FACTORS LIMITING GERMINATION IN TRIPLOID WATERMELON

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Factors Limiting Germination in Triploid Watermelon (May 2016)

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Seedless watermelon, a major crop in Texas, grows from sterile triploid seeds. The triploid seed varieties have poor germination rates and are typically more expensive. Identifying techniques that increase vigor and rates of germination could decrease the amount of labor and cost of cultivation required to grow seedless watermelons. To identify the cause of low vigor, the seeds underwent stress tests using modified State Seed Laboratory germination techniques to measure sensitivity to moisture and reduced gas exchange. Triploid seeds also have embryo deformations in high frequency, which were hypothesized to play a role in low vigor. The seeds were germinated on blue blotter paper in 9 cm petri dishes with punched holes to allow for greater gas exchange. Seeds were inspected for deformations upon germination, and seeds that failed to grow were inspected at the end of each repetition. It was found that increased levels of water diminished rates of germination in triploid varieties, but had no significant effect on the diploid control variety. Testing in elevated gas exchange conditions promoted germination rates in triploid varieties significantly. The rate of deformation in triploid seed embryos did not alter the seeds rate of germination. High sensitivity to limited gas exchange appears to play a larger role in germination success of triploid seeds than the deformations originally identified. This explains the poor vigor from seeds planted directly into the soil and calls for techniques that allow for greater gas exchange to promote germination.

CHAPTER I

INTRODUCTION

The abnormal ploidy levels in triploid seeds leads to a variety of defects, making germination difficult (Malepzy, 1998). We have been examining triploid watermelon varieties *Citrullus lanatus* (Thunb.) to identify various factors that affect the rate of germination. The watermelon industry in Texas, with 23,500 acres in 2012, ranked first in total cultivated acres (Thornsbury *et al*., 2013). Triploid watermelon seed sensitivity to water and oxygen stress can be partially alleviated by several techniques such as increasing the oxygen concentration, treating with H_2O_2 or scoring the seed coat (Duval *et al.*, 2000; Grange *et al.*, 2000; Grange *et al.*, 2003). Structurally triploid watermelon seeds have thicker seed coats (Grange *et al*., 2003) and possess smaller starch grains and lipid bodies than diploid seeds (Wang *et al*., 2003). Wang *et al*. (2011) reported on the composition of triploid and diploid watermelon seeds and showed that triploid seeds have altered fatty acid profiles with higher levels of linoleic acid (C18:2) and significantly reduced starch levels than diploids.

Seedless fruit are commercially valuable due to their convenience to the consumer. Due to the difficulty involved with germinating and growing them as a crop, triploid watermelons are much more expensive to produce at the commercial scale. The objective of this study is to identify patterns in the germination rates of assorted varieties of triploid watermelon seeds and to determine if these rates are associated with water sensitivity, oxygen concentration sensitivity, and rates of malformations that are common to the triploid varieties of watermelon. This study

will provide meaningful and useful data that can be utilized to improve the harvest of seedless watermelons and lower the expense of this crop to producers in Texas.

CHAPTER II

METHODS

Seed materials

The low vigor triploid varieties ‘Melody’ and ‘Fascination’ were use in this experiment. The high vigor diploid ‘Sugar Baby’ variety served as the control.

Germination Conditions

Seventy-five seeds of each variety were used for each replication. Twenty-five seeds per replication were placed into 9 cm petri dishes with blue blotter paper. 8 mL of water was added to each petri dish. This quantity of water saturated the blotter paper, leaving a small excess reserve of free water that the paper could continue to wick up as the experiment progressed to prevent changes in available water. To avoid flooding the seeds and to prevent oxygen depravation, the dishes were placed at a slight angle during incubation. The seeds were incubated at 25 °C for one week and inspected at 24-hour intervals. At every inspection, seeds with a radicle emergence of 5 mm were recorded as successfully germinated for that 24- hour period and removed from the petri dish to be visually examined for deformations, such as the cleft deformation in Figure 1. After the germination period has ended, seeds that failed to germinate were also visually examined for deformations. Deformations in the seeds were then recorded for each variety and the date of germination was noted. Some replications required the seeds to be treated with the antifungal thiram to prevent the spread of fungus that would infect the seeds at certain months of the year. This was a preferred method to sterilizing the seeds beforehand. In parallel repetitions, the 9 cm petri dishes were altered to allow for greater gas exchange. In order

to allow for this, the lids of the dishes were punctured with a hot metallic rod five times in a cross pattern to leave holes. The dishes were then prepared the same as the control, with blue blotter paper and 8 mL of water before seeding. During incubation, seeds were still inspected for malformations.

