A-5 Effect of Enhanced Ultraviolet-B (UV-B) Radiation on Organizers (Abstract of the Final Report)

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Total Budget for FY 1999 – FY 2001 62,934,000 Yen (**FY 2001**; 16,304,000 Yen)
Key Words: Ozone, UV-B, PAR, Ultraviolet Radiation Absorbing Compounds, Mycosporine-like amino acids, DNA, Photorepair Enzyme, Forest, Seedling, Crops, Phytoplankton, Eggs, Zooplankton, Larval Fish, Wild Plants

1. Introduction

Ozone depletion above the Antarctica is estimated to continue for another twenty years at least. A similar ozone depletion has been observed from 1995 above the Arctic. Once ozone depletion is accelerated, ultraviolet radiation- B (UV-B) with 280 – 320 nm would be enhanced drastically. Those enhanced UV-B radiation may depress all biological activity on the Earth. It is known to have wide-ranging biological effects that can include mutagenesis, embryonic development, larval mortality, and acute physiological stress that may ultimately result in death. All plant and animal groups appear to be susceptible to UV-B, but to a highly variable extent that depends on the individual species and environment.

This variability in response has resulted in a great deal of controversy and condition about the implications of increasing UV-B for specific ecosystem. It has been, therefore, desirable to develop more standard method to be able to evaluate the effect of increased UV-B radiation on living organisms.

2. Research Objectives

Plant ecosystem and marine ecosystem are identified as the most susceptible to UV-B radiation. Damage and repair systems are significantly different between those two ecosystems. The effectiveness of UV-B radiation on plant growth is strongly influenced by the prevailing microclimate. Since field mircoenvirinments undergo considerable annual variation and the effects of UV-B radiation are influenced by microclimate, field validation studies conducted over multiple growing seasons are crucial to any assessment of potential UV-B impacts upon agricultural productivity. One purpose of this study is to determine if UV-B radiation affects yield of cauliflower and spinach by multiple field experiments.

Part of the variation in UV-B responsiveness may be dependent upon the genetic composition of the cultivars themselves. Consequently, conclusions based upon the UV-B susceptibility of only one or two cultivars of any crop or native species may be misleading. The other purpose of this study is to estimate diversities of UV-B sensitivity among four to ten cultivars of cauliflower, broccoli and spinach in dry weight increase, UV-absorbing pigments concentration and antioxidative activity in leaves.

Methodological study on the photoprotective mechanism to UV-B exposure and the accumulation mechanism of DNA lesions are carried out to establish more comprehensive evaluation methods for terrestrial plants and marine organisms. To estimate frequency of mutation in the plant genome under increased UV-B environment, we prepare a transgenic plant to evaluate the level of genetic mutation. A common feature in plant responses to different types of environmental stress might be the generation of reactive oxygen species (ROS) and an increase in intracellular oxidative stress. Accumulation of large amounts of ROS induces 8-Hydroxy-2'-deoxyguanosine (8-OHdG), a major oxidative lesions formed by ROS-induced DNA damage. In the this study, 8-OHdG content in a DNA extracted from cucumber cotyledons that has been irradiated with UV-C or UV-B is quantified. The present study is consisted of the following five objectives:

(1) Effect of increased UV-B radiation on forest ecosystem,

(1) Effect of increased UV-B radiation on crops in term of growth, yields, and quality,

(2) Effect of increased UV-B radiation on ultraviolet radiation absorbing materials in marine ecosystem,

(2) Effect of increased UV-B radiation on DNA damage in marine phytoplankton,

(3) Evaluation of the accumulation mechanism of DNA lesions due to UV-B radiation.

3. Research Methods

UV-B exposure experiments were conducted with seedling, crops, marine plankton, and fish larvae under UV-B radiation with photosynthetically active radiation (PAR) in the natural condition and laboratory conditions. Ultraviolet radiation absorbing compounds were identified and quantified with spectrophotometer and HPLC. Extraction methods of DNA from various organisms were examined. New method was developed to determine the accumulation of DNA lesions and the relative occurrence mutation in plant cells exposed to UV-B radiation.

