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The Effect of Acute Gamma Ray Irradiation through RbcL Gene in Thai Sweet *Tamarindus indica* L.

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Abstract

This experiment investigated in sequence base of partial chloroplast rbcL gene in three cultivars of Thailand sweet tamarinds(Prakaitong, Seetong, and Srichoompoo)planted in Phetchabun, Thailand, through acute gamma ray irradiation 5 levels (0,5,10,15 and. 20 Kr). Look for the replacement of sequence due to radiation and morphology of growth in tamarind received such radiation. The results showed that seedling sprouted from seeds that have been irradiated to all treatments. The first set of true leaves form a mosaic pattern. The mosaic gradually lost when the true original seried leaves came forth. When examining the sequences of the rbcL gene found a replacement site, up 43 points from 564 bp, representing 7.62% of the dose 15 Kr in Prakaitong cultivar, Seetong cultivar was found a replacement site at 13 points, representing 2.08% of 625 bp from 20 Kr treated treatment and Srichompoo cultivar was found a replacement site at 65 points, representing 11.11% of 585 bp from 10 Kr treated dose. For the other doses were not significance to the controls. Furthermore , there were found that, such radiation of the those three cultivars form similarly morphology changes. At the first year in soil in the pots, it was found that the diameter of trunk greater than the control group (8.72 $\pm$ 0.37 mm., while the control was 5.04$\pm$ 0.46mm.) Length of the leaflet was as long as two time when compared to the control group (2.02$\pm$ 0.23cm.,while the control group was 1.08$\pm$ 0.13 cm.), But the height of the stems shorter than the control group (33.00$\pm$ 4.47 cm., while the control group was 47.20 $\pm$ 10.68 cm.) And all of treatments are statistically significant difference at the 0.05 level, and when compared the shape of

the trunk. The exposure such radiation treatments were shaped like the branches of the tree in reproductive stage, while the control shaped like a cylinder, which is a Juvenile stage of growth. After planting all treatments in soil, 4 years later. All of irradiated pants of 3 cultivars become bigger and taller than the controls, but still in the same sequence base. Some of those such treaded treatments were grafting on native tamarind in the farm. It was found that, they were flowering fallow the mother plant but still the same sequence base in *rbcL* gene. These data indicated that the partial *rbcL* gene range in this research should be act as molecular marker for cultivar identification, and *rbcL* gene did not control in morphology expression of sweet tamarind. Acute gamma ray cause morphology changes in Thai sweet tamarinds.And 15-20 Kr acute gamma ray irradiation could be used for crop improvement in sweet tamarind cultivars .

Keywords: Acute gamma ray irradiation, Sweet Tamarinds in Thailand, *rbcL* gene, *Tamarindus indica* L., single nucleotide analysis

Introduction

Thailand may be only one country in the word that reported, there are sweet Tamarinds. They were identification into 19 cultivars with DNA Barcodes using partial cds of *rbcL* gene in 2015[1]. They are in the same scientific name *Tamarindus indica* L.The famous cultivar is Prakaitong,then Seetong cultivar and Srichompoo cultivar, respectively.

Tamarinds are economic plant of Phetchabun province, Thailand. Since climate changes,all tamarind fruits were infected with fungi like white color fungi name *Pestalotiopsis sydowiana* (Bres.) B. Sutton, green color fungi name *Cladosporium cladosporioides* (Fresen.) G.A. de Vries) and orange color fungi name *Fusarium solani* (Mart.) Sacc. [2] And also tamarind leaves were infected with fungi name Cladosporium sp., Aspergillus sp., Geotrichum sp. and Nodulisporium sp.[3]. Researcher tried to solve fungal infected problems. The fungi named *Pestalotiopsis sydowiana* (Bres.) B. Sutton was found that it is an endophyte specy in vascularbundle of the tamarind trunk [2]. Researchers tried to use so many medicinal plants [2] and several methods for reducing the fungal effects like Soil Management [4], Water management [5], Pest and insects controlling [6,7 and 8]. But the tamarinds still infected with fungi. This study aim to make anti-fungal effect of sweet tamarind plants by mean of gamma ray irradiation. Due to tamarinds are long live and slow growing plants, for 10 years up just can give fruits/pods, for reducing the time, so some irradiated plants were grafting on native mother tamarind plants in the farm. The chloroplast *rbcL* gene for ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, was used as molecular marker. The single nucleotide analysis and clustal X /W program [9] were used to investigation in this study.

