Ethylene-, Light-, and Prechill-enhanced Germination of *Echinacea angustifolia* Seeds

S.M. Javad Feghahati¹ and R. Neil Reese²

Department of Biology and Microbiology, Box 2140 D, South Dakota State University, Brookings, SD 57007

Additional index words. coneflower, seed dormancy, scarification, prechill, ethephon

Abstract. *Echinacea angustifolia* DC., the common coneflower of the western Great Plains, is difficult to propagate by achenes due to inherent seed dormancy. The effects of light and prechilling on seed germination were examined, alone and combined with scarification (mechanical, acid) and ethylene (ethephon) treatments. The results showed that a 2-week prechill treatment combined with ethephon and continuous light, followed by a 2-week germination period in light (16 hours per day) at 25C, could induce >95% seed germination in *E. angustifolia*. This was a significantly higher percentage of germination over a shorter period of time than any other method examined or previously described. This treatment also synchronized germination, with most viable seeds germinating in <1 week after being placed at 25C in the light. Chemical name used: 2-chloroethylphosphonic acid (ethephon).

The genus *Echinacea* (Asteraceae) is comprised of nine species, commonly referred to as purple coneflowers, that are indigenous to North America (McGregor, 1968). All of the species are perennials, with many-flowered heads. A single series of ray flowers is much elongated and sterile. The stems arise from vertical or horizontal root stocks that are generally well developed and are very pungent to the taste (Foster, 1991; McGregor, 1968). The three most common species are widely distributed east of the Rocky Mountains: *E. purpurea* (L.) Moench, the common purple coneflower, is found from Michigan south into Georgia and west into Kansas; *E. pallida* (Nutt.) Nutt., the pale purple coneflower, is found from Wisconsin and Indiana south to Arkansas and Texas; and *E. angustifolia*, the narrow-leafed purple coneflower of the western Great Plains, is distributed on dry prairies from Texas to Saskatchewan and from west of the Rocky Mountains to Minnesota (Foster, 1991; McGregor, 1968).

Echinacea can be propagated by achenes, division of off-shoots from the crown, or by planting 10- to 12-cm root sections. Propagation by achenes is limited by embryo or seed-coat dormancy and usually requires scarification or prechilling (stratification) to increase germination (Baskin et al., 1992; Smith-Jochum and Albrecht, 1987). Reports indicate that scarification without prechilling (Sorensen and Holden, 1974) or prechilling without scarification (Baskin et al., 1992; Blake, 1935; Hesse, 1973) enhanced germination of *Echinacea* achenes. Emal and Conard (1973) indicated that light exposure enhanced germination. Light and prechill combined have been shown to increase the overall germination of all *Echinacea* spp. examined (Baskin et al., 1992; Smith-Jochum and Albrecht, 1988). Of the three widely distributed species, *E. angustifolia* has been the most resistant to prechill and scarification. Smith-Jochum and Albrecht (1987) were able to obtain only ~50% germination of *E. angustifolia* achenes using a 15-min hypochlorite treatment followed by water soaking or stratifying for 1 or 2 months. Their data showed that a prechill

period longer than 1 month did not improve *Echinacea* seed germination and may have slightly reduced total germination. In contrast, Baskin et al. (1992) showed that a higher percentage of germination of *E. angustifolia* seeds could be obtained with 12 weeks of stratification at 5C followed by 2 weeks at 15/6C in a daily 14-h light/10-h dark cycle. Light during the prechill period clearly increased the germination of *E. angustifolia* seeds under most of the experimental conditions examined by Baskin et al. (1992).

Mechanical scarification and chemical treatments have been shown to break dormancy in many types of seeds. Breaking the seed coat physically, imbibing seeds with water or acid solutions, and applying hormones, alone or in combinations, have all been shown to enhance germination of seeds from various plant species (Bradbeer, 1988; Ross, 1984; Smith, 1973; Wareing, 1982).

Endogenous ethylene production was demonstrated to be important for germination of many dormant and postdormant seeds (Abeles and Lonski, 1969; Corbineau et al., 1990; Logan and Stewart, 1991; Wareing, 1982). Ethylene has been shown to be synthesized in seeds, to be involved in radical elongation, and to increase germination of after-ripened embryos (Ketring and Morgan, 1969; Ross, 1984). The role of ethylene and the mechanisms by which it acts have not been elucidated. However, several members of the Asteraceae family have been shown to require ethylene to break seed dormancy (Abeles and Lonski, 1969; Corbineau et al., 1990; Katoh and Esashi, 1975).

