Seedcoat Structure and Oxygen-enhanced Environments Affect Germination of Triploid Watermelon

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ABSTRACT. Triploid or seedless watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] cultivars often have erratic germination and low seedling vigor. The morphology of the seedcoat on two triploid cultivars—Tri X 313 and Tri X Sunrise—was examined by scanning electron microscopy (SEM) to identify structural differences compared to diploid seeds. Triploid seeds incubated with oxygen-enhanced treatments that included nicking, 1% hydrogen peroxide (H₂O₂), and 40% oxygen were investigated at low and high medium moisture levels. Triploid seed has a thicker seedcoat with a dense endotesta layer and a larger and highly variable air space surrounding the embryonic axis as compared with diploid seed. All cultivars rapidly imbibed water (≈50% of the original weight) during the first hour of imbibition, with a faster increase for triploids than for diploids. High moisture affected germination to a lesser extent in diploid than triploid seeds. Triploid germination under low medium moisture ranged from 96% to 76%, but was severely reduced to <27% under high medium moisture. Triploid seed germination was significantly improved at high moisture by H₂O₂ and by 40% oxygen. Triploid watermelon seed is very sensitive to submerged conditions, possibly due to a combination of physiological and morphological defects. The rapid imbibition and excess water collected in the seedcoat and air space surrounding the embryo, could reduce oxygen diffusion and impair metabolic pathways leading to normal germination and seedling development.

Triploid watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] represents a significant share of the total watermelon market in the United States (Maynard, 2001). Although the consumer demand is increasing, growing triploids is risky compared to diploids. The high price of the seed, about \$180 per 1000 seeds, is attributed to the high labor cost of producing the seed. The process of creating triploid seed is long and labor intensive. A diploid watermelon is treated with colchicine at the apical meristem during the seedling stage to induce a tetraploid plant. The tetraploid is then selfed or sibbed until a stable line is developed. The number of seeds of a tetraploid are often 50 to 100 per fruit, and as low as 0 to 5 seeds, compared to 200 to 800 seeds for the diploids (Wehner et al., 2001; Kihara 1951). Tetraploid seeds generally have a low germination percentage, which has been related to the small size of the embryo relative to the seedcoat (Nerson et al., 1985). The tetraploid (female) is then crossed with a diploid (male) to create an F₁ cross triploid. The number of triploid seeds obtained from this cross is also in the range of 50 to 100 per fruit. Triploid seed germination is generally ≈60% to 80%, compared to up to 95% for diploid seed. They also have less uniformity and seedling vigor after emergence. Therefore, most triploid watermelons are grown as transplants in greenhouses, with an additional cost (\$80 to \$100 per 1000 plants) to the grower.

Variation within a seed lot is also a common problem in cucurbit-

its, such as muskmelons (Welbaum, 1999) and triploid watermelon (T. Williams, Syngenta Seeds, Inc., personal communication).

The final structure of the seedcoat in mature seeds from Cucurbitaceae species may be different from the developing seed due to processes that include cell division, cell enlargement, differentiation, absorption by the growing endosperm, and seed desiccation (Werker, 1997). The mature seedcoat of triploid watermelon is hard and thick, like the tetraploid parent (Kihara, 1951). A thick seedcoat could be a physical barrier to radicle protrusion and contribute to seedcoat adherence to cotyledons after emergence (Maynard, 1989). Kihara (1951) also noted that triploid embryos do not completely fill the seed cavity, possibly due to a weak and poorly developed embryo. Limited research has been reported on improving germination of triploid watermelon seeds and in understanding the factors limiting seed germination. One procedure most studied to improve and advance germination of several vegetable species is priming or osmoconditioning (Bradford, 1986). In muskmelon (*Cucumis melo* L.), priming improved seed quality of seed lots that had not reached physiological maturity (Welbaum and Bradford, 1991). However, priming was ineffective in restoring muskmelon seed quality lost due to seed aging caused by delayed fruit harvesting (Oluch and Welbaum, 1996). So far, studies using priming or other seed conditioning treatments show limited success in improving germination of triploid watermelon seeds. In one study, emergence of triploid seed from 'Genesis' was improved when seeds were mechanically scarified with very coarse sand to abrade the seedcoat (Duval and NeSmith, 1999). Another study subjected the same cultivar to decoating and clipping the seedcoat opposite the radicle end, followed by incubation in hydrogen peroxide (H₂O₂) at a range of 0% to 8% (Duval and NeSmith, 2000). In the absence of H₂O₂, decoated and clipped seeds germinated better than the intact seeds.

