HortScience 35(1):85–86. 2000.

Treatment with Hydrogen Peroxide and Seedcoat Removal or Clipping Improve Germination of 'Genesis' Triploid Watermelon

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Additional index words. Citrullus lanatus, seedless watermelon

Abstract. **Seeds of triploid watermelons [*Citrullus lanatus* (Thunb.) Matsum & Nakai] often germinate poorly, which prevents adequate stand establishment in both field and greenhouse environments. Methods of improving germination and emergence of these expensive seeds would reduce overall risk to growers, thus increasing the crop's market prominence. Seeds of 'Genesis' triploid watermelon were subjected to three treatments: 1) seedcoat removal; 2) clipping the seedcoat opposite the radicle end; or 3) no seedcoat alteration; and were germinated on agar in the presence of a 0%, 1%, 2%, 4%, or 8% aqueous H_2O_2 at constant 28 °C in the dark. Seedcoat removal, clipping, and all levels of H_2O_2 increased final germination percentages relative to the control (no seedcoat alteration, no H_2O_2) by as much as 70%. Hydrogen peroxide levels >2% resulted in severe injury to germinating seeds. These findings suggest that germination barriers of triploid watermelon are seedcoat related, and that seedcoat alteration and H_2O_2 can overcome these barriers.**

Triploid watermelon seeds often exhibit poor germination and emergence. Kihara (1951) reported that triploid seedcoats are hard and thick, like those of their tetraploid parent, and this may inhibit germination. The author further suggested that removing part of the seedcoat should improve germination. Splitting the seedcoat of tetraploid watermelons laterally improved germination (Nerson et al., 1985). Recently, Duval and NeSmith (1999) confirmed that mechanical scarification improved and increased the consistency of emergence of 'Genesis' triploid watermelon; however, emergence was still not at an acceptable level. Alternative mechanical pretreatments, with or without chemical treatment, of triploid watermelon seed could improve germination and stand establishment.

Hydrogen peroxide (H_2O_2) has been reported to improve germination of seeds of *Vagueria infausta* Robyns. (Msanga and Maghembe, 1989), *Paspalum distichum* L. (Huang and Hsiao, 1987), *Fragaria×ananassa* Duch. (Negi and Singh, 1972), *Anthyllis cytisoides* L. (Ibanez and Passera, 1997), *Tripsacum dactyloides* L. (Kindiger, 1994), and *Cinnamomum camphora* L. (Chien and Lin, 1994). Successful methods were highly variable and ranged from seeds being soaked in high H_2O_2 concentrations (30%) for short periods of time (2 h) to germination of seeds in solutions of low H_2O_2 concentrations (1%). These studies suggest that H_2O_2 can be beneficial in promoting germination of hard-seeded species. This research was conducted to determine the effectiveness of seedcoat alteration and H_2O_2 treatments on improving germination dynamics of triploid watermelon.

Materials and Methods

'Genesis' seeds (Shamrock Seed Co., Salinas, Calif.) were purchased for use in various seed treatment tests. Six hundred seeds were surfaced sterilized by placing them in a 1-L beaker with 500 mL of 1% NaOCl for 5 min with constant stirring. After sterilization, seeds were rinsed thoroughly under running tap water for 5 min. A 3 × 5 factorial experimunt was established with two methods of seedcoat alteration, plus unaltered seed, and five levels of H_2O_2. Seedcoat alterations included clipped seedcoats and excised embryos. Clipping consisted of using common nail clippers to remove a portion of the seedcoat opposite the radicle end, resulting in an opening ≈3 × 1 mm. Embryos were excised by carefully splitting the seedcoat and gently prying it away from the embryo and cotyledons. They were not surfaced sterilized. Following seedcoat alteration, seeds were placed on 20 mL of a 0.8% (w/v) sterile agar media in 100 × 15 mm petri dishes. Five milliliters of a 0%, 1%, 2%, 4%, or 8% H_2O_2 solution (J.T. Baker Chemical Co., Phillipsburg, N.J.) were added to the petri dishes and allowed to remain in the dish throughout the experiment. Five dishes containing 10 seeds each for each seedcoat alteration and H_2O_2 treatment combination were used in each of three experiments. Each experiment was considered a replication. For each replication, dishes were placed in a dark growth chamber (model E15; Conviron, Pembina, N.Dak.) at 28 ± 0.5 °C. Germination data were taken daily for 10 d (the point at which no additional germination over three consecutive days was observed). Seeds were considered germinated when the radicle had protruded 2 mm. Seed quality was rated after 7 d in the chambers. Quality of each seed was assessed on a 1 to 10 scale, with 1 being the lowest rating and 10 the highest. Quality points were deducted from growing seedlings for stunted growth, discoloration, and other abnormalities.

