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Contributed Paper

Effects of Plasma Technique and Gamma Irradiation on Seed Germination and Seedling Growth of Chili Pepper

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ABSTRACT

The effects of plasma technique and gamma irradiation on seed germination, seedling growth and desirable traits of chili pepper for breeding program were investigated. In the plasma technique, the seeds were treated with the argon plasma. The argon plasma was driven by high voltage DC pulse at 25 kHz, plasma dissipated powers were 0.41 to 0.61 watt and argon gas flow rate of 3 standard liters per minute for 15 sec. The treated seeds including control were sown in the tray and kept in a green house in completely randomized design (CRD) with 3 replications. At 1 week after sowing, the highest of seed germination percentage was found on plasma dissipated powers 0.48 watt, significantly. Seedlings at 8 weeks were shown non-significantly in height, diameter of canopy and number of leaf. These results indicated that plasma treatment might promote the seed germination while, the growth of seedling was not different with control. In gamma irradiation, the seeds were treated with gamma ray in 150 to 600 gray. The results showed that seed germination percentage and survival percentage were different significantly. The growth of seedlings in height, diameter of canopy and number of leaf were differed with control. Plasma treatments and gamma irradiation were effects on seed germination and seedling growth of chili pepper. This data will be useful for chili pepper improvement further.

Keywords: plasma technique, gamma irradiation, chili pepper, seed germination, seedling growth

1. INTRODUCTION

Chili pepper belongs to the genus *Capsicum*, which is a member of the Solanaceae family. It is an important vegetable and condiment that is consumed for food and used for pharmaceutical purposes due to the specific secondary metabolites, capsaicinoids, which confer the pungency in fruits and have various medicinal effects [1]. It is widely planted in Southeast Asia, South Asia, Korean Peninsula and Latin America [2].

The focus in chili pepper breeding worldwide, its genetic basis is relatively narrow and significantly constrained the progress of chili pepper breeding. Induced mutagenesis is an important breeding tool to improve the desirable characters among the existing commercial varieties.

Plasma technique is a fast, economic and pollution-free method to improve seed performance and crop yield. It has essential roles in a broad spectrum of developmental and physiological processes in plants, including reducing the bacterial bearing rate of seed, changing seed coat structures, increasing the permeability of seed coats, and stimulating seed germination and seedling growth [3]. Plasma treatment could significantly increase crop yields such as wheat, grain and legume [4]. Regarding the use of plasma technology, recent published works indicate the positive effect of plasma treatment on seed germination and plant development [5-9]. Jiayun et al. [10] showed that cold plasma treatment promotes *Andrographis paniculata* germination and seedling emergence. Sera et al. [6] also found that wheat and oat seedling growth was enhanced by a cold plasma treatment. Moreover, Li et al. [3] report that a cold plasma treatment with an appropriate energy level promoted the soybean seedling growth, while much lower or higher energy levels did not show any promoting effects. The influence of the dielectric barrier discharge plasma jet has been found to be a very useful technique indicate the positive effect on seed germination and on plant development and growth in the field of agricultural science [3].

Gamma ray is one of the most important physical mutagens used for inducing beneficial as well as harmful cytogenetic effect in many crop plants [11]. There belong to ionizing radiation and interact to atoms or molecules to produce free radicals in cells. 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 depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds [1], [12-13].

There are several usages of nuclear techniques in agriculture. In plant improvement, the irradiation of seeds may cause genetic variability that enable plant breeders to select new genotypes with improved characteristics such as precocity, salinity tolerance, grain yield and quality [14]. Ionizing radiations are also used to sterilize some agricultural products in order to increase their conservation time or to reduce pathogen propagation when trading these products within the same country or from country to country [15]. However, reports in the effects of plasma technique and gamma irradiation on chili pepper are limited. The aim of the study was to investigate the effects of plasma technique and gamma irradiation on seed germination, seedling growth and desirable traits of chili pepper for breeding program.

2. MATERIAL AND METHODS

Chili pepper seeds cultivar 'Superhot' were chosen for investigation to the effect of plasma technique and gamma irradiation on seed germination, seedling growth and desirable traits of chili pepper.

