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Seed Germination of Seabeach Amaranth (*Amaranthus pumilus*) in Response to Temperature, Light, and Gibberellin A₃ Treatments¹

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Abstract

Seeds of seabeach amaranth (*Amaranthus pumilus* Raf.) stored at 4C (39F) for approximately 1 year (harvested September 2003) and freshly harvested seeds (October 2004) were soaked in November 2004 in solutions of the potassium (K) salt (K-salt) of gibberellin A₃ (K-GA₃) at 0, 100, 500, or 1000 mg/liter (ppm) for 24 hr in darkness. After treatment, seeds were germinated at 25C (77F) or at an 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness) or 16 hr. Germination was recorded every 3 days for 30 days. Stored and fresh seeds responded similarly. However, the 2003 seeds had greater viability (percent germination) and vigor (germinated faster) and these data are presented. Regardless of germination temperature and photoperiod, nontreated seeds [0 mg/liter (ppm) K-GA₃] did not germinate. When germinated at 25C (77F) the response of seeds to K-GA₃ treatment was linear for both photoperiods with significantly greater total (30-day) germination occurring in the dark for seeds treated with 100, 500, or 1000 mg/liter (ppm) K-GA₃. At 25C (77F), the greatest total germination (84%) was observed for seeds treated with 1000 mg/liter (ppm) K-GA₃ and maintained in darkness, whereas for seeds exposed to a 16-hr photoperiod, maximum germination was 72%. At 30/20C (86/68F) the response to K-GA₃ was quadratic with maximum germination at predicted rates of 882 and 875 mg/liter (ppm) K-GA₃ (88 and 92%, respectively) for photoperiods of 0 and 16 hr, respectively. Treatment of nonstratified seeds of seabeach amaranth with K-GA₃ removed physiological (embryo) dormancy and eliminates the need for stratification (moist-prechilling). Treatment also reduced sensitivity of the seeds to light, and appeared to broaden the range of temperatures for germination.

Index words: sexual propagation, beach restoration, seed dormancy, seed stratification, Amaranthaceae, recovery plans, threatened species, dune species.

Significance to the Nursery Industry

To remove physiological (embryo) seed dormancy of seabeach amaranth (*Amaranthus pumilus*), a species federally listed as 'threatened,' the seeds must be stratified (moist-prechilled) for 84 to 120 days. Results herein indicate treatment of seeds for 24 hr with a solution of 1000 mg/liter (ppm) K-GA₃ will remove physiological dormancy without the need of lengthy stratification. Treatment with K-GA₃ also eliminates the need for light to maximize germination and may broaden the range of temperatures at which germination occurs.

Introduction

Seabeach amaranth is a summer annual native to the beaches and barrier islands of the Atlantic Coast. The plant once ranged from Massachusetts to South Carolina (9). By 1990 it no longer occurred in six of the nine states of its original range with the remaining populations occurring in New York, North Carolina, and South Carolina (9). Elimination of two-thirds of its historic range, and vulnerability of the plant to various threats,

both natural and human, caused seabeach amaranth to be listed as 'threatened' by the U.S. Fish and Wildlife Service in 1993 (7). As a result of the threatened status of the plant, a recovery plan was developed by Weakley et al. (9).

One concern regarding loss of the species is that seabeach amaranth plays an important role in the initial stages of the development of sand dunes by trapping and binding sand on the beach (8, 9). Ecologists also view the plant as an indicator of the vitality and vigor of a beach ecosystem. Thus, various state and federal agencies are interested in restoring the species to areas where it once grew. In addition, beach restoration and sand renourishment projects have created a demand for seedling transplants of seabeach amaranth that are currently unavailable.

To establish seabeach amaranth in locations where it was once endemic and to meet the demand for transplants will require protocols for propagation and culture. One approach may involve production of seedlings that can then be planted in suitable beach environments. Some research has been reported regarding sexual propagation, specifically seed germination (2, 4), but more research is needed. If production protocols are developed, these would provide opportunities for growers to produce and sell plants to federal, state, and private agencies for recovery efforts.

