# Seed germination under UV-B irradiation

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Ultraviolet-B rays (UV-B) were studied how it affected on seed germination in nineteen plant species. Seed germination rates under UV-B (U), Light (L) and Dark (D) conditions were compared. The reactions to the three conditions in seed germination rates will be putatively classified into 13 types in order of germination rates indicated by the three letters including no difference among the three conditions (type ---). Seven types, ---, --U (U was the lowest; non significant difference between the other two conditions), L-- (L was the highest; non significant difference between the other two conditions), LUD (L was the highest, D was the lowest, U was the middle), DUL (D was the highest, L was the lowest, U was the middle), D-- (D was the highest; non significant difference between the other two conditions), and --D (D was the lowest; non significant difference between the other two conditions) were detected in this study. U suppressed the seed germination of celery, eggplant and Swiss chard (type --U), but no species was accelerated by U. However, U did not affect seed germination rates in type ---, i.e. buckwheat, cabbage, Chinese cabbage, parsley, potherb mustard and turnip, and type --D, i.e. carrot, Chinese chive and komatsuna. On the other hand, L accelerated the seed germination rate of burdock (type L--), but suppressed that of radish (type DUL). D accelerated those of onion, pea, spinach and welsh onion (type D--) and radish. In radish (type DUL), there were significant differences among the three conditions. D was the highest, L was the lowest, and U was the middle. U, L and D worked interactively. D predominantly restored seed germination rate from the suppression of U, and L predominantly suppressed the effect of D.

Key words: seed germination, ultraviolet rays, UV-B.

#### **INTRODUCTION**

Ultraviolet rays were classified into three partitions according to their wavelengths, i.e. UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm). The radiation amount of ultraviolet ray to the earth, especially of UV-B, is increasing by the depletion of ozone layers in the stratosphere caused by the emission of chlorofluorocarbon into the air, and it is still remaining in the air without resolution in spite of the regulation of its usage. The meticulous UV-B monitoring research work has been accomplished (Kon et al. 2003), and according to the atmospheric environmental research (Sasaki 2006; Japan Meteorological Agency 2007), total ozone values were relatively low in most regions of the world in 2007, and somewhat increasing trends in UV radiation have been seen.

UV-B was reported as an environmental stress to retard the growth of dwarf bean (Tamai and Tanabe 2001a) and the cucumber (Takeuchi et al. 1989; Tamai and Tanabe 2001b) by suffocating photosynthesis. Ultrastructural damages were also induced by UV-B (Brandle et al. 1977; He et al. 1994; Santos et al. 1998). On the other hand, it was effective to control excessive elongation in lettuce seedlings (Hayashida et al. 2004). It was also reported that UV-B had a slight positive effect on the vegetative biomass production of *Bromus* (Deckmyn and Impens 1998).

Previous studies were all of the effect on plants level not on seed germination. The size of plant population is firstly determined by seed germination that would affect on the yield of plant production as well as the dynamics of the ecosystem on a large scale. It could be an applicable result for weed control that common purslane (*Portulaca oleracea* L.) was significantly reduced by UV-B radiation (Matsuo et al. 1999). The effects of UV-B on pollen germination (Feder and Shrier 1990; Torabinejad et al. 1998) and spore germination of fungus were reported (Fargues et al. 1997; Smits 1991; Ohbayashi 1983), whereas UV-B was the detrimental factor to conidia. However, there is no report on the effects of UV-B radiation on plant seed germination.

The objectives of this study were to evaluate how UV-B rays affect on seed germination, especially in plants adapting to cooler climates which are assumed to be more susceptible to higher temperature, and to detect differences among plants in seed germination reactions to UV-B radiation.