In plasma technique, the seeds of chili pepper were treated with the argon plasma. The argon plasma was driven by high voltage DC pulse at 25 kHz., plasma dissipated powers were 0.41 to 0.61 watt and argon gas flow rate of 3 standard liters per minute for 15 second. The plasma treatments were in 0.41, 0.48, 0.55, and 0.61 watt.

In gamma irradiation, the seeds of chili pepper were treated with gamma ray in 150, 300, 450 and 600 gray using Gammacell 220 (Cobalt-60 source) at the Program in Applied Physic, Faculty of Science, Maejo University (Chiang Mai, Thailand).

The treated seeds including control were sown in the tray and kept in a green house at the Program in Horticulture, Faculty of Agricultural Production, Maejo University (Chiang Mai, Thailand). Water was applied manually to maintain the soil moisture.

The experiments were designed as completely randomized design (CRD) with three replications, fifty-two seeds per replication. Data on seed germination percentage was recorded at 1 week and 2 weeks after sowing by visually counting the number of germinated seeds for each treatment. Survival percentage was counted at 8 weeks after sowing. Germination percentage and survival percentage were calculated using the following formula Eq (1) and Eq (2), respectively. Also, the growth of seedlings in height, diameter of canopy and number of leaf were measured at 2 weeks to 8 weeks after sowing.

2.1 Germination Percentage

The germination percentage was caculated according to following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seed}}{\text{Total of seed}} \times 100 \qquad \text{Eq (1)}$$

2.2 Survival Percentage

The survival percentage was caculated according to following formula:

$$\text{Survival (\%)} = \frac{\text{Number of germinated seed at 8 weeks}}{\text{Total of seed}} \times 100 \qquad \text{Eq (2)}$$

2.3 The Growth of Seedlings

Seedlings were measured in height, diameter of canopy and number of leaf at 2 weeks to 8 weeks after sowing by record in the 30 randomly selected seedlings from each plasma treatment and control. The experiments were designed as completely randomized design (CRD) with three replications, ten seedlings per replication.

For gamma irradiation, only the survival seedlings in each gamma treatment were recorded in height, diameter of canopy and number of leaf at 8 weeks after sowing. The representative of control was showed in the one calculated from the average of 30 randomly selected seedlings.

- Height (cm.): measuring by Vernier Caliper for the length of stem to shoot of plant.
- Diameter of canopy (cm.): measuring by Vernier Caliper for the width of plant.
- Number of leaf (leaves): counting in the visual leaf of plant.

2.4 Statistical Analysis

Data was subjected to ANOVA test and "SAS version 9.0" statistical software was used for statistical analysis. Differences among the average were tested at P = 0.01 significance levels. Means in each treatment were compared by Duncan's Test.

3. Results and Discussion

3.1 Effects of Plasma Technique on Seed Germination and Seedling Growth of Chili Pepper

The effect of the dosage of plasma treatment on the seed germination percentage, the survival percentage and the growth of seedling was shown in Table 1 and Table 2. At 1 week after sowing, the plasma treatments in 0.41, 0.48, 0.55, and 0.61 watt were shown the seed germination percentage in 86.53%, 100%, 95.51% and 94.23%, respectively. These plasma treatments were higher than control (24.35%) in the seed germination percentage significantly. The highest of seed germination percentage was found on plasma dissipated powers 0.48 watt. Nevertheless, there was non-significantly at 2 weeks after sowing averaged from 97.43% to 100% (Table 1). This result indicated plasma technique had positive effects on seed germination that increasing the permeability of seed coats, and stimulating seed germination and seedling growth [3].

For the survival percentage of each plasma treatments and control, at 8 weeks after sowing, there were in the range of 98.71% to 100% with non-significantly (Table 1). In the growth of seedling at 8 weeks after sowing, there also were shown non-significantly, especially in height of seedling, diameter of canopy and number of leaf

Table 1. Plasma technique on the seed germination percentage and the survival percentage in chili pepper.

