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SEED TREATMENTS AND ORIENTATION AFFECTS GERMINATION AND SEEDLING EMERGENCE IN TETRAPLOID WATERMELON

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Abstract

Polyploid watermelon has poor germination and low seedling vigor mainly due to thick seedcoat and seedcoat adherence to emerged cotyledons. Seed treatments nicking at radicle end and soaking in distilled water, hydrogen peroxide (1 or 2%), gibberellic acid (0.5 or 5 mM), benzyladenine (0.5 or 5 mM) and potassium nitrate (3%) were applied to seeds of SS-8 and SS-11 lines of tetraploid watermelon. Seeds were soaked for 4 hours followed by drying for 5 days at 20 °C with 40% relative humidity and germinated in Petri plates at 30°C. Seed treatments, nicking, presoaking in distilled water or H_2O_2 enhanced the germination of tetraploid seeds but showed genotypic variation. Early seedling emergence after 4 days occurred in line SS-8 with nicking, GA_3 (5mM) and H_2O_2 (2%) treatment but line SS-11 acquired comparatively more time for seedling emergence. Seed planting orientation affected both seedling emergence rate and seedcoat adherence. Seed positioned with radicle up reduced the seedcoat adherence to cotyledons. A genotypic variation was observed regarding growth characteristics; however, 2% H_2O_2 treated seeds showed better growth of seedlings.

Introduction

Use of triploid hybrids has provided a method for production of seedless fruit. The development of triploid watermelon cultivars adds extra time for the development of tetraploid watermelon and additional selection against sterility (Kihara, 1951). Breeders, interested in the production of seedless triploid hybrids, need to develop tetraploid inbred lines and colchicine is probably the most widely used chemical for induction of watermelon tetraploids. Besides low fertility and seed yield, poor seed germination is another problem with tetraploids (Andrus *et al.*, 1971). Hence a small number of tetraploid inbred lines are available.

Poor seed germination in polyploid watermelon is generally correlated with thick seedcoat, poor embryo and high moisture content (Grange *et al.*, 2000). In many seeds, germination can be inhibited by mechanical restriction exerted by the seedcoat. Permeability limitation of water and gases is typical due to hard seedcoat. The imbibed coat and large seed cavity in the tetraploid watermelon form a continuous wet layer around the embryo by which the oxygen must transverse (Grange *et al.*, 2003).

Seed treatments have enhanced germination in various field crops and vegetables. Combined application of ethephon and GA_{4-7} has improved germination in diploid watermelon (Nelson & Sharples, 1980). Germination and emergence of watermelon seed was also improved by priming in salt solution (Sachs, 1977) and redrying after priming was a critical step for maintaining seed quality (Parera & Cantliffe, 1992). Seed priming permits pre-germination physiological and biochemical changes to occur (Bradford, 1986). Mechanical weakening of the seedcoat structure such as scarification, seed nicking and seedcoat removal has been reported to successfully enhance germination of triploid watermelon seed (Grange *et al.*, 2000). Seedcoat adherence to cotyledons in polyploid seeds is another problem in seedling emergence; however, seed orientation with the radicle end up decreased seedcoat adherence (Maynard, 1989) but did not improve emergence.

Effect of priming and seed treatments on germination of diploid and triploid watermelons has been studied (Sung & Chiu, 1995) but little information is available regarding seed treatment effects on tetraploid seed germination, emergence and growth. Because of reduced viability with polyploid versus diploid watermelon seeds, growers use transplants from seeds that are germinated with some seed treatments in incubators to facilitate uniform germination and improved plant production. Even if polyploid plants are produced with seed enhancements, seedling emergence and growth may still be reduced. It is well known that the establishment of a good seedling stand is a prerequisite for improved yield and quality (Wurr & Fellows, 1983). This research was conducted to determine the effectiveness of seed alteration and chemical treatments on tetraploid watermelon seed germination and seedling stand.

Materials and Methods

Seed material: The diploid watermelon seed of SS-8 and SS-11 line was obtained from National Horticulture Research Institute, Korea. The tetraploid genotypes of these diploid lines were developed by injecting colchicine into meristem of seedlings at true leaf emergence stage (Jaskani *et al.*, 2004).

