EFFECTS OF PRIMING AND STAGE OF DEVELOPMENT ON VIGOR AND LONGEVITY OF MUSKMELON (*CUCUMIS MELO* L.) SEEDS

by

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Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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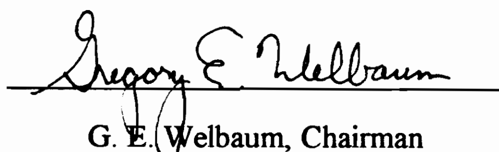

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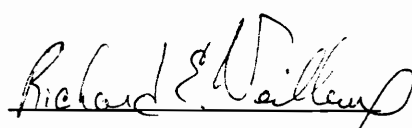

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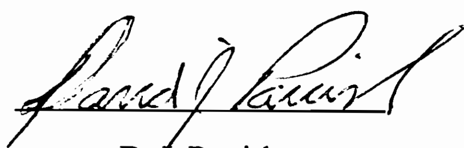

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(ABSTRACT)

Muskmelon seeds were harvested at eight stages of development to determine the optimal harvest time for best seed quality and long-term storage. Several types of osmotic and matric priming treatments were investigated to determine the optimal treatment for maximal seed vigor and to test the effects of priming on seed storage life. The most effective priming treatments for improved germination of muskmelon seeds occurred at water potentials (Ψ) between –1.5 and –1.8 MPa and at priming durations of 4 to 7 days. Osmotic priming in KNO_3 and PEG 8000 solutions was more effective than matric priming with calcium silicate, vermiculite, and Hayter loam soil. Priming increased the storage life of newly matured 40 and 45-days after anthesis (DAA) stored seeds but decreased the storage life of 55 DAA seeds. Fifty, 55, and 60-DAA seeds showed the greatest tolerance to adverse storage conditions and water stress and had the greatest seedling vigor. The highest quality seeds were attained 50 to 60 DAA from fruit harvested after edible maturity but before the onset of severe decomposition. After 6 years of storage, seeds washed in water at harvest were more vigorous and resistant to accelerated aging than unwashed seeds at most stages of development. Primed seeds retained some beneficial effects after 9 years of storage at optimal conditions but lost viability, vigor, and

uniformity of germination more rapidly than nonprimed seeds following controlled deterioriation. Instron analysis showed that priming weakened the perisperm envelope tissue prior to radicle emergence. Less force was required to puncture primed seed pieces than nonprimed. The penetration force and energy required to puncture the perisperm envelope tissue decreased gradually during imbibition, increased steadily during seed development and dry storage, and was negatively correlated with seed vigor. Priming accelerates enzymatic breakdown of perisperm envelope during germination. Endo-β-mannanase-like activity declined steadily during imbibition and was higher in nonprimed seeds than primed seeds, indicating that enzymatic activity in the perisperm peaked during priming. Flow cytometry revealed that the beneficial effects of priming and seed maturity do not correlate with DNA replication activity in muskmelon seeds. This study has shown that seed maturation inside the fruit until just after edible maturity is necessary for highest muskmelon seed vigor. Priming treatments should be performed as close to the planting date as possible, because primed seeds exhibit reduced storage life compared to nonprimed seeds.

To my Parents Obed and Dinah Oluoch ....

I dedicate this work.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS v

LIST OF TABLES ix

LIST OF FIGURES xi

CHAPTER ONE

General Introduction 1

Effects of Seed priming on Viability and Vigor 2

Viability and Vigor of Muskmelon Seeds during Development and Storage 4

Viability and Vigor during Priming and Storage 6

Role of the Endosperm and Enzymatic Activity during Germination 8

Effects of Priming on Nuclear Replication Activity during Germination 12

Purpose of Current Study 14

CHAPTER TWO

OSMOTIC AND MATRIC PRIMING OF MUSKMELON SEEDS

Abstract 16

Introduction 17

Materials and Methods 19

Results 21

Discussion 38

TABLE OF CONTENTS (cont:)

CHAPTER THREE

EFFECT OF POSTHARVEST WASHING AND POST-STORAGE PRIMING ON VIABILITY AND VIGOR OF 6-YEAR-OLD MUSKMELON SEEDS FROM EIGHT STAGES OF DEVELOPMENT

Abstract 43

Introduction 44

Materials and Methods 45

Results 48

Discussion 58

CHAPTER FOUR

VIABILITY AND VIGOR OF OSMOTICALLY PRIMED MUSKMELON SEEDS AFTER 9 YEARS OF STORAGE

Abstract 64

Introduction 65

Materials and Methods 66

Results 69

Discussion 77

TABLE OF CONTENTS (cont:)

