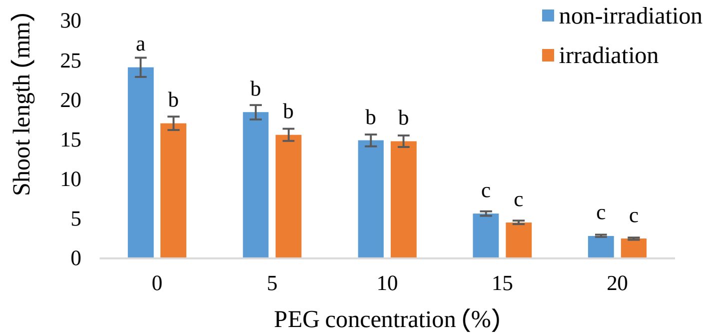

Figure 3. Mean values of shoot length of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

Roots length of seedlings

The mean value of root length increased in the irradiated seedling compared with that in the non-irradiated ones (Fig4). The length of the roots at 5, 10, 15 and 20% PEG decreased for non-irradiated and irradiated genotypes compared with 0% PEG. The interaction between irradiation and PEG% showed larger root length in 15% PEG with irradiation than in the non-irradiation treatment with the same concentration. As the data, all genotypes resulted in PEG stress for irradiated and non-irradiated treatments. The plant height and root length decreased with increased PEG concentration (Fig.5).

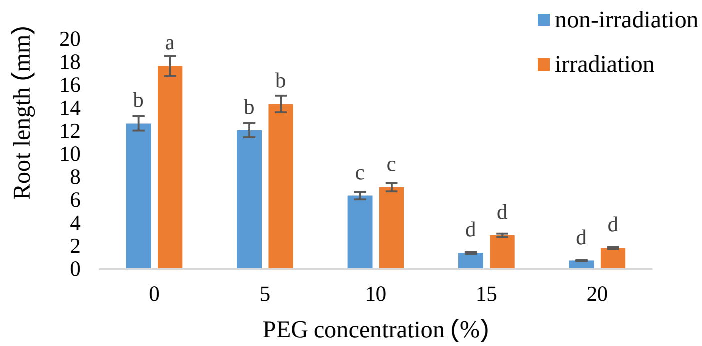

Figure 4. Mean values of root length of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

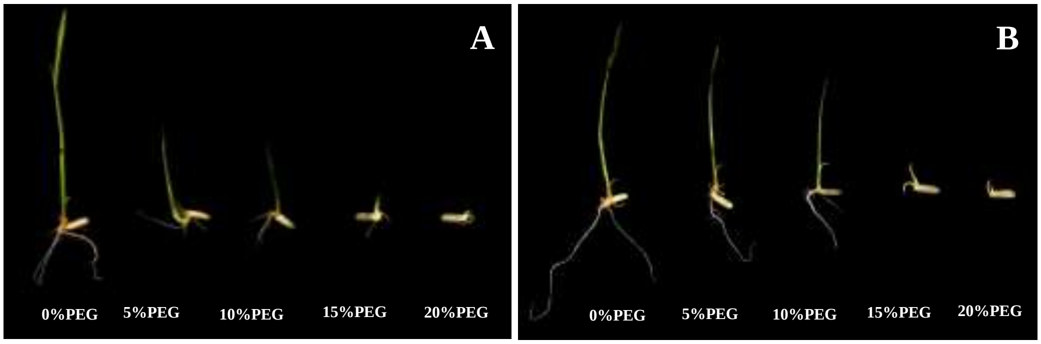

Figure 5. The morphology of non-irradiated rice seedling (A) and irradiated rice seedling (B) after culture on NB medium enhanced with 0, 5, 10, 15 and 20% of PEG6000

Fresh weight of seedling

The mean fresh weight increased in the non-irradiated sample compared with irradiated seedling (Fig.6). The data indicated that a similar response was generated under PEG stress by irradiated and non-irradiated. Fresh weight decreased with a high level of PEG. The interaction between irradiation and PEG% revealed that higher fresh weight (40.92 mg) was recorded for non-irradiation treatment with 0% PEG than for irradiation treatment (37.64 mg) at the same concentration.