Treatment	Germination (%)		Survival (%)
	1 week	2 weeks	8 weeks
Control	24.35[b]	100.00[a]	100.00[a]
Plasma 0.41 W	86.53[a]	98.71[a]	98.71[a]
Plasma 0.48 W	100.00[a]	100.00[a]	100.00[a]
Plasma 0.55 W	95.51[a]	98.71[a]	99.36[a]
Plasma 0.61 W	94.23[a]	97.43[a]	98.71[a]
F-test	**	ns	ns
CV.(%)	13.77	1.73	1.50

** The mean difference is significant at the 0.01 level.
ns Non significant at the 0.01 level.

Table 2. Plasma technique on the growth of chili pepper seedling at 8 weeks.

Treatment	Height of seedling (cm.)	Diameter of canopy (cm.)	Number of leaf (leaves)
	8 weeks		
Control	16.70[ab]	14.16[ab]	6.33[a]
Plasma 0.41 W	16.84[ab]	15.27[ab]	6.36[a]
Plasma 0.48 W	17.22[ab]	14.51[ab]	6.53[a]
Plasma 0.55 W	19.33[a]	16.08[a]	7.00[a]
Plasma 0.61 W	15.08[b]	12.75[b]	7.13[a]
F-test	ns	ns	ns
CV.(%)	11.73	9.12	11.53

** The mean difference is significant at the 0.01 level.
ns Non significant at the 0.01 level.

that were in average from 15.08 cm. to 19.33 cm., 12.75 cm. to 16.08 cm. and 6.33 leaves to 7.13 leaves, respectively (Table 2). These results indicated that plasma treatment might be promoted the seed germination while, the growth of seedling was not different with control.

The overall results in the growth of chili pepper seedling treated with plasma treatments during the 8 weeks after sowing were shown in Figure 1, Figure 2 and Figure 3. As compared to control, the plasma treatments have enhanced the seedling growth in height of seedling (Figure 1), diameter of canopy (Figure 2) and number of leaf (Figure 3). It can be suggested that the plasma treatments are promoted the seedling growth. Several researches have demonstrated that the plasma treatment could promote seedling growth of plants. Zhou et al. [16] observed that the tomato seedling growth was improved by an atmospheric pressure plasma treatment. The plasma treatment induces changes on the seed surface and allows radicals to penetrate into the seed and affect the metabolic process of plant growth as shown by Sera et al. [6] for wheat and oat treatment. Moreover, the quality of water may play a major role on improving the plants

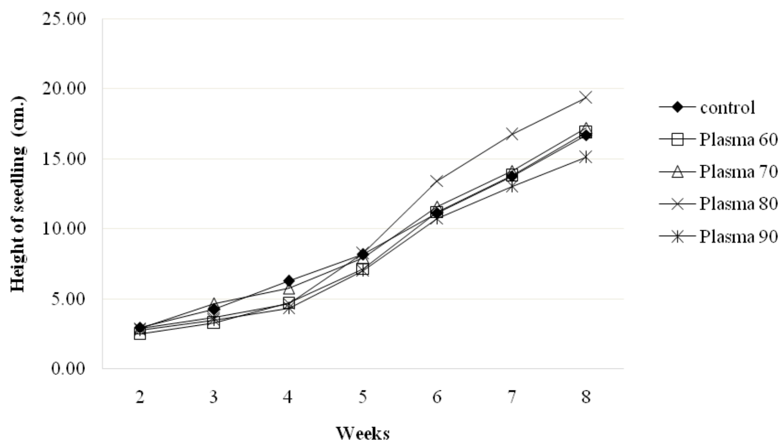

Figure 1 Plasma technique on the growth of chili pepper seedling in the height at 2 weeks to 8 weeks.