4. Results

Responses of seedling to increased UV-B radiation were characterized by the inverse U shape. Those results indicated that seedling required some UV-B radiation to achieve better growth than one under the present solar UV-B radiation. Japanese cypress grown naturally at the attitude of 800 m indicated a poor growth without the solar UV-B radiation. Relative contribution of UV-B radiation to total solar radiation was increased with attitudes.

Cauliflower "Shiratama" was grown with or without the UV-B supplement under field conditions in six growing seasons during three years. Four spinach cultivars were grown with or

without the supplemental UV-B under field conditions in two growing seasons during a year. UV-B radiation was provided by Q-panel UVB-313 sunlamps suspended above plants. UV sunlamps were filtered with 0.13 mm thick cellulose diacetate (transmission down to 290 nm) or lumiror (no transmission below 320 nm). When weighted for biological effectiveness of UV-B (UV-B_{BE}), the UV-B dose used in these experiments was equivalent to that which would be received at Fukuyama (329'54"N, 133 23'24"E) with an anticipated 40 % ozone depletion . This UV-B irradiation did not affect yield of both cauliflower and spinach. This UV-B irradiation increased 1,1-diphenyl-2-picrylhydrazyl scavenging activity (antioxidative activity) in curds of the cauliflower significantly. But foliage antioxidative activity of spinach cultivars was not affected by the increased UV-B except one cultivar in only one of two growing seasons. To estimate diversities of UV-B sensitivity among cultivars, seedlings of four to ten cultivars of cauliflower, broccoli and spinach were grown under three or four levels of UV-B radiation in multiple field experiments. The proportions of UV-B_{BE} to solar radiation were 1, 2, 3 or 1, 2, 3,4 in each level of treatment. UV-B radiation was provided by modified lamp system of Nouch et al. Seedlings of four cauliflower cultivars showed diversity of UV-B sensitivity in dry matter increase, UV-absorbance and antioxidative activity of 80%-methanol extracts from leaves. Two cultivars with a white curd were more sensitive in depression of dry matter increase by UV-B than other two cultivars with an orange or purple curd. Concentration of UV-absorbing pigments and antioxidative activity in leaves were less stimulated by UV-B in these two white-curd cultivars than in others with a colored curd. These less stimulation concerning protective mechanism could cause greater sensitivity of the two white-curd cultivars in dry matter. UV-B did have little effects on dry matter increase in four broccoli cultivars and ten spinach cultivars tested more than once. In most cultivars of them, increased UV-B stimulated UV-absorbance and antioxidateive activity of 80% methanolic extracts and the degree of these stimulation varied among their cultivars.

To determine if foliage antioxidants induced by UV-B exposure are transported into another leaf, seedlings of two spinach cultivars were grown for 5 or 9 days in the field with or without UV-B supplement when their fifth or sixth leaves were expanding. Except just expanded and expanding leaves, all other leaves were removed. Of each seedling, one leaf was exposed to solar or supplemental UV-B and the other leaf was covered with lumiror film (no transmission below 320 nm) not to be exposed to UV-B radiation. Antioxidative activity in leaves exposed to UV-B were increased according to their exposed UV-B dose. But that in covered leaf was not affected by the UV-B dose the other leaf was exposed to. The result indicated that foliage antioxidants induced by UV-B exposure are not transported into another leaf.

Both marine phytoplankton and zooplankton were shown to depress their physiological processes such as growth, hatching rate, and survival rate. However neustonic copepods were found to accumulate the ultraviolet radiation absorving compounds in their body to protect from UV-B damage. Phytoplankton also responsed to increased UV-B radiation with production of ultraviolet radiation absorving compounds such as mycosporine-like amino acids. Amounts of damaged DNA were related with the increased UV-B radiation.