Preparing of Plant Materials

The 500 seeds of each sweet tamarind cultivar .They are Prakaitong, Seetong, and Srichoompoo cultivars .then they were subjected in five ravels of acute radiation(0,5 10,15 and 20 kilorad =Kr),then all 15 treatment combinations (3cultivars x 5level of irradiation)
With 3 replications each were cultured in the soil at green house of Phetchabun RaJabhat University, Phetchabun sProvince,Thailand. Tamarind leaves were collected from the green house. Leaves were harvested from control groups(0 Kilo–rad = 0Kr., none irradiated plants) and treatment groups with 4 levels of acute gamma ray(5,10,15 and 20Kr) greenish, hardened and fully expended, the fourth leaf from the shoot tip (Figure 1). All harvested leaves were placed in an ice-box and kept away from sunlight before transporting to the laboratory. They were kept at -20°C

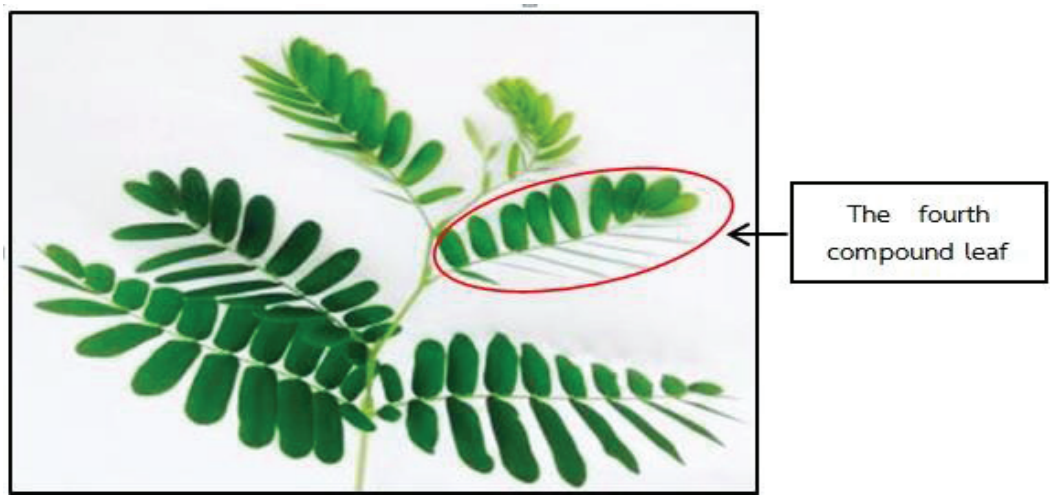

Figure 1. The fourth compound leaf from the shoot tip of tamarind leaves were optimum used for DNA extraction in this study.

DNA extraction by CTAB medthod.
The solutions and solvents used for the assay include Chloroform : Isoamyl alcohol (24:1), 5 M NaCl, Isopropanol, 70% ethanol, 95% ethanol, RNase A (0.1 µg/µl), TBE buffer [1 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)], CTAB buffer [1.0 M Tris-base, 0.5M ethylene diamine tetra Acetic acid (EDTA), 5 M NaCl, 2% w/v hexadecyl trimethyl-ammonium bromide (CTAB)], 1% w/v polyvinyl pyrrolidone (PVP) (MW 40,000), 1% v/v beta-mercapto ethanol[10].

DNA isolation and analysis.
Leaves were washed gently with distilled water in order to remove all surface particles. All midribs and secondary veins were removed using an alcohol-sterilized scissors, 500 µl of DNA isolation buffer was added into each 1.5 ml microfuge tube. 100 mg. of leaf sample was weighed and pulverized in liquid nitrogen. It was then transferred into each buffer-added microfuge tube. After a quickspin, another 300 µl of DNA isolation buffer was added. All tubes were gently flicked and inverted thoroughly to mix the sample, and were incubated at 65°C for 40 min. 700 µl of

