J Plant Growth Regul (1992) 11:203–208

Journal of
Plant
Growth
Regulation

Inhibition of Red Light-Induced Seed Germination by Indole-3-Acetic Acid in *Hygrophila auriculata*

Dilip Amritphale, Bharat Singh, and Aarti Gutch

School of Studies in Botany, Vikram University, Ujjain 456 010, India

Received May 18, 1992; accepted August 10, 1992

Abstract. Seed germination in *Hygrophila auriculata* (Schumach.) Haines was found to be under phytochrome control. Exogenous indole-3-acetic acid (IAA) application at concentrations greater than 1×10^{-6} M inhibited germination in the dark, as well as in the light. Red light-induced radicle growth, prior to radicle protrusion through seed coverings and measured as an angle formed by the radicle with the seed axis, was found to be inhibited by IAA. Delay in application of IAA to red light-irradiated seeds resulted in a gradual increase in percent germination, which probably corresponded to the time-course of Pfr action. It is suggested that exogenously applied IAA probably reimposes dormancy in red light-induced seeds of *Hygrophila auriculata*.

Robertson et al. (1976) questioned any regulatory role of indole-3-acetic acid (IAA) in the germination of lettuce seeds. On the other hand, Kopecky et al. (1975) and Nikolaeva (1977) suggested that IAA is involved in the regulation of seed dormancy. Tillberg and Pinfield (1981) and Tillberg (1984), using more sensitive assays for determination of endogenous levels of IAA, disagreed with the observations made by Nikolaeva (1977) which correlated a decrease in the level of endogenous IAA to dormancy removal in *Acer tartaricum* seeds. Lewak (1984) maintained that the role of IAA in dormancy may be restricted to certain species.

Besides conducting studies to correlate endogenous levels of a specific hormone to an observed physiological response, there is also a need to use sophisticated physiological approaches, for example, using isolated embryos with surgical excision of various parts or reducing endogenous hormone levels by leaching (Wareing 1982), to understand the role of hormones in regulation of seed dormancy and germination. Following such an approach, Karssen (1976a,b) recognized four morphological stages in the seed germination of *Chenopodium album* and used them to demonstrate that ABA had no function in regulating dormancy.

In a study on phytochrome control of seed dormancy and germination in *Hygrophila auriculata*, exogenously applied IAA was found to inhibit red light-induced germination of seeds. The seeds of *Hygrophila*, when dry, show a tightly appressed radicle against one of the cotyledons. Interestingly, growth of the radicle before protrusion was found to be under the control of red light and could be quantified by measuring the angle formed by the radicle relative to the seed axis. This offered an opportunity to examine whether exogenously applied IAA inhibits germination by influencing red light-induction and thus plays some role in seed dormancy in *Hygrophila*.

Materials and Methods

Hygrophila auriculata (Schumach.) Haines (Acanthacea) is an annual growing in low-lying areas around Ujjain (23°11′N and 75°43′E). Seeds were collected in March 1991 from the University Campus at time of seed dispersal. After desiccating them in paper bags for 3 days, they were stored in air-tight polyethylene bottles at −10°C in the dark.

Germination Procedure

All germination tests were conducted with 25 seeds per replicate and five replicates per treatment. Seeds were placed in 10-cm glass Petri dishes on cellulose filter papers each moistened with 5 ml of glass distilled water or a solution of IAA (Sigma), which was prepared by dissolving the required quantity in water by the addition of a little NNaOH to pH 7. The dishes were kept at 28 ± 1°C for germination in light-proof polyethylene bags. Germination tests were repeated at least twice and the criterion of germination was emergence of the radicle through the seed coat.