Visual Embryo Inspection

Due to the delicate nature of the seeds embryos and the properties of the strong seed coat, the seeds required soaking in ethanol in order to split them easily for observation. Two weeks prior to inspection, 150 seeds of each variety were placed into large test tubes with 95% ethanol to soak. During the two-week period, the containers were frequently disturbed to ensure mixing was occurring within the tube. After the two week soaking period, the seeds were individually split open using needle nose pliers and a spatula to carefully expose each embryo. We recorded the rates of embryo deformation to compare to the rate of germination seen in each variety. An example of the typical cleft deformation in a triploid seed can bee seen below in Figure 1, compared to a normal diploid seed in Figure 2.

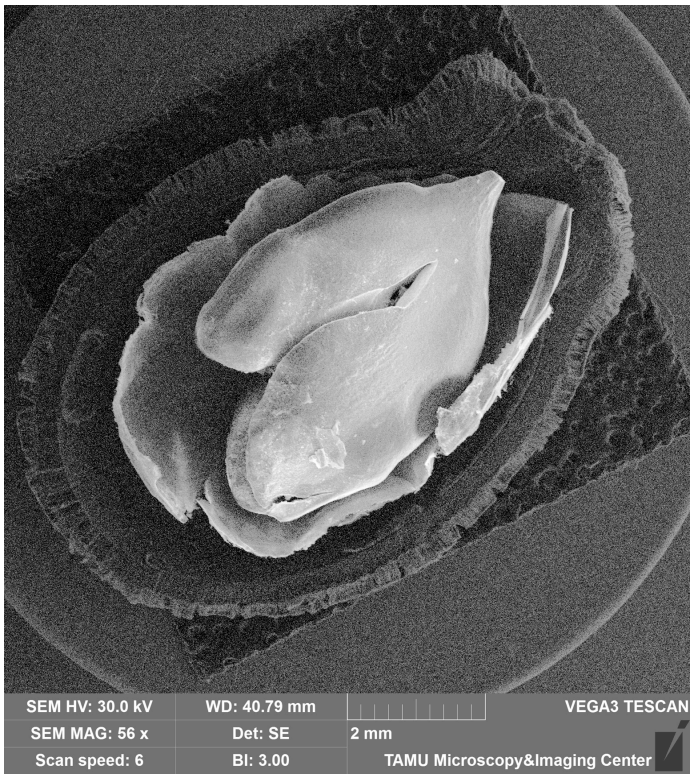

Figure 1: ESEM image of triploid embryo with cleft deformation

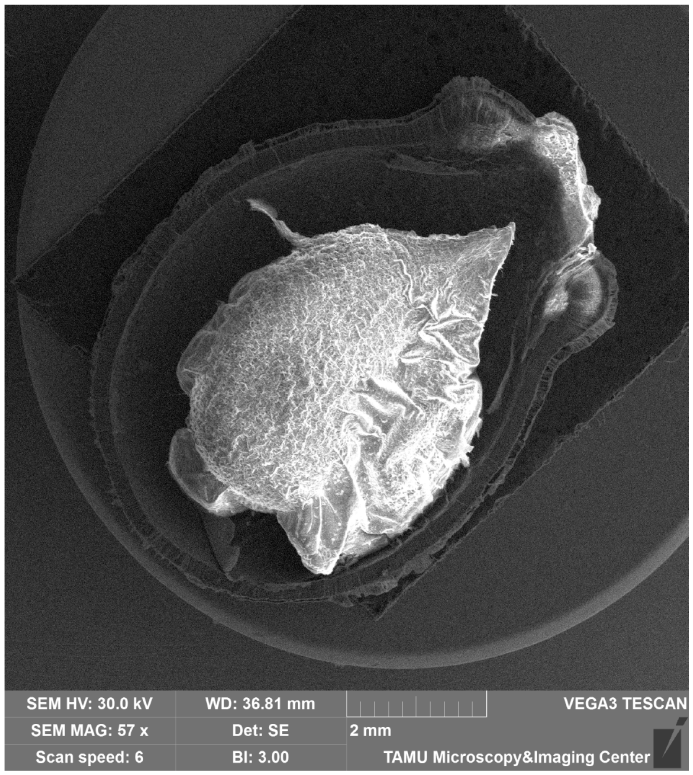

Figure 2: ESEM image of normal diploid embryo with perisperm

CHAPTER III

RESULTS

Figure 3 shows the percent germination of the diploid variety and the two triploid varieties under control conditions. On average, on the third day of incubation, the germination percentage of diploid seeds was higher, and most of the diploids had germinated by the fourth day. The triploid varieties had a much poorer rate of germination, even after the full week of incubation. Figure 4 shows the percentage germination for the same seeds with the aerated disk arrangement. Under these conditions, the diploid seeds behaved similarly to the control. The triploid seeds saw a significant increase in the rate of germination, typically germinating over 50 percent of the seeds by the end of the incubation period.

