In Vitro Resistant - Induction Of Tamarillo (Solanum Betaceum Cav.) Applied By UV-B Radiation Against Anthracnose Disease By Determination Of Peroxidase And Polyphenoloxidase Activity

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ABSTRACT: The aim of the research was to acquire resistance callus of Tamarillo (Solanum betaceum Cav.) which was applied by UV-B radiation against the antrachnose disease. The study was designed by Factorial randomized design, consisting of two factors: UV-B light power; 10, 20 and 30 watts, and exposure time 30, 60 and 90 seconds. The Colletorichum filtrate added into medium (25-125 ppm) as resistant selection. Callus gained by selection were analyzed by peroxidase and polyphenoloxidase activity changing. Analysis of both enzymes activity showed decreased of protein levels at all samples. But after selection of Colletorichum filtrate, both enzymes showed increasing of theirs protein levels. The samples after selection showed more tolerant against filtrate compared to control, the highest value obtained in the treatment of UV-B radiation of 20 watts and exposure time of 30 seconds for peroxidase and 20 watt and 60 seconds for polyphenoloxidase. The lowest protein content for both enzyme found on control treatments.

Key word: UV-B Rays, Tamarillo, Solanum betaceum Cav, Peroxidase, Polyphenoloxidase, antrachnose.

1. INTRODUCTION:

It is well known that increasing chloroflourocarbon (CFC), cholorocarbons (CCs) and others pollutants led to depleting to stratospheric ozone layer. After a long time, it causes ultraviolet radiation-B (UV-B; 280-320 nm) on the earth surface. In some parts of the earth, UV radiation increase clearly due to the missing function of the ozone layer (Martinez-Ghersa, 2004). Ultraviolet is adverse problems of environment including cause plants to stress. The disturbances of UV radiation will lead new problems into the plant later on (Martinez-Ghersa et al., 2004). Consequently, there is an increasing of DNA damage and alter the genome integrity that may led to mutation on all organisms as well as plant (Mazza et al., 2000). All types of UV radiations are known to damage various plant processes. Such damage can be classified into two categories: DNA damage which can cause heritable mutations and damage to physiological processes (Stapleton, 1992).

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Ultraviolet present in sunlight thus expose to plant through photosynthesis. In the past this ultraviolet rays emitted out by the ozone layer (Snustad et al., 1984). With the onset of the ozone destruction, then more and more ultraviolet radiated to earth. Unlike X-rays, ultraviolet radiation does not have a strong energy to induce ionization but it can be absorbed by certain substances such as purine bases (guanine and cytosine) and the pyrimidine (adenine and thymine). Because ultraviolet energy is low, the UV can only penetrate the surface of cells of multicelluler organism (Mc. Kenzie et al, 2003). Certain UV-absorbing pigments are produced by some organisms as defense mechanisms but unable to avoid UV-radiation completely from reaching its DNA (Rastogi et al., 2010) Genes exposed to mutagens in the right conditions will mutate and change the phenotype, for example by changes in color, shape, behavior, or physiological changes (Snustad et al., 1997). Changes in DNA caused by radiation can cause genetic changes in somatic or others, depending on the cell type in question (Ulm and Nagy, 2005). In general, genetic mutagens can increase frequency of mutations but if the organisms can overcome the lethal effect of the UV radiation in some point, it will lead increasing the number of mutant-organisms. Mutagenesis UV radiation will pave the way for the discovery DNA repair (Muller-Xing et al., 2014). Various studies on UV effect on plants has been studied both in the field and glass house especially its influence on plant productivity (Kakani et al, 2003). In the soybean (Glycine max L.), UV-radiation gave significant variation of its pollen germination, ranges from 92% to 72%, but the UV- radiation did not give effect to the anther length (Koti et al., 2004). The effect of UV on the plant highly depends on the levels of phenols compounds from the leaves. The higher phenol compound of plant, the higher possibility of plant can overcome the effect of UV radiation (Mazza et al., 2000). In higher plants, the compounds of flavonoids and its derivate such as phenylpropanoid, accumulated in epidermal cells of leaves and is very effective to reduce the