Researchers have determined that freshly harvested seeds of seabeach amaranth are physiologically dormant (have embryo dormancy) and require a period of stratification to break (release) dormancy (2, 4). Stratification of 84 to 120 days is necessary to remove physiological dormancy completely followed by germination at high temperatures [e.g., 8/16-hr thermoperiod of 30/20 (86/68F)] with light (e.g., a daily 16 hr photoperiod) to achieve maximum germination (2, 4). The benefit of stratification raises the issue of whether a growth regulator such as gibberellic acid (GA) can ease

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germination by eliminating the need to stratify. Physiological seed dormancy of many species can be removed by treatment with various gibberellins, most notably GA_3 or GA_{4+7} (3, 5). Therefore, the following research was conducted to study the influence of temperature, light, and GA treatment on seed germination of seabeach amaranth.

Materials and Methods

Mature utricles (one-seeded, small, indehiscent, bladderly fruit) of seabeach amaranth were collected from plants growing on Oak Island, North Carolina, on September 15, 2003. The plants were growing on the beach berm in the area between the high tide line of the Atlantic Ocean and the toe of the frontal dune. As the utricles were collected they were placed in a paper bag, and left to dry at 21C (70F). On October 6, 2003, the utricles, still in the paper bag, were transported to Raleigh and the fruit were placed in a plastic dish pan for additional drying at 21C (70F). From October 16 to October 20, 2003, the seeds were extracted by hand by rubbing them between the palms. Extracted seeds were placed in a covered glass petri dish and stored in the dark at 21C (70F). After extraction, seeds were cleaned manually by removal of chaff and other debris, and left in the dark at 21C (70F). On November 18, 2003, the seeds were placed in a glass bottle, the bottle was sealed, and the bottle placed in a refrigerator maintained at 4C (40F).

On October 22, 2004, mature fruit of seabeach amaranth were collected from plants growing on Emerald Isle, North Carolina. These plants were also growing in a beach envi-

ronment like that of the plants from which fruit were collected in September 2003. The utricles were handled like those harvested in September 2003 and they were transported the same day collected to Raleigh for drying, seed extraction, and cleaning as described for the 2003 seeds. Following cleaning, these seeds were stored in the dark at 21C (70F).

On November 3, 2004, the 2003 and 2004 seeds were removed from storage and graded under a dissecting scope to remove abnormal or damaged seeds and any debris not removed by previous cleaning. From the graded seeds, four lots consisting of 800 seeds per lot of the year 2003 seeds, and four lots consisting of 800 seeds each of the year 2004 seeds were removed from the graded seeds. Each lot of 800 seeds was placed in a 125-ml Erlenmeyer flask containing 50 ml of solution of the potassium (K) salt (K-salt) of GA_3 (K- GA_3) at 0, 100, 500, or 1000 mg/liter (ppm). The solutions were prepared by dissolving K- GA_3 in distilled water (pH of distilled water = 6.3) and the control [0 mg/liter (ppm)] was distilled water. Flasks were wrapped with aluminum foil to exclude light, and placed on a rotary shaker (100 revolutions per minute) for 24 hr. Next, seeds were removed from the flasks and placed in covered, 9-cm diameter glass petri dishes (50 seeds per dish) with each dish containing two germination blotters uniformly moistened with tap water. The blotters had been soaked previously in tap water for 48 hr. Dishes were placed in black sateen cloth bags and left overnight at 21C (70F). The next day, dishes were randomized within two growth chambers [C-chambers (6)] at the Southeastern Plant Environment Laboratory (NC State University Phytotron, Raleigh). The chambers were maintained at 25C