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Germination of intact seeds was improved with 1% to 2% H₂O₂, but seeds were injured at >2%. Grange et al. (2000) reported that germination of triploid seeds ('ASM 121', 'ASM 1616', and 'ASM 1614') incubated at high medium moisture condition was severely reduced to 15%, while nicking or clipping improved germination to ≈40%. Maynard (1989) performed experiments to increase triploid germination and decrease seedcoat adherence by orienting the seeds with the radicle ends up at 90° and 45° angles, horizontally, or with radicle end down at 90° and 45° angles. Although positioning the seeds with the radicle up at either 45° or 90° significantly decreased seedcoat adherence, it did not improve emergence. Nerson et al. (1985) conditioned tetraploid and diploid seeds with various treatments, including lateral splitting of the seedcoat, soaking them in either H₂O (aerated and nonaerated) for 24 h at 20 °C, GA₄₊₇ or BA (5, 0.5, and 0.05 mM) for 24 h at 20 °C, or in 3% KNO₃ for 5 d at 20 °C. After treatment seeds were dried for 6 d at 25 °C and germination evaluated at three incubation temperatures, 17, 21, and 25 °C. Lateral splitting improved the germination of the tetraploid seed but had no effect on the diploid seed. Soaking in water (aerated and nonaerated), GA₄₊₇, BA, or KNO₃ for 24 h improved the germination percentage of the tetraploid at all temperatures, and the diploid at 17 °C. The tetraploids absorbed more water in the seedcoat and the seed cavity than diploids.

Yang and Sung (1994) compared germination rate and mean germination time of triploid seed 'Phong Sen No. 1' in response to the weight, classified as either light or heavy. Heavy seeds germinated better but light seeds had greater water uptake per gram of seed. Light seeds also had less seed coat tissue. Botha et al. (1984) investigated oxygen uptake in diploid watermelon seeds exposed to water stress and found that stressed seeds did not have a respiratory lag phase compared to the nonstressed seeds.

Seed structural components and germination responses to environmental conditions are not well known in triploid watermelon cultivars. The objectives of our work were to characterize seedcoat morphology of dry and imbibed triploid seeds, and to determine germination responses to seedcoat alteration, medium moisture content and oxygen-enriched environments. This information may be useful in developing seed treatments, such as liquid or solid matrix priming (SMP), to improve triploid watermelon seed germination, seedling vigor and emergence uniformity.

Materials and Methods

SEED MORPHOLOGY. Diploid 'Sunsweet', 'Allsweet', and 'Sugarlee' and triploid 'Tri X 313' with low (L), medium (M) and high (H) germination lots, and triploid 'Tri X Sunrise' with low (L) and high (H) germination lots were used to characterize seed morphology. Seed lot germination ratings were provided by the supplier (proprietary information from Syngenta Seeds, Inc. Boise, Idaho).

Total seed weight and seed components were measured by randomly selecting 10 seeds from each cultivar and lot. Each seed was cut longitudinally and placed under a 18× magnification stereomicroscope. Thickness of the seedcoat and the distance between the edge of the cotyledons and the innermost layer of the seedcoat were measured with an eyepiece micrometer. The embryo was then excised, the seedcoat weighed, and the weight of the embryonic axis calculated. Seed weight for each cultivar was standardized to the same water content.