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### **MATERIALS AND METHODS**

Seed germination reaction to UV-B. Seeds of buckwheat 'Shinano Oosoba', burdock 'Takinogawa', cabbage 'Okina', carrot 'Kuroda Gosun', celery 'Top Seller', Chinese chive 'Hirohaba-nira', Chinese cabbage 'Kyoto 3', egg plant 'Chikuyo', lettuce 'Great Lake', mizuna (*Brassica rapa* L. Japonica Group) 'Kyoshigure', komatsuna (*B. rapa* L. var. *perviridis* L. H. Bailey) 'Syousai', onion 'OL Ki', radish 'Minowase', 'Comet' and 'Hamadaikon (collected in Takanabe, Miyazaki)', parsley 'Curly paramount', pea 'Hyogo Kinusaya', perilla 'Chirimen Aoshiso', spinach 'Alright', turnip 'Syogoin Oomarukabu', Swiss chard 'Umaina', and Welsh onion 'Kujo Futonegi' were used for this experiment.

Three conditions of UV-B (U), Light (L) and dark (D) were examined. In U condition, materials of seeds were continuously irradiated by UV-B lamps (GL20SE 20w, Sankyo Denki Co., Ltd.) of which wave lengths were 280 nm and over with the peak of 306 nm. The lamps were set 15 cm above seeds, with the intensity of 2 W/m<sup>2</sup> referring to the maximum observational data in Tokyo, Japan (Komine et al. 2002). In L condition, fluorescence lamps were used for irradiating continuously with the intensity of 33.6  $\mu$ mol  $\cdot$  m<sup>-2</sup>  $\cdot$  s<sup>-1</sup>. In D condition, stainless steel boxes were used to shade light. The dark condition was set as the control plot.

Polystyrene dishes (60 mm in diameter and 12 mm in depth) were used, with No. 2 Whatman filter paper laid inside and containing 2 ml distilled water in each dish.

Twenty-five seeds were inoculated per dish. Germinating seeds were counted every day until the cumulative germination rate became a plateau. As there are light-stimulated or light-inhibited seed germination, in order to avoid influences of light on seed germination during counting the number of germinating seeds, dishes with seeds inoculated were prepared individually for each plot (for everyday counting). Each condition was consisted of four repetitions. The dishes were incubated at 20°C which was within the range of the optimum temperature for seed germination for all plants tested. Data were shown in figures with averages and their confidence intervals of population mean at the 95% level.

Interactive effect of UV-B, light and dark condition. The radish varieties, 'Miyashige' and 'Sakurajimaoomaru', were used for further experiment to examine interactive effects between U and D (U/D), and, L and D (L/D) conditions. For the U/D condition, UV-B was irradiated for 12 hrs followed by 12 hr-dark a day. The L/D condition was irradiated by fluorescent lamps for 12 hrs followed by 12-hr dark a day. The U, L and D conditions were tested for comparison. The other experimental conditions were the same as the previous test.

### **RESULTS AND DISCUSSION**

The results were summarized in Table 1. Seven different types of seed germination reactions were detected in this study (at the 5 or 1% level) on seed germination

Plants	Among light conditions	Among days after sowing	Туре	Fig. no. for reference
Buckwheat 'Shinano Oosoba'	1.535	66.751** <sup>z</sup>		1
Cabbage 'Okina'	3.149	317.666**		
Chinese Cabbage 'Kyoto 3'	0.001	0.141		
Parsley 'Curly paramount'	0.736	404.395**		
Mizuna 'Kyoshigure'	2.409	567.035**		
Turnip 'Syogoin Oomarukabu'	2.445	213.911**		
Celery 'Top Seller'	9.283**	23.091**	U	2
Eggplant 'Chikuyo'	7.418**	42.123**	U	
Swiss chard 'Umaina'	10.501**	2.219	U	
Burdock 'Takinogawa'	15.545**	3.708**	L	3
Lettuce 'Great Lake'	10.019**	15.685**	LUD	4
Perilla 'Chirimen Aoshiso'	13.301**	3.815**	LUD	
Radish 'Minowase'	34.323**	9.542**	DUL	5
'Hamadaikon'	19.292**	1.700	DUL	8
'Comet'	46.797**	31.290**	DUL	
'Miyashige'	23.498**	3.049*	DUL	
'Sakurajima-oomaru'	90.503**	5.710**	DUL	
Onion 'OL Ki'	13.255**	26.221**	D	6
Pea 'Hyogo Kinusaya'	6.690**	23.323**	D	
Spinach 'Alright'	26.512**	122.576**	D	
Welsh onion 'Kujo Futonegi'	8.757**	17.875**	D	
Carrot 'Kuroda Gosun'	17.175**	85.037**	D	7
Chinese Chive 'Hirohaba-nira'	7.054**	31.651**	D	
Komatsuna 'Syousai'	19.242**	3.946**	D	

