Industrial Crops and Products 32 (2010) 111–117

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Effect of light, gibberellic acid and abscisic acid on germination of guayule (*Parthenium argentatum* Gray) seed

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ARTICLE INFO

Article history:
Received 5 January 2010
Received in revised form 28 March 2010
Accepted 30 March 2010

Keywords:
Guayule
Dormancy
Light quality
Gibberellic acid
Abscisic acid
Seed coat

ABSTRACT

Guayule (*Parthenium argentatum* Gray) produces high quality, low-allergenic rubber which has commercial potential. The species has small seeds with a high level of dormancy which was investigated in a series of germination experiments. Many researchers have investigated the effect of gibberellic acid and light on guayule seed dormancy but there have been no reports on light quality. In this study, increased germination was found for yellow (82.0%) and red (65.3%) light compared to green (55.3%) and blue (25.3%) light. Effect of light quality indicates evidence of phytochrome-mediated germination and dormancy in guayule. A higher ratio of red to far-red radiation in yellow and red light activates phytochrome that stimulates production of endogenous gibberellins to promote germination. The highest level of far-red radiation found in blue light had similar inhibitory effects as complete darkness (21.3%). An intermediate level of red to far-red radiation for green light produced intermediate germination. Seed coat and light also affected germination of freshly harvested guayule seed. Embryos responded to light compared with darkness (68.0% vs. 34.7%) albeit to a much lower level than intact seed (57.3% vs. 8.6%) indicating the possible presence of inhibitors in the seed coat. Light and GA_3 strongly interact to overcome dormancy in guayule and appear to act on the same pathway. The optimum concentration of GA_3 for seed used in this experiment ranged from 250 to 500 ppm.

1. Introduction

Guayule (*Parthenium argentatum* Gray) is a potential source of commercial natural rubber. It not only produces rubber with high performance properties but also high quality latex suitable for manufacture of hypoallergenic products (Siler and Cornish, 1994). The high cost of establishment is a primary factor restricting commercialisation (Foster and Coffelt, 2005; Dissanayake et al., 2008); a key factor affecting establishment is percentage of germinable seed.

Guayule flowers and sets seed continuously when days are longer than 9.5 h (Backhaus et al., 1989), temperatures are over 15 °C and there is adequate soil moisture (Tipton et al., 1981). Because flowering lasts from late spring until autumn (Thompson and Ray, 1989), maturity of seeds harvested at any one time varies. This affects seed quality and frequently, a high percentage of seeds are empty or non-viable with the normal range of viable embryos being 10–45% (Benedict and Robinson, 1946; Thompson and Ray, 1989). Jorge and Ray (2005) used X-ray analysis to discriminate between filled, partially filled and unfilled seed and found the presence of internal structures plays a major role in germination of guayule seed. Seed colour was an important indicator in determining seed quality (Jorge et al., 2007). Bekaardt et al. (2010) found that quality of guayule seed was severely decreased through empty achene production due to genetic variability as well as the effect of rainfall, wind speed and temperature during flowering.

Dormancy is another important factor which may negatively influence germination and establishment especially fresh guayule seed. Two types of dormancy have been reported for freshly harvested guayule seed, a 6–12-month long seed coat dormancy and about a 2-month long endogenous dormancy (Benedict and Robinson, 1946). Benedict and Robinson (1946), Emparan and Tysdal (1957) and Hammond (1959) found that light was important for germination of guayule seed. Emparan and Tysdal (1957) reported that the combined treatment by 0.75% NaOCl and exposure to light for 3–4 days during germination completely overcame dormancy of freshly harvested guayule seed. Hammond (1959) found that continuous exposure of moist seed to daylight for 3 weeks completely broke dormancy of freshly harvested guayule seed. However, Jorge et al. (2006) found that conditioning had little effect on germination of three-year-old seed and concluded that seed quality was more important for establishment.

Seeds of some species sensitive to light require a specific light quality to induce germination. In the visible spectrum, red light (660–665 nm wavelengths) has been found to be the most important in breaking seed dormancy in many species. The effect of red light is mediated through phytochrome (Bewley and Black,

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doi:10.1016/j.indcrop.2010.03.012

1994; Smith, 2000). Phytochrome stimulates or inhibits germination according to the level of red or far-red radiation in the light. Generally, light with high levels of far-red light (light filtered through a plant canopy) inhibits germination, while light with high levels of red light stimulates germination (Gorski, 1975; Taiz and Zeiger, 2002). While some species are affected by brief exposure to light, others require intermittent illumination or require exposure for long periods to induce germination (Bewley and Black, 1994; Taiz and Zeiger, 2002).

Ahlawat et al. (1979) found that seed germination of *Parthenium hysterophorus* was inhibited under continuous red, blue, green and white light but not under yellow light. In one lettuce variety germination was reduced by blue and slightly reduced by red light, whereas in another variety green light markedly reduced and yellow light tended to increase germination (Favilli, 1967). In studies with radish seeds, germination of all cultivars was inhibited by far-red light, and that of one cultivar, Punjabi, was inhibited by blue light (Swarnkar and Kumar, 1977).