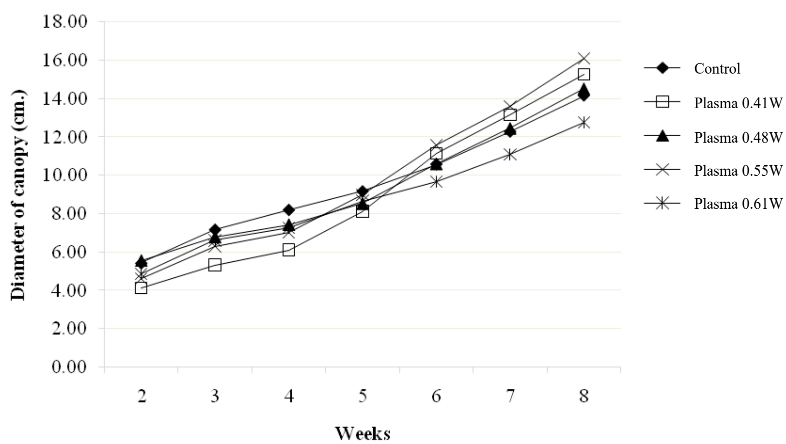

Figure 2. Plasma technique on the growth of chili pepper seedling in the diameter of canopy at 2 weeks to 8 weeks.

development by providing chemical species [8, 17]. The plasma-induced actions on the seed coat could lead to the penetration of active species such as reactive ions and UV into seeds, which probably affect the physiological reactions, seed germination and growth [18-22].

3.2 Effects of Gamma Irradiation on Seed Germination and Seedling Growth of Chili Pepper

The effect of the dosage of gamma irradiation on the seed germination percentage, the survival percentage and the growth of seedling were shown in Table 3 and Table 4. The germination percentage with gamma irradiation at 1 week after sowing, it could not determine because of no seed germination. At 2 weeks after sowing, the seed germination percentage in the gamma treatments was decreased and differed significantly with the control. The average was from 55.13% to 100% (Table 3). There might be the main cause of germination reduction in plants has been attributed to the occurrence of seeds without completely developed embryos that may be credited

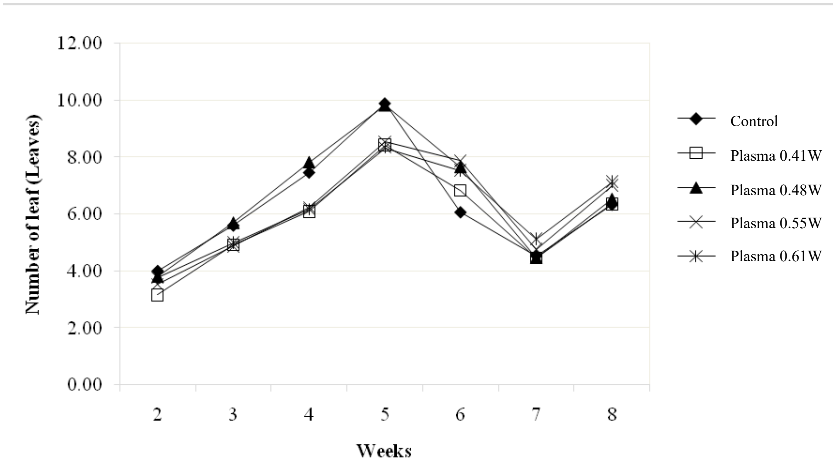

Figure 3. Plasma technique on the growth of chili pepper seedling in the number of leaf at 2 weeks to 8 weeks.

Table 3. Gamma irradiation on the seed germination percentage and the survival percentage in chili pepper.

Treatment	Germination (%)		Survival (%)
	1 week	2 weeks	8 weeks
Control	-	100.00[a]	100.00[a]
150 gray	-	55.13[b]	11.53[b]
300 gray	-	71.15[b]	1.28[c]
450 gray	-	55.77[b]	0.00[c]
600 gray	-	73.08[b]	0.00[c]
F-test	-	**	**
CV.(%)	-	13.61	17.18

** The mean difference is significant at the 0.01 level.

ns Non significant at the 0.01 level.

Table 4. Gamma irradiation on the growth of chili pepper seedling at 8 weeks.