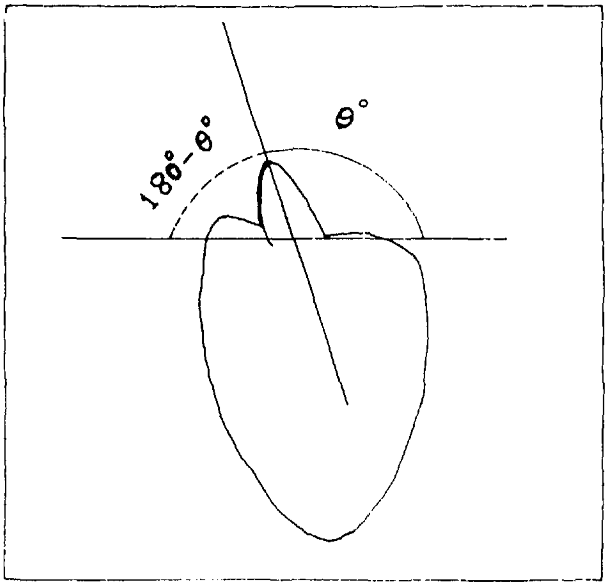

Fig. 1. Diagram showing the method of measuring the radicle angle (θ°) formed by the radicle to the seed axis.

Percent reversibility was calculated in accordance with Mohr (1972, 1984). Standard errors for the percentage germination were calculated after arcsin transformation of the data.

Light Sources and Filters

Light sources and filter combinations have been described elsewhere (Amritphale et al. 1984). The spectral ranges for red and far-red filters were 620–700 nm and 720–800 nm, respectively. The photon flux for red light, 1.5 μmol m^{-2} s^{-1} at 660 ± 5 nm, and for far-red light, 7.5 μmol m^{-2} s^{-1} at 730 ± 5 nm, was measured with a SKYE 660/730 nm Ratio Measuring System—SKR 110/100.

Measurement of Radicle Angle

Seeds were imbibed in the dark in distilled water or in a solution of IAA. After 24 h imbibition, germinated seeds were discarded and the remaining seeds were allowed to continue in the same medium with or without red light. Seeds without light treatment were decoated for radicle angle (θ°) measurement after a total 72-h period of darkness, irrespective of chemical treatment, whereas red light irradiated seeds were decoated 9 and 48 h after light treatment in distilled water and IAA treatment, respectively. Radicle angles were also measured in 25 dry viable seeds as well as in heat-killed seeds imbibed in distilled water for 24 h. The method of measuring the radicle angle is depicted in Fig. 1 and the data represent an average for 25 seeds per treatment.

Effect of Desiccation on Viability of Imbibed Seeds

Seeds were imbibed in the dark in distilled water for 24 h. After discarding the germinated fraction, remaining seeds were exposed to a 10-min red light regime. Nine hours after light exposure (a time period selected to coincide with about 100° radicle angle in nearly 60% seeds), seeds were blotted dry between two layers of filter paper and air-dried in the dark at 28 ± 1°C for 3 days. The seeds were either used immediately for the germination test or stored for 1 month in the dark at 28 ± 1°C.

Results

Phytochrome control of seed germination in *Hygrophila* is known (Amritphale et al. 1989) and the germination response of the present seedlot was found to conform to it (Fig. 2). Concentration of IAA greater than 1×10^{-6} M inhibited the germination of seeds in the dark as well as in the light (Fig. 3). At lower concentrations of IAA, which failed to reduce percent germination, slight to modest retardation of radicle growth was observed (data not shown).

Figure 4a shows the tightly appressed radicle of a dry decoated seed of *Hygrophila*. Noticeable movement of the radicle, which could be attributed to imbibition was observed in dead water imbibed seeds, but it was much less than the growth-associated movement shown by radicles of viable seeds allowed to germinate in distilled water in the dark Fig. 4b and c. Still more significant growth of the radicle was noted in seeds allowed to germinate in distilled water after red light irradiation (Fig. 4d). Figure 4e clearly shows the inhibitory effect of IAA on red light-induced growth of the radicle.

An attempt was made to quantify the effect of IAA on radicle growth by measuring the angle formed by it with the axis of the seed (Table 1). Radicles of dry viable seeds displayed an angle of about 165°, whereas heat-killed seeds showed 144° angle after 24 h imbibition in distilled water. Seeds kept in distilled water in the dark for 72 h (excluding those which had already germinated) showed a growth movement of radicles from 165 to 125°. Nearly 60% of red light-treated seeds, which were preimbibed in distilled water for 24 h in the dark, showed radicle movement from 165 to 100° after 9 h of light exposure, and in the next 9 h radicles had protruded in more than half of the seeds. On the other hand, radicles of the seeds preimbibed in the dark for 24 h in IAA failed to respond to red light as evident from the 131° angle, even 48 h after the light treatment.