To establish an *E. angustifolia* breeding nursery, an efficient method for germinating large numbers of seeds was needed. The enhancement of germination by light, scarification, and ethylene treatment in conjunction with prechilling was examined and a seed germination protocol was developed.

Materials and Methods

Mature seed heads were collected from plants growing in wild populations after stems had dried but before achenes were released. Heads from randomly chosen individual plants growing in North Dakota were collected in October and November 1989 and bulked to provide a uniform source of seeds for use in preliminary germination studies and development of DNA probe libraries. Achenes that were released from dried heads with little or no manual stimulation were judged to be mature and were used in the experiments. Additional bulked seeds were collected from the South Dakota population in September 1993.

Received for publication 21 June 1993. Accepted for publication 27 Dec. 1993. South Dakota Agricultural Experiment Station publication 2747. Research funded in part by grants from the South Dakota Agricultural Experiment Station, NSF-EPSCOR grant no. OSR-9108773, and the Midwest Plant Biotechnology Consortium. Mention of specific brand names does not imply endorsement of that product over those of other manufacturers. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Graduate student, Plant Science Dept.

²To whom reprint requests should be addressed.

Table 1. Locations of *Echinacea angustifolia* populations.

Population	Location
North Dakota Grassland Station	Kidder Co., T 137 N, R 70 W
Wyoming (Dayton)	Sheridan Co., T 56 N, R 86 W
South Dakota (Sioux Valley Prairie)	Moody Co., T 107 N, R 50 W
Kansas (Tuttle Creek)	Riley Co., T 9 S, R 7 E
Nebraska (Meadowlark Prairie)	Lancaster Co., T 12 N, R 5 W

More than 30 heads were collected randomly in late August and early September 1991 from individual plants found growing in each of five populations in North Dakota, Wyoming, South Dakota, Kansas, and Nebraska (Table 1). The heads were desiccated and stored individually in paper bags at 5C for use in germination studies and subsequent planting into a breeding nursery.

In initial experiments, the effects of light and prechill on germination of *E. angustifolia* seeds were examined for 0, 1, or 2 weeks. In subsequent experiments, prechilling for 2 weeks in the light was standard unless otherwise noted. In all experiments, mature fruit were coated with 10% Vitavax 200 (Gustafson Co., McKinney, Texas) using 0.5 ml/100 achenes to protect them from fungal contamination, and were then evenly distributed on 11 × 11-cm blotters (Anchor Paper Co., St. Paul, Minn.) that were saturated with deionized water or various aqueous solutions. Fifty achenes were placed in each 12 × 12-cm plastic germination box (Tristate Molded Plastics Co., Albany, Ore.). The achenes were prechilled at 4C with constant light [4.2×10^{-6} mol·m⁻²·s⁻¹ photosynthetically active radiation (PAR)] from cool-white fluorescent tubes. Moisture levels were maintained in the boxes by adding the appropriate solutions as needed.

After the prechill period, the boxes were transferred to a growth chamber at 25C, with light provided by cool-white fluorescent tubes (1.65×10^{-4} mol·m⁻²·s⁻¹ PAR) for 16 h/day. Deionized water was added as needed. Percent germination of seeds was determined at 7 and 14 days. Seeds were considered germinated when the radicle and cotyledons became visible.

The second set of experiments examined the effects of water leaching, mechanical scarification, and ethephon, in addition to prechilling and light, on seed germination in *E. angustifolia*. Achenes were leached with water by placing them in an 8 × 15-cm cotton sample bag in a plastic bucket and rinsing continuously with cold tap water for 14 h per the procedure of Lambert (1989). The achenes were blotted on paper towels and transferred to the germination boxes.

Mechanical scarification was accomplished by gently rubbing mature achenes between two sheets of no. 200 grit sand paper and removing the chaff with a gentle stream of air. Seeds showing visible damage to the embryo were removed.

Achenes were acid-scarified by soaking in 10 ml of 40% or 50% H₂SO₄ for 5 min in a beaker, as described by Rubida and Gutormson (1989). After acid scarification, the achenes were transferred to a wire mesh strainer, rinsed with running tap water for 4 to 5 min, and spread out on paper towels for 12 to 16 h to air dry before being placed on blotters in the seed germination boxes.