A scanning electron microscope (SEM) was used to examine dry and imbibed seeds. Dry seeds were cut in half with a #10 scalpel blade and the halves were placed in a 9-cm petri dish. The cut seeds were placed in a microwave at the highest power for 30 sec to kill

any living cells and then transferred to a vacuum oven at 60 °C for 48 h. The samples were mounted on small stubs using a superglue-type gel. The samples were then sputter coated with AuPd for 5 min at 10 mA. Samples were stored in a 9-cm petri dish in a desiccator until use.

Imbibed seeds were placed in distilled water in plastic tubes overnight. After draining off excess water the tubes were kept at room temperature for 24 h. The seeds were then manually cut into small sections and halves with a #10 scalpel. Each section, considered a sample, was placed in a 15-mL plastic tube and filled with liquid nitrogen. The tubes were placed under a vacuum on dry ice overnight. The seed sections were mounted on stubs, coated, and stored in the same manner as the dry seed samples.

Dry and imbibed seeds processed by the above methods were examined using a JSM-T330A SEM (Joel, Peabody, Mass.) at 15 kV. Control, dry seeds were observed at magnifications from 75× to 1000× and micrographs taken at 200×. Imbibed seeds were also observed at these magnifications, but due to the larger size caused by the expansion of tissue, most micrographs were taken at 150× magnification. Images were captured on either 400 ISO film (Polaroid, Bedford, Mass.) or Ilford HP5 Plus 400 negative film and contact prints were made. All photos were taken in black and white and enhanced using Photoshop 3.0 (Adobe System Inc., San Jose, Calif.).

Further observations were made using an Environmental SEM E-3 (ESEM, ElectroScan, Wilington, Mass.). This equipment allows for materials to be viewed in their native state, thus eliminating seed preparations as needed for the JSM-T330A SEM. Seeds were cut with a #10 scalpel blade and samples were attached to a metal stub with double-sided tape and observed in the ESEM at 15 kV at magnifications ranging from 100× to 1000×. Digital images were enhanced using the same graphics program as that used for the JSM-T330A SEM images.

SEED IMBIBITION. Diploid seed from 'Allsweet' and 'Sugarlee', and triploid seed of low (L) and high (H) germination lots from 'Tri X 313' and 'Tri X Sunrise' were placed on moistened seed germination paper in 9-cm petri dishes. A total of 100 seeds per cultivar, with 25 seeds per petri dish was used. Seeds were germinated in a growth chamber at 25° C. Initially, 5 mL of water was added followed by an additional 2 mL at 60 h. Weights were taken at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 32, 40, 48, 56, 64, 72, 80, 106, and 125 h. To record seed weight gain, seeds were placed on paper towels to absorb any extra moisture, weighed on an analytical balance, and then returned to the petri dishes. Gains over the original weight were calculated.

MOISTURE, NICKING AND HYDROGEN PEROXIDE EXPERIMENT. This experiment used 'Sunsweet' as diploid, and 'Tri X 313' and 'Tri X Sunrise' with low (L) and high (H) germination lots as triploids. A total of 100 seeds per cultivar, with 25 seeds per petri dish was used. Germination was at 30° C in a dark growth chamber. Two levels of medium moisture were used as described in Grange et al. (2000). For the first level, 5 mL water was added to each petri dish and was considered as low or optimal moisture. In a previous hydration experiment with polyploid watermelon seeds, Sung and Chiu (1995) used as a control treatment 4 mL of dionized water for 20 seeds placed in a 9-cm petri dish. For the second level, 10 mL water was added to each dish and was considered excessive medium moisture. The intent was to expose germinated seeds to a hypoxic stress due to submerged incubation conditions. AOSA (2001) indicates that optimum seedling development occurs when medium moisture is kept on the 'dry side'; therefore we viewed submerged conditions as a stressful environment for testing triploid watermelon seeds. Nicking was done by carefully cutting a 1 mm hole in the edge of the seedcoat opposite the radicle end. A 1% H₂O₂ solution (5 and

10 mL) was used in place of water for the low and high levels medium moisture, respectively. Germination data were collected daily for 12 d.