Table 1. F-values from analysis of variance for seed germination among the three light conditions of UV-B, light and dark

<sup>Z</sup>\*\*,\*: significant at the 1% and 5% level by analysis of variance.

according to the analysis of variance (ANOVA). If the significant differences in the cumulative germination rate among the three conditions (U, L and D) are detected, the three letters were placed orderly from the left (the highest) to the right (the lowest). There supposed to be six categories of type ULD (U: the highest, L: the middle and D: the lowest), UDL, LUD, LDU, DUL and DLU. If there is a significant difference in one condition from the other two conditions, there should be six categories of type U--, --U, L--, --L, D-- and, --D (the left letter is the highest, or the right one is the lowest). In the case that no significant difference in the seed germination rate was detected among the three conditions, it was shown as '---'. There

supposed to be 13 types in total.

There was no species whose seed germination was accelerated by UV-B rays in this study, but there were species whose seed germination rate were not affected by U, i.e. type --- and --D. In buckwheat, cabbage, Chinese cabbages, parsley, mizuna and turnip (Fig. 1), there was no significant difference in seed germination rates among the three conditions (type ---). In celery, eggplant and Swiss chard (Fig. 2), the seed germination rates were restrained or delayed by the UV-B rays (type --U) irradiation. It is also considerable that the effect of environmental conditions during seed formation would affect the seed germination (Choi and Takahashi 1979)



Fig. 1. Comparison of cumulative seed germination rates in U, L and D (type ---). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 2. Comparison of cumulative seed germination rates in U, L and D (type - - U). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 3. Comparison of cumulative seed germination rates in U, L and D (type L - - ). Vertical vars indicate confidence intervals of population mean at the 95% level.

In burdock (Type L-- shown in Fig. 3), lettuce and perilla (Type LUD shown in Fig. 4), L showed the highest seed germination rates among the three conditions. On the other hand, seed germination rates of radishes, 'Minowase', 'Hamadaikon' and 'Comet' were suppressed by L (type DUL shown in Fig. 5). There were significant differences among the three conditions one another. D was the highest, L was the lowest among the three conditions, and U was in the middle. For further research, photo periodic response should also be taken into consideration.

In lettuce (Fig. 4), there was not a significant difference in the final seed germination rates in 7 days among the three conditions, however, L reached a plateau the fastest among the three conditions, and D was the latest to the rate of L. As the synthesis of endogenous gibberellic acid was suppressed by UV-B in spinach (Long et al. 1988), it is suggested that the seed germination in lettuce might be delayed by the suppression of the level of endogenous gibberellic acid by UV-B irradiation like as under the dark condition.

In onion (Fig. 6), on the other hand, there was not a significant difference in the final seed germination rates in 7 days among the three conditions, however, the germination rate in L reached a plateau the latest among the three conditions. In pea, there was no difference in the final seed germination rate in 10 days among the three conditions, however, the germination rate in D was higher than those of the other conditions in the first 6 days from the start. As mentioned above, D accelerated seed germinations in onion, pea, spinach and welsh onion (type D--), and radish (type DUL). On the contrary, D suppressed seed germination in carrot, Chinese chive and komatsuna (Fig. 7).