For treatment with ethephon, mature achenes were transferred into germination boxes containing two blotters saturated with 12 ml of 1.0 mM ethephon (Ruud, 1976). The achenes were prechilled for 1 or 2 weeks in the light as indicated. Ethephon solution was added as needed to maintain moisture and ethylene levels.

To examine the relationship between seed size and germination, achenes (about one-third of the total) were selected using a seed blower (model 757; Seedburo, Chicago). Seed samples were

blown for 3 min with the air regulator opened to 3.5 cm. The heavier achenes that remained in the hopper were collected and germinated in the presence of ethylene with or without prechill and light treatments.

A fourth set of experiments was conducted to determine if the effects of ethephon were the result of ethylene, low pH, or increased phosphate availability. Blotters were treated with H₂O, the 1 mM ethephon solution, 1 mM phosphoric acid, or water acidified to pH 3.8 with HCl to match the acidity of the ethephon. Ethylene concentrations in the seed germination boxes were measured by removing 1 ml of air with a gas-tight syringe and injecting it into a gas chromatograph (model 6000; Varian, Palo Alto, Calif.) equipped with a Chromosorb 101 column. Ethylene standards were purchased from Supelco, Bellefonte, Pa.

To establish a breeding nursery, achenes were collected from 30 to 40 individual plants from each of the five populations (Table 1). All samples were germinated as described previously, except that incubation at 25C was allowed to continue for 3 weeks to ensure that all germinating seeds were collected. Seedlings were transferred into 2.5 × 15-cm tubes containing a porous soil mix (50% perlite) for transplanting into the field. Viability of some seed lots was determined by a standard tetrazolium test (Grabe, 1970).

One-way analyses of variance of the germination data were conducted using SAS's (Cary, N.C.) general linear model (GLM) procedure. Differences in main effects were detected using the least-squares means procedure.

Results and Discussion

The mechanisms controlling seed dormancy in *Echinacea* are not well understood. Embryo dormancy and dormancy imposed either mechanically or chemically by the pericarp, testa, or other surrounding tissues may be involved (Bradbeer, 1988). Initial attempts to germinate *E. angustifolia* seeds showed that seed maturity was an important factor in germination. Achenes that released from heads without some form of mechanical stimulation consistently germinated more readily than seeds that had to be physically removed from the heads. Germination percentages of the mature *E. angustifolia* seeds were relatively low in the absence of some form of scarification or prechilling. When *E. angustifolia* seeds were prechilled for up to 2 weeks in the dark, percent germination did not increase significantly above those of controls that were not prechilled (Fig. 1). Highest germination in any replication was only ≈15%. Continuous light during the prechill period significantly increased the percentage of germinating seeds. One week prechill in light resulted in ≈20% germination, and 2 weeks of treatment increased germination to 30% (Fig. 1). The data from this study (Fig. 1) agree with the findings of Smith-Jochum and Albrecht (1987) and Baskin et al. (1992), both in regard to the synergistic effects of light and low temperatures on germination, and that a 2-week prechilling induced <20% germination. Prechilling and light treatments have been used singly and together to break seed dormancy in many plant species (Bradbeer, 1988). The brief course of treatment (2 weeks) is extremely short compared to that needed for seeds of most wild plant species, especially with regard to the cold treatment (Baskin et al., 1992; Bradbeer, 1988; and Wareing, 1982).

Imbibing and leaching-potential germination inhibitors from achenes with running water did not increase the percent germination of *E. angustifolia* seeds compared to control seeds given only the prechill treatment (Fig. 2). These results suggest that the pericarp does not prevent germination by releasing water-soluble inhibitors.

Fig. 1. Effects of light and prechill on the percent germination (mean $\pm$ SE) of *Echinacea angustifolia* seeds; 0/-, no prechill; 1/-, 1 week prechill in the dark; 2/-, 2 weeks prechill in the dark; 1/+, 1 week prechill in the light; 2/+, 2 weeks prechill in the light.