influence of UV. However, increasing intensity of UV will also increase phenylpropanoid compounds (Landry et al., 1995 and Reuber et al, 1996 in Mazza et al, 2000). On the other hand, peroxidase enzyme activity increased in line with the high level of tolerance of Spirodela punctata plants and thus decreasing its auxin level (Jansen et al, 2001) Tamarillo has an economic value mainly because it can be used as a syrop, dried fruit, pickles, and others but production of tamarillo especially in Indonesia is still low due to many constraints. Tamarillo has environmental limits to growth and to develop, like sun exposure and disease, especially of antrachnose disease caused by the fungus Colletotrichum. Base on the survey, the symptoms and damage caused by the Coletotrichum sp can only visible from the stems, leaves and fruit organs. The time of Colletotrichum effects into the organs is not at the similar times, thus it not easy to see the resistance level between plants and between the different plant organs. Therefore, the variation of attack is very high to occur. The Information on the effects of UBV-B radiation to the tamarillo and its impact to the anthracnose is still very limited. So, this study was conducted to obtain in vitro of tamarillo resistance through UV-B mutation induction especially through its enzyme activity, peroxidase and polyphenoloxidase.

2. MATERIALS AND METHODS

2.1 Sampling

Physiologically ripe fruits of Tamarillo were collected from the healthy trees at Berastagi, Karo district of North Sumatra. In the laboratory, the seeds sorted, cleaned by washing under running water and soaked them for 15 minutes in a solution of detergent and then rinsed with distilled water (Mathius and Nurhaimi, 1995). The seeds were growth in the moisture chambers for 3 days.

2.2 Ultraviolet-B Radiation Applications

UV induction performed by using UV lamp (10, 20 and 30 watts, and exposure time 30, 60 and 90 seconds) on the 3-day-old sprouts. The distance of the UV lamp to the sample is 30 cm. UV lamps and samples placed in a dark box. Once the irradiation is completed, UV will be turned off and the samples was left inside the dark box for 10 minutes to prevent photoreactivation (Mazza et al, 2000 and Ueda et al., 2005)

2.3 Tissue culture of Tamarillo

The medium used for callus initiation is the basic MS (Murashige and Skoog, 1962) medium with the addition of 1.0 mg/L 2,4-D and 1.0 mg/L BAP. Planting explants performed in a laminar air flow that has been cleaned with 70% alcohol and was turned on for 15 minutes. Bottles containing explants culture placed on shelves at temperatures ranging from 25-27^{0}C, with 1000 lux light intensity and illumination for 16 hours of light and 8 hours of dark. Culture room sprayed with 70% alcohol every day to prevent contamination.

2.4 Callus Induction and Selection

To obtain optimal growth of callus induction a modified - MS medium was used for the treatment (Murashige and Skoog, 1962). Then, a total of 1.0 mg/L 2,4-dichlorophenoxyacetic acid and 0.5 mg/L BAP were added to the medium as a growth regulator substances medium for callus selection, and coupled with mushroom filtrate based on the treatment. Colletotrichum filtrates which added gradually by increasing the concentration of each sub-cultures, started from 25, 50, 75, 100 to 125 ppm concentration (modification of Widiyanto, 1992).

2.5 Callus Extraction

Callus extraction was done according to the method used by Widiyanto (1992). Callus of each sample was taken as 400 mg fresh weight. Callus was grinded in mortal by adding liquid nitrogen and homogenized with 2 ml of buffer Tris-HCl 0.05 M (pH 8, temperature 00C). The homogenate was centrifuged at 14,000 rpm for 20 minutes at a temperature of 0^{0}C. Then, the supernatant used for subsequent analysis

2.6 Determination of protein content

The protein content in the extract was determined using the dye reagent from Bio-Rad (Richmond California), based on the method of Bradford (1976); Sedmak and Groosberg (1977). Dye reagents contain Coomassie Blue G-250. Absorbance of the solution formed was measured with a spectrophotometer at a wavelength of 595 nm. The protein content is determined based on the equation of the line is made of protein standard "Bovine Serum Albumin (BSA)".