Air-drying the seeds of *Hygrophila*, after the radicle had attained an angle of about 100° in nearly 60% seeds, did not cause any loss of viability as evident from a high percentage of germination when allowed to reimbibe in distilled water (Table 2). Furthermore, extending dry storage at 28°C for 30 days after air-drying had no apparent adverse effect on

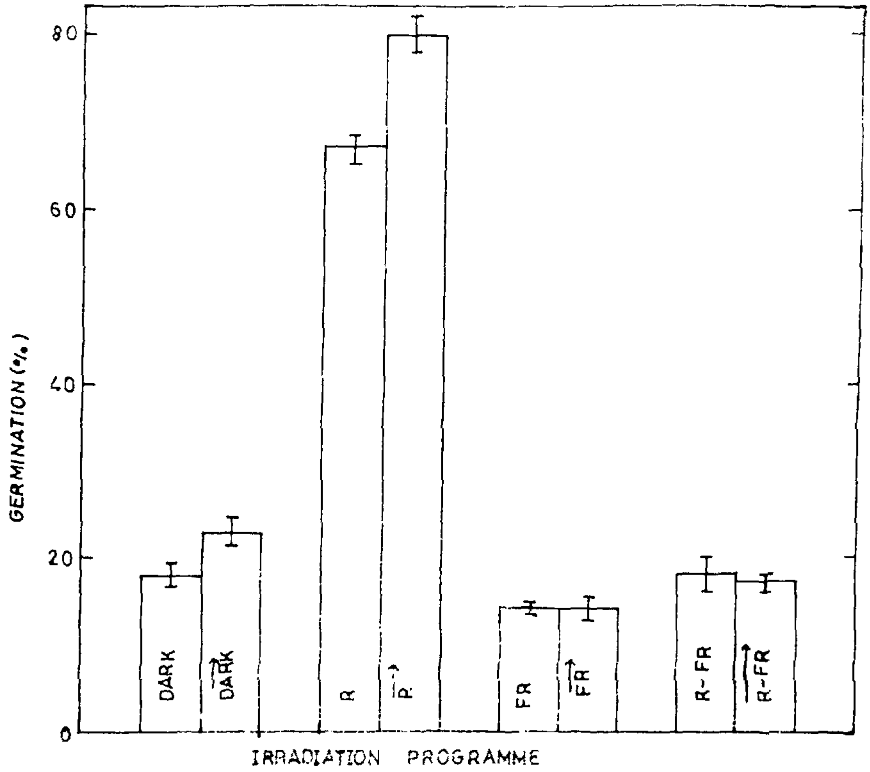

Fig. 2. Red (R)-far red (FR) reversibility and effect of seed transfer in dormancy-breaking. "→" indicates seed transfer.