OXYGEN-ENRICHMENT EXPERIMENT. Diploid cultivars used for this experiment were ‘Sunsweet’, ‘Allsweet’ and ‘Sugarlee’. Triploid cultivars were ‘Tri X 313’ and ‘Tri X Sunrise’ each from a low (L) and high (H) germination lot. Seeds were incubated at low (5 mL water) and high (10 mL water) medium moisture levels with 21% or 40% oxygen. A total of 100 seeds per cultivar, with 25 seeds per petri dish were used. The petri dishes were placed in 3.75 L jars with tightly fitted lids and inlet and outlet tubes. Forty percent oxygen was introduced into the jars by mixing purified oxygen (99.995%) and nitrogen (99.995%) with a mass flow controller set to deliver 40% oxygen through a humidification chamber before entering the jars. The outlet tube was connected to an air stone submerged in a beaker of water to provide a slight positive pressure and thus ensure that gas flow was occurring. The system remained closed for the duration of the experiment. The 21% oxygen control utilized the same general system except that an air pump was used as the gas source to deliver ambient air. A final germination count was made at 5 d. All experiments were performed at 25° C in a dark growth chamber.

STATISTICS. Imbibition, seedcoat alteration and oxygen-enhancement experiments were arranged in a randomized complete block design, and each cultivar was replicated four times. Each replication consisted of 25 seeds placed in a 9-cm petri dish, with a total of 100 seeds used per cultivar. Seeds were considered germinated when the radicle protruded (≥ 2 mm) from the seedcoat. Percentage of germination was transformed to square root arcsine prior to performing an analysis of variance. Data were analyzed using PROC GLM and PROC REG of SAS (SAS Institute, Inc., Cary, N.C.) and means were separated by LSD (0.05).

Results and Discussion

SEED MORPHOLOGY. Triploid seeds were heavier with a thicker seedcoat than the diploid ‘Sunsweet’ and ‘Allsweet’, but not compared to ‘Sugarlee’ (Table 1). Across cultivars and seed lots, the average seedcoat weight was 57% and 48% of the total seed weight for the triploid and diploid, respectively. The largest difference in seed characteristics among cultivars was the greater air space in triploid seeds (0.57 to 1.12 mm), compared to the diploid (0.03 to 0.15 mm). The greater air space and thicker seedcoat was also reported for the triploids ‘ASM 121’, ‘ASM 1614’, and ‘ASM 1616’

(Grange et al., 2000). The air space for those triploid cultivars ranged from 0.89 to 2.27 mm, compared to 0.07 mm for the diploid ‘Charleston Gray’. The thicker seed coat and large air space surrounded by the endotesta layer could partially restrict oxygen uptake under high moisture conditions by allowing water to collect and form a layer around the embryo. The large air space in triploids also results in the embryo having to expand more before splitting the seedcoat, with the potential reduction of seedling vigor and uniformity, particularly under wet conditions.

Morphological differences were found between diploid and triploid seeds when examined by SEM. There were also structural differences between imbibed seed and dry seed, and for those triploid seeds that germinated during the 24-h incubation period compared to nongerminated imbibed seeds. There are two main layers in the seedcoat of the watermelon seed. The first layer is called the exotesta and is composed of the sclerotic tissue (Sl), seed hypodermis (Sh) and seed epidermis (Se). The sclerotic tissue is the innermost layer and varies in thickness, from one to two cells around the seed. The thickest part of the exotesta layer is the seed hypodermis, which ranges from 5 to 7 cells. The seed epidermis is a one-cell layer thick and marks the outside of the seedcoat. The layer between the exotesta and the embryo (E) is referred to herein as the endotesta (Ed).