Seed germination treatments: For each line, seed treatments included were control, nicking, and soaking (non-aerated) in distilled water, gibberellic acid (GA_3, 0.5 or 5 mM), benzyl adenine (BA, 0.5 or 5 mM), hydrogen peroxide (H_2O_2, 1 or 2%), and potassium nitrate (KNO_3, 3%) solution for 4 hr at 24ºC. In case of nicking, seeds were nicked at radicle end avoiding any damage to embryo. Each treatment was replicated four times. After each soaking treatment, seeds were washed thoroughly in running tap water and dried at 20°C with 40% humidity for 5 days before germination tests were conducted. Distilled water (4 ml) was added for 20 seeds placed in 9 cm Petri dishes and incubated at 30°C in a dark growth chamber. Germination was observed for three days after incubation. Seeds were considered germinated when the radicle protruded (≈2 mm) from the seedcoat.

Seedling emergence and seedcoat adherence: The plastic pots (7x7x6 mm) were filled 24 hours before seeding with a commercial watermelon media (Broker Inc.) having coconut bark (75%), vermiculite (15%) and perlite (10%), and applied water to field capacity. No additional nutrition was applied; however, the potting media contained available PO_4 (200-400 mgl^{-1}), NH_4 (150 mgl^{-1}) and NO_3 (200-400 mgl^{-1}). The germinated seeds were sown in moist media (2 cm deep) with two seed orientations, radicle side up or horizontal placement to observe any effect on seedling emergence and seedcoat adherence. The pots were then covered with polythene film and placed in greenhouse at 25±2°C. The total emerged seedlings and the number of seedcoats adhered to cotyledons in both seed orientations were counted up to 12 days of seeding.

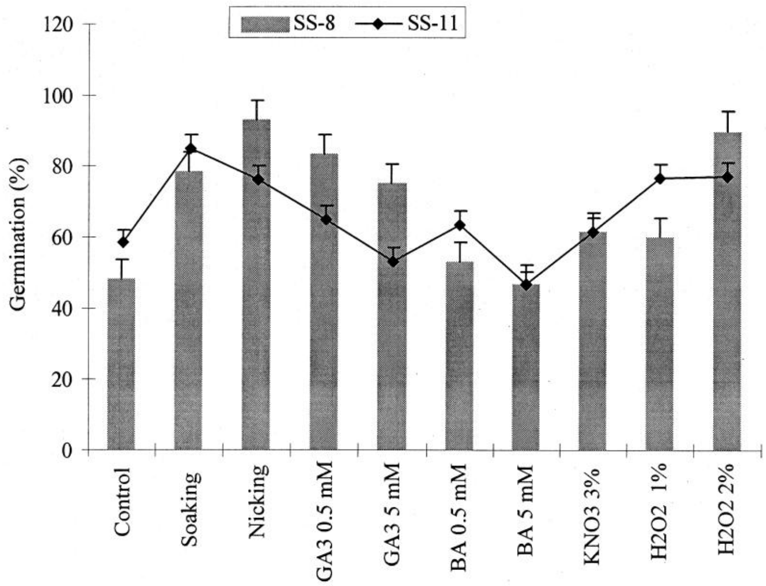

Fig. 1. Effect of seed treatments on germination of SS-8 (LSD0.05=12.7) and SS-11 (LSD0.05=10.1) tetraploid watermelon lines. Vertical thin lines indicate SE.

Vegetative growth of seedlings: Fifteen plants from four replications of each treatment were randomly harvested for growth measurement at 30th day of emergence. Growth measurements included leaf count, leaf area (model LI-3000; LI-COR, Lincoln, Neb.) and dry weight of shoot and root tissues (after washing off the medium) that were obtained after samples were dried in a forced-air oven at 65 °C for 48 hours. Tissue dry weights were measured to the nearest 0.1 mg by using an analytical balance (A&D, GR-200). Root: shoot dry weight ratios were calculated.

Statistical design: The experiment was arranged in a completely randomized design, and each line was replicated four times. Twenty seeds per replication, a total of 80 seeds, in each treatment and line were investigated for seed germination. For seedling emergence and growth, 60 germinated seeds in each treatment and line were investigated. Data were analyzed using statistiXL software and means were compared by $LSD_{(0.05)}$.