chloroform: isoamyl alcohol (24:1) was added to each microfuge tube and gently inverted until emulsion was formed. Samples were centrifuged at 10,000 rpm for 15 min and the aqueous phase was gently transferred into a new 1.5 ml microfuge tube. The chloroform: isoamyl alcohol procedure was repeated. Half volume of 5M NaCl was added followed by two volume of cold isopropanol (- 20°C) and contents were gently inverted before incubating on ice for 10 min. Samples were centrifuged at 13,000 rpm for 5 min. Supernatant was discarded. DNA pellets were washed twice with 500 µl of cold 70% and ethanol (-20°C). The resulting genomic DNA pellets were air-dried at room temperature and dissolved in 30 µl of TE buffer. RNase A was added to each sample (1/10 µl DNA sample) and was incubated at 37°C for 1 h. Samples/Genomic DNA were stored in -20°C.

PCR - Polymerase Chain Reaction technique for amplify *rbcL* gene and data analysis.

PCR master mix was performed by using 15 genomic DNA samples as templates.

Forward primer: *rbcL*-Tam-F03, sequences from 5' to 3' are CAT GGG AAG AAA TGA TAA AAA and reverse primer: *rbcL*-Tam-R04, sequences from 5' to 3' are GCA GCA GCT AAT TCA GGA CTC for amplify *rbcL* gene[11], using Thermocycler , ESCO ,Swift™ MaxPro Thermal Cycler. PCR products were quantified by using a spectrophotometer at A260, while the purity of the DNA was checked through A260/A280. And PCR products were analyzed through 1% agarose gel electrophoresis using Mupid-EXU® Gel Electrophoresis System. Then they were sent to FIRST BASE co Ltd, Singapore for sequencing. The data of nucleotide sequence base were computed for % Identity and sequence alignment by using Clustal W2 program[9] and conducted single nucleotide analysis by BioEdit compare to BLASTn of NCBI DATA BASE.

Results and discussion.

The 500 seeds of each sweet tamarind cultivars, were subjectived in 5 doses of acute gamma ray irradiations (100 seeds/dose/cultivar),then culture in the soil. It was found that, one month seeding of all doses of irradiated tamarinds were found to be a mosaic similar of leaf mosaic disease [12]and Figure 2, but this symptom will disappear when the plant produced the second true leaves. After one years of cultured in the soil, we observed the morphology change of treatments compare to the control. It was found that the irradiated plants are bigger than control in trunk dimension, wider and longer of leaflet, more compound leaves, but shorter in plant height, while the controls are small in trunk dimension, no branching and still in juvenile stage. All treatments are significant differences at p-value < 0.05, as shown in Figure 3–5.

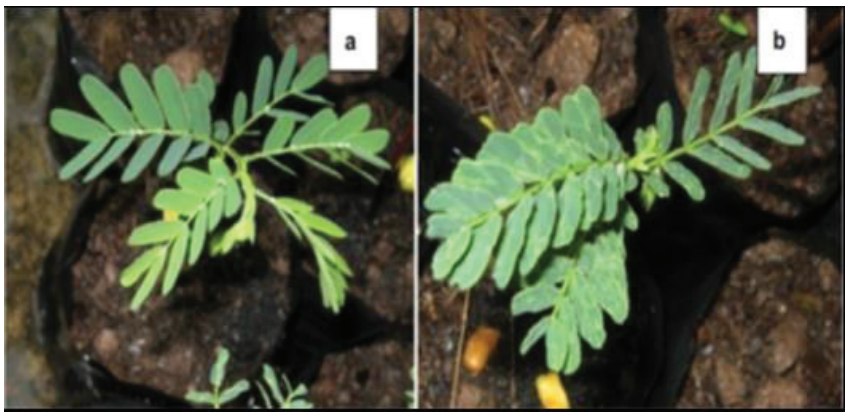

Figure 2. One month seeding of Tamarind (a) Non–irradiated tamarind seedling, (b)The irradiated tamarind seedling was found to be a mosaic similar of leaf mosaic disease.

