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# Effect of UV-C Radiation on Seed Germination and Some Ultrastructure in Water Convolvulus (*Ipomoea aquatica* Forssk. 'Reptan')

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**ABSTRACT**

The objective of this study was to investigate the effects of UV-C radiation on seed germination percentage, germination rate, root length, root thickness and some ultrastructure of water convolvulus (*Ipomoea aquatica* Forssk. 'Reptan'). The water convolvulus seeds were exposed to UV-C (254 nm) for period of 2 (1.4 $J.cm^{-2}$), 4 (2.9 $J.cm^{-2}$), 8 (5.8 $J.cm^{-2}$) and 12 (8.7 $J.cm^{-2}$) hours. The number of germinated seeds was observed every 12 hours for 168 hours. After that, the length and the thickness of the root were measured and studied by semi-thin and ultrathin technique under a light microscope (LM) and a transmission electron microscope (TEM) respectively. The result showed that the UV-C treatment increased germination rate, root length and root thickness. The treatment of 2 hours irradiation had the highest seeds germination percentage (98.89 ± 1.92 %), germination rate (2.49 ± 0.40 seed/hr) and root length (33.60 ± 1.68 mm) when compared with non-irradiated and other UV-C treatments. In addition, the ultrastructure in the root of the 2 hours UV-C treatments under LM and TEM showed an increase in vacuoles in zone of cell division compared to those of the control roots.

## INTRODUCTION

Water convolvulus (*Ipomoea aquatica* Forssk. 'Reptan') is a member of Convolvulaceae family. It is a commonly grown vegetable in Africa, tropical Asia, Malaysia and Australia [1]. Water convolvulus is also widely to cultivated and consumed in Thailand, it can grow in every season of the year and ready to harvest 20-25 days after sowing [1,2]. In addition, water convolvulus also is high nutritional value because it contains vitamin A, vitamin C, calcium and phosphorus [3]. Moreover, in 2020, Thailand exported water convolvulus seeds about 2,604.41 tons, valued at 207.78 million baht [3]. During seed storage, the seeds will produce free radical, resulting in seed deterioration. Furthermore, long-term seed storage can lead to a reduced germination [5]. From previous study, there have been studies on the growth of water convolvulus seed by various methods. Porramain *et al.* (2019) reported that seeds were exposed to cold plasma irradiation had a higher germination than non-exposed [5]. In addition, according to a study reported by Nopphol (2006), increasing water temperature tended to increase hypocotyl length and fresh weight of seedling [6].

The depletion of the ozone layer is causing more ultraviolet radiation (UV) to reach the Earth's atmosphere, and those UV can affect plant. UV radiation have three types, UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). UV-A radiation is the least harmful and energy among UV radiation. UV-B can induce changes morphological, physiological processes and chemical composition in plants [7-9]. Viviana *et al.* (2014) reported that UV-B increased height and weight of melon plants which were grown from irradiated seeds. In contrast, irradiation with UV-B decreased the amount of phosphorus (P), calcium (Ca) and sodium (Na) in leaves when compared with non-irradiated treatments [10]. UV-C radiation has the highest energy and the shortest wave length among UV radiation. UV-C can have positive and negative effect on plant [7-9]. UV-C radiation increased germination rate, radicle length and plumule length of the maize [8]. Moreover, UV-C also increased the length of root and shoot, the number of branches and leaves in groundnut seedling [11]. In contrast, UV-C reduced seed germination in Andean lupin [12]. Wheat seeds treated with UV-C radiation showed decrease the growth rate and biomass weight of shoot and root and also reduced chlorophyll a, b and total chlorophyll contents in wheat seedling [13]. Thus, this study aimed to investigate the effect of UV-C radiation on seed germination and some ultrastructures of water convolvulus seedling.

## METHODOLOGY

### Seed Germination study

Water Convolvulus seeds were sterilized with 1% sodium hydroxide for 10 min. After that they were rinsed three times with distilled water. Then, seeds were placed in sterilized petri dishes with 5 ml distilled water before exposed to UV-C [8]. The UV-C wavelength was 254 nm and intensity of irradiation was 0.5 $mJ.cm^{-2}s$. The UV treated seeds were exposed to UV-C for different period at 2 hrs. (1.4 $J.cm^{-2}$), 4 hrs. (2.9 $J.cm^{-2}$), 8 hrs. (5.8 $J.cm^{-2}$) and 12 hrs. (8.7 $J.cm^{-2}$). Each treatment was done in 3 replicates (30 seeds per replicate). After exposure to UV-C, the seeds were planted on filter paper moistened with tap water in 9 cm sterilized petri dishes (between paper (BP) method) and kept at room temperature in darkness [8]. Seeds were watered every 12 hrs. to maintain moisture. Seeds were considered germinated when the radicle tip had extended 1 mm out of seed coat.