Results

Seed germination: The tetraploid seeds of two watermelon lines responded differently against germination treatments and significant differences were detected at $p \leq 0.05$. Final germination percentage in SS-8 tetraploid line was the highest (93.3%) in nicking at radicle end treatment followed by H_2O_2 (2%) and GA_3 (0.5 mM) (Fig. 1). SS-11 line seeds presoaked in distilled water showed the highest germination (85%). H_2O_2 (1 or 2%) was the second best seed germination improvement treatment (76.6% and 78.3%, respectively). Germination of seeds treated with BA (0.5 or 5 mM) did not improve germination and showed similar results as control for both watermelon lines (Fig. 1). In contrast, GA_3 (5 mM) treatment was ineffective in SS-11 line but improved germination (80%) in SS-8 line. Moreover, the radicle became swollen as callus tissue and lacked further elongation (Fig. 2a,b).

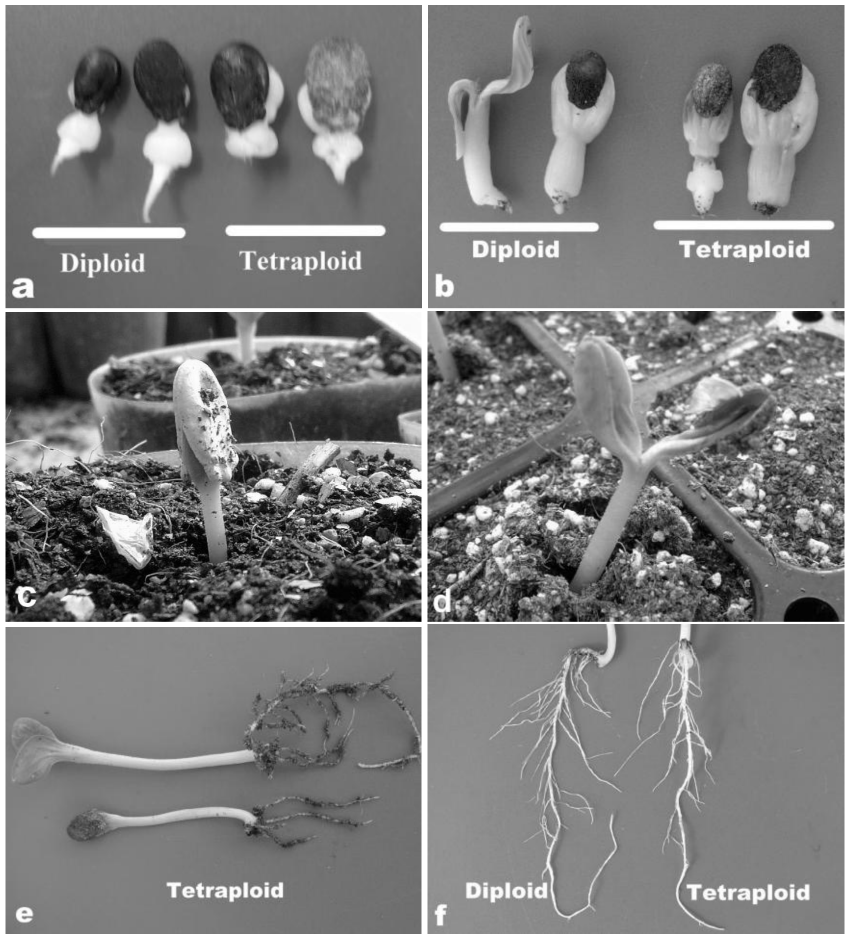

Fig. 2. Effect of seed treatments and seedcoat adherence on germination and seedling growth in tetraploid watermelon. a) Bulbous radicle in germinated seeds and b) no root induction or seedling emergence in diploid and tetraploid SS-8 and SS-11 lines, respectively, with BA (5 mM) treatment, c) adhered seedcoat and d) folded cotyledon due to seedcoat adherence in tetraploid seedling of SS-11 line, e) seedcoat adherence opposed root growth and f) lower root branching in tetraploid as compared with diploid seedling in line SS-8.