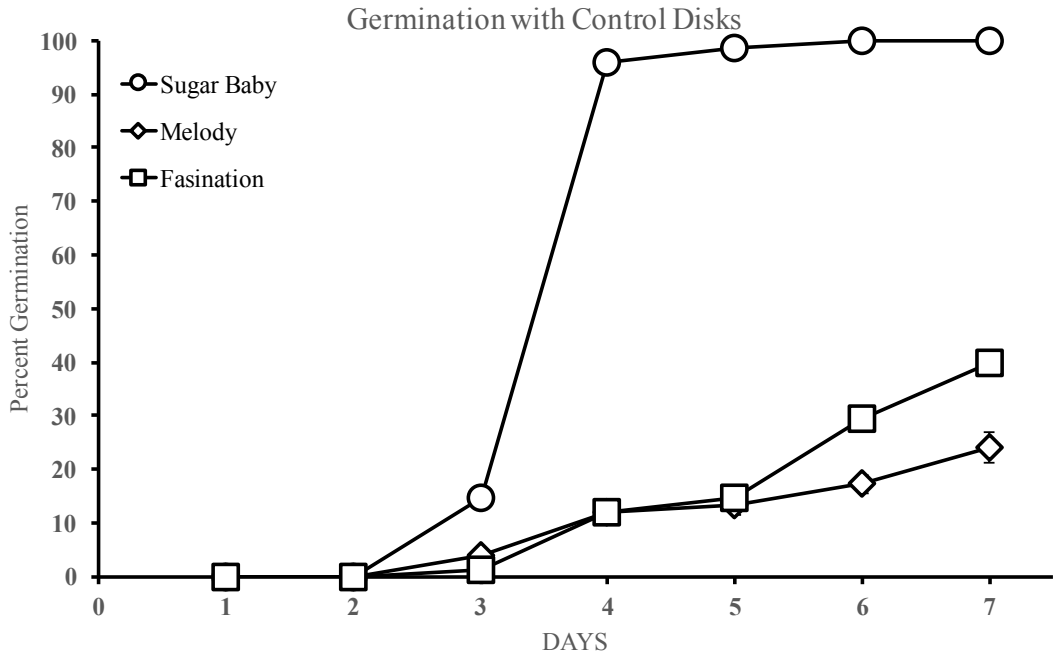

Figure 3: Average percent germination for control repetitions

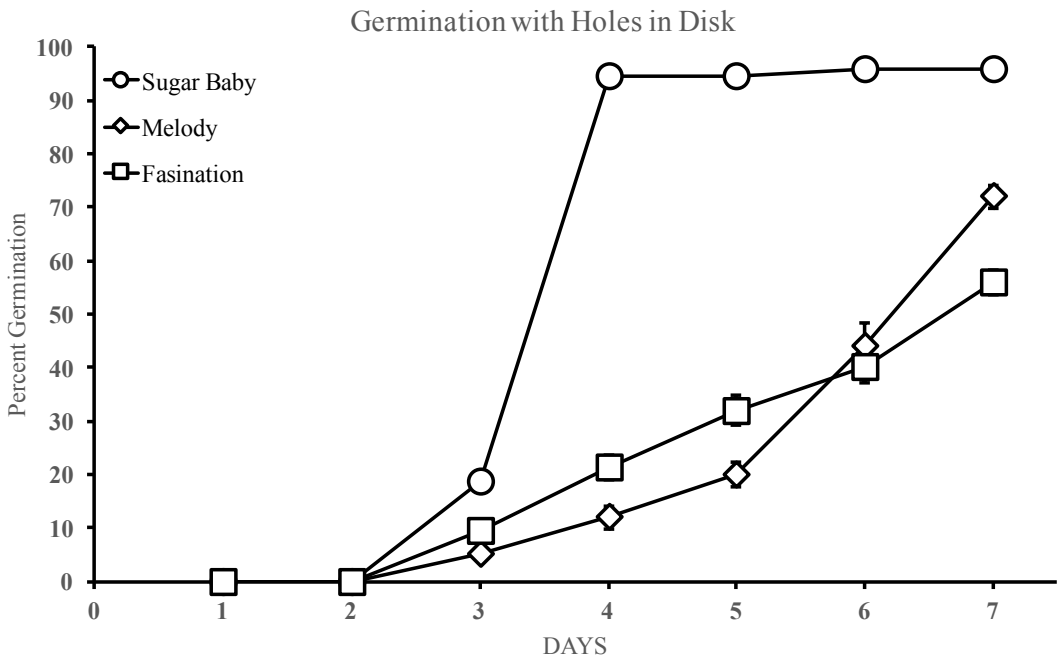

Figure 4: Average percent germination for aerated disks

150 seeds of each variety were soaked in ethanol to identify the percentage of deformed seeds in each population. Table 1 compares the average germination rates to the rate of cleft embryos recorded during the visual inspection in percentages. No abnormal embryos were observed in the diploid seeds during experimentation.

Table 1: Triploid cleft deformation rates versus rates of germination

	Cleft Embryo Deformation Rate	**Control Germination Rate**	**Holes Germination Rate**
Melody (3n)	42.0	24.0	72.0
Fascination (3n)	30.7	40.0	56.0

CHAPTER IV

CONCLUSION

While it was originally hypothesized that the cleft deformations seen in triploid seeds were a likely contributor to poor vigor, the data from Table 1 shows the relationship between the variables is limited. Instead, we found that the seeds are sensitive to conditions within the regular petri dish. The petri dish used in the experiment is designed to allow for sufficient gas exchange for microbes, however, we believe the lid limits the triploid seed respiration to inadequate levels. Figure 5 and Figure 6 directly express the increase in germination rates for the two triploid varieties, Melody and Fascination, respectively, compared to the closed dish control.