A gene encoded *E. coli* cytocine deaminase (*codA*) under the control of cauliflower mosaic virus 35S promoter was integrated to *Arabidopsis thaliana*. This transgenic plant showed sensitivity to 5-fluorocytocine (5-FC) while the wild type plants were resistant. The number of 5-FC resistant progenies propagated from UV-B irradiated transgenic plants was larger than that from white light irradiated ones.

The contents of H_2O and 8-OHdG in UV-C irradiated cucumber cotyledons were increased in a dose-dependent manner. UV-B irradiation slightly increased H_2O_2 content, but considerably 8-OHdG content.

5. Discussion

Physiological responses to the increased UV-B radiation are shown to be distinctly different between terrestrial plants and marine plankton. Seedlings grow better if they are exposed to the increased UV-B radiation. However their growth is depressed if too much UV-B radiation is provided. Crops do not indicate significant difference with and without UV-B radiation. The present study shows that marine plankton are more susceptible than terrestrial plants. Their growth rate, hatching rate of eggs, and survival rate of juvenile stage are greatly reduced once they are exposed to the increased UV-B radiation.

Ultraviolet radiation absorbing compounds are detected from both terrestrial plants and marine plankton and their responses are examined in relation with the increased UV-B radiation in the present study. The supplemental UV-B had no effects on yield of a cauliflower cultivar and four spinach cultivars tested. Farther more studies in regard to its reduction during storage and possibility of its uptake into human body are required to determine if antioxidative activity stimulated in a curd of the cauliflower by increased UV-B makes sense of nutrition as a edible part. If UV-B sensitivity was defined as negative susceptibility in biomass, two white-curd cultivars were more sensitive than other two colored-curd cultivars among four cauliflower cultivars tested. But concerning degrees of stimulation in concentration of UV-absorbing compounds or antioxidative activity, colored-curd cultivars were more effected by the enhanced UV-B. Most studies performed under field conditions demonstrated the increased UV-B radiation as consequence of suspected ozone layer depression might cause little effects on biomass of terrestrial higher plants. The results of this study supported these implication. Because of this little susceptibility of plant biomass to UV-B, UV-B sensitivity might be preferable to be discussed in respects to factors which cause their UV-B tolerance such as degree of stimulation in foliage concentration of UV-absorbing compounds or antioxidative activity. Farther more studies are required concerning possibility of change in taste and nutrition of their edible parts caused by increase of UV-B absorbing compounds concentration, of antioxidative activity, induced by the increased UV-B. Marine phytoplankton are shown to produce mycosporine-like amino acids under the increased UV-B radiation. Some marine zooplankton indicate the accumulation of mycosporine-like amino acids in their body to exploit new niche at the surface skin layer of the ocean.

Methodological problems in extraction of DNA from tissue of terrestrial plants and marine

plankton are recognized during the present study. More specific extraction methods should be developed to specific samples and the results should be standardized for more comprehensive application. The present study confirms that the amounts of DNA destructive substances are increased with the increased UV-B radiation, suggesting the dependable and precise evaluation of increased UV-B radiation effect on living organisms in general. Phenotype of the transgenic a plant was 5-FC sensitive, indicating that cod A was suitable for a marker gene for the evaluation of genetic mutation. The number of 5-FC resistant progenies propagated from UV-B irradiated transgenic plants was larger than that from white light irradiated ones but the number of seeds used for our experiment was not enough for the static evaluation. This new method is promised to be able to detect the relative occurrence of mutation in any cells exposed to UV-B radiation. Both UV-B and UV-C irradiated cucumber cotyledons containe 8-OhdG and they are increased in a dose-dependent manner. But H_2O_2 content is not increased same manner by UV-B irradiation, indicating that ROS generated in plant cells exposed to UV irradiation result in the formation of oxidative DNA damage, and suggest the possibility that the reaction manner of UV-B might be different from that of UV-C.