CHAPTER FIVE

CHANGES IN PERISPERM STRENGTH AND ENZYMATIC ACTIVITY DURING DEVELOPMENT, AFTERRIPENING, GERMINATION, AND PRIMING

Abstract	82
Introduction	83
Materials and Methods	86
Results	93
Discussion	99

CHAPTER SIX

EFFECTS OF PRIMING AND SEED DEVELOPMENT ON NUCLEAR REPLICATION ACTIVITY IN MUSKMELON SEEDS

Abstract	107
Introduction	108
Materials and Methods	111
Results	114
Discussion	123

CONCLUSIONS	126
LITERATURE CITED	129
VITAE	147

LIST OF TABLES

Table 2.1. The best osmotic and matric priming treatments for improving muskmelon seed vigor.......... 31

Table 3.1. Germination of washed and unwashed muskmelon seeds during development after 6 years of storage.......... 49

Table 3.2. Effects of post-storage priming and controlled deterioration on mean time to loss of viability, the initial probit viability, and the slope of the probit viability loss regression line.......... 53

Table 4.1. Germination of primed and nonprimed seeds after 9 years of storage.......... 70

Table 4.2. Seedling emergence percentage and mean log time to emergence (log $\bar{t}$) after 9 years of storage.......... 72

Table 4.3. The effect of priming on germination percentage, mean log time to germination (log $\bar{t}$), and standard deviation (σ) after 9 years of storage.......... 75

Table 4.4. The effect of priming after controlled deterioration on germination percentage, log $\bar{t}$, and σ after 9 years of storage 76

Table 6.1. Germination percentages, log $\bar{t}$, and σ in germination time of nonprimed, osmotic primed, and matric primed seeds.......... 115

Table 6.2. A cell cycle analysis of nuclear DNA replication activity showing the ratio of 4C/2C nuclear signals in the embryonic axes cells during imbibition.......... 117

LIST OF TABLES (cont:)

Table 6.3. Cell cycle stage analysis showing nuclear DNA replication activity expressed as percentages of total nuclei in embryonic axes cells during imbibition.. 118

LIST OF FIGURES

Fig. 2.1. Longitudinal planar area (mm^2) of dry seed testa, embryo, and embryonic axis 23

Fig. 2.2. Planar area (mm^2) between the periphery of the seed coat and the embryo; and of the cotyledons 24

Fig. 2.3. X-ray images of nonprimed and primed seeds 25

Fig. 2.4. Representative X-ray images illustrating longitudinal planar dimensions of dry primed and nonprimed seeds 26

Fig. 2.5. Solid carrier moisture content versus water potential during the priming treatment 27

Fig. 2.6. Germination percentage, log $\bar{t}$, and σ in germination times versus duration of priming treatment 28

Fig. 2.7. Germination percentage and log $\bar{t}$ versus priming water potential using PEG 8000 solutions 30

Fig. 2.8. Germination percentage, log $\bar{t}$, and σ in germination times versus decreasing Ψs for seeds primed in calcium silicate 32

Fig. 2.9. Germination percentage, log $\bar{t}$, and σ in germination times versus duration of priming treatment for seeds primed in calcium silicate 33

Fig. 2.10. Germination percentage, log $\bar{t}$, and σ in germination times versus decreasing Ψs for seeds primed in soil 35

LIST OF FIGURES (cont:)

Fig. 2.11.	Germination percentage, log $\bar{t}$, and σ in germination times versus duration of priming treatment for seeds primed in soil	36
Fig. 2.12.	Germination percentage, log $\bar{t}$, and σ in germination times versus decreasing Ψs for seeds primed in vermiculite	37
Fig. 2.13.	Germination percentage, log $\bar{t}$, and σ in germination times versus duration of priming treatment for seeds primed in vermiculite	39
Fig. 3.1.	Probit percentage germination versus hours of controlled deterioration for 40, 45, 50, 55, and 65 DAA seeds after 6 years of storage	52
Fig. 3.2.	Log $\bar{t}$ versus hours of controlled deterioration for 45, 50, 55, and 65 DAA seeds after 6 years of storage	54
Fig. 3.3.	Radicle emergence times (σ) versus hours of controlled deterioration for 45, 50, 55, and 65 DAA seeds after 6 years of storage	55
Fig. 3.4.	Ψ_{50} at 30°C for washed or unwashed 30, 35, 40, 45, 50, 55, 60, and 65 DAA seeds after 6 years of storage	57
Fig. 3.5.	Comparison of washed and unwashed postharvest seed treatments on seedling vigor for 6 stages of development after 6 years of storage	59
Fig. 4.1.	Effects of controlled deterioration on primed and nonprimed seed germination percentage, log $\bar{t}$, and σ of germination	73

LIST OF FIGURES (cont:)

Fig. 4.2. Instron analysis of the force required to penetrate seed pieces during imbibition 79