In all radish varieties tested, 'Minowase', 'Comet', 'Hamadaikon', 'Miyashige' and 'Sakurajima-oomaru', were type DUL (Fig. 5 and 8). there were significant differences



Fig. 4. Comparison of cumulative seed germination rates in U, L and D (type LUD). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 5. Comparison of cumulative seed germination rates in U, L and D (type DUL). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 6. Comparison of cumulative seed germination rates in U, L and D (type D - - ). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 7. Comparison of cumulative seed germination rates of radishes in U, L and D (type -- D). Vertical vars indicate confidence intervals of population mean at the 95% level.



Fig. 8. Interactive effect among U, L, D, U/D and L/D on seed germination rates in radish. Vertical vars indicate confidence intervals of population mean at the 95% level.

among the three conditions. D was the highest, L was the lowest among the three conditions, and U was the middle. In 'Comet', there was also significant difference between U and D, however, U was close to D (Fig. 5). This result showed that 'Comet' was highly tolerant to UV-B, which was consistent with the previous studies (Krupa and Kickert 1989; Nouchi 1991).

In order to examine how D interacts with U or L on seed germination in radishes, conditions of U with D (U/D), and L with D (L/D) were additionally tested in the second experiment. There were also significant differences among L, D and U one another in both cultivars of 'Miyashige' and 'Sakurajima-oomaru' as the other radish varieties showed in previous experiment. The interactive effects in both U/ D and L/D were detected. In 'Sakurajima-oomaru', there was not a significant difference between L and L/D nor D and U/D (Fig. 8). This suggested that L predominantly suppressed seed germination to D, and dark condition covered up the negative effect of UV-B radiation on seed germination. In 'Miyashige', there was not a significant difference between L and L/D similarly to 'Sakurajima-oomaru', but there were significant differences among D, U/D and U. There were differences between the two cultivars in the degree of the compensation of seed germination rates by dark condition against UV-B. The germination rate were compensated for UV-B effect with the dark condition in both two varieties. However, there was difference in the degree of the effect by dark condition against UV-B between the two cultivars. As D covered up inadequately the effect of UV-B in 'Miyashige', this suggests that 'Miyashige' is more sensitive than 'Sakurajima-oomaru' which is adapted to lower latitude where the intensity of UV-B is relatively higher. Photo periodic response should also be taken into consideration for further research.

The effect of UV-B on germinating energy was observed in some species. In buckwheat, the germination energy was higher than the other light conditions. On the other hand, those of lettuce and perilla were lower than L condition, and that of celery was lower than the other light conditions. In order to put UV-B rays to practical use for weed control or nursery, further detailed studies on the effect of UV-B on seed germination, however, these results showed the possibility of usability of UV-B for plant growth regulation.

It is known that there are light germinating seeds or

dark germinating seeds. Cabbage, Chinese cabbage, carrot, turnip, celery, lettuce, perilla and burdock are known as the former. Onion, Welsh onion, egg plant and radish belong to the latter. The results of light-inhibited seeds in this study were consistent with that mentioned above, including egg plant (type --U) of which germination rate in D reached plateau earlier than L (Fig. 2).

As the difference in the same species (*Brassica rapa* L.), i.e. between mizuna (Fig. 1) and komatsuna (Fig. 7) was detected, there might be some different seed germination reaction to U, L and D among varieties. It was reported that there were differences in reaction to UV-B radiation on the plant growth among species and even among varieties (Teramura 1983; Runeckles and Krupa 1994). It is necessary to investigate by expanding the range of plant species as well as varieties whether there are plant species or varieties belonging to the rest of germination types (ULD, UDL, LDU, DLU U-- and --L) that were not detected in this study. It is also necessary to examine the mechanisms how UV-B rays work on seed germination and to control plant growth or the size of plant population by using UV-B rays.