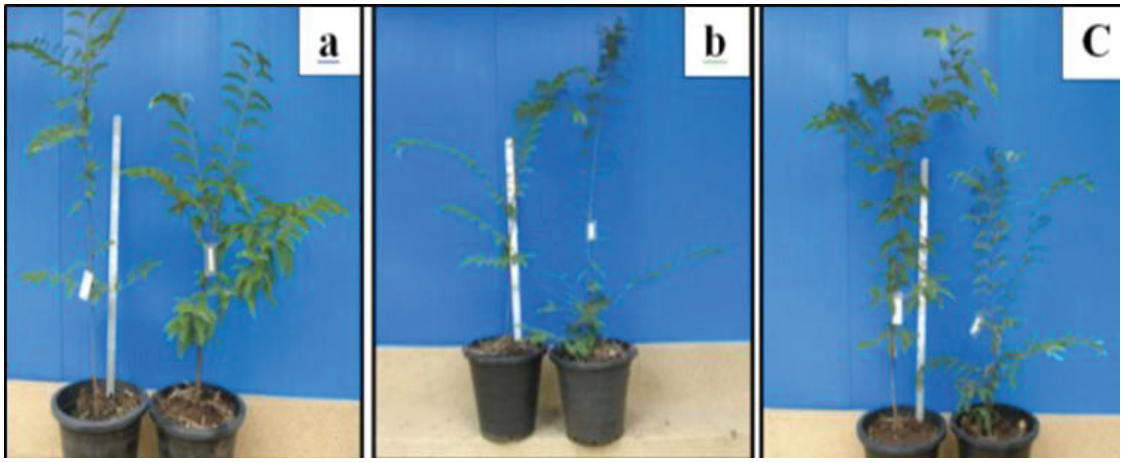

Figure 3. The comparisons of non-irradiated plants (left) and irradiated plants (right) of 3 sweet tamarind cultivars at one year old in soil culture.

a. Prakaitong cultivar.
b. Seetong cultivar.
c. Srichoompoo cultivar.

From the results in Figure 3 indicated that the effect of acute gamma ray irradiation at dose of 15Kr to Prakaitong (PKT),20Kr to Seethong(ST)and 10Kr to Srichompoo(SCP), induced morphology changes. At the first year in soil in the pots, it was found that the diameter of trunk greater than the control group (8.72 + 0.37 mm., while the control was 5.04+ 0.46mm.) Length of the leaflet was as long as two time when compared to the control group (2.02+ 0.23cm.,while the control group was 1.08+ 0.13 cm.), But the height of the stems shorter than the control group (33.00+ 4.47 cm., while the control group was 47.20 + 10.68 cm.) And all of treatments are statistically significant difference at the 0.05 level, and when compared the shape of the trunk. The exposure such radiation treatments were shaped like the branches of the tree in reproductive stage, while the control shaped

like a cylinder, which is a Juvenile stage of growth.

Nevertheless, we identified the sequences of *rbcL* gene in chloroplast genome[13,14] to characteristic of nucleotide sequences of irradiated and non-irradiated plants at one year old and 4 years old of Tamarind plants.(Figure 4, 6 and 8). It was found that they were still in the same sequence bases, but different in plant height, shape and dimension of trunk (Figure5,7and 9).The irradiated plant, nucleotide substitutions were found at in all doses of gamma ray irradiations as shown in Table1,2 and 3) as follows:

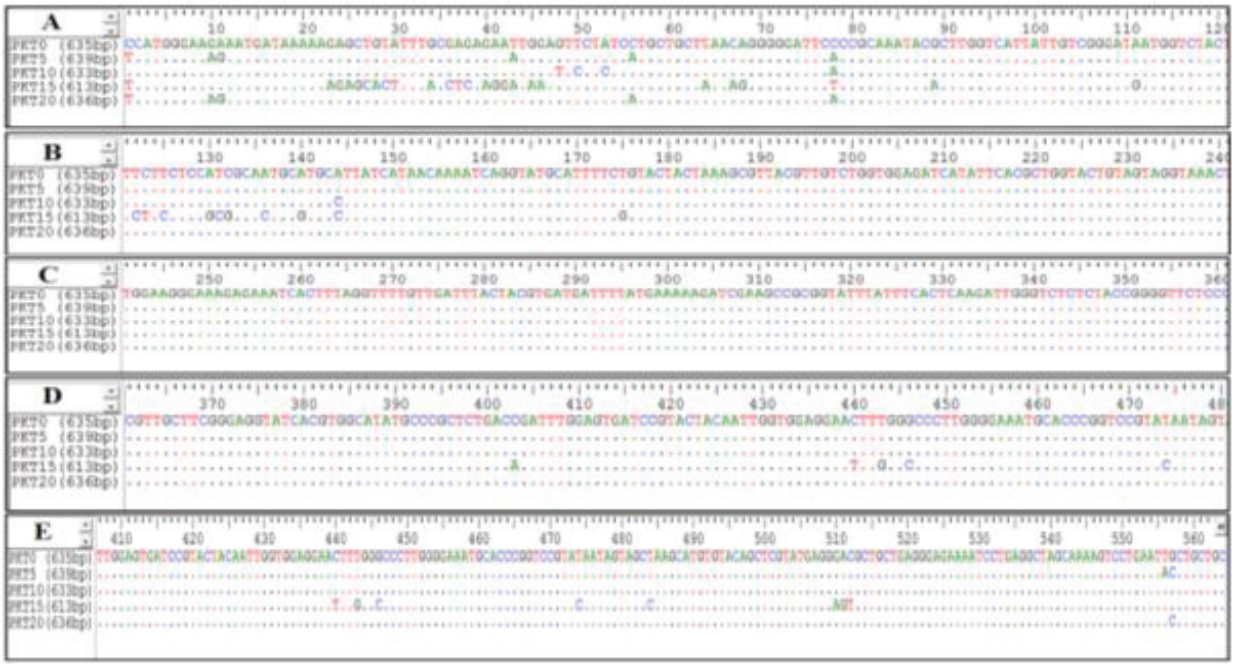

Figure 4.Features and similarities in alignment window of 5 Tamarind Prakaitong cultivar sequences, present number of base 1-564 bp. DOT(.)=view conservation by plotting identities to a standard control (PKT0=no irradiation) as a dot. (PKT0 = Normal Prakaitong, PKT5 = Treated with 5Kr, PKT10 = Treated with 10Kr,PKT15 = Treated with15Kr, PKT20=Treated with 20Kr.)

Figure 5.The comparison of control plant (left) and irradiated plant (right) of treated 15 Kr Prakaitong cultivar at 4 years old in soil culture.The height become taller , bigger trunk, more compound leaves than the control and reproductive stage in branches and shape. Giving flower when grafting on the native mother plant in during of 8 months. (Figure11. A)