Seedling emergence rate: The two lines showed different genotypic response against seed treatments for seedling emergence rate (Table 1). Line SS-8 showed maximum seedling emergence in treatment H_2O_2 (1 or 2%) with radicle up placement. GA_3 (0.5 or 5 mM), soaking in distilled water and control produced statistically similar results for seedling emergence. Horizontal seed placement showed maximum seedlings emergence (93.3%) with nicking and GA_3 (0.5 mM). BA (0.5 mM or 5 mM) had toxic effect and did not show seedling emergence with both seed orientations.

In tetraploid watermelon line SS-11, all seed treatments with radicle up placement affected positively on seedling emergence except BA (5 mM) (Table 2). However, seedling emergence was lower with horizontal seed placement except nicking. Line SS-11 was somewhat tolerant to lower concentration of BA (0.5 mM) and showed 60% emergence with radicle up but lower (26.6%) with horizontal placement.

Seedcoat adherence: Significant differences ($p \leq 0.05$) were recorded for seedcoat adherence with respect to seed treatments and seed orientations. Seed placement with radicle up reduced the seedcoat adherence to cotyledons (Table 1). Only 0.3% cotyledons were observed with adhered seedcoat in line SS-8 with GA_3 (0.5 mM) and KNO_3 (3%) treatment. Seed treatments GA_3 (0.5 mM) and H_2O_2 (1 and 2%) with horizontal seed orientation emerged significantly minimum cotyledons with adhered seedcoat. In line SS-11, 100% cotyledons emerged without seedcoats with radicle up seed orientation except control (13.3%) and soaking (20%) treatment (Table 1). In contrast to line SS-8, H_2O_2 did not remove seedcoat adherence to cotyledons but KNO_3 (3%) and BA (0.5 mM) showed significantly the lowest (1%) seedcoat adherence.

Seedling growth: Seedlings emerged from seeds treated with 2% H_2O_2 in line SS-8 yielded maximum number of leaves (3) and leaf area (10.8 cm^2) but were statistically at par with control and GA_3 (0.5 mM) (Table 2). The higher concentration of GA_3 (5 mM) affected negatively on shoot dry weight (62.9 mg) but enhanced root dry weight (21.6 mg). This was in contrast to H_2O_2 seed treatment which produced higher shoot dry weight (153.1 mg) but lower root dry weight (9.1 mg).

Seeds of line SS-11 soaked in distilled water produced the seedlings with lower leaf number (1.2), leaf area (4.9 cm^2), shoot (52.2 mg) and root (13.5 mg) dry weight as compared with other treatments (Table 2). Although 2% H_2O_2 yielded higher number (2.6) and area (17.4 cm^2) of leaves but it was non significantly different with 0.5 mM BA. The different concentrations of H_2O_2 produced variable results for shoot and root dry weight (Table 2). Shoot dry weight (175.6 mg) was higher in 2% H_2O_2 whereas root dry weight (24.4 mg) was higher in 1% H_2O_2 treatment. Moreover, shoot dry weight of line SS-11 with 2% H_2O_2 treatment was significantly higher than control (117.1 mg) but at par for root dry weight.

It was also observed that the seedcoat adherence to cotyledons restricted root growth (Fig. 2e). Moreover, lower root branching was observed in tetraploid as compared to diploid seedlings of same age (Fig. 2f). Incidence of abnormal seedling growth increased with increasing BA concentration. Radicle swelling and lack of elongation after protrusion from the seedcoat at BA levels of 0.5 or 5 mM was very pronounced.

Discussion

In Korea generally watermelon transplants are produced and the transplant production requirements are more precise with polyploid watermelon than diploid watermelon. The reduced seed germination in polyploid watermelon results in uneven transplant size and weaker plants resulting in yield reduction.