As a synergistic effect of UV-B radiation with a high temperature caused poor growth (Inaba 2005), and the effect of UV-B was eased by applying with moderate light condition for photosynthesis (Nouchi and Kobayashi 1995), further studies on the interaction between UV-B and light and/or high temperature may lead to a better understanding. As one of the mechanisms of UV-B resistance is the accumulation of UV-absorbing compounds in leaf tissues (Sato and Kumagai 1997), which might be related to the differences of the reaction in seed germination to UV-B among the plant species. The differences in the reaction of seed germination under UV-B radiation revealed in this study would suggest potentialities for controlling seed germination, which, in turn, could be informative for weeding. The introduction of UV-B rays into nurseries or weeding system could be of use.

This study could not detect positive effect of UV-B rays in seed germination. It has been reported that UV-B rays are detrimental to plants and retarded the growth of lettuce (Hayashida et al. 2004), dwarf bean (Tamai and Tanabe 2001a), rice, cucumber (Tamai and Tanabe 2001b), and crop plants (Teramura 1983). However, there were also species in natural plant population which

increased biomass under UV-B radiation (Sullivan et al. 1992). Monocotyledonous species were more sensitive to UV-B treatment than dicotyledonous species in pollen germination (Torabinejad et al. 1998), whereas dicotyledonous species were more sensitive to UV-B than monocotyledonous species in plant growth (Krupa and Kickert 1989). A specific UV-B photoreceptor relating to morphological responses was demonstrated (Ballare et al. 1992). It was also reported that UV-B deteriorated photosynthesis activity (Iwanzik et al. 1983; Takeuchi et al. 1989; Teramura 1983; Vu et al. 1982). The level of increasing polyphenol synthesis stimulated by being exposed to UV-B for the protection (Caldwell et al. 1983; Teramura 1983) could differ in plant species. Tropical plant species which were highly exposed to solar UV-B even without ozone depletion showed less reduction of leaf length against UV-B radiation (Searles et al. 1995). As the content of superoxide radical was enhanced by UV-B (Prasad et al. 2005), the differences among the plants species or varieties could be caused by the differences in the level of superoxide dismutase and catalase activities (Balakumar et al. 1997), and accumulation of phenols and anthocyanins under UV-B radiation (Ebisawa et al. 2008).

The seed germination is also a basic requirement for an efficient breeding to realize various genotypes through cross method of breeding. In the case of introducing wild species into the nursery system, usually their seed germinations are low or slow because of the seed dormancy. If UV-B rays accelerate their seed germinations, they are expected to contribute to the usage of them for plant breeding and cultivation. On the contrary, if UV-B suppress seed germinations, it could be usable for weed control.

It is needed to find plant species showing positive reaction to UV-B on seed germination by expanding the range of plant species including tropical or alpine plants which would be adapted to much more intensive UV-B radiation in their natural surroundings, since latitudinal and elevation gradients may have resulted diverse sensitivity in plants (Searles et al. 1995; Sullivan et al. 1992). There were species of which seed germination rate were not affected by the UV-B. The mechanism of the reaction should be investigated genetically in the successive studies. The information about the seed germination reaction to UV-B would offer a solution for how to cope with the recent abnormal climate to plant production.

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# 紫外線UV-B照射下での種子発芽 杉本和宏 南九州大学環境園芸学部

#### 要 約

紫外線 UV-B が植物に及ぼす影響について,UV-B 照射条 件(U),明条件(L),暗条件(D)における種子発芽を比較した. この3条件下の発芽率を高い順に左から上記略号で表すと有 意差の認められない場合(---)を含めて13タイプが想定さ れる.本研究では---,--U(Uが最小,他2条件間に有意 差なし),L--,D--,--D,LUD,DULの7タイプが検出さ れた.UV-Bによって,セロリ,ナス,フダンソウ(--U型) では発芽が抑制され,促進されたものはなかった.---型の ソバ,キャベツ,ハクサイ,パセリ,ミズナ,カブ,および --D型のニンジン,ニラ,コマツナの発芽には,UV-Bは特 に影響を及ぼさなかった.ダイコンでは3条件間に有意差が 認められ,これら3条件の間に相互作用がみられ,UV-Bの 抑制作用は暗条件により回復した.