Seedcoats of Cucurbitaceae species follow a typical pattern of development (Singh and Dathan, 1990). The inner seedcoat is comprised of a chlorenchymatous region, which becomes mucilaginous, drying into a papery membrane and unspecialized inner epidermis (endotesta). In muskmelon, the endosperm is a single suberized cell layer covered with callose that is deposited around 30 to 35 d after anthesis (Yim and Bradford, 1998). In watermelon, whether the endotesta layer may be part of remnant endosperm, either single or few cell layers which may have either been absorbed during seed development and seed desiccation is unknown. In mature Cucurbit seeds, the endosperm is very poorly represented (Singh, 1953). It may form a single layer just outside the embryo or persists near the cotyledonary tips. In some species, such as *Luffa cylindrica* no trace of endosperm occurs (Singh, 1953).

The endotesta was different between diploid and triploid cultivars. In the diploid ‘Sunsweet’, (Fig. 1A) the endotesta was very porous and thin as compared to that of the triploid seed ‘Tri X 313’ from the H germination lot (Fig. 1B), or from ‘Tri X 313’ from the L germination lot (Fig. 1C). The endotesta on the triploids, particularly from ‘Tri X 313’ L germination lot was thick with less intercellular spaces. It is interesting to note that seeds of ‘Tri X 313’

Table 1. Seed characteristics of diploid (2x) and triploid (3x) watermelon cultivars. Values are means $\pm$ SE (n = 10).

Cultivar ²	Germination ³ (%)	Seed dry wt (mg)			Seed length (mm)	Coat thickness ^x (mm)	Air space ^x (mm)
		Whole	Coat	Cotyledon + embryo			
Sunsweet (2x)	92 $\pm$ 1.2	43.15 $\pm$ 1.51	20.36 $\pm$ 0.73	22.79 $\pm$ 1.07	8.90 $\pm$ 0.15	0.34 $\pm$ 0.03	0.03 $\pm$ 0.01
Sugarlee (2x)	80 $\pm$ 1.4	98.25 $\pm$ 2.41	47.25 $\pm$ 1.74	51.00 $\pm$ 1.84	12.94 $\pm$ 0.18	0.42 $\pm$ 0.03	0.10 $\pm$ 0.03
Allsweet (2x)	96 $\pm$ 0.4	42.14 $\pm$ 0.88	21.27 $\pm$ 1.37	20.88 $\pm$ 1.58	8.70 $\pm$ 0.10	0.37 $\pm$ 0.01	0.15 $\pm$ 0.03
Tri X 313 L (3x)	67 $\pm$ 1.8	57.08 $\pm$ 2.22	32.46 $\pm$ 0.80	24.62 $\pm$ 1.56	9.31 $\pm$ 0.11	0.45 $\pm$ 0.03	0.83 $\pm$ 0.29
Tri X 313 M (3x)	95 $\pm$ 0.7	70.41 $\pm$ 1.82	41.62 $\pm$ 1.35	28.79 $\pm$ 1.15	9.72 $\pm$ 0.09	0.44 $\pm$ 0.02	0.57 $\pm$ 0.18
Tri X 313 H (3x)	81 $\pm$ 1.0	67.40 $\pm$ 1.35	36.27 $\pm$ 3.85	31.13 $\pm$ 3.07	9.74 $\pm$ 0.13	0.45 $\pm$ 0.02	0.94 $\pm$ 0.30
Tri X Sunrise L (3x)	60 $\pm$ 1.7	58.87 $\pm$ 2.27	34.37 $\pm$ 1.20	24.50 $\pm$ 1.17	9.19 $\pm$ 0.15	0.46 $\pm$ 0.02	0.89 $\pm$ 0.20
Tri X Sunrise H (3x)	93 $\pm$ 0.8	61.27 $\pm$ 2.24	34.51 $\pm$ 1.51	26.76 $\pm$ 1.03	9.30 $\pm$ 0.14	0.52 $\pm$ 0.02	1.12 $\pm$ 0.29
CV (%)		16.4	23.5	21.2	4.9	19.3	99.8

²L, M, H = Low, medium and high germination lots. Ratings were provided by the supplier (Syngenta Seeds, Inc.).

³Mean germination percentage under laboratory conditions at 27 °C.