Gibberellic acid affects both seed germination as well as establishment of guayule. However, researchers have reported different results for the effect of gibberellic acid on seed germination. Hammond (1959) reported that gibberellins substitute for light by completely overcoming both embryo and inner seed coat dormancy in fresh guayule seed. However, later studies by Naqvi and Hanson (1980) and Chandra and Bucks (1986) reported that gibberellic acid did not completely replace the light requirement. Therefore, conclusions from these later researchers were at variance with Hammond's and thus further research is needed to clarify the effect of gibberellins on germination of guayule seed. The effect of osmopriming and gibberellic acid on establishment has been shown by Dissanayake et al. (2008) who found enhanced seedling growth in direct seeded guayule. This is especially important as the crop is slow growing in the early growth stages.

Even though the effect of light on germination of guayule seed is known, light quality studies have not been reported. Therefore, the main objective of this study was to gain a greater understanding of mechanisms affecting dormancy and germination of guayule seed with special emphasis on the effects of light quality, and growth promoter and inhibitor. This understanding would aid development of conditioning regimes to overcome dormancy and enhance establishment of guayule.

2. Materials and methods

Five experiments were conducted to investigate the effect of light and its quality, GA_3, ABA and seed coat on germination of guayule seed.

2.1. Seed collection and cleaning

Seed used in all germination tests was harvested from AZ-2, one of the promising lines evaluated in germplasm trials in Australia (Dissanayake et al., 2007). AZ-2 was released jointly by the USDA-ARS and the University of Arizona (Ray et al., 1999). Seed was harvested at Gatton, Queensland on three occasions, November 2003, April and October 2004, when plants were two to three years of age. Mature seed heads were harvested by manually stripping them from the stalks. Harvested seeds were air-dried for a few days and then hand threshed to minimize seed damage. Round holes sieves (1.18 and 2.36 mm diameter) and a laboratory seed blower were used to clean seed. Later, the seeds were separated manually from other particles of similar size using a magnifying lamp. The seed blower was used again to remove most of the empty and half-filled seed. The procedure produced 75–86% filled seeds. Cleaned seed was stored at room temperature during the period of study. Seed used in Experiment 1 was stored for 10 months during which it was subjected to monthly minimum and maximum temperatures of 6 and 22 °C during winter and 18 and 33 °C during summer. Seed for Experiments 2, 4 and 5 was stored for 32 days and subjected to average minimum and maximum temperatures of 13 and 30 °C. Seed used in Experiment 3 was stored for 35 days and subjected to average minimum and maximum temperatures of 13 and 26 °C.

2.2. Viability test

Each clean seed lot was tested for viability before germination testing. Three samples each of 100 seeds were selected and soaked overnight in water between paper towels surrounded by cotton cloth. Then under a magnifying lamp, seed coats were removed using a scalpel blade. The number of embryos obtained from 100 seeds was counted, placed in a container with 1% tetrazolium solution and then into an oven at 40 °C for 3 h according to seed testing procedures of the Queensland Department of Primary Industries (Low, 1993). Finally, viable embryos were counted based on pigmentation. Viability was affected mainly by the presence of partially filled (immature) seed that could not be removed by cleaning.

2.3. Seed decoating

Samples of 75–100 seeds were soaked overnight in water between paper towels surrounded by cotton cloth to facilitate decoating. Under a magnifying lens, seed coats were carefully removed using a scalpel blade. A sample of 50 embryos was selected for testing germination. Seeds intended for use in dark germination were decoated within 15 s under very low levels of room light. Soon after decoating, these embryos were placed on moistened filter paper in petri dishes in the dark (sealed metal container).

The guayule achene has two coats: the outer one is the fruit coat (pericarp) which is brittle and easy to remove (Benedict and Robinson, 1946). The inner coat is the seed coat which is thin and somewhat difficult to remove without damaging the embryo. Measures were taken to either remove this inner seed coat or to make a small cut at the cotyledon end to enable exchange of gases and water.

2.4. Germination tests

Five germination experiments were carried out in the Queensland Seed Technology Laboratory located on the Gatton Campus of the University of Queensland. Experiment 1 was conducted to investigate effect of light while Experiment 2 examined the effect of light quality. Experiments 3–5 were designed to gain greater understanding of the effect of growth promoters and inhibitors together with their interactions with light in guayule seed germination. Unless specified, a sample size of 50 randomly selected seeds/embryos was used and replicated three times. Germination tests were carried out in 10 cm diameter glass petri dishes with three layers of 90 mm diameter Whatman No. 1 filter papers. Seed germinated under light was exposed to 8/16 h of light and dark daily. Dark germination was conducted in a separate cabinet with no light and petri dishes were placed in sealed metal containers which were wrapped in dark cotton towels to prevent light contamination. The temperature optimum for germination of guayule seed of 20 °C (Da Cruz, 2003) was maintained for all tests. Germination tests were conducted for a period of 12–15 days. The night before testing, all coat intact seed was soaked between paper towels surrounded by cotton cloth. This was to simulate the same soaking treatment as was undertaken for decoated seed.