Enhancement of Seed Germination by Aging, Cold-stratification, and Light Quality during Desiccation in Burcucumber (*Sicyos Angulatus* L.)

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ABSTRACT : Seeds of burcucumber were treated with accelerated aging, cold-stratification, and light quality illuminated during desiccation to enhance their germination and seedling emergence. The germination was increased by aging and cold-stratification although the latter treatment showed greater effectiveness than the former one. In the combined treatment of aging 6 days at 45°C and cold-stratification, the germination was promoted under longer period of cold-stratification to reach nearly 100% in 3 week cold-stratification on the ninth day from sowing. In the sequentially combined treatment of aging, cold-stratification, and light quality during 24 hour desiccation at 35°C, no-stratified seeds showed the highest rate in red light treatment but the lowest in far-red light. This implies that the phytochrome action run during the desiccation of imbibed seeds. The red light exposure during drying for the cold-stratified seeds after aging accelerated the germination even more than the dark treatment and germinated 100% on the next day of sowing. It is concluded that the sequential treatment of aging, cold-stratification, and red light illumination during desiccation can highly promote percentage and speed of burcucumber seed germination.

Keywords : burcucumber (*Sicyos angulatus*), aging, cold-stratification, drying, light quality, germination

Burcucumber has been classified as a noxious weed in USA and Europe. Its seeds, however, have been used as a medicine in orient, and recently in some countries including Korea it has been used as rootstocks of *Cucurbitae* since a species was known to be resistant to root-knot nematode and low-temperature (Bulder *et al.*, 1991; Kim *et al.*, 1997; Mann *et al.*, 1981). Despite of the above advantage, its seeds show the poor germination to limit its utilization. Its seed treatment techniques to elevate the germination, therefore, have to be developed. The seed treatments reported so far were confined not only to the environment control including temperature, air composition and moisture, after-ripening, cold-stratification, physical and chemical scarifications but also to single treatment of the above factors (Lee *et al.*, 1991; Lim *et al.*, 1994; Mann *et al.*, 1981).

The seeds stored at room temperature for 1 to 2 years after removal of either of their arils attached around their micropyle or physically scarified seeds after cold-stratification of over 8 weeks showed germination of 87% or 72%, respectively. The seed immediately after harvesting or non-scarified seeds, however, nearly did not germinate (Lim *et al.*, 1994; Walker, 1973). In addition, Mann *et al.* (1981) and Jones (1971) reported that optimum germination temperature in the scarified seeds was from 20 to 30°C although their germination was possible from 15 to 35°C and there was no inhibitor in seed coat and embryo but disruption of the seed coat was necessary for their germination.

Seed germination is controlled by the ratio of phytochrome red (Pr) and phytochrome far- red (Pfr) which can be interconverted by red and far-red lights, respectively. Greater proportion of Pfr to Pr promotes seed germination. It is possible to increase the Pfr ratio through the light treatment during the desiccation of treated seeds as well as they should be dried to be sold out into commercial markets (Bewley & Black, 1994). A specific light, therefore, given during desiccation after seed treatments such as aging and cold-stratification may influence its seed germination. This study was done to examine the effect of aging, cold-stratification and light quality illuminated during the desiccation of imbibed seeds on the seed germination of burcucumber.

MATERIALS AND METHODS

Test seed preparation

Fruit clusters of burcucumber collected in Andong, Korea were supplied by the Andong Agricultural Technology Center in November 1998, and then cleaned by huller and hands. After the cleaned seeds were dried to about 10% moisture content and separated into mature medium-sized seeds weighing about 6.5 g per 100 seeds, they were dried indoor, packed into polyethylene bags and then stored at 3°C until using as experimental materials. An aril around their micropyle was cut off using nail clippers immediately before each treatment was initiated.

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Germination tests and calculation of T_{50}.