Fig. 2. Effects of mechanical and chemical treatments on germination (mean $\pm$ SE) of *Echinacea angustifolia* seeds after a 2-week prechill treatment with continuous light. All treatments except ethephon were conducted before prechilling. Ethephon was applied concomitantly with the prechill. The 1-week ethephon treatment differed from the others in that the total prechill period was only 7 days. Con = no additional treatment; Water = leaching in running water for 14 h; Mech = scarified with sandpaper; 40% and 50%, 5 min soak in 40% or 50% sulfuric acid, respectively; Eth-1 = ethephon-treated in the light at 4C for 1 week; Eth-2 = ethephon-treated in the light at 4C for 2 weeks.

Fig. 3. Germination (mean $\pm$ SE) of selected heavy *Echinacea angustifolia* seed; 0/-, no prechill; 2/+, 2 weeks prechill with continuous light; e0/-, ethephon applied on day of planting, no prechill period; e1/+, 1 week ethephon and prechill treatment; e2/+, 2 weeks ethephon and prechill treatment.

Fig. 4. Ethylene concentration in the head space in unsealed germination boxes containing *Echinacea angustifolia* seeds treated with 1 mM ethephon during a 2-week prechill period in the presence of light.

Fig. 5. The effect of ethephon, phosphate, and low pH on *Echinacea angustifolia* seed germination, with a 2-week prechill in the light. Con = water, pH 7; Phos = 1 mM phosphoric acid, pH 3.8; HCl = hydrochloric acid, pH 3.8; Eth = 1 mM ethephon, pH 3.8.

The effects of mechanical scarification on germination were similar to that of treatment control. However, mechanical scarification damaged the seeds such that 10% of the achenes could not be used. Mechanical scarification did not increase germination levels significantly above those of controls that were merely prechilled in the light for 2 weeks. Therefore, inhibition of germination of *E. angustifolia* seeds is probably not the result of physical limitations to imbibition imposed by the seed coat.

Scarification of seeds with strong acid has proven effective when used on seeds from many species that are difficult to germinate. In a preliminary trial, *E. angustifolia* achenes were treated with different concentrations of sulfuric acid (30%, 40%, 50%, 60%, 70%, or 80%) for 5 min as described by Rubida and Gutormson (1989). The data (not shown) indicated that <40% acid had no effect on germination and >50% damaged the seeds severely. Forty percent acid treatment for 5 min did not significantly enhance seed germination in this study (Fig. 2), and the higher concentrations of acid actually reduced germination. These data are consistent with those above and suggest that dormancy in *E. angustifolia* is probably not imposed by tissues surrounding the embryo.

Ethylene has been shown to effectively stimulate seed germination, with administration of ethephon being a simple and direct method for increasing ethylene level (Adkins and Ross, 1981; Mekki and Leroux, 1991). In wild oats (*Avena fatua* L.), ethylene increased germination only when the treatments were applied at cool temperatures, and warm temperatures reduced or overcame the ethylene-induced germination.

Ethylene treatment of *Echinacea* seeds differed from the other treatments in this set of experiments in that ethephon was applied during the prechill period and both 1- and 2-week prechill treatments were made. Two weeks of prechilling in the light with ethephon resulted in ~80% germination (Fig. 2), whereas the

Table 2. Mean percent germination of *Echinacea angustifolia* seeds from five populations treated as described in the ethylene protocol.

Population	N ^z	Germination (%)	Max (%) ^y	Min (%) ^y	Abnormal (%) ^x
North Dakota	32	56.4 ab ^w	98	2	9.2 ab
Wyoming	41	63.1 a	95	0	3.0 a
South Dakota	33	46.0 b	93	0	10.6 b
Kansas	38	30.3 c	64	0	13.8 b
Nebraska	30	28.6 c	65	0	28.1 c

^zN = the number of individual seed heads.

^yMax and Min percentages refer to percentage of germination of seeds from individual heads within the population indicated.

^xAbnormal = percentage of total number of germinating seeds that failed to develop normally, usually dying within a few days of germinating.

^wMeans in the same column not having the same letters are significantly different at $P = 0.05$ by LSD.

1-week prechill with ethylene did not increase germination above controls or above the germination induced by 1 week of prechill in the light (Figs. 1 and 2). Ethephon treatment accompanying prechilling of *E. angustifolia* achenes for 2 weeks in the light was the most effective treatment for inducing germination in these seeds.