2.7 Determination of Enzyme Activity

Peroxidase activity (PO) and Polyphenoloxidase (PPO) were determined by Kar and Mishra (1976) method. The oxidation process of PO in catalyzing the reactions using H2O2 (Kar and Mishra, 1976: Maehly and Chance, 1964), whereas the oxidation of the PPO is not using H2O2. One unit of enzyme activity is proportional to the change in absorbance at a wavelength of 420 nm per minute per mg protein (units/mg protein).

3. RESULTS AND DISCUSSION

3.1 Morphogenesis of tamarillo callus applied by UV-B radiation.

One type of explant was the formation of callus proliferation. Coleoptile callus growing from the base or from the cut explants. Callus types may vary for different explants, but can also have the same type though its different explants sources

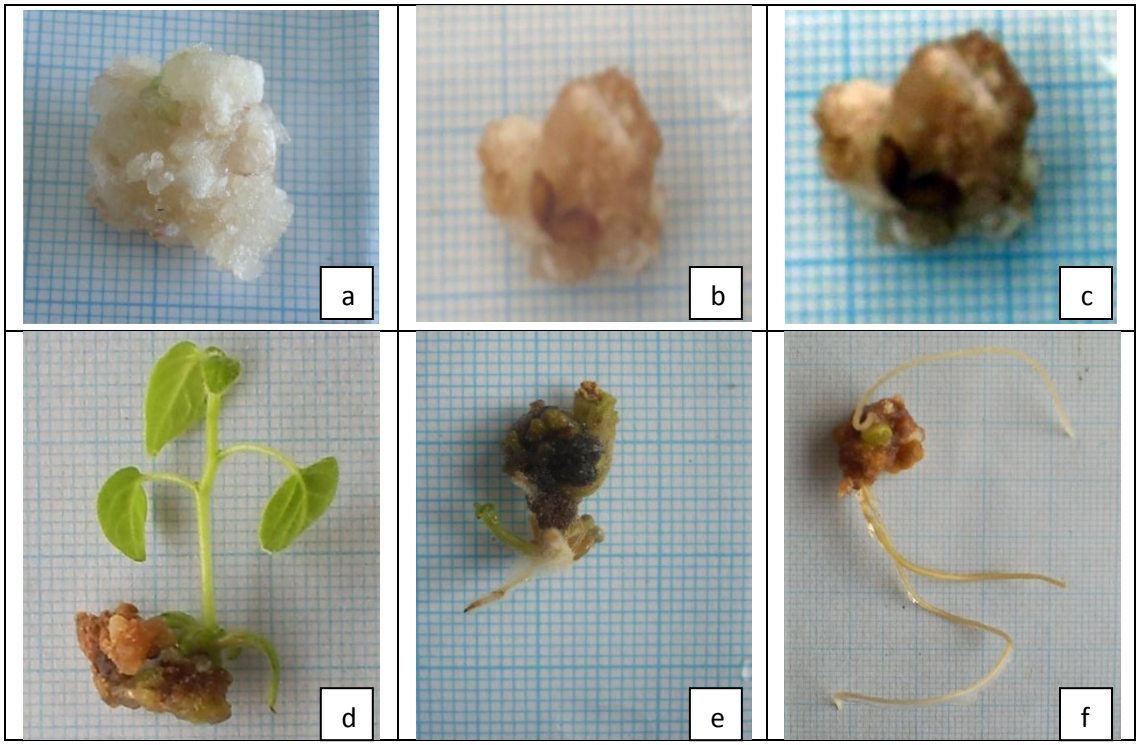
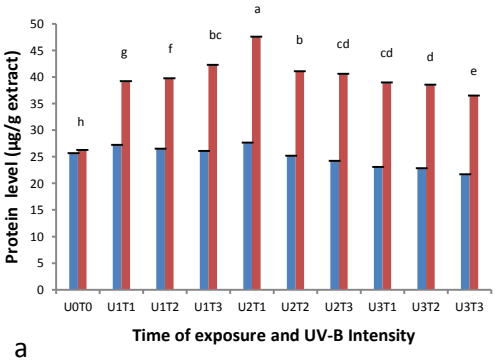