All the experiments were conducted twice with three replications of 30 seeds in a completely randomized design unless noted otherwise. Germination tests of every experiment were done with 9 cm petri dishes in which the seeds were placed on two sheets of filter papers, and kept under darkness and 30°C constant temperature. Water was supplied twice a day with a mist sprayer. Germinated seeds were counted daily up to ninth day after initiation of the germination test on the basis of individual seeds showing the radicle extrusion of over 1 mm and calculated into percent germination. The time to get 50% germination rate (T_{50}) were calculated according to the Coolbear *et al.* (1984). The procedures not mentioned here, moreover, followed ISTA rule (1985).

Aging treatment.

Aril-removed seeds were aged for 0, 3 or 6 days at a dark incubator controlled at 45°C constant temperature and 100% relative humidity before the germination test. The aging treatment was forced by accelerated aging method (Delouche & Baskin, 1973) using the Magenta Vessels with membrane rafts (Sigma Chemical Co., USA).

Cold stratification.

At one week interval, aril-removed seeds were placed into wet commercial bed soil contained in a plastic box and kept moist and dark at a low temperature incubator of 3°C. After 3 weeks, all the seeds chilled for 1, 2 or 3 weeks were removed from the bed soil and then washed by tap water for 5 minutes. The experiment was conducted with 4 levels by adding non-treated control.

Combination of aging and cold stratification.

To assess the combining effect of aging and cold stratification, two experiments consisting of cold-stratification after aging or vice versa were done by considering the results of above aging and cold-stratification tests. Aging treatment of aril-removed seeds was done for 6 days with the same method of above aging test, but their cold-stratification treatment was chilled for 0, 1 or 3 weeks using the same procedure of above cold-stratification test.

Drying.

After aril-removed seeds was aged for 6 days and cold-stratified for 3 weeks as mentioned above, they were dried in a dark incubator of 35°C until 24 hours after desiccation to determine the time required to return the original seed moisture content. The moisture content was measured with Infrared Moisture Meter (MB 300, Ohaus Co.). Germination test was carried out to compare the percent germination of non-dried seeds and seeds dried for the determined time.

Light quality treatment.

Aril-removed test seeds were chilled for 0, 1 or 3 weeks after aging for 6 days as mentioned above. To check the effect of light quality given during their desiccation on percent germination, dark, blue, red or far-red light was treated by inserting glass filters (Melles Griot Co., USA) into commercial halogen lamps. Lights were illuminated for 16 hours and their spectra were shown in Fig. 1.

Seedling emergence.

Presowing treatments combined the best results of aging, cold stratification or light quality treatment during desiccation of treated seeds. After aril-removed seeds aged for 6 days were chilled for 1 week, they were dried under red light illumination or darkness as mentioned above. Their percent germination was checked prior to investigating the seedling emergence. The emergence trial was carried out in a glasshouse and a treated seed was sown into every cell of plastic trays containing a commercial bedsoil Tosile. Seedling emergence was measured everyday on the basis that cotyledons were fully expanded.

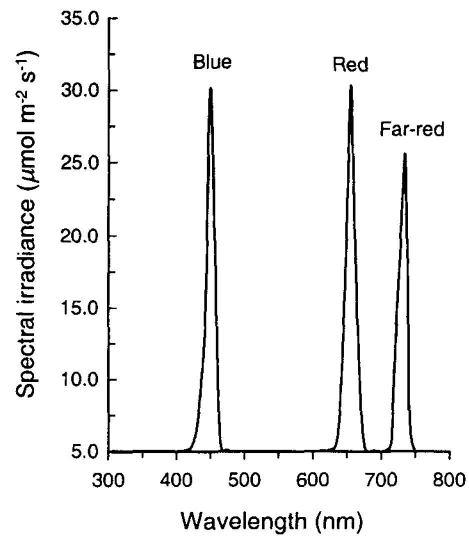

Fig. 1. Light spectrum used for light quality treatment. Measurement was done by the LI-1800 spectroradiometer (LI-COR Co., USA).