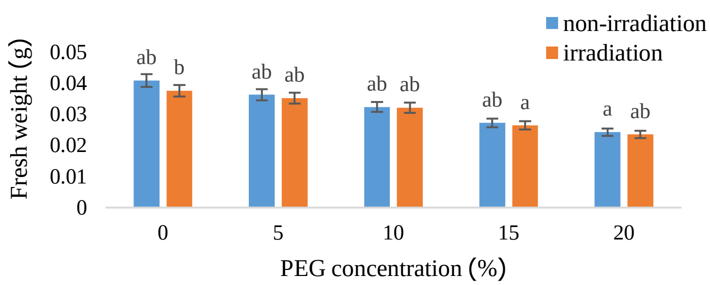

Figure 6. Mean values of fresh weight of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

Dry weight of seedling

There were no differences in mean dry weight when observed in the non-irradiated and irradiated samples (Fig.7). Moreover, the mean dry weight decreased all concentration of PEG compared with 0% PEG. The interaction between irradiation and PEG% irradiation sample revealed a higher dry weight in non-radiation treatment at weight in radiation treatment at 15% PEG concentration than in non-irradiation treatment at the same concentration.

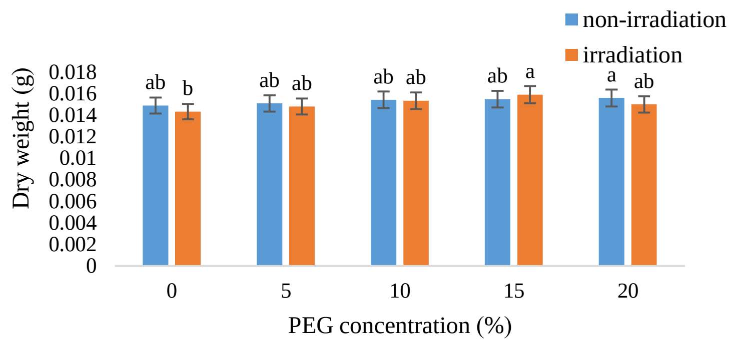

Figure 7. Mean values of dry weight of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

Total chlorophyll of leaf

Regardless of the applied PEG concentration, no differences in total chlorophyll content were observed in the non-irradiated and radiated samples. The non-irradiated and irradiated genotypes compared with 0% PEG were decreased (Fig.8). The interaction between irradiation and PEG irradiation treatments showed higher total chlorophyll for irradiation treatment than for non-irradiation treatment at 15% and 20% PEG concentration.

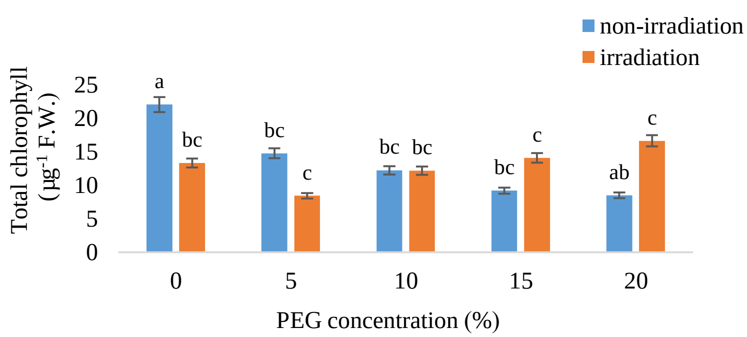

Figure 8. Mean values of total chlorophyll of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

Proline concentrations of leaf

The mean value of proline concentrations increased in the irradiated genotypes compared with the non-irradiated ones (Fig. 9). The proline concentrations exhibited in increasing at 5, 10, 15 and 20% PEG compared with 0% PEG. The interaction between irradiation and PEG showed higher proline concentrations in the radiation treatment at 0% PEG than in the non-irradiation treatment at 0% PEG. The data showed higher proline concentrations in the radiation treatment at 15% PEG concentration than in the non-irradiation treatment at the same concentration.