Figure 1. *Morphogenesis of tamarillo callus applied by UV-B radiation. a) a yellowish white callus, b) browning white callus, c) black and white callus, d) plantlets, e) shoots and, f) roots.*

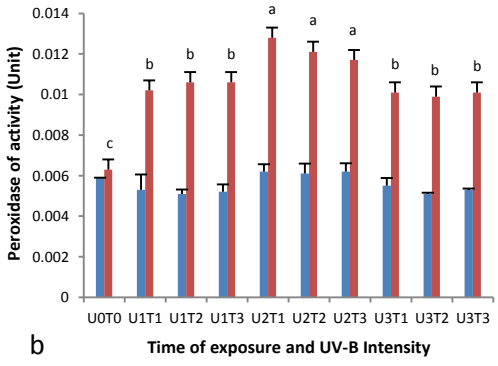
The ability of each explant in callus varies, this indicates that the explants have different properties to respond to any provision of plant growth regulators or mutagenic compounds were added to the initiation media. The callus proliferation differences due to the nature of embryogenic callus which has significant potential to form plantlets or shoots and roots. Formed on the polarization properties of embryogenic callus result in the growth of explants into plantlets

3.2 Peroxidase and Polyphenoloxidase enzyme activities of Tamarillo Callus

In general, the research showed that UV-B radiation was able to change its enzymatic activity both peroxidase as well as polyphenoloxidase. Changes in protein levels and activity of peroxidase and polyphenoloxidase of tamarillo callus after applied by of UV-B radiation shown in Figure 1.



a



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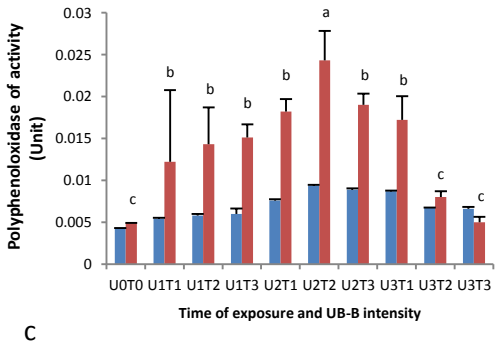

c

Figure 2. *Changes in levels of protein (a), the activity of peroxidase (b) the activity polyphenoloxidase (c) tamarillo callus induced UV-B radiation: before and after selection by Colletotrichum filtrate*

Generally, initial protein content of mutation tamarillo prior to Colletotrichum - filtrate selection tends to decline (Figure 2-a). The stronger of UV radiation, the lower protein contains of the callus. This is apparently due to mutation properties of UV-B radiation that cause changes at the DNA level then led to change of protein level. The significant interference of UV-B shown in the metabolism of protein, so that the protein content of the cells of the callus is reduced. According to Wang and John (1991), UV radiation will merge adjacent thymine to form a covalent bond result in errors during transcription. The formation of thymine dimers can reduce or end the ability of structural and regulatory proteins to bind and function properly. The protein content after the selection shown to rise in line with increased UV radiation power applied. The highest protein content was found in the treatment U2T1 while the lowest found in treatment U0T0. High levels of a protein found on some treatments because of the content of other compounds dissolved in the extract such as enzymes and metabolites. Kazuko and Uritami (1973) reported high levels of protein in the resulting callus caused by glutamate dehydrogenase activity (GDH). Glutamic acid not only serve as building blocks of protein, but also form other amino acids that are essential for the growth and development of plants. Low levels of the protein may be related to the changes pattern of physiological differences such protein synthesis and general degradation occur continuously during the protein metabolism. According Simhian (1998), a formation of protein of a plant can be inhibited if there is one thing or another that could interfere with the synthesis process in plants, that processes can be analyzed biochemically. In Figure (2-b), it can be seen that the peroxidase activity of UV–B mutation tamarillo before and after the selection has a similar pattern. Peroxidase activity in the control treatment (U0T0) was lower compared to other treatments and was significantly different from others. Increasing UV radiation power as well as time of exposure gave the peroxidase activity increase until U2T1 treatmen (20 watt, 30 second). Then the peroxidase activity began to decline until U3T3 treatment. The highest peroxidase activity of callus found at U2T1 which reached 87% compared to before selection. Therefore, exposing the tamarillo by 20 watt UV for 30 seconds gave the best result of antrachnose tolerant. From Figure (2-c), it can be seen that the highest PPO activity before and after the selection found on U2T2 treatment and the lowest found at U0T0. The tamarillo- polyphenoloxidase activity applied by UV-B radiation found increase after the selection which reach into 107%. Measurement of the specific activity of peroxidase and polyphenoloxidase shown in Table 1.