The number of germinated seeds was observed at every 12 hours for 168 hours. The equation to calculate germination rate is:

$$GR = \frac{X_1}{Y_1} + \frac{X_2}{Y_2} + \frac{X_3}{Y_3} + \cdots \frac{X_n}{Y_n}$$

$X_1$, $X_2$, $X_3$ and $X_n$ are the number of seed germinated on first, second, third and $n^{th}$ hour, respectively, and

$Y_1$, $Y_2$, $Y_3$ and $Y_n$ are the number of hours after seed were planted to first, second and $n^{th}$ count, respectively [14].

The length and thickness of root were measured after 168 hours. The data were analysed by SPSS version 21. Ultrastructure of root was studied by LM and TEM.

**Ultrastructure study**

The roots that grow from seeds which were UV-C irradiated for 2 hours were selected to study the ultrastructure because they had more positive result than other irradiated treatments such as germination percentage, germination rate and root length.

Root samples were pre-fixed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer pH 7.2 at 4°C for 12 hrs. Then, they were washed three times in 0.1M sodium phosphate buffer pH 7.2, and subsequently post-fixed in 2% osmium tetroxide in distilled water for 2 hrs. at room temperature. After that, the samples were washed thrice with distilled water, and dehydrated with acetone (30%, 50%, 70%, 90% and 100%) for about 15 mins for each. The samples were infiltrated with Spurr's and acetone using ratios 2:1, 1:1 and 1:2 respectively and then the samples were transferred to pure Spurr's resin (3 hrs. for each). Then, they were incubated at 80°C in oven for 7 hrs. and then the sample were cut into semi-thin section (1,000 nm) and Ultra-thin section (70 nm) by ultramicrotome (Leica, ultracut UC7). Semi-thin sections were stained with 1% toluidine blue O in 2.5% sodium carbonate and ultrathin section were stained with 5% uranyl acetate and lead citrate (Reynolds) [15]. Semi-thin section and ultra-thin section were examined under LM (Carl Zeiss, Axiostar Plus) and TEM (Hitachi, HT7700) respectively.

## RESULTS AND DISCUSSION

**Seed germination**

All of UV-C irradiation treatments showed no significantly differences on germination percentage when compared with control, it may be due to the seeds have a high germination percentage even though they were not expose to UV-C. However, germination percentage of seed was the highest in the 2 hours UV-C radiation treatment than the other treatments due probably to low dose of UV radiation stimulated the process of germination which activated by many enzyme activities or translocation of storage food in the endosperm (Table 1) [16].

Moreover, from Figure 1 show the cumulative seed germination every 12 hours, the treatment of 2 hours exposure to UV-C showed the highest cumulative seed germination in comparison with other treatments (Figure 1).

In the same way, the result showed that germination rate of seed of all treatments with UV-C was higher when compared with control. The 2 hours and 8 hours of UV-C exposure treatments led to the highest germination rate of water convolvulus seeds (2.49 seed/hr), which was 0.74 higher than control (Table 1). These results were in agreement with Ramesh *et al.* (2015) and Pouria *et al.* (2019), seeds of groundnut treated with UV-C showed an increase in germination percentage and UV-C radiation increased germination rate in maize and sugar beet seeds [8,11 ].

The higher of germination percentage and germination rate of the water convolvulus seeds irradiated with UV-C due to UV-C radiation induced cracking the seed coat and increased temperature resulted in higher and faster oxygen and water can to pass through in seeds which were important factors required for seed germination. Furthermore, Samuel *et al.* (1984) reported that increased temperature increased respiration rate, mitochondrial activities, germination rate and radical elongation in grain sorghum seeds [17].

**Root length and root thickness**

In comparison with control, both root length and root thickness of water convolvulus seeds showed the results in same direction, all of UV-C irradiated treatments had higher root length and root thickness than the control (Table 1). UV-C radiation may be induced gibberellin and ethylene production [18,19]. As previous reported, gibberellin stimulated root growth and seed germination [20-23]. Moreover, ethylene also increased root diameter and inhibits root elongation [24,25]. Although, ethylene inhibits root elongation, it had gibberellins that helped to induce root elongation.