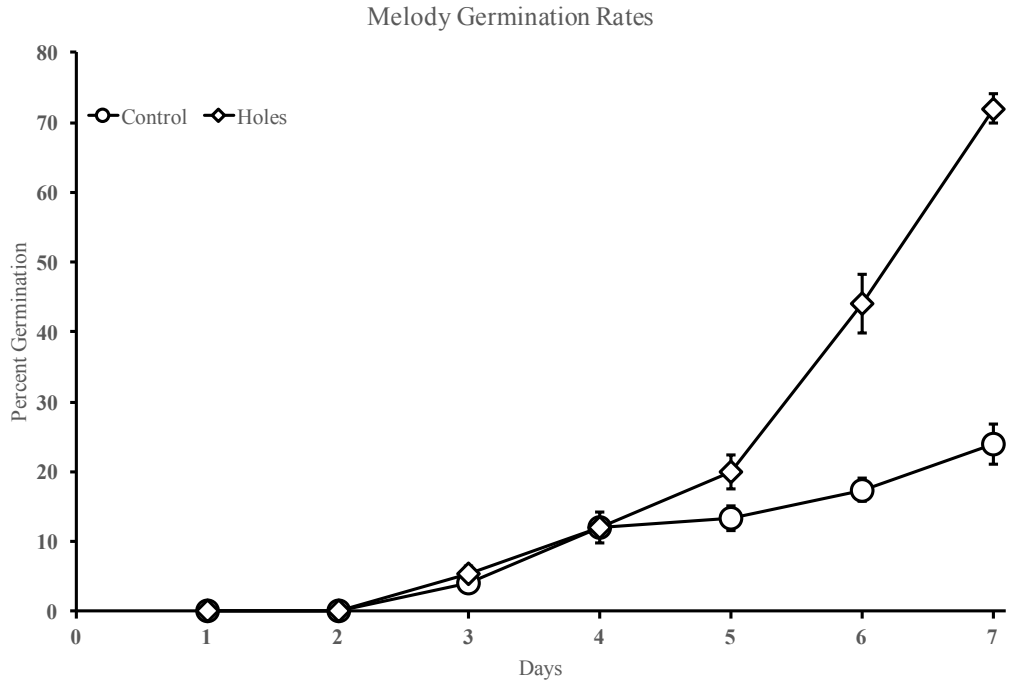

Figure 5: Average percent germination for Melody (3n) seeds

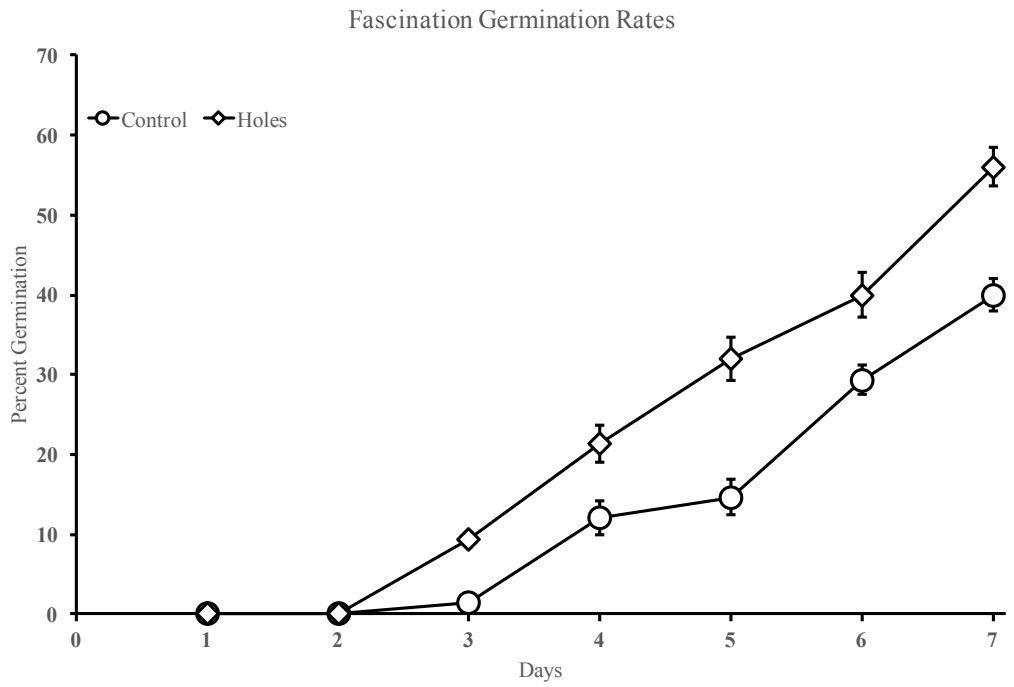

Figure 6: Average percent germination for Fascination (3n) seeds

We believe that the results strengthen the hypothesis that seed germination is limited by the lower levels of starch and lipid stores within triploid seeds (Wang *et al*., 2003). By using the aerated petri dishes, seed germination increased significantly, suggesting that humidity and concentration of oxygen or CO_2 likely play a large role in germination success of low vigor seeds. Deformations commonly seen within the triploid seeds did not appear to significantly affect germination alone, however, there may be unstudied roles at the food storage level. We hope this data will contribute to techniques that allow for more consistent and successful rates of germination in triploid watermelon varieties to lower costs associated with them.

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