RESULTS AND DISCUSSION

Accelerated aging treatment after aril removal increased the germination rate in comparison with aril removal but no-aging treatment. The germination rate of burcucumber seeds aged for 6 days was the highest among the three aging treatments. The longer treatment promoted the germination speed as indicated by decreased T_{50} (Fig. 2 A). Cold-stratification treatment also enhanced the germination. Although there were no big differences among stratification periods from one to three weeks, three-week stratification showed the highest percent germination and the shortest T_{50} (Fig. 2 B). The two experiment results indicate that in case of the aril removal cold-stratification and aging treatments are effective in promoting germination, showing greater effectiveness in cold-stratification.

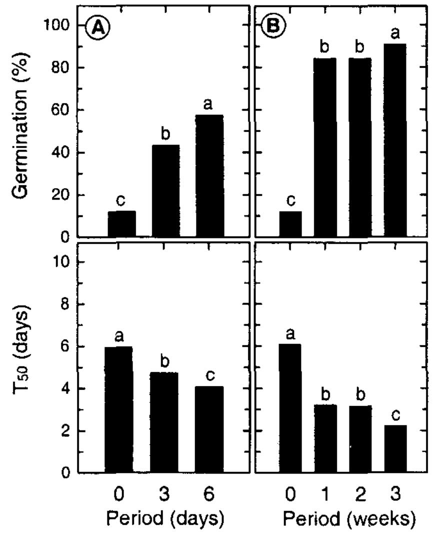

Fig. 2. Effect of accelerated aging (A) and cold stratification (B) on burcucumber seed germination. Aging and cold-stra tification treatments were done by the accelerated aging procedure at 45°C and 3°C, respectively. The bars having the same letters were not significantly different by LSD.05.

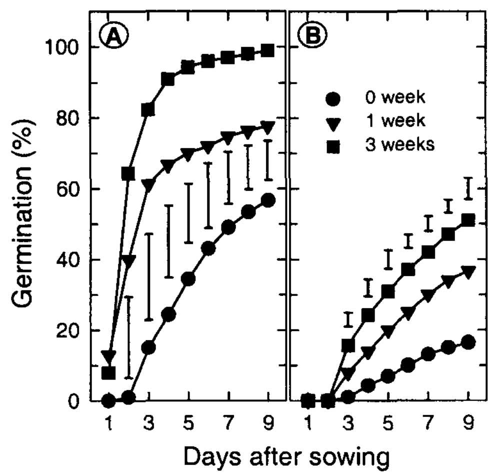

Fig. 3. Combining effect of aging after cold-stratification treatment (A) or vice versa (B) on burcucumber seed germination. Aging and cold-stratification treatments were done at 45°C for 6 days and at 3°C for above 3 periods, respectively. The vertical bars indicate values of LSD.05.

In the experiments combining the best results from the above aging and cold-stratification treatments, cold-stratification after the aging treatment showed higher germination than the reverse treatment and the effect was greater in longer stratification period. The former combined treatment, moreover, enhanced the germination percentage and speed compared to single treatment of the above aging or cold stratification. The germination reached nearly 100% on the 9th day after sowing in three-week stratification after 6-day aging treatment (Fig. 3).

The combined treatment of aging and then three-week stratification was more effective than the reported single treatment of aril removal, after-ripening (Lim *et al.,* 1994), and cold-stratification (Mann *et al.*, 1981). The cause might be ascribed to that the combining treatment broke the seed dormancy more effectively and/or reduced the mechanical resistance to the entrance of water and oxygen compared to the treatment of single factor mentioned above (Lim *et al.*, 1994; Mann *et al.*, 1981; Walker, 1973). Therefore, the combined treatment of aging and cold-stratification for the seeds within one year after harvesting can be adopted in the commercial seedling mass production system.