Germination data were modeled and analyzed using the following logistic function (Eq. 1) (SAS Institute, 1990):

$$Y = \frac{\beta_0}{1+\left[(1-p)/p\right] e^{-\beta_1(day-\beta_2)}} \quad [1]$$

Where β_0 = asymptote of final germination percentage, β_1 = relative rate of germination, β_2 = time until asymptote of p is reached, and p = point where a predetermined value of maximal germination is obtained (90%).

Significant differences among parameters were determined based upon 95% confidence intervals. Seed quality data were subjected to linear and quadratic analysis.

Results and Discussion

Significant interactions between seedcoat and H_2O_2 were detected ($P \leq 0.01$) for germination; thus, data are presented for the different seedcoat alteration methods at each level of H_2O_2 (Table 1). Final germination percentage in water alone (Fig. 1a, Table 1) was higher for embryos than for clipped seeds and lowest for intact seeds. No differences were observed among treatments in relative rate of germination or in time until 90% of maximum germination was reached.

At 1% H_2O_2, final germination percentage for clipped seeds was significantly less than for excised embryos or intact seeds (Fig. 1b, Table 1). Other model parameters were nonsignificant. No differences among seedcoat treatments were significant at the 2% concentration of H_2O_2 (Fig. 1c, Table 1). Final germination percentage of intact seeds was greatly enhanced by all concentrations of H_2O_2 (Fig. 1), although 8% was less effective than lower concentrations.

When 4% H_2O_2 was applied, toxic effects became evident. Germination percentage of embryos was lower than that of clipped or intact seeds (Fig. 1d, Table 1). No other differ-

Received for publication 9 Nov. 1998. Accepted for publication 10 May 1999. A contribution of the Univ. of Georgia Agricultural Experiment Station, Georgia Station, Griffin. This research was supported by state and Hatch Act funds allocated to the Georgia Agricultural Experiment Stations. 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.

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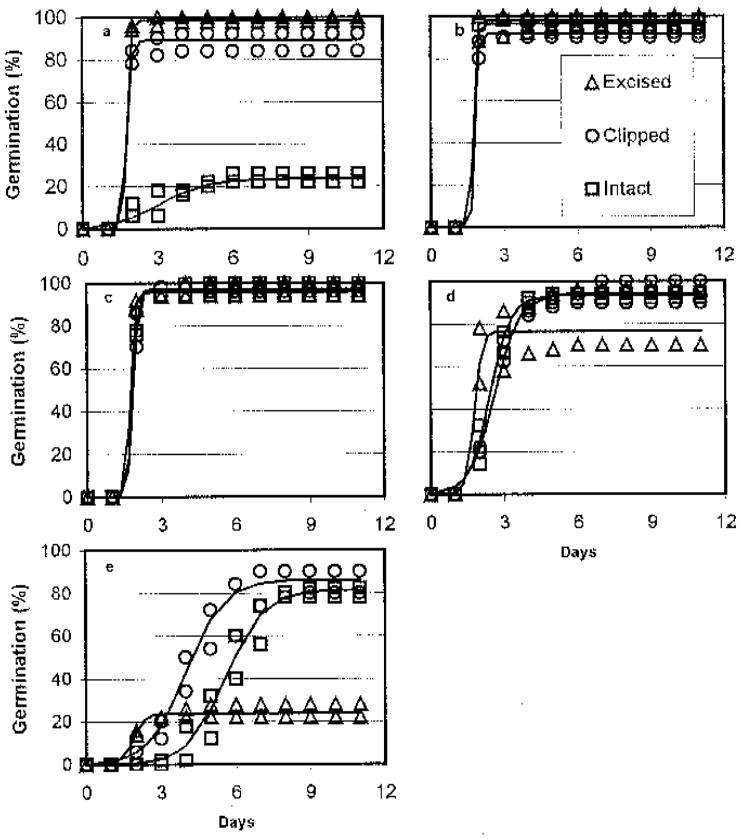

Fig. 1 (a–e). Germination of excised embryos, clipped, and intact seeds exposed to (**a**) 0%, (**b**) 1%, (**c**) 2%, (**d**) 4%, or (**e**) 8% H_2O_2. Lines representative of modeled germination. Symbols represent individual data points from 3 experiments. Many points maybe hidden by overlapping. Model parameter estimates can be found in Table 1.

Table 1. Model parameter estimates for germination of 'Genesis' triploid watermelon after seedcoat alteration and exposure to hydrogen peroxide.