Treatment	Height of seedling (cm.)			Diameter of canopy (cm.)			Number of leaf (leaves)		
	1-8	9-16	17-24	1-9	10-18	19-27	1-6	7-12	13-18
Control	-	1	-	-	1	-	-	1	-
150 gray	5	9	4	3	11	4	2	9	7
300 gray	0	1	1	0	1	1	0	1	1
450 gray	0	0	0	0	0	0	0	0	0
600 gray	0	0	0	0	0	0	0	0	0

to activation of RNA or protein synthesis during the early stage of germination [23-24].

Survival percentage in gamma irradiation at 8 weeks after sowing was decreased significantly comparing with the control (100%). Dose of gamma ray in 150 and 300 gray were showed the survival percentage in 11.53% and 1.28%, respectively, while the gamma ray in 450 and 600 gray could not survive in the time (Table 3). The results of this research showed in the poor growth and development of chili pepper seedlings. At a certain level of radiation, the plant can grow at early stage of growth but they cannot survive at certain duration probably due to DNA breakage and inability to repair them [25]. A similar of survival percentage was reported by Norfadzrin and et al. [26] in which increasing gamma ray dose decreased survival percentage of tomato.

At 8 weeks after sowing, the seedlings treated with gamma irradiation were found in 20 survival seedlings with the different growth of height, diameter of canopy, and number of leaf. The separated levels for each growth were due to the control as the representative of the average one. The height of seedling was separated to 3 levels at low (1-8 cm.), medium (9-16 cm.) and high (17-24 cm.). The result showed 18 seedlings of 150 gray of irradiation were 5 seedlings at low (1-8 cm.), 9 seedlings at medium (9-16 cm.) and 4 seedlings at high (17-24 cm.). At 300 gray of irradiation had 2 seedlings at medium (9-16 cm.) and high (17-24 cm.) (Table 4 and Figure 4). Gamma irradiation have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose [27]. The result of Mendoza and et al. [28] have shown that plant height at 30 days after transplanting significant difference between the control and gamma irradiation in chili pepper (*Capsicum annuum* L.). For the diameter of canopy, it also was separated to 3 levels at small (1-9 cm.), medium (10-18 cm.) and large (19-27 cm.). The present study showed that 18 seedlings were 3 seedlings at small (1-9 cm.), 11 seedlings at medium (10-18 cm.) and 4 seedlings at large (19-27 cm.) for 150 gray and there were 2 seedlings at medium (10-18 cm.) and large (19-27 cm.) for 300 gray (Table 4 and Figure 5). The number of leaf also was separated to 3 levels at less (1-6 leaves), medium (7-12 leaves) and more (13-18 leaves). The result was 18 seedlings for 150 gray which in 2 seedlings at less (1-6 leaves), 9 seedlings at medium (7-12 leaves) and 7 seedlings at more (13-18 leaves), while in 1 seedling at medium (7-12 leaves) and 1 seedling at more (13-18 leaves) for 300 gray (Table 4 and Figure 6).

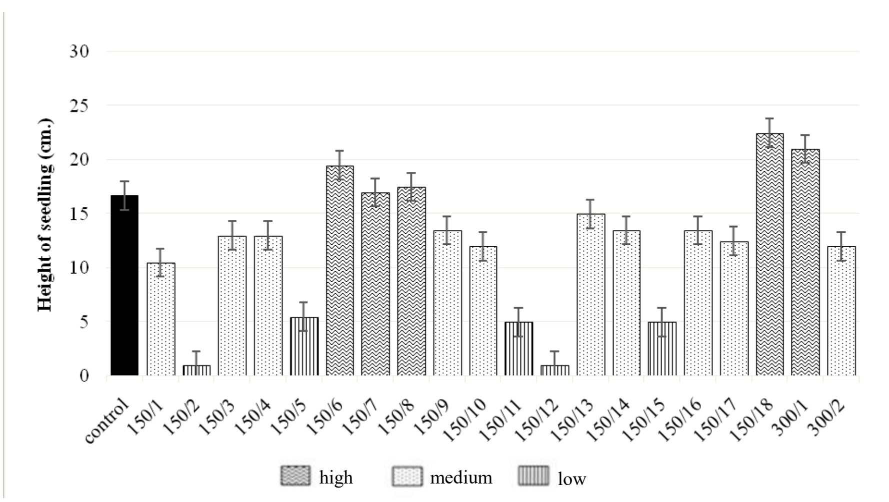

Figure 4. Gamma irradiation on the growth of chili pepper seedling in the height at 8 weeks.