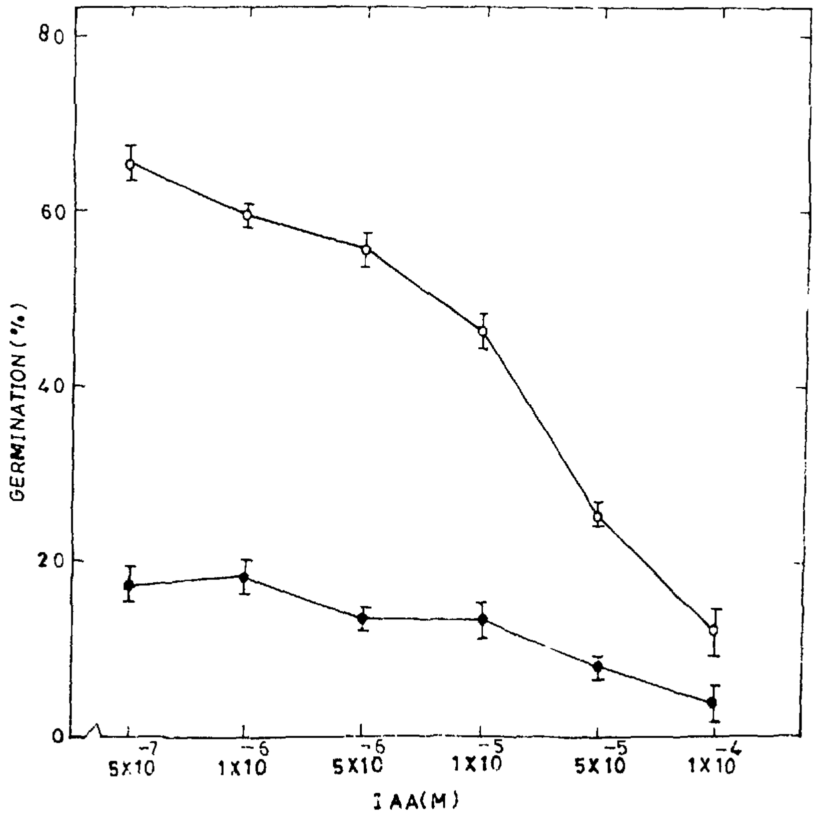

Fig. 3. Percent germination of seeds treated with IAA. Dark (●—●); red light (○—○).

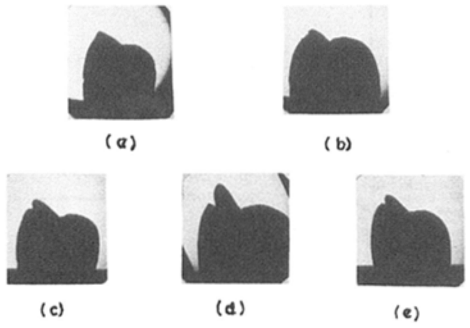

Fig. 4. Seeds decoated and photographed to show the angle formed by the radicle to the seed axis: (**a**) Embryo from dry viable seed. (**b**) Embryo from dead seed allowed to imbibe in distilled water for 24 h. (**c**) Embryo from seed allowed to germinate in distilled water in dark for 72 h. (**d**) Embryo from seed exposed to a 10-min red light period after 24 h imbibition in distilled water in dark. Growth movement of radicle 9 h after red light irradiation. (**e**) Embryo from seed exposed to a 10-min red light period after 24 h imbibition in IAA (1×10^{-4} M) in dark. Growth movement of radicle 48 h after red light irradiation. Magnification, ×26.

Table 1. Radicle angle (degree) in dry viable seeds and 24-h water-imbibed dead seeds and IAA-treated seeds with or without a 10-min red light irradiation.

Dry viable seed	165 ± 5
Dead imbibed seed	144 ± 4

	Light treatment	
IAA (M)	Dark	Red light
0.0	125 ± 2	100 ± 5
1×10^{-5}	122 ± 2	113 ± 2
1×10^{-4}	129 ± 4	131 ± 4

Twenty-five seeds were imbibed for 24 h in distilled water or IAA solution prior to light treatment. Angle (θ°) was measured 9 and 48 h after red light in distilled water and IAA solution, respectively. In the corresponding dark controls, the angle (θ°) was measured after a total 72-h period of darkness.

seed viability. In contrast, seeds were found to become completely nonviable if air-dried after radicle protrusion as shown by triphenyl tetrazolium chloride and germination tests (data not given).

Escape from photoreversibility on insertion of a dark period between red and far-red light is known in various systems under phytochrome control. *Hygrophila* seeds also showed a gradual escape from red–far-red reversibility and the act of transferring the seeds markedly accelerated its rate (Fig. 5). Since IAA was found to inhibit red light-induced movement of radicle in *Hygrophila*, experiments were conducted where application of IAA was delayed after red light irradiation to allow sufficient time for Pfr action.

Table 2. Effects of desiccation and dry storage on seed viability.

	Germination (%)	
Dry storage (days)	Dark	Red light
0	56 ± 3	73 ± 2
30	53 ± 2	78 ± 2

Seeds were imbibed for 24 h in the dark in distilled water and were given a 10-min red light exposure. After 9 h, when the radicle had attained an angle of about 100°, seeds were air-dried in the dark for 3 days and tested in five replicates of 25 seeds each for viability either immediately or after 30 days dry storage.