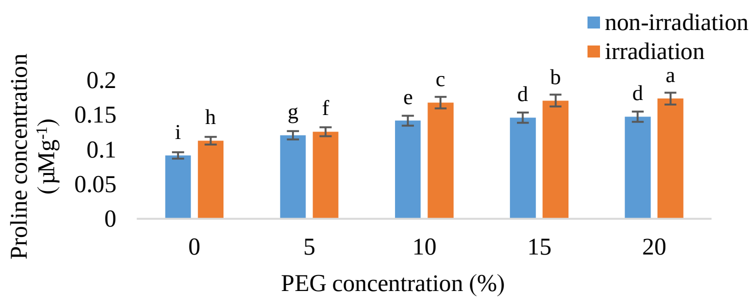

Figure 9. Mean values of proline concentration of riceberry rice obtained from seeds exposed to acute gamma ray at 0 krad (non-irradiation) and 28 krad (irradiation) and PEG treatments at concentration of 0, 5, 10, 15 and 20%

Discussion

It has been long known that plants are sensitive to ionizing radiations. Plant have been irradiated in order to investigate the effects of these radiations on crop plants. Studies have shown that radiation of different types at various intensities can either inhibit growth or kill the plant completely (Kumar, 2013). Escorted by (Ismachin, 1972) found that the high doses 8 krad will inhibit the growth of rice. It will block the process of dividing cells or cause different changes depending on the dose received. While radiation at less than 2 krad does not cause death of rice. In this studiy showed that the morphology of rice plants was change color from green to brown. The length of shoot and root was decreased. Since, the effect of gamma ray influence plant growth and development by inducing cytogical, genetical, biochemical and physiological changes in cells and tissue (Hagen *et al*., 1961). These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depening on the radiation dose (Ashraf *et al*., 2003). So the result of the experiment is LD_{50} values was 28 krad, expected that plant would be the highest survival rate and mutation.

Proline is a type of amino acid, made up of glutamic acid. Generally, the plants have proline accumulation at a low level (Gzik, 1996). The results of this study appears that the proline accumulation were increased at a high degree concentration of %PEG obtained from rice leaves cultivated on drought conditions. At 20% PEG, Irradiation plant had the highest proline content compared with non-irradiation. In addition, proline content increased in the irradiated compared with the non-irradiated. These results are consistent with previous research (Waranya, 1998) they found that RD23 rice drought tolerant genotypes exhibited higher proline content than wildtypes. It chould be used as an index for drought tolerant rice. It can be said that irradiated rice varieties have been drought resistant than the non-irradiated rice. There have been studies of proline accumulation (Delauney and Verma, 1993) described Proline synthesis is controlled by pyrroline-5-carboxylate synthetase (P5CS) enzyme is controlled by the process of feedback inhibition. The crop is water stress, the crop loses feedbacks inhibition. For this reason, plants were high stimulated for proline synthesis.

The results of root length increased in the irradiated seedling compared with that in the non-irradiated ones. Similarly the result of Keokene and Pattanagul (2006) observed the root length of Kao Dawk Mali rice cultured on drought condition increased compare with normal condition. In plant sensitive to water shortfall adapt to drought by reducing growth. The plants will reduce the surface area of the leaves for the available water content and the root length high proliferation (Farooq *et al*., 2009). The large reduction in all characteristics with the increase in PEG percentage, except forthe mean values of proline concentration was in line with the results of Soni *et al*. (2011) who observed a decrease in root and shoot fresh weight under stressed circumstances in all investigated genotypes. Additionally, most of the tolerant genotypes gathered more dry matter than the vulnerable ones. By adding PEG to the culture medium, the water of the medium decreased.

The decrease in seedling fresh and dry weight with cumulative PEG levels might have occurred because of the osmotic pressure influencing the accumulation of soluble elements in cells; consequently, it affected the other physiological functions in the cells. The physiological significance of gamma radiation is due to the formation of free radicals over the hydrolysis of water that can haste to the modulation of an antioxidative scheme, accumulation of phenolic combinations and chlorophyll pigments affecting shoot and root distance and fresh and dry heaviness (Borzouei *et al*., 2010 and Ashraf, 2009). Drought enhances senescence by alleviating chlorophyll degradation, nitrogen damage and lipid peroxidation (Swain *et al*., 2014). Drought decreases photosynthesis by decreasing stomatal and mesophyll conductance (Aroca *et al*., 2012). The decrease in chlorophyll standards because of drought stress could be related to the typical symptom of oxidative stress and could be the result of pigment photo-oxidation and chlorophyll degradation (Farooq *et al*., 2009). The opposing consequence of water shortfall stress on chlorophyll content and comparative water content overlaps with the general trend of the consequence of drought on physiological procedures in plants. Photosynthetic pigments might be destroyed by high doses of gamma irradiation and with the concomitant damage of photosynthetic volume (Jan *et al.*, 2012).

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