**Ultrastructure study**

The ultrastructure in the root of the 2 hours UV-C treatment showed an increase accumulation both of vacuoles and starch grains compared to those in control roots (Figure 2-3). In contrast, in red pepper leaf found that chloroplast of UV exposed cells have starch grains less than non UV exposed cells [26]. The higher accumulation of starch grain was due to UV radiation inhibits synthesis of amylolytic enzymes which are group of enzymes for starch degradation [26]. Starch is the major storage of carbohydrate which composed of sugar and derived metabolites to support plant growth [27,28]. The immobilization of starch by UV-C radiation resulted that starch cannot provide carbon and energy for plant growth.

UV-C radiation as an abiotic stress, can stimulate seeds to produce vacuoles. The vacuoles are crucial role for plant growth and development, and have multifunction such as maintaining acidity in cell, responding to injury and regulating the transport for substances. Moreover, they are also the storage organelles for proteins, soluble

**Table 1.** Germination percentage, germination rate, root length and root thickness of water convolvulus seeds which exposed and non-exposed to UV-C radiation. (Tukey's HSD, $p < 0.05$).

| Treatment | Germination percentage | Germination rate (seed/hr) | Root length (mm) | Root thickness (mm) |
|---|---|---|---|---|
| 0 hrs. (control) | $94.44 \pm 5.09^a$ | $1.75 \pm 0.09^a$ | $24.83 \pm 14.68^a$ | $0.71 \pm 0.03^a$ |
| 2 hrs. | $98.89 \pm 1.92^a$ | $2.49 \pm 0.40^a$ | $33.65 \pm 16.80^b$ | $0.72 \pm 0.04^{ab}$ |
| 4 hrs. | $90.00 \pm 0.00^a$ | $2.33 \pm 0.17^a$ | $29.86 \pm 17.06^{ab}$ | $0.75 \pm 0.04^b$ |
| 8 hrs. | $94.44 \pm 1.92^a$ | $2.49 \pm 0.6^a$ | $30.32 \pm 17.10^{ab}$ | $0.80 \pm 0.04^c$ |
| 12 hrs. | $91.11 \pm 5.09^a$ | $2.35 \pm 0.47^a$ | $32.17 \pm 18.52^b$ | $0.82 \pm 0.02^c$ |

*Different letters within a column show a statistically significant difference.