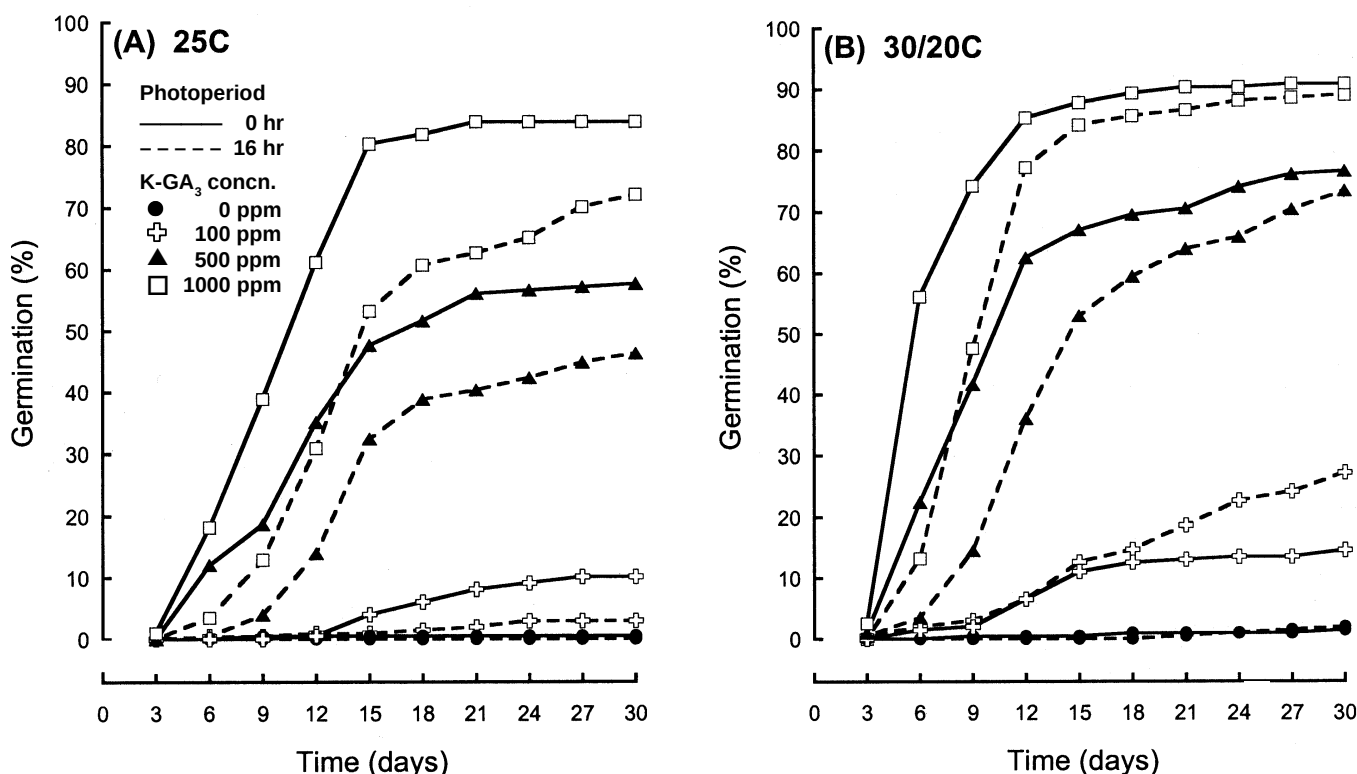


Fig. 1. Influence of temperature, photoperiod, and K- GA_3 treatments on time course of seed germination of nonstratified seeds of seabeach amaranth. Following treatment with 0, 100, 500, or 1000 mg/liter (ppm) K- GA_3 for 24 hr, seeds were germinated at (A) 25C (77F) or at an (B) 8/16-hr thermoperiod of 30/20C (86/68F) with daily photoperiods at each temperature of 0 (total darkness) or 16 hr. Legend in (A) also applies to (B). Symbols in (A) and (B) represent mean percent germination of four petri dishes each containing 50 seeds.

(77F) or an 8/16-hr thermoperiod of 30/20C (86/68F). Chamber temperatures varied within 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected to daily photoperiods of 0 (total darkness) or 16 hr. Regardless of temperature, the 16-hr photoperiod treatment was imposed the same time each day. The photoperiod treatment for the alternating temperature of 30/20C (86/68F) began with the transition to the high temperature portion of the cycle.

Growth chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400–700 nm) of approximately 32 $\mu\text{mol}/\text{m}^2/\text{s}$ (2.4 klx) as measured outside the dishes at dish level with a cosine-corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE). Regardless of photoperiod, temperature within the petri dishes, as measured with a thermocouple, never exceeded ambient temperature by more than $\pm 0.5\text{C}$ (0.9F) of the set point. The constant darkness treatment was imposed by keeping the petri dishes in black sateen cloth bags throughout the experiment, and all germination counts and moistening of the blotters for this treatment were performed utilizing a fluorescent lamp equipped with a #122 Roscolux green diffusion filter (Rosco Laboratories, Inc., Stamford, CT). Germination blotters were kept moist throughout the experiment. Seeds showing signs of decay were removed from the dishes when recording germination.