Fig. 5.1. Cross-sectional diagram of a muskmelon seed.... 88

Fig. 5.2. Instron analysis showing changes in pressure of the seed tissues during penetration 90

Fig. 5.3. Gel diffusion assay showing endo-β-mannanase-like activity.... 92

Fig. 5.4. Germination percentages and log $\bar{T}$ versus stage of seed development for newly harvested and afterripened seeds.... 94

Fig. 5.5. The force and energy required to penetrate muskmelon seed pieces during seed development at harvest, and after 5 months of storage.... 96

Fig. 5.6. The force and energy required to penetrate muskmelon seed pieces during imbibition and development.... 97

Fig. 5.7. The mechanical stiffness of muskmelon seed pieces during imbibition and development.... 98

Fig. 5.8. Ψs measured for cotyledons and embryonic axes for primed and nonprimed seeds during imbibition.... 100

LIST OF FIGURES (cont:)

Fig. 5.9. Endo-β-mannanase-like activity of primed and nonprimed seeds during imbibition.. 101

Fig. 6.1. Germination percentages, log $\bar{t}$, and σ in germination time versus stage of seed development 116

Fig. 6.2. Imbibition time versus 4C/2C ratio for 30, 40, 50, and 65 DAA seeds.. 120

Fig. 6.3. Percentage of nuclei in 2C, 4C, 8C, and S fraction for embryonic axes cells from 30, 40, 50, and 65 DAA seeds........... 121

Fig. 6.4. Percent nuclei versus hours of imbibition for 30, 40, 50, and 65 DAA seeds.. 122

Chapter 1.

General Introduction

Muskmelon (*Cucumis melo* var. *reticulatus*) is a thermophilic crop that requires a long growing season to complete its life cycle. Commercial muskmelon production is mainly on irrigated land in semi-arid regions. Farmers in northern latitudes plant crops in early spring in an attempt to obtain high returns from early-season production. Early planting is problematic, because establishment of the crop is limited by low soil temperatures, seedling diseases, and soil crusting (Bradford et al., 1988). Muskmelon seed germination decreases rapidly at temperatures below 20°C (Nelson and Sharples, 1980; Nerson et al., 1982). The minimal germination temperature for muskmelon seed is 15°C, while the optimal temperature is 30 ± 2°C (Lorenz and Maynard, 1980). Although melon seeds require relatively high temperatures for germination, different cultivars vary greatly in their response to sub-optimal temperatures (Nerson et al., 1982). Various types of seed treatments, geared towards improving germination of melon crops at low-temperature, have been reported. Nerson et al. (1985) showed that higher germination at low temperatures could be obtained in watermelons (*Citrullus lanatus* L.) by seed coat splitting. Seed coat removal in melon seeds improved germination at low water potential (Dunlap, 1988) and low temperature (Edelstein and Kigel, 1990; Edelstein et al., 1995). Pretreatments with hormone solutions have also proved effective in enhancing germination of melons (Nerson et al., 1982; Edelstein and Kigel, 1990). On the other hand, seed priming has been used extensively to improve low-temperature germination of many species (Bradford, 1986). Improving muskmelon seed germination under supra- and sub-optimal temperatures is necessary to improve stand establishment under adverse

conditions. The establishment of a good seedling stand is a prerequisite for improved yield and quality (Wurr and Fellows, 1983).

A) Effect of Seed Priming on Viability and Vigor

The term priming has historically been used to describe a controlled hydration seed treatment followed by redrying. According to Khan (1992), the term was coined by Malnassy (1971) to describe a treatment to improve germination at low temperature. Seed priming involves exposing seeds to low water potentials that restrict germination but permits pregerminative physiological and biochemical changes to occur (Bradford, 1986; Khan, 1992; Heydecker and Coolbear, 1977). Upon rehydration, primed seeds may exhibit faster rates of germination, more uniform emergence, greater tolerance to environmental stress, and reduced dormancy in many species (Bradford, 1986; Khan, 1992; Welbaum and Bradford, 1991a). The optimal duration for the priming process varies according to the type of osmoticum, osmotic potential, temperature, and seed species (Parera and Cantliffe, 1994). Matric and osmotic priming are the two most common types of treatments. For osmotic priming, seeds are incubated in solutions of inorganic salts such as KNO_3, K_2HPO_4, K_3PO_4, NaCl, $CaCl_2$, or Na_2SO_4 or solutions of mannitol, sorbitol, or polyethylene glycol (Khan, 1992). It has been reported that priming tomato (*Lycopersicon esculentum* Mill.) (Pill et al., 1991; Frett et al., 1991; Haigh et al., 1986; Argerich et al., 1990; Alvarado et al., 1987; Barlow and Haigh, 1987), carrot (*Daucus carota* L.) (Szafirowska et al., 1981; Haigh et al., 1986) and onion (*Allium cepa* L.) (Haigh and Barlow, 1987) seeds in solutions of potassium salts reduced the time to 50% germination. Similarly, osmotic priming in solutions of inorganic salts or organic solutes has improved the emergence of asparagus (*Asparagus officinalis*) (Pill et al., 1991; Frett et al., 1991), beet (*Beta vulgaris* L. var. *crassa*) (Khan et al., 1983), celery (*Apium graveolens* L.)