Seeds preimbibed for 24 h in dark in 1×10^{-4} M IAA and transferred to the same medium after a 10-min red light period did not show any noticeable increase in germination (Table 3). In contrast, seeds allowed to preimbibe in distilled water for 24 h in dark and transferred to 1×10^{-4} M IAA, immediately after a 10-min red light irradiation period, showed considerably higher percent germination. Three or more hours delay in the transfer of seeds after red light irradiation from distilled water to IAA resulted into a further increase in percent germination.

Discussion

While a few workers, for example, Khan and Tao (1978) understand the germination of seeds to mean resumption of growth of the embryo, which may or may not result in the protrusion of the radicle through the seed coverings, many investigators regard such protrusion as a manifestation of germination. Following the latter concept, Karssen (1976a,b) and Khan and Samimy (1982) precisely located the site of ABA action at the various visible stages of radicle growth preceding germination in *Chenopodium album* and *Rumex crispus* seeds, respectively. They found that ABA effectively prevented light-mediated growth of the radicle, but failed to inhibit light-dependent induction of radicle growth in these species.

Since *Hygrophila* seeds showing a radicle angle of about 100° could be desiccated and stored for 1 month without a loss of viability (Table 2), the stage, considering Karssen's observation (1976a), may be that of growth prior to germination. Figure 4 and the data in Table 1 clearly show, then, that the

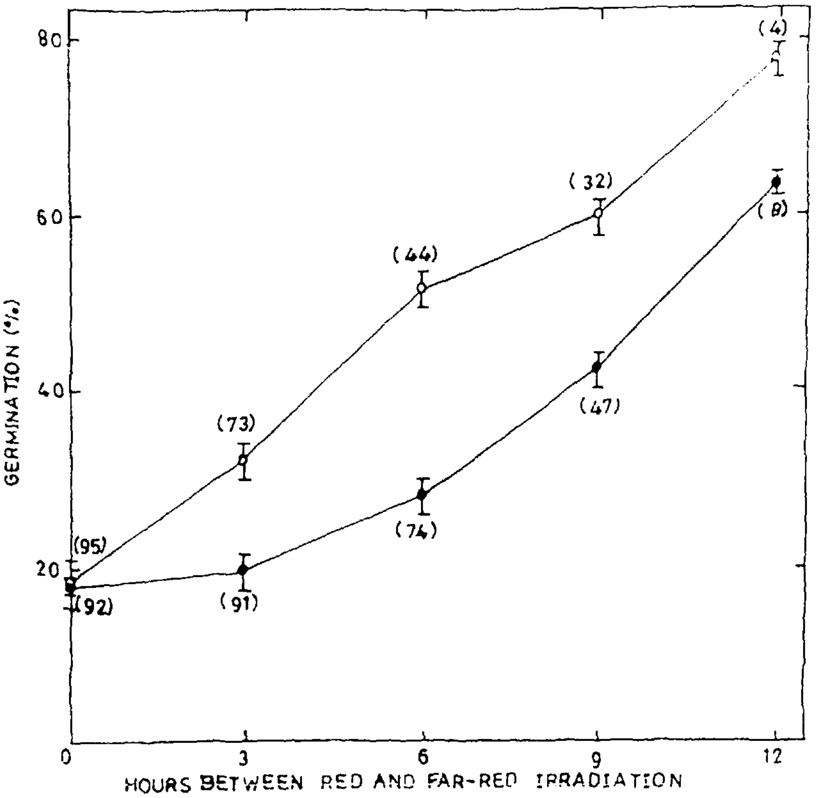

Fig. 5. Effect of seed transfer on escape from red–far red reversibility. % germination: without transfer (●—●); with transfer (○—○). Data in parentheses represent percent reversibility.

Table 3. Effect of the delay in transfer of 10-min red light irradiated seeds to IAA (1×10^{-4} M) on percent germination.