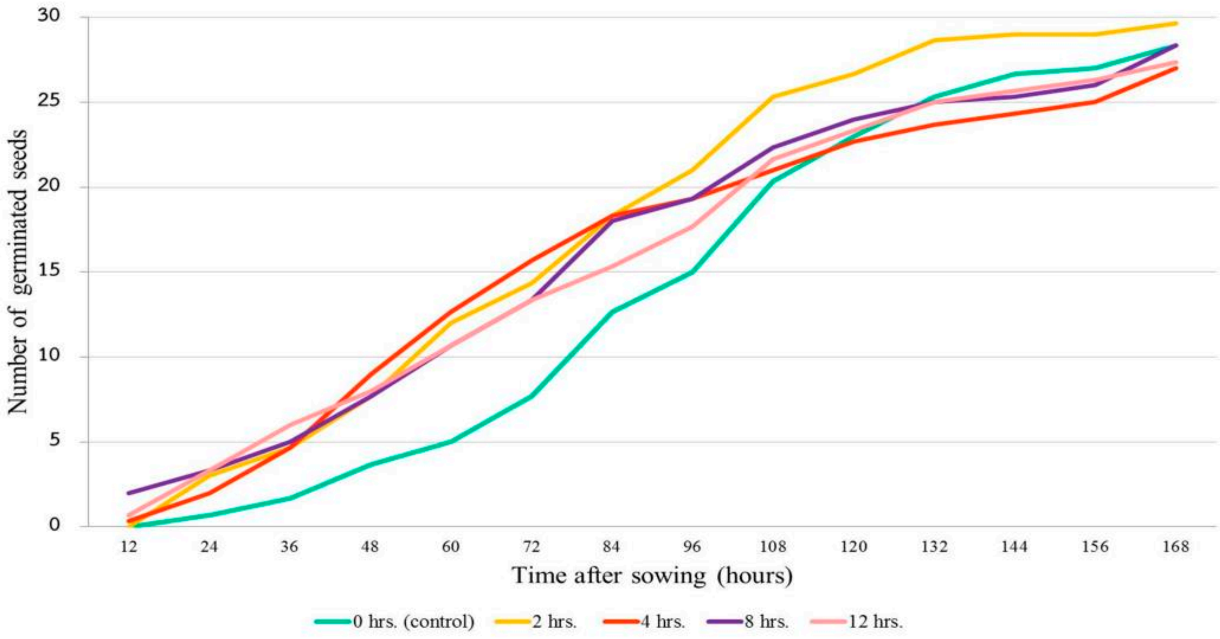


**Figure 1.** Cumulative seed germination of water convolvulus affected by the UV-C radiation treatments.

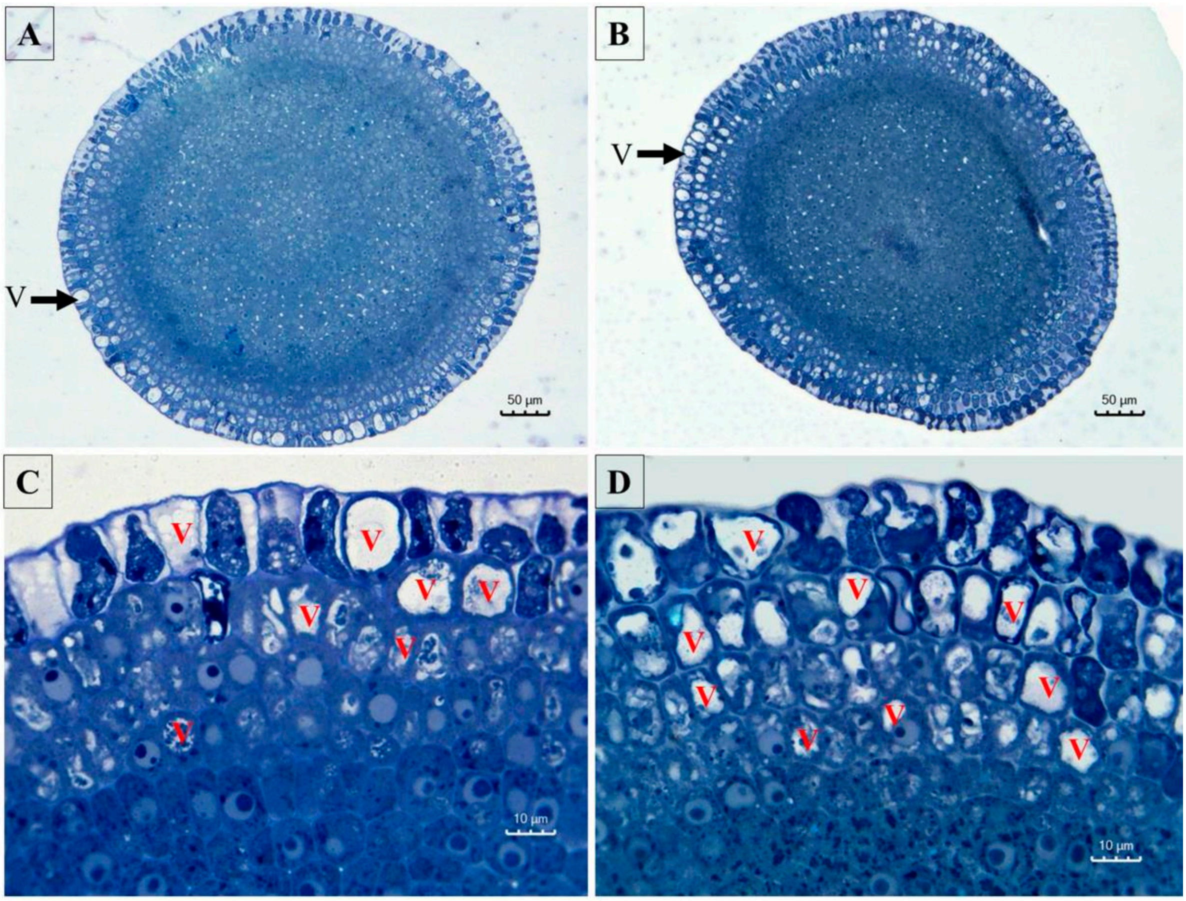


**Figure 2.** Transverse light micrograph of water convolvulus root A and C: non-irradiated, B and D: irradiated with UV-C for 2 hrs. Images were analyzed by using 20x and 100x magnification for A-B and C-D respectively. (V = vacuole).