^xThickness of the seedcoat and the distance between the edge of the cotyledons and the innermost layer of the seedcoat (air space) were measured with an eyepiece micrometer.

labeled as medium (M) germination lot by the supplier showed a more porous and disrupted endotesta (Fig. 1D), which was similar to that of the diploid 'Sunsweet'. Subsequent germination tests conducted on these seeds, so called M germination lot, found them to have a higher total germination to that of the 'Tri X 313' from the H germination lot (Table 1). The thickness and porosity of this endotesta layer could determine the ability of the seed to germinate. In addition, the variability in the endotesta thickness between genotypes, seed lots, or even within seed lots, could be a factor in the large inconsistencies of germination rates and seedling vigor. In a study by Edelstein et al. (1995), SEM was used to examine the intercellular spaces on the surface seedcoat of melon (*Cucumis melo* L.). In 'Noy Tizre'el', which germinated poorly at low temperatures, the intercellular spaces were completely sealed compared to the prominent intercellular spaces in 'Persia 202'. Edelstein et al. (1995) hypothesized that the most probable cause for the poor germination at low temperature in certain melon varieties was a combination of lower seedcoat permeability of oxygen and greater embryo sensitivity to hypoxia. Whether the structure of the endotesta layer in triploid watermelon is affected by environmental or cultural conditions during development is unknown. As in muskmelon

seeds (Welbaum, 1999), several factors could affect seedcoat morphology and the endotesta layer, including the nutrition of the mother plant, maturity at harvest, final seed size, mechanical integrity, seed desiccation, and aging.

After 24 h imbibition there seems to be no major difference in the condition of the seedcoat except for a large expansion of the seed epidermal layer for both diploid (Fig. 2A) and triploid (Fig. 2B) seed. This layer expands upon contact with water within 12 h, and does not appear to shrink when dried. In hydrated primed seeds this seed epidermis is already expanded (not shown). Another distinctive morphological characteristic in triploid seeds is the amount of airspace. During the incubation period of the triploid and diploid seed, a few seeds of the 'Tri X Sunrise' from the low germination lot were observed to have enough radicle protrusion from the seedcoat to be considered germinated. These seeds were observed with the SEM and compared to seeds that did not germinate during the first 24 h (Figs. 2C and D). The airspace of a diploid seed was similar to that of the germinated triploid (not shown). The amount of air space in the nongerminated seed cavity is clearly evident (Fig. 2D), and not observed for the germinated triploid. Our first hypothesis is that a very small triploid embryo encased in the tetraploid seedcoat could

Fig. 1. Cross sections at 200 $\times$ magnification of a dry 'Sunsweet' diploid seed (A), dry 'Tri X 313' triploid seed from a high (H) germination lot (B), dry 'Tri X 313' triploid seed from a low (L) germination lot (C), and dry 'Tri X 313' triploid seed from a medium (M) germination lot (D). E = embryo, Ed = endotesta, Sl = sclerotic layer, Sh = seed hypodermis, Se = seed epidermis.

run out of storage reserves, which in turn will produce malformed hypocotyls (e.g., curled, shortened) and cotyledons (e.g., convoluted or with unshed seed coats). Even when the radicle breaks the seed coat, root morphological abnormalities (e.g., taproot missing, minimum root branching) may also be present. Second, the large air space may allow a layer of water to form around the embryo under high moisture conditions, creating an anaerobic environment during the early phases of germination.

In some triploid seeds examined with the ESEM, the cotyledons were folded abnormally inside the seedcoat (Fig. 3). Instead of the cotyledons laying flat against each other, as in the diploid seeds, these cotyledons were folded in half. Some cotyledons appear as if they have a fold or crease down the center after emergence. This defect during development could also lead to difficulty in breaking open the seedcoat during cell expansion to allow for radicle emergence. Abnormal triploid seedlings in commercial greenhouse plantings have also shown three or fused cotyledons on one seedling. These defects may have occurred as a pregermination event, probably during embryo developmental expansion within the seedcoat.