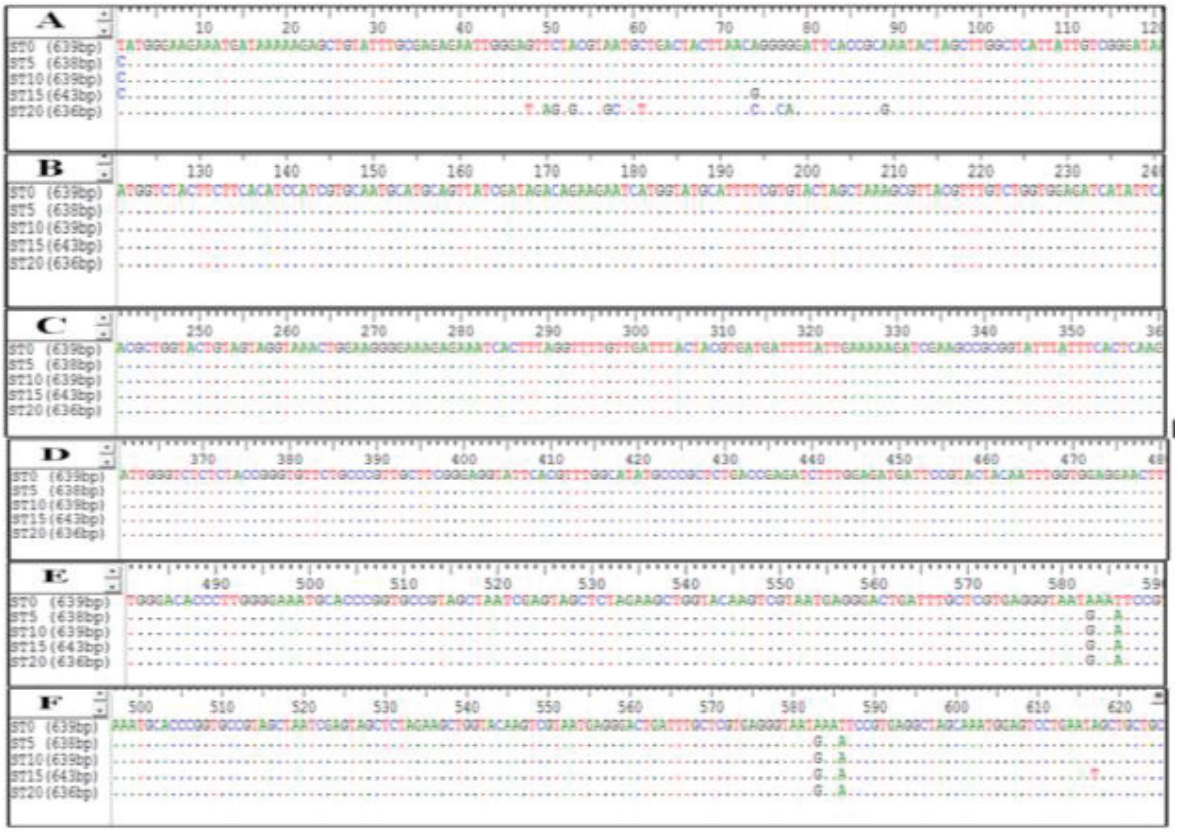

Figure 6.Features and similarities in alignment window of 5 Seetong cultivar sequences, present number of base 1-625 bp. DOT(.)=view conservation by plotting identities to a standard control (ST0=no irradiation) as a dot.(ST0 = Normal Seetong, ST5 = Treated with 5Kr, ST10 = Treated with 10Kr, ST15 = Treated with15Kr,ST20 = Treated with 20Kr.)

Figure7. The comparison of non-irradiated plant (left) and irradiated plant (right) of treated 20 Kr Seetong cultivar at 4 years old in soil culture. The height become shorter, but bigger trunk, more compound leaves than the control and reproductive stage in branches and shape. Giving flower when grafting on the native mother plant in during of 8 months.(Figure.11B)