	β_0[z]	β_1[y]	β_2[w]	r^2
		0% H_2O_2		
Excised embryo	98.5a[x]	14.0a	1.9a	0.99
Clipped	89.1b	10.0a	2.0a	0.98
Intact seed	23.6c	1.1a	5.2a	0.90
		1% H_2O_2		
Excised embryo	96.4a	16.1a	1.9a	0.99
Clipped	91.6b	11.1a	2.0a	0.99
Intact seed	98.0a	14.8a	1.9a	0.99
		2% H_2O_2		
Excised embryo	95.8a	11.0a	1.9a	0.99
Clipped	97.2a	9.5a	2.1a	0.99
Intact seed	95.8a	9.9a	2.1a	0.99
		4% H_2O_2		
Excised embryo	76.6a	6.1a	2.1a	0.77
Clipped	94.4b	1.9a	3.8a	0.98
Intact seed	93.4b	2.3a	3.4a	0.99
		8% H_2O_2		
Excised embryo	23.7a	4.1a	2.4a	0.90
Clipped	86.1b	1.3a	5.8b	0.96
Intact seed	81.3b	1.3a	7.3c	0.97

[z]Asymptote of final emergence percentage.
[y]Relative rate of emergence.
[w]Time (days) until asymptote of 90% emergence is reached.
[x]Parameter separation among seedcoat treatments within each level of H_2O_2. Parameter separation determined by 95% confidence intervals.

ences were noted. The highest level of H_2O_2 (8%) was detrimental to all seeds (Fig. 1e, Table 1). Final germination percentage was lower for embryos than for either clipped or intact seeds, and time to reach 90% of final germination was significantly different among treatments (naked < clipped < intact). No differences in relative rate of germination were observed.

Incidence of abnormal seedling growth increased with increasing H_2O_2 concentration for all seedcoat treatments (data not shown). Analysis showed significant linear ($P \leq 0.01$) and quadratic ($P \leq 0.01$) effects of increasing concentrations of H_2O_2. Radicle damage (browning, cracking of the epidermis, and lack of elongation after protrusion from the seedcoat) at H_2O_2 levels of 2% and 4% was very pronounced. At 8% H_2O_2 severe bleaching of tissue occurred and radicles failed to elongate more than 3 mm.

Clipping the seeds or completely removing the seedcoat apparently relieved any mechanical restraint and/or barriers to gas exchange, as these treatments greatly improved germination of 'Genesis' when compared to the intact seeds in water. This evidence suggests that any barriers hindering triploid watermelon germination are associated with the seedcoat. Germination of intact seeds in 1% or 2% H_2O_2 equaled that of other seed treatments, and far exceeded germination of intact seeds in the absence of H_2O_2. This improved germination in the presence of 1% or 2% H_2O_2 may result from weakening of the seedcoat or from evolution of O_2 as H_2O_2 reacts with the seeds. In *Camphora* (Chien and Lin, 1994) seed cracking along the hilum was observed when seeds were exposed to 15% H_2O_2 for 25 min. Chien and Lin proposed that this weakening of the seedcoat enhanced germination. Cracking was not observed for the triploid watermelon seedcoats in the current experiment. Nerson et al. (1985) discounted O_2 impermeability of the seedcoats of tetraploid watermelon as the barrier to germination based on responses in aerated vs. non-aerated solutions. Hydrogen peroxide has been evaluated as an additive to polymer gels for seeding cotton with a hydraulic planter (Bordovsky et al., 1991). It was discovered that peroxide reacted with seeds and, to a lesser extent, the starch in the planting gel to produce O_2. Bordovsky et al. (1991) concluded the amount of O_2 produced was sufficient to meet the respiration requirements of cotton seeds in a polymer gel. Evolution of O_2 from H_2O_2 in the current study would have enriched the atmosphere within the closed petri dish, and the increased O_2 concentration might have favored its diffusion through the seedcoat. In addition, the H_2O_2 that was absorbed by the seed and reacted within the seed itself could supply O_2 directly to the growing embryo. At concentrations >1% H_2O_2, significant damage to growing seedlings negated any benefit in germination rate. Therefore, long-term seed exposure to concentrations > 1%H_2O_2 should not be considered.

In conclusion, clipping, removing the seedcoat, and the presence of H_2O_2 greatly enhanced the germination of 'Genesis' triploid watermelon seed. Further research needs to be conducted to determine the permeability of triploid watermelon seedcoats to O_2. Also, the practicality of seed pretreatments, such as priming to enhance germination in a production setting, need to be explored.

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