For each temperature and year of seed collection (2003 and 2004), all photoperiod and K-GA₃ treatments were replicated four times with a replication consisting of a petri dish containing 50 seeds. Germination counts were recorded every 3 days for 30 days and germinated seeds were removed from the dishes. A seed was considered germinated when radicle emergence was ≥ 1 mm (0.04 in). Percent germination was calculated as a mean of four replications per treatment. Within each temperature regime, the experiment was a $2 \times 2 \times 4 \times 10$ factorial in a completely random design. The main plots were 2 collection dates/locations, 2 photoperiods, 4 concentrations of K-GA₃, and 10 germination counts (every 3 days for 30 days). All data were subjected to analysis of variance (ANOVA) procedures. Data were analyzed following arsin transformation and results were similar to nontransformed data. Hence, results presented are based on nontransformed data.

Results and Discussion

Even though seeds differed in location and year of collection, and duration of storage, statistical analysis revealed both groups responded similarly although the 2003 seeds had greater viability (percent germination) and vigor (germinated faster). Therefore, subsequent statistical analysis focused on data of the year 2003 seeds and are discussed and presented.

ANOVA showed that within each temperature regime, light, K-GA₃, time (days), and their interactions were highly significant ($P < 0.001$). Thus, within each temperature, K-GA₃ concentration data were fitted to simple linear and quadratic equations within each time $\times$ photoperiod combination. The maximum of the polynomial curve was calculated as a first order derivative of the independent variable where the dependent variable equaled zero. Likewise, within each temperature, cumulative (30-day) germination as influenced by photoperiod within each K-GA₃ concentration was separated by the F test.

Nontreated seeds [0 mg/liter (ppm) K-GA₃] did not germinate regardless of temperature and photoperiod (Figs. 1

and 2). At 25C (77F) the response of seeds to K-GA₃ was linear with significantly greater germination occurring in darkness for seeds treated with 100, 500, or 1000 mg/liter (ppm) K-GA₃ compared to the 16-hr photoperiod (Fig. 2). The greatest total germination at 25C (77F) was 84% for seeds treated with 1000 mg/liter (ppm) K-GA₃ and kept in darkness, whereas for seeds exposed to a 16-hr photoperiod, 72% germination was realized at 1000 ppm K-GA₃. Previous research by Blazich et al. (4) demonstrated seed germination of seabeach amaranth is negligible at 25C (77F) whether or not the seeds are first stratified prior to being placed at 25C and whether or not stratified seeds are subjected to a 16-hr photoperiod at 25C (77F). When nonstratified seeds of seabeach amaranth were treated in the present investigation with K-GA₃, they not only germinated at 25C (77F) but greater germination occurred in darkness as opposed to seeds exposed to light.