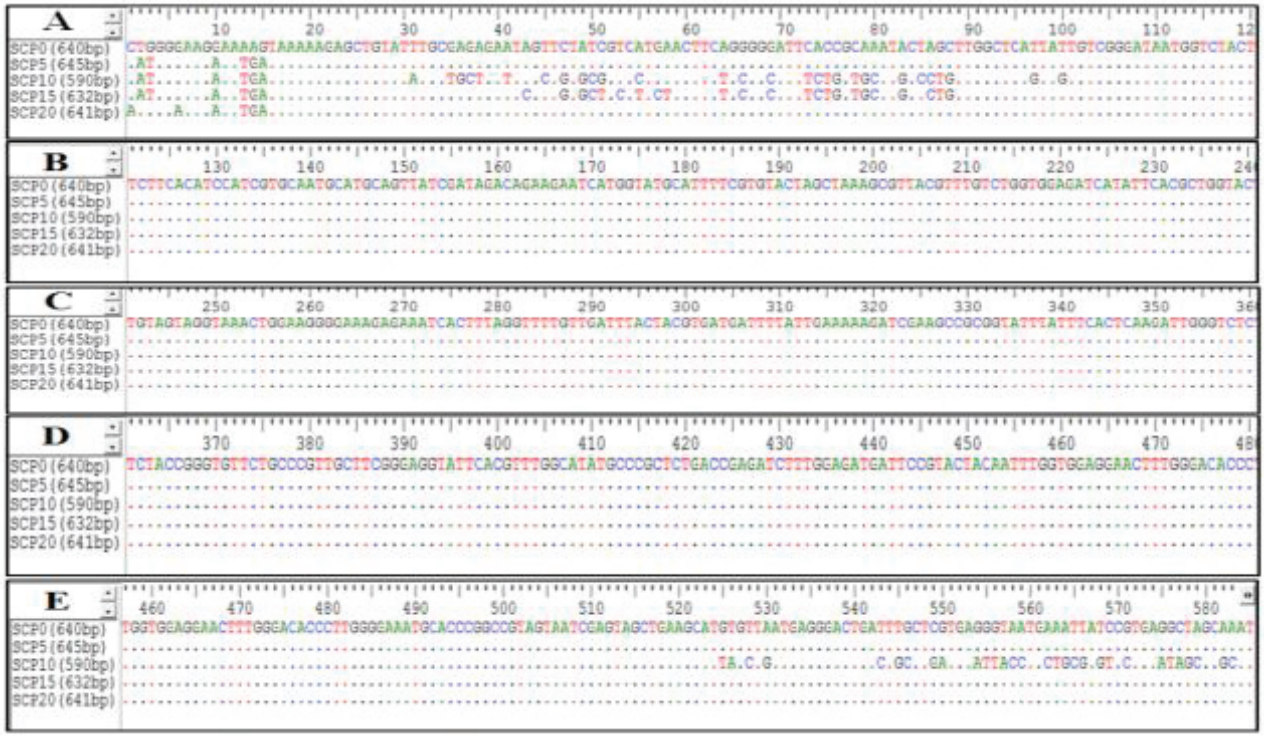

Figure 8. Features and similarities in alignment window of 5 Srichompoo cultivar sequences, present number of base 1-585 bp. DOT(.)=view conservation by plotting identities to a standard control (SCP0=no irradiation) as a dot. (SCP0 = Normal Srichompoo, SCP 5 =

Treated with 5Kr, SCP10 = Treated with 10Kr, SCP15 = Treated with15Kr, SCP 20 =Treated with 20Kr.)

Figure 9.The comparison of non-irradiated plant (left) and irradiated plant (right) of treated 10 Kr Srichompoo cultivar at 4 years old in soil culture.The height become a litle bit eqoaul , but bigger trunk, more compound leaves than the control and there were reproductive stage in branches and shape. No flowering yet, when grafting on the native mother plant in during of 8 months.

Table 1. The conclusion of single nucleotide analysis in Sweet Tamarind Prakaitong 5 treatments suing data in Figure 6 by BioEdit software in the present of treatments, number of points that were substitution, % mutation and induction of flowering.

Treatments	Number of points were substitution	% mutation	Flowering on mother plants when 4 years old, within 8 months
PKT 0 Kr	–	–	–
PKT 5 Kr	8	1.42	–
PKT 10 Kr	5	0.87	–
PKT 15 Kr	43	7.62	Flowering as shown in Figure11A
PKT 20 Kr	6	1.06	–

Table 2.The conclusion of single nucleotide analysis in Sweet Tamarind Seetong 5 treatments suing data in Figure 8 by BioEdit software in the present of treatments, number of points that were substitution, % mutation and induction of flowering.

Treatments	Number of point mutation	% mutation	Flowering on mother plants when 4 years old, within 8 months
ST 0 Kr	–	–	–
ST 5 Kr	3	0.48	–
ST 10 Kr	4	0.64	–
ST 15 Kr	5	0.80	–
ST 20 Kr	13	2.08	Flowering as shown in Figure11B

Table 3. The conclusion of single nucleotide analysis in Sweet Tamarind Srichompoo 5treatments suing data in Figure 8 by BioEdit software in the present of treatments, number of points that were substitution, % mutation and induction of flowering.

Treatments	Number of point mutation	% mutation	Flowering on mother plants when 4 years old, within 8 months
SCP 0 Kr	–	–	–
SCP 5 Kr	6	1.05	–
SCP 10 Kr	65	11.11	–
SCP 15 Kr	29	4.96	–
SCP 20 Kr	6	1.05	–

Figure 10 Grafting plant of sweet tamarind.
A. Mother plant of native tamarind, growing in the farm.
B. Explant of gamma ray treated sweet tamarind, sample in this research.

Figure 11.The induced flowering of explant by grafting techniques in sweet tamarind trees
A. Flowering of 15 Kr treated Prakaitong cultivar.
B. Flowering of 20 Kr treated Seetong cultivar.

All resulting indicated that *rbcL* gene in representative of primer F03 (CAT GGG AAG AAA TGA TAA AAA) and primer R04(GCA GCA GCT AAT TCA GGA CTC) could be act as molecular marker in tamarind species and concordance with the research of Sukrong,S. also Khunthapok,P.[15,16]. For the next generation of crop improvement in Thai sweet tamarind or any long live and slow growing trees, suggest that should be used in combination of acute gamma ray irradiation in dose of 15-20 Kr techniques with grafting techniques[Figure 10,11]. Simultaneously, marker technology should be conducted together.