IMBIBITION RESPONSE. All seeds, particularly triploids, had the most rapid weight gain (about 50% of the original weight) during the

first hour of imbibition (Fig. 4A and B). The weight of 'Allsweet', 'Tri X 313' L and 'Tri X 313' H germination lots leveled out at 12 h (Fig. 4A), while the weight of 'Tri X Sunrise' L and H germination lots did not appear to level off until 24 h (Fig. 4B). The weight of 'Sugarlee' did not level off until 64 h, probably due to having the largest seedcoat and embryonic tissue of all tested cultivars. 'Tri X Sunrise' L germination lot gained the most weight in the first 24 h. The assumption is that water is being taken up through the outer seedcoat tissues into the seed cavity and expanding the endotesta layer of the seedcoat. The seedcoat thickness in 'Tri X Sunrise' L germination lot was statistically lower (0.46 mm) than the H germination lot (0.52 mm) (Table 1). The thicker seedcoat and larger seed cavity in triploids compared to diploids would allow for more water to be stored first inside the seedcoat, followed by filling the seed cavity. This excess water could slow oxygen diffusion to the embryo. A rapid increase in water uptake could also increase the potential for imbibitional damage to the embryo. Other studies have indicated that water uptake of imbibing tetraploid and triploid whole seeds was 50% during the first 4 h and was completed within 24 h (Nerson et al., 1985; Sung and Chiu, 1995; Yang and Sung, 1994). Water gain for triploid 'Phong Sen No. 1' seed was also much higher

Fig. 2. Cross sections of an imbibed 'Sunsweet' diploid seed at 150 $\times$ (A), imbibed 'Tri X 313' triploid seed from a low (L) germination lot at 150 $\times$ (B), germinated 'Tri X Sunrise' triploid seed from a low (L) germination lot at 75 $\times$ (C), and imbibed 'Tri X Sunrise' triploid seed from a low (L) germination lot at 100 $\times$ magnification (D). E = embryo, Ed = endotesta, Sl = sclerotic layer, Sh = seed hypodermis, Se = seed epidermis.

Fig. 3. Cross section of an imbibed 'Tri X 313' triploid seed from a low (L) germination lot at 98 $\times$ magnification. E = embryo, Ed = endotesta, Sl = sclerotic layer, Sh = seed hypodermis, Se = seed epidermis.

than the diploid and tetraploid parent seeds (Chiu et al., 1995).

MOISTURE AND OXYGEN ENHANCEMENT. High moisture did not affect seed germination of the diploid 'Sunsweet' intact seeds. Hydrogen peroxide and nicking decreased germination compared to the low moisture intact seeds (Fig. 5). Overall 'Sunsweet' diploid germination ranged from 85% to 95%. Germination in high moisture medium was severely impaired in all triploid cultivars. Under optimal moisture, germination ranged from 96% (high germination lot) to 76% (low germination lot), but was severely reduced to less than 27% under high moisture. For three of the four triploid genotypes incubated under high moisture conditions, nicking significantly improved germination, but only from 2%–3% to 12%–

25%. In a previous study with other triploid cultivars, high medium moisture reduced germination to 15%, while nicking increased germination up to 40% (Grange et al., 2000). High moisture with H_2O_2 enhanced germination of all triploids, ranging from 36% to 69%. Duval and NeSmith (2000) found that final germination of intact seeds of triploid 'Genesis' incubated in agar was significantly increased by 1% H_2O_2 . Comparing low germination lots of triploids, both 'Tri X 313' and 'Tri X Sunrise' had the same germination when incubated at low moisture (Fig. 5). At high moisture, nicking or H_2O_2 significantly enhanced germination of 'Tri X 313' low germination lot, compared to the high moisture intact seeds, but not 'Tri X Sunrise' low germination lot, indicating that treatment response was cultivar dependent. Even though the germination improvements were significant, the levels obtained were lower than commercially accepted by industry standards.