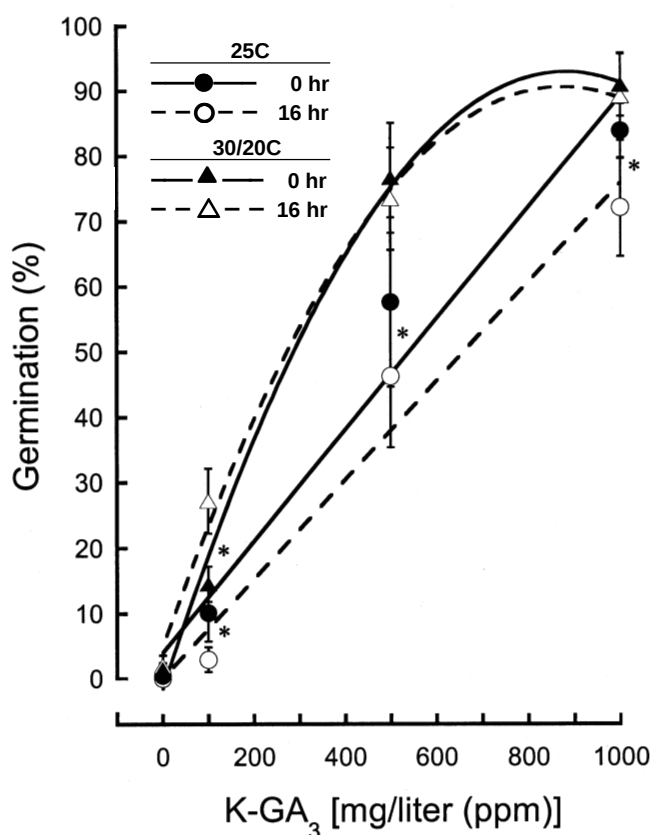


Fig. 2. Cumulative (30-day) seed germination of nonstratified seeds of seabeach amaranth as influenced by temperature, photoperiod, and K-GA₃ treatments. Following treatment with 0, 100, 500, or 1000 mg/liter (ppm) K-GA₃ for 24 hr, seeds were germinated at 25C or at an 8/16-hr thermoperiod of 30/20C with daily photoperiods of 0 (total darkness) or 16 hr. Symbols represent mean percent germination of four petri dishes each containing 50 seeds and vertical bars = ± 1 SE. An asterisk denotes a significant difference at $P \leq 0.05$ between means of dark (0-hr photoperiod) and light (16-hr photoperiod) germination within each K-GA₃ rate at 25C (77F) or 30/20C (86/68F). Regression equations are as follows: 25C (77F), 0 hr photoperiod, $y = 3.92 + 0.085x$, $R^2 = 0.98$; 25C (77F), 16 hr photoperiod, $y = -0.001 + 0.076x$, $R^2 = 0.99$; 30/20C (86/68F), 0 hr photoperiod, $y = -1.5 + 0.214x - 0.0001x^2$, $R^2 = 0.99$; 30/20C (86/68F), 16 hr photoperiod, $y = 4.8 + 0.196x - 0.0001x^2$, $R^2 = 0.99$.

At 30/20C (86/68F) the response to K-GA₃ was quadratic with maximum total germination at predicted rates of 882 and 875 mg/liter (ppm) K-GA₃ (88 and 92%, respectively) for seeds exposed to photoperiods of 0 or 16 hr, respectively (Fig. 2). Photoperiod had no influence on germination of seeds treated with 500 or 1000 mg/liter (ppm) K-GA₃ and germinated at 30/20C (86/68F), but seeds treated with 100 mg/liter (ppm) K-GA₃ and germinated in light had significantly greater germination than seeds in darkness. These results, like those at 25C (77F) are also intriguing because Blazich et al. (4) reported that following seed stratification of seabeach amaranth for 120 days and germination in the dark at 30/20C (86/68F), germination of 49% was observed by day 12 increasing to 50% by day 30, whereas for seeds exposed to a daily 16-hr photoperiod, 82% germination was realized by day 12 and increased to 85% by day 30.

As mentioned previously, the present research included seeds collected in 2003 and stored dry for approximately a year at 4C (40F) and seeds collected in 2004 which were stored briefly under dry conditions at 21C (70F) and can be regarded as fresh seeds. Statistical analysis revealed both groups responded in a similar manner although the 2003 seeds had greater viability and vigor. Since the 2003 seeds did not germinate unless treated with K-GA₃, indicates physiological seed dormancy of seabeach amaranth persists over time.

Treatment of seeds with 100 mg/liter (ppm) K-GA₃ was only slightly effective in removing physiological dormancy of seabeach amaranth in comparison to treatment of seeds with 500 or 1000 mg/liter (ppm) K-GA₃ (Figs. 1 and 2). Reduced effectiveness of K-GA₃ may have simply been a dose response. Another possible explanation may have been related to the pH of the 100 mg/liter (ppm) K-GA₃ solution. Following preparation of the K-GA₃ solutions with distilled water (pH = 6.3), the pH of the 100, 500, and 1000 mg/liter (ppm) K-GA₃ solutions was 5.8, 3.9, and 3.7, respectively. Arnold et al. (1) reported removal of embryo dormancy of seeds of dwarf snapdragon [*Chaenorrhinum minus* (L.) Lange] by treatment with various gibberellins was influenced by many factors including solution pH, concentration of the

solutions, duration of treatment, and the kind/type of gibberellin used to treat the seeds. They also observed pH optimum varied between seeds stored for 10 months at room temperature and freshly harvested seeds.

Since a seed was considered germinated when radical emergence was ≥ 1 mm (0.04 in), the authors did not observe subsequent development of the germinated seeds to determine if the K-GA₃ treatments, particularly at the higher concentrations of 500 and 1000 ppm, had any deleterious effects on subsequent seedling growth. However, observations from a later nonreplicated study indicated seedlings resulting from seeds treated with 1000 ppm K-GA₃ grew normally.

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