In addition to significantly increasing the mean percent germination, the 2-week ethephon treatment accelerated and synchronized germination of the seeds after they were transferred from the prechill chamber to the growth chamber. At 25C, the ethephon-treated seeds started to germinate 2 to 3 days after being transferred, with most of the seeds germinating within 1 week, whereas, germination of the seeds after the other treatments generally occurred slightly later and was more evenly distributed over the 2-week period.

Germination of seeds selected by specific density using the seed-blower is shown in Fig. 3. These results clearly demonstrate that the heavier (and typically larger), more developed seeds have a greater potential to germinate than the seed population as a whole. Selection of seeds by weight increased germination significantly in controls and in the ethephon treatments (10% vs. 25% germination in controls without prechill; 77% vs. 97% for 2-week ethephon-treated seeds). The ethephon treatment induced nearly all of the heavier *E. angustifolia* seeds to germinate after only a 2-week prechill treatment. Careful scrutiny of the lightweight achenes left behind in the blower showed that, although many contained damaged or nonviable seeds, many seeds were viable.

Ethylene and phosphate are both slowly released during the degradation of ethephon at low pH (Ernst et al., 1992). Acidic conditions and either of the degradation products could contribute to the increased seed germination. Therefore, a set of experiments was completed to examine the effects of these factors on seed dormancy and germination. Ethylene production in the seed boxes in response to ethephon application occurred during the entire 2-week prechill period (Fig. 4), with the highest levels being measured during the first week. No measurable ethylene was detected in control or acid-treated seed boxes, and no ethylene was detected in the ethephon-treated boxes after deionized water was added and the seeds were placed into the 25°C growth chamber. Figure 5 shows the percent germination of seeds treated with ethylene compared to low pH or phosphate. The increased percent germination of *E. angustifolia* seeds in response to ethephon seems to be due to the effects of ethylene, as phosphate or low pH alone had no effect on total germination.

The ultimate goal of developing a germination protocol for *Echinacea* was to maximize the number of individuals in seed families derived from each of the seed-heads collected as representatives of the five midwestern populations (Table 2). In this study, all of the achenes produced by an individual plant, without selecting for maturity or size, were placed in a seed box and germinated using the ethephon protocol. The results show that the production of germinating seed varied greatly between and within the five populations, with germination ranging from 28% to 63%. The Kansas and Nebraska populations had seeds that showed the lowest mean percent germination at 30% and 28%, respectively (Table 2).

The cause of the low percentage of seed germination in the Kansas and Nebraska populations was examined with seeds from several samples not used in the original germination study. Tetrazolium viability tests revealed that the low percentage of germination was matched by low seed viability (47.6% $\pm$ 7.2% and 37.3% $\pm$ 4.5% viable seed, Kansas and Nebraska populations, respectively).

In addition to low seed viability, seedling vigor was also much lower in the collections from Kansas and Nebraska. The last column in Table 2 shows the mean percentage of seedlings that germinated but that did not develop normally. These seedlings were twisted and failed to develop normal roots and shoots. Although occurring in all of the populations, the number of abnormal seedlings was especially high in the Kansas and Nebraska populations, in which 14% and 28% of the germinating seeds failed to grow, respectively. Examination of fruit from the five populations revealed a higher incidence of insect damage in achenes from Kansas and Nebraska; this may have significantly lowered germination percentages. Additionally, preliminary examination of two *E. angustifolia* populations near Brookings, S.D., suggests that some populations may produce inherently low levels of viable seeds. Germination of seeds from these two populations

has differed significantly over the past 2 years, with germination of seeds from one location being consistently <50% while germination of seeds from the second population has been >80%.

Summary

The data demonstrate that dormancy can be released in seeds of *E. angustifolia* by prechilling (4°C) mature seeds for 2 weeks with ethephon and continuous light. All three treatment conditions must be used, as lack of any one of them resulted in significantly lower germination levels.

The lack of induction of germination by mechanical and chemical scarification and the ineffectiveness of water leaching the fruit suggests that dormancy of the *E. angustifolia* seeds is probably not imposed by physical or chemical factors located in the pericarp, seed coat, or other surrounding structures. This conclusion is further supported by the release from dormancy and resulting increase in germination of the seeds exposed to ethylene during the prechill treatment.