Table 1. *Specific Activity Values of peroxidase and polyphenoloxidase enzymes induced UV-B radiation of tamarillo, before and after selection by the fungus Colletotrichum sp.*

Treatment	Specific activity of PO enzyme (Units / mg protein)		Specific activity of PPO enzyme (units / mg protein)	
	Early	End	Early	End
U0T0	$1{,}60 \times 10^{-4}$	$1{,}64 \times 10^{-4}$	$1{,}68 \times 10^{-4}$	$1{,}87 \times 10^{-4}$
U1T1	$1{,}58 \times 10^{-4}$	$4{,}39 \times 10^{-4}$	$1{,}98 \times 10^{-4}$	$3{,}63 \times 10^{-4}$
U1T2	$2{,}00 \times 10^{-4}$	$4{,}43 \times 10^{-4}$	$2{,}19 \times 10^{-4}$	$3{,}85 \times 10^{-4}$
U1T3	$2{,}38 \times 10^{-4}$	$5{,}11 \times 10^{-4}$	$2{,}30 \times 10^{-4}$	$4{,}31 \times 10^{-4}$
U2T1	$2{,}97 \times 10^{-4}$	$5{,}11 \times 10^{-4}$	$2{,}65 \times 10^{-4}$	$4{,}67 \times 10^{-4}$
U2T2	$2{,}82 \times 10^{-4}$	$5{,}11 \times 10^{-4}$	$3{,}73 \times 10^{-4}$	$5{,}18 \times 10^{-4}$
U2T3	$2{,}65 \times 10^{-4}$	$3{,}62 \times 10^{-4}$	$3{,}68 \times 10^{-4}$	$4{,}93 \times 10^{-4}$
U3T1	$1{,}80 \times 10^{-4}$	$3{,}48 \times 10^{-4}$	$3{,}33 \times 10^{-4}$	$4{,}30 \times 10^{-4}$
U3T2	$2{,}23 \times 10^{-4}$	$3{,}01 \times 10^{-4}$	$2{,}93 \times 10^{-4}$	$2{,}07 \times 10^{-4}$
U3T3	$2{,}45 \times 10^{-4}$	$2{,}86 \times 10^{-4}$	$3{,}05 \times 10^{-4}$	$1{,}30 \times 10^{-4}$

UV-B Lamp Power
U0 = 0 watts
U1 = 10 watts
U2 = 20 watts
U3 = 30 watts

The intensity of radiation
T1 = 30 seconds
T2 = 60 seconds
T3 = 90 seconds

Evaluation of chemical processes in the callus can be done by analyzing the changes in the protein content, such as the activity of peroxidase (PO) and polyphenoloxidase (PPO). Both PO and PPO enzyme are critical enzyme in plants and are found in every organ of the plant. Changes in the PO and PPO enzyme could indicate the occurrence of physiological changes in the callus. PO enzyme plays an important role in controlling the growth of any plant, from the level of cells, tissues or the organ However, PPO enzyme closely relates to the nature of the defense of a plant, to protect plants from the external mechanical/physical pressures as well as from other organisms like pathogenic threats.

4. CONCLUSION

Base on the research conducted, it can be summarized as follows : initial protein content reduce at all samples, the stronger the UV radiation, the lower protein contains of the callus. The protein content, peroxidase and poliphenoloxidase enzyme activities were then increased in the tamarillo callus of antrachnose tolerance. The protein content after the selection shown to rise in line with increased UV radiation power applied

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