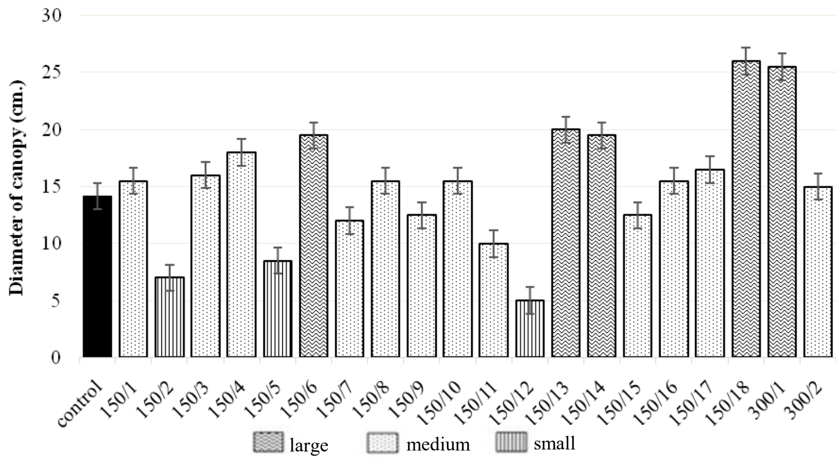

Figure 5. Gamma irradiation on the growth of chili pepper seedling in the diameter of canopy at 8 weeks.

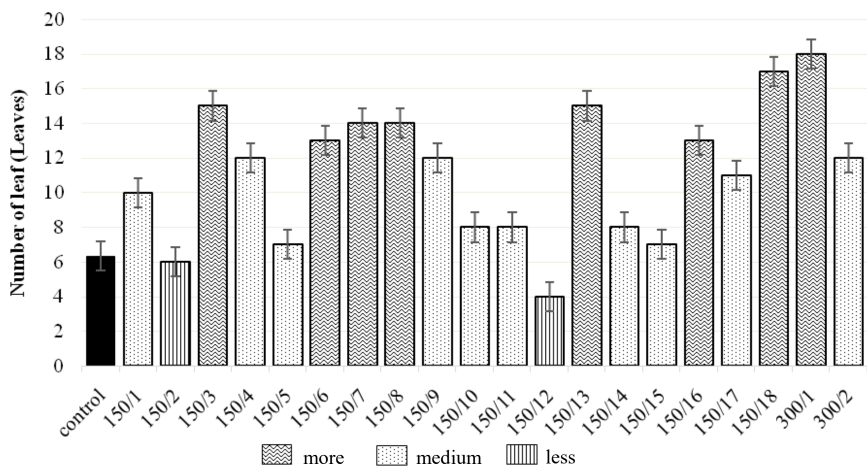

Figure 6. Gamma irradiation on the growth of chili pepper seedling in the number of leaf at 8 weeks.

In the results of gamma irradiation, dose of gamma irradiation was caused to decreasing of seed germination and survival percentage compared with the control. It was led to the new characteristic of chili pepper [27]. Gamma rays are common used in plant breeding program because there are known for their simple applications, good penetrations, reproducibility, high mutation frequency and less disposal problems. Besides breeding, they are also used as an alternative for improvement of desired characters in agricultural crops [23].

4. Conclusions

Plasma treatments and gamma irradiation were effects on seed germination and seedling growth of chili pepper. Plasma powers 0.48 watt could promote 100 percentage of seed germination at 1 week after sowing. Gamma irradiation dose caused to decreasing of seed germination and survival percentage compared with the control and induced to the new characteristic of chili pepper. All data will be useful for chili pepper improvement and breeding program further.

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