Literature Cited

- Abeles, F.B., and J. Lonski. 1969. Stimulation of lettuce seed germination by ethylene. *Plant Physiol.* 44:277–280.
- Adkins, S.W., and J.D. Ross. 1981. Studies in wild oat dormancy. I. The role of ethylene in dormancy breakage and germination of wild oat seeds (*Avena fatua* L.). *Plant Physiol.* 67:358–362.
- Baskin, C.C., J.M. Baskin, and G.R. Hoffman. 1992. Seed dormancy in the prairie forb *Echinacea angustifolia* var. *angustifolia* (Asteraceae): Afterripening pattern during cold stratification. *Intl. J. Plant Sci.* 153:239–243.
- Blake, A.K. 1935. Viability and germination of prairie forb seed. *Ecol. Monogr.* 5:405–460.
- Bradbeer, J.W. 1988. Seed and germination. Chapman & Hall, New York. p. 38–94.
- Corbineau, F., S. Bagniol, and D. Come. 1990. Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. *Israel J. Bot.* 39:313–325.
- Emal, J.G. and E.C. Conard. 1973. Seed dormancy in Indiangrass as effected by light, chilling and certain chemical treatments. *Agron. J.* 65:383–385.
- Ernst, R., J.F. Bjornsen, and J. Arditti. 1992. Effects of ethephon, its nonethylene-generating analog ethylphosphonic acid, and phosphorus acid in aseptic culture of orchid seedlings. *Amer. J. Bot.* 79:275–278.
- Foster, S. 1991. *Echinacea: Nature's immune enhancer*. Healing Arts Press, Rochester, Vt.
- Grabe, D.F. 1970. Tetrazolium testing handbook for agricultural seeds. AOSA Publications, Contrib. 29. p. 62.
- Hesse, M.C. 1973. Germination of seven species of wildflowers as affected by different pregermination conditions. MS thesis. Univ. of Nebraska, Lincoln.
- Kato, H. and Y. Esashi. 1975. Dormancy and impotency of cocklebur seeds. I. CO₂, C₂H₄, O₂ and high temperature. *Plant Cell Physiol.* 16:687–696.
- Ketring, D.L. and P.W. Morgan. 1969. Ethylene as a component of the emanations from germinating peanut seed and its effect on dormant Virginia-type seeds. *Plant Physiol.* 44:326–330.
- Lambert, H. 1989. Cal West Seed Co. and Interstate Seed Co. Brochure 8/21/89.
- Logan, D.C. and G.R. Stewart. 1991. Role of ethylene in the germination of the hemiparasite *Striga hermonthica*. *Plant Physiol.* 97:1435–1438.
- McGregor, R.L. 1968. The taxonomy of the genus *Echinacea* (Compositae). *Univ. Kansas Sci. Bul.* 48.
- Mekki, M. and G.D. Leroux. 1991. False chamomile seed germination requirements and its enhancement by ethephon and nitrate. *Weed Sci.* 39:385–389.
- Ross, J.D. 1984. Metabolic aspects of dormancy, p. 45–76. In: D.R. Murray (ed.). *Seed Physiology*, vol. 2. Germination and reserve mobilization. Academic Press, San Diego.
- Rubida, A.L. and T.J. Gutormson. 1989. Determining viability of green needlegrass (*Stipa viridula* Trin.). *J. Seed Technol.* 12:107–112.
- Ruud R. 1976. The use of ethephon to break dormancy of sunflower seeds in a germination test. *AOSA Nwsl.* 50:43–44.
- Smith-Jochum, C. and M.L. Albrecht. 1987. Germination of three *Echinacea* species. *Acta Hort.* 208:115–120.
- Smith-Jochum, C. and M.L. Albrecht. 1988. Transplanting or seeding in raised beds aids field establishment of some *Echinacea* species. *HortScience* 23:1004–1005.
- Smith, H.L. 1973. Ethephon versus predrying for testing dormant peanut seed. *AOSA Nwsl.* 47:58.
- Sorensen, J.T. and D.J. Holden. 1974. Germination of prairie forb seed. *J. Range Mgt.* 27:123–126.
- Wareing, P.F. 1982. Hormonal regulation of seed dormancy—Past, present and future, p. 185–202. In: A.A. Khan (ed.). *The physiology and biochemistry of seed development, dormancy and germination*. Elsevier Biomedical Press, Amsterdam.