The thick seedcoat and a large airspace between the underdeveloped embryo and seedcoat tissues appear to have a major role in limiting seed germination of tetraploid watermelon. However, seed nicking or decoating results indicate that polyploid seed germination is not inhibited by the seedcoat alone (Grange *et al.*, 2003) but also is very sensitive to increased moisture contents (Grange *et al.*, 2000). Although presoaking or nicking the seedcoat significantly increased tetraploid seed germination but was partly related to genetic factors associated with the different ploidy level of the seed e.g., variable response of two lines (Grange *et al.*, 2003). H_2O_2 was the best among other treatments as it increases availability of oxygen to seeds at high temperatures by providing supplemental oxygen for respiration and metabolic activities (Katzman *et al*., 2001). The improved germination in the presence of 1% or 2% H_2O_2 may result from weakening of the seedcoat (Chien & Lin, 1994) as H_2O_2 reacts with the seedcoat. Cracking was not observed in tetraploid watermelon seedcoats by H_2O_2 treatment; however, the H_2O_2 solution reacted with seedcoat and changed from transparent to brown. Batak *et al*., (2002) reported that nitrogenous compounds, such as potassium nitrate, potentiate germination of different species of light-requiring seeds; however, in our case KNO_3 did not improve germination of tetraploid watermelon seed. Variable genotypic response was evident in tested tetraploid lines and Kihara (1951) noted that improvement of triploid watermelon germination following seed treatments were inconsistent.

Successful seedling emergence generally depends on the seedling emergence force that varies with seed size and seed weight (Sung, 1992). Triploid and tetraploid seeds had a lower emergence percentage than diploid seeds, possibly due to their higher seedcoat splitting strength and weak seedling emergence strength (Sung & Chiu, 1995). Weak development of the embryo in tetraploid watermelon is another causative factor of poor germination and subsequent seedling emergence (Kihara, 1951). Energy content of a given seed depends on the composition and amount of stored material within the seed. This stored material is presumably utilized to support seedcoat splitting and subsequent seedling emergence. With the weak embryo, the reserve material to generate energy for overcoming resistance exerted by thick seedcoat and overlying growth media would be limited to some extent, resulting in a lower emergence percentage. Seed treatments using GA3, benzyl adenine (BA), potassium nitrate, phosphate and chloride (Cantliffe *et al*., 1987), polyethylene glycol (Dearman *et al*., 1987), ABA (Finch & McQuistan (1989) is known to promote germination of vegetable seeds to different degrees.

Emergence and seedcoat adherence, however, improved with seed orientation in both tetraploid watermelon lines. Previously, diploid cucumber and watermelon emergence was reported lower when the radicle of the seed pointed up rather than down or placed horizontal (Nettles, 1971). It was further noted that if the seed depth was less than 2 cm with radical up seed orientation, the hypocotyl emerged instead of epicotyl from the media. However, fewer seedcoats adhered to cotyledons when seeds were positioned with radicle up. Maynard (1989) also reported that seed orientation greatly affect adherence of seedcoats to emerged cotyledons. The seedlings with adhered seedcoats showed poor growth with malformed and distorted cotyledons (Fig. 2c,d).

Sporadic and delayed seedling emergence has been a major problem in transplant production that results in a reduced crop stand, directly lowering yield and quality (Wurr & Fellows, 1983). Although germination and vigor is gene controlled but seed size, viability, sowing depth, soil moisture, oxygen concentration and temperature all can influence germination, emergence and vigor (Bewley & Black, 1982). We also evidenced genotypic variation in seedling growth in response to seed treatments. Line SS-11 showed overall better growth of seedlings than line SS-8. Seed priming has been reported to promote uniform emergence in some vegetable crops (Bradford, 1986). GA_3, applied during and after stratification to pistachio seed, significantly increased the length, trunk diameter, internode length, leaf area and fresh and dry weight of seedlings (Rahemi & Baninasab, 2000). However, in current studies the application of GA_3 (5 mM) resulted in lower shoot but higher root dry weight. It is also evident that the seed treatment with H_2O_2 produced the lower root dry weight at 2% as compared with 1% concentration in line SS-11. Similarly, exogenous application of hydrogen peroxide has been reported to stimulate seed germination and growth by increasing mass and length of sprouts and roots in carrot (Narimanov & Korystov, 1997).

The results of the present studies would suggest that seed soaking, nicking, H_2O_2 or GA_3 enhanced tetraploid seed germination but H_2O_2 and seed orientation with radicle up resulted in better seedling emergence and overcome seedcoat adherence. However, variation in genotypic response needs to be further explored.

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