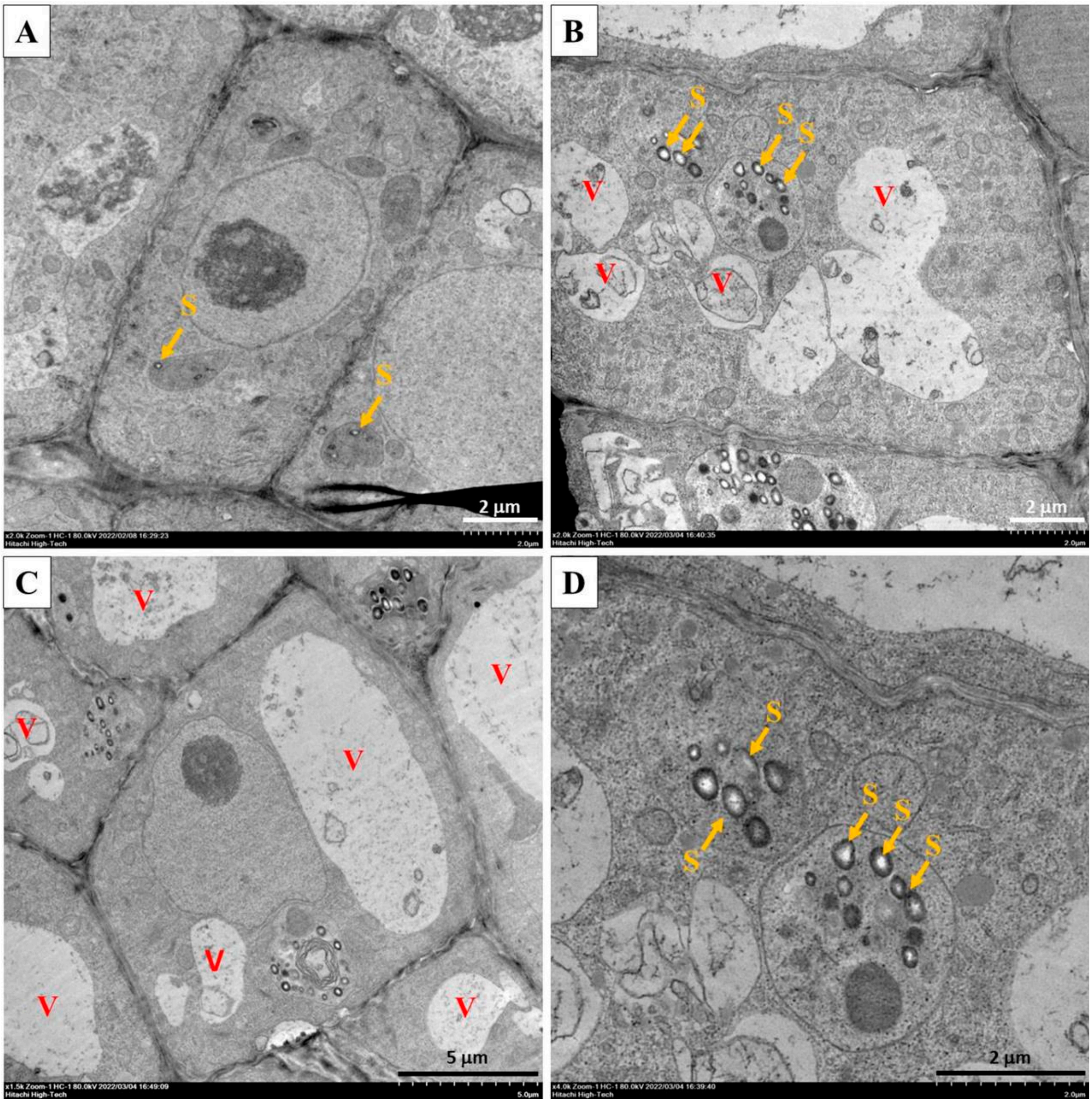


**Figure 3.** Transverse electron micrograph of water convolvulus root A: non-irradiated (magnification 2000x). B-D: Showing increased vacuoles and starch grains in irradiated with UV-C for 2 hrs. (magnification 2000x, 1500x and 4000x respectively). (S = starch, V = vacuole).

carbohydrates, water and sugars that are important requirement for plant growth [29,30].

Although the increasing of starch grains may reduce seed germination, there were other positive effects from irradiated with UV-C such as increasing of number of vacuoles and hormones to promote seed germination or plant growth.

## CONCLUSION

The treatment of 2 hours irradiation has the highest seeds germination percentage (98.89 ± 1.92 %), germination rate (2.49 ± 0.40 seed/hr) and root length (3.36 ± 1.68 mm) when compared with non-irradiated and other UV-C treatments. Moreover, the ultrastructure in the root of the 2 hours UV-C treatments under LM and TEM showed an increase in vacuoles and starch grains comparing those in control roots.

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