Pretreatment	Light and IAA treatment	Germination (%)
24-h dark imbibition in 1×10^{-4} M IAA	R	12 ± 3
	R → IAA	16 ± 1
24-h dark imbibition in distilled water	R → IAA	27 ± 2
	R—3 h dark → IAA	45 ± 2
	R—6 h dark → IAA	46 ± 1
	R—9 h dark → IAA	55 ± 2
	R—9 h dark—FR → IAA	49 ± 3

Twenty-five seeds in five replicates each were imbibed for 24 h in the dark either in distilled water or IAA prior to a 10-min red light irradiation. "→" indicates seed transfer.

exogenous application of IAA prevents light-dependent induction of radicle growth in *Hygrophila*. Moreover, a gradual increase in percent germination, when IAA was administered to red light irradiated seeds at specific physiological times, probably corresponded to the time-course of Pfr action, again indicating the IAA action on Pfr-induced dormancy breaking.

Thus, our data for radicle growth (Table 1) and the increased percent germination ensuing from a delay in transfer of seeds after light exposure to IAA (Table 3) suggest that exogenously applied IAA reimposes dormancy in red light irradiated seeds of *Hygrophila*.

Acknowledgments. The authors are grateful to Dr. R. C. Verma and Miss Suman Arora for their skillful technical cooperation.

References

Amritphale D, Iyengar S, Sharma RK (1989) Effect of light and storage temperature on seed germination in *Hygrophila auriculata* (Schumach.) Haines. J Seed Technol 13:37–42

Amritphale D, Mukhiya YK, Gupta JC, Iyengar S (1984) Effect of storage, photoperiod and mechanical scarification on seed germination in *Ocimum americanum*. Physiol Plant 61:649–652

Karssen CM (1976a) Uptake and effect of abscisic acid during induction and progress of radicle growth in seeds of *Chenopodium album*. Physiol Plant 36:259–263

Karssen CM (1976b) Two sites of hormonal action during germination of *Chenopodium album* seeds. Physiol Plant 36: 264–270

Khan AA, Samimy C (1982) Hormones in relation to primary and secondary seed dormancy. In: Khan AA (ed) The physiology and biochemistry of seed development, dormancy and germination. Elsevier Biomedical Press, Amsterdam, pp 203–241

Khan AA, Tao KL (1978) Phytohormones, seed dormancy and germination. In: Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds—a comprehensive treatise, vol. II. Elsevier/North Holland Biomedical Press, Amsterdam, pp 371–422

Kopecky F, Sebanek J, Blazkova J (1975) Time course of the changes in the level of endogeneous growth regulators during the stratification of the seeds of the "Panenskeceske," apple. Biol Plant 17:81–87

Lewak S (1984) Hormones in seed dormancy and germination. In: Purohit SS (ed) Hormonal regulation of plant growth and development. Agro Botanical, India, pp 95–144

Mohr H (1972) Lectures on photomorphogenesis. Springer-Verlag, Berlin, pp 1–12

Mohr H (1984) Criteria for photoreceptor involvement. In: Smith H, Holmes MG, (eds) Techniques in photomorphogenesis. Academic Press, London, pp 13–42

Nikolaeva MG (1977) Factors controlling the seed dormancy pattern. In: Khan AA (ed) The physiology and biochemistry of seed dormancy and germination. North Holland, Amsterdam, pp 51–74

Robertson J, Hillman JR, Berrie AMM (1976) The involvement of indole acetic acid in the thermodormancy of lettuce fruits, *Lactuca sativa* cv. Grand Rapids. Planta 131:309–313

Tillberg E, Pinfield NJ (1981) The dynamics of indole-3-acetic acid in *Acer platanoides* seeds during stratification and germination. Physiol Plant 53:34–38

Tillberg E (1984) Levels of endogenous indole-3-acetic acid in achenes of *Rosa rugosa* during dormancy release and germination. Plant Physiol 76:84–87

Wareing PF (1982) Hormonal regulation of seed dormancy—past, present and future. In: Khan AA (ed) The physiology and biochemistry of seed development, dormancy and germination. Elsevier Biomedical Press, Amsterdam, pp 185–202