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References

[1] Srisuvoramas, B, Sansenya,S., Aiumsamang,S., Piamalung,T. and Sripanom,L. DNA Barcodes for cultivar identification of Thailand *Tamarindus indica*.L. The International Conference on Science and Technology 2015 (TICST02015) .November 4–6,2015. Rajamanggala University of Technology Thunyaburi, Thailand. Proceeding. Full paper.[Online].Available: IEEE

[2] Srisuvoramas,B., Snamchaiskhul,C and Jantaramungkorn,N. Studies on the Fungal Infected

Stages and the Fungal Inhibitor Effect of Trichoderma sp., Fermented Medicinal Plants, Azadirachtin extract and Wood Vinegar on Prakaithong SweetTamarind *in Vitro*. Thailand Research Funding in 2009 through Phetchabun Rajabhat University.[Online].Available:ThaiLIS.

[3] Srisuvoramas,B. and Khoomsab, K. Identification and Classification of Some Fungi and the Fungal Inhibitor Effect of Fermented *Terminalia sp*. on *Tamarindus indica* L.Thailand Research Funding in 2014 through Phetchabun Rajabhat University.[Online].Available: ThaiLIS.

[4] Piamalang, T. et al. Soil Management for Sweet Tamarind Orchard of Community Economy Network, Farmers in Phetchabun Province.Thailand Research Funding in 2011 through Phetchabun Rajabhat University. Thailand.

[5] Piamalang, T. et al. Effects of water to increase productivity in Sweet tamarind variety Si Thong. Thailand: Phetchabun Rajabhat University, 2010.

[6] Piamalang, T. et al. Effect of storage products Sweet Tamarind Variety Si Chom Phu at room temperature to the destruction of the Beetles legs Grow. Thailand: Phetchabun Rajabhat University, 2010.

[7] Piamalang, T. et al. Pest Management of Sweet Tamarind for Self Sufficiency Economy of Community Enterprise of Banplum, Maung District, Phetchabun Province. Thailand: Phetchabun Rajabhat University, 2010. 2009.

[8] Piarmalang,T and Sanamchaisakul, J. et al. Optimizing The Management of The Garden Sweet Tamarind Geographical Indications of Farmer Networks Phetchabun Province. Thailand: Phetchabun Rajabhat University, 2013.

[9] Jeanmougin,F. Thompson,J.D. GonM,M. Higgins, D.G. and Gibson,T.J. Multiple Sequence Alignment with Clustalx.Trends in Biochemical Sciences, Southampton : International Centre for Underutilized Crops,1998.

[10] Doyle,J.J. and Doyle,J.LA. Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue," Photochemical Bulletin, vol. 19, 1987, pp. 11–15.

[11] Dieffenbach. C.W. and Dveksler,G.S.. PCR primer : Laboratory manual, 2nd ed. New York: Cold Spring Harbor, 2003.

[12] Kress, W.J., Erickson,D.L., Jones, F.A., Swenson, N.G., et al. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot inPanama.Proc.Natl.Acad.Sci. U. S. A.,Vol.106, 2009,pp.18621-18626.

[13] Kass,E. and Wink,M. Phylogenetic relationships in the Papilionoideae (family Leguminosae)based on nucleotide sequences of cp DNA (rbcL) and ncDNA(ITS1and 2) Molecular Phylogenetics and Evolution, vol. 8,1997, pp.65-88.

[14] D.L.Swofford, PAUP*:Phylogenetic Analysis Using Parsimony (* and Other Method).Beta Version4.0610. Sunderland, Massachusetts.Semina.Associats, 2003.

[15] Sukrong,S. gi|219687663|dbj|AB378727.1,*Tamarindus indica* chloroplast *rbcL* gene for ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit, complete cds, specimen voucher: PCU:TI-SP/P5," NCBI, 2007.

[16] Khunthapok,P. DNA Fingerprint and chemical assessment of selected Tamarind Cultivars with high laxative activity. Master of Science Program in Biotechnology, Faculty of Science, Chulalongkorn University, Vol.23, 2007, pp.403-405.