Increasing the oxygen concentration to 40% had no significant effect on the germination of diploid 'Sunsweet' seeds incubated at either low or high medium moisture levels (Fig. 6). 'Allsweet' and 'Sugarlee' had a 30% and 65% decrease in germination, respectively, at high medium moisture and 21% oxygen. However, 40% oxygen slightly increased germination of both diploid seed lots incubated at high moisture (Fig. 6). The triploid cultivars showed no significant change in germination between 21% and 40% oxygen environments under low moisture conditions. For 'Allsweet' and 'Sugarlee' under high moisture, enhanced oxygen increased germination 6% to 10% over the high moisture control. Similar trends, but with a larger response, were found in all triploid cultivars incubated at high moisture. For example, at 21% oxygen 'Tri X Sunrise' H germination decreased from 93% at low moisture to 0% under high moisture, but at 40% oxygen germination increased to 18%. Low germination was reported for cucumber seeds submerged in water and under oxygen deficiency, but increased after oxygen content was raised (Rabie et al., 1989).

The inhibition of germination in stressed watermelon seeds was related to the reduction of the key metabolic pathways, such as glycolysis, pentose phosphate pathway and the TCA cycle (Botha and Small, 1985). The major substrates for seed respiration are carbohydrates, lipids and proteins. Triploid watermelon seeds (same lots as in our study) had lower starch reserves (12% to 27% vs. 42%, dry weight basis) and larger lipid bodies than diploid seeds (Wang et al., 2001). Diploid and triploids with high germination percentages had a larger increase in oxygen uptake relative to triploids with low germination capacity (Wang, 2002). Under submerged or saturated conditions the reduction of oxygen levels might reduce the activation of storage tissues for the cellular events precluding germination. As reported by Wang (2002), if lipids constitute an important energy substrate for respiration in triploids, they will require more oxygen since lipids are more reduced than carbohydrates. The relative larger triploid vs. diploid germination response at 40% oxygen at high media moisture, provides evidence in support to this hypothesis.

The thick endotesta in the seedcoat and a large airspace between the underdeveloped embryo and seedcoat tissues appears to have a major role limiting seed germination of triploid watermelon. However, the results obtained by nicking the seed indicate that triploid seed germination is not inhibited by the seedcoat alone. Triploid watermelon seed is very sensitive to increased moisture conditions, possibly due to a combination of physiological and morphological defects. The size of the cotyledons, embryo, seedcoat and consequently seed cavity, are components inherited from both the diploid male and tetraploid female parent. We hypothesize that part of the seed germination problem of triploid seeds is related to genetic

Fig. 4. Seed moisture increase (fresh weight/original weight) for diploid 'Allsweet', and triploids 'Tri X 313' low (L) and 'Tri X 313' high (H) germination seed lots (A), and diploid 'Sugarlee', and triploids 'Tri X Sunrise' low (L) and 'Tri X Sunrise' high (H) germination seed lots (B).

Fig. 5. Germination of diploid 'Sunsweet' and triploid 'Tri X 313' and 'Tri X Sunrise' watermelon with low (L) and high (H) seed germination lot in response to moisture (M), hydrogen peroxide (H_2O_2), and nicking. Each bar represents a mean $\pm$ SE (n = 4).

Fig. 6. Germination of diploid 'Sunsweet', 'Allsweet', and 'Sugarlee', and triploid 'Tri X 313' and 'Tri X Sunrise' with low (L) and high (H) seed germination lot in response to moisture (M) and oxygen. Each bar represents a mean $\pm$ SE (n = 4).

factors associated with the different ploidy level of the seed, e.g., triploid embryos being encased by tetraploid seedcoats. Increasing oxygen, either by oxidation with 1% H_2O_2 or 40% oxygen increased germination under excessive medium moisture, but total germination levels were lower than the intact seeds incubated at low medium moisture. We could not correlate total protein with germination capacity of triploids (unpublished data). Correlations between seed quality and other storage reserves such as starch, lipids, and carbohydrates are not yet fully established.

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