The cold-stratified seeds after aging should be dried to be sold through the commercial markets. Thus, a proper drying method is needed to be set up. The treated seeds were required to be dried for at least 18 hours at 35°C (Fig. 4 A) to

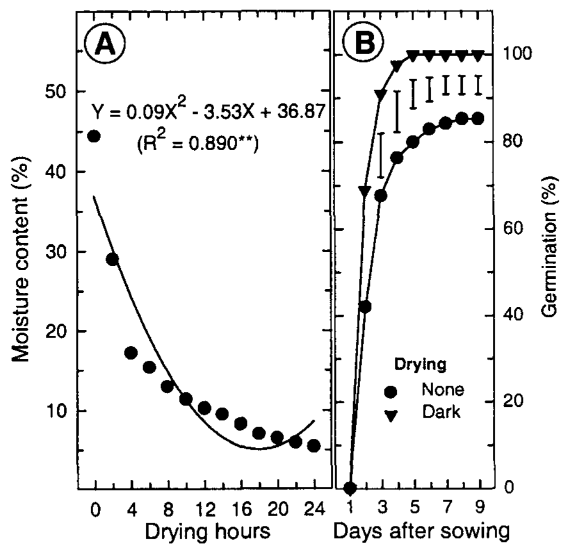

Fig. 4. Change of seed moisture content according to drying period (A) and drying effect on burcucumber seed germination (B). Seed drying of Fig. A was done at 35°C. The vertical bars indicate values of LSD.05.

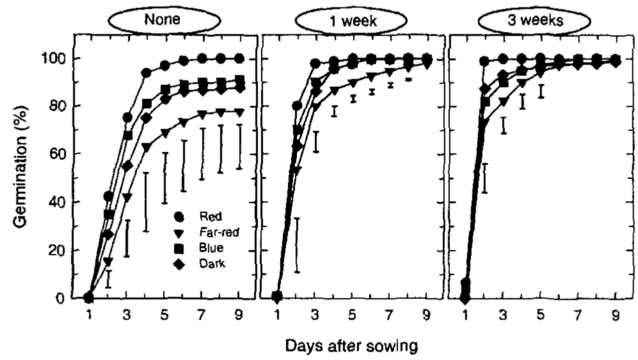

Fig. 5. Effect of cold-stratification after aging and light quality treatment during desiccation after stratification on burcucumber seed germination. The vertical bars indicate values of LSD.05.

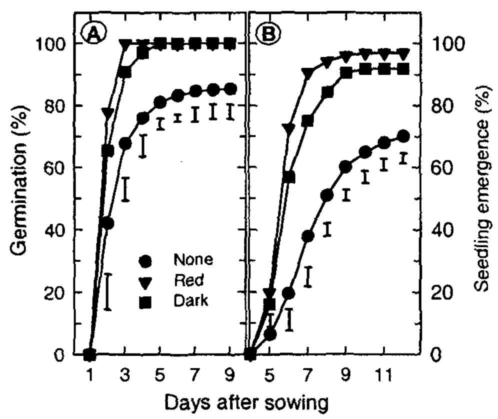

Fig. 6. Effect of desiccation and light quality treatments after aging and cold-stratification on burcucumber seed ger mination (A) and seedling emergence (B). The aged and cold-stratified seeds were dried under no or red light illumination, or not dried (none). The vertical bars indicate values of LSD.05.

return to the moisture level prior to the seed treatments. In addition, the cold-stratified seeds must be dried because the seeds dried under darkness showed better germination than the non-dried seeds (Fig. 4 B). The seeds non-stratified after aged at 45°C for 6 hours germinated faster in red light treatment than in dark and far-red light ones during seed drying. The combined treatment of cold-stratification and light quality speeded up the germination. The seeds cold-stratified for 3 weeks and red light-illuminated during the desiccation germinated 100% within 2 days (Fig. 5). The desiccation of the aged and cold-stratified seeds improved the seedling emergence as well as the germination. Moreover, red light exposure during the desiccation enhanced the germination percentage and speed even compared to the dark treatment, being expected to be effective for the higher and more uniform seedling production (Fig. 6). In conclusion, the sequential treatments of aging, cold-stratification, and red light illumination during desiccation can be applied effectively in treating the commercial seeds of burcucumber.

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