(Atherton and Farooque, 1983; Brockelhurst and Dearman, 1983), parsley (*Petroselinum crispum* L.) (Rabin et al., 1988; Pill, 1986), pepper (*Capsicum annuum*) (Bradford et al., 1990; Smith and Cobb, 1991), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*) (Khan, 1980/81); cabbage (*Brassica oleracea* L. var. *capitata*) (Khan et al., 1981), kale (*Brassica oleracea* L. var. *acephala*) (Rao et al., 1987), turnip (*Brassica rapa* L. var. *rapifera*) (Rao et al., 1987), lettuce (*Lactuca sativa* L.) (Cantliffe et al., 1981), cucumber (*Cucumis sativus* L.) (Passam et al., 1989), cantaloupe (*Cucumis melo* L. var. *cantalupensis*) (Bradford, 1985), and watermelon (Sachs, 1977). In muskmelon, priming in solutions of KNO_3 markedly improved the germination rates and final germination percentages, particularly at sub-optimal germination temperatures (Nerson and Govers, 1986; Bradford et al. 1988; Welbaum and Bradford, 1991a).

Solutes reduce the solution water potential (Ψ), preventing germination during osmotic priming. However, matric priming depends upon the properties of moistened solid carriers such as calcium silicate, zonolite vermiculite, calcined clay, or leonardite shale, which have a high matric potential and little or no osmotic potential (Khan, 1992; Kubik et al., 1988; Harman and Taylor, 1988; Taylor et al., 1988). Ideal solid matrix priming agents have high matric potential, are insoluble in water, have no phytotoxicity, have a high water-holding capacity, have a large surface area, and are easy to remove from seeds after treatment (Parera and Cantliffe, 1994; Khan, 1992; Taylor et al., 1988). Wallace (1960) demonstrated that seed priming by pre-equilibration in unsaturated soil could also stimulate germination. Matric priming treatments have successfully increased the germination rates of tomato, onion, carrot (Taylor et al., 1988), and broccoli (*Brassica oleracea* L. var. *italica*) (Jett et al., 1995) seeds.

B) Viability and Vigor of Muskmelon Seeds during Development and Storage

The production of good quality seed requires timely harvest for maximal seed vigor. Seed vigor is a qualitative term encompassing the properties that determine the potential level of activity and performance of the seed or seedlot during germination and seedling emergence (Perry, 1978). Investigations relating maize (*Zea mays* L.) seed quality to maturity showed that maximal germination occurred at high moisture levels early in development before the seed reached physiological maturity, although seed dry weight was a better indicator of seed vigor than seed moisture content (MC) (TeKrony and Hunter, 1995). In many orthodox seeds, such as cereals or other grain crops, natural desiccation coincides with the attainment of maximal dry weight and physiological maturity (Rosenberg and Rinne, 1986). In many cases, drying coincides with discoloration of the plant and seed, allowing harvest dates to be easily determined based on appearance or seed MC. Determining the optimal time of harvest for seeds that develop inside fleshy fruits is more complicated, because seed maturation may precede fruit maturity (Coombe, 1976). For example, muskmelon seeds were held inside fruits for several weeks after maximal dry weight accumulation at high MC before the seeds were released during fruit decomposition (Welbaum, 1993). In some cultivars, a high percentage of seeds were either dead or had very low vigor if harvest was delayed until fruits were severely decomposed, because seed MC was high enough to cause rapid aging but too low to repair subcellular damage (Welbaum, 1993). On the other hand, when fruits were harvested prematurely seeds required dry storage, referred to as afterripening, or priming to increase vigor to the same level as older seeds, even though maximal seed dry weight had been obtained (Welbaum and Bradford, 1991a, b). Both harvesting after fruit

maturation and dry afterripening increased germination by overcoming seed dormancy in various *Capsicum* species (Randle and Honma, 1981; Edwards and Sundstrom, 1987).

Seed development may be characterized by various stages of maturity. The first phase begins with fertilization and rapid cell division followed by a second phase, which is characterized by cell expansion and accumulation of reserve materials by the embryo and endosperm. The third phase is characterized by water loss from the seed as seeds pass into a quiescent and sometimes dormant state before metabolic systems are re-activated upon imbibition (Bradford, 1986).

Muskmelon seeds must be harvested at the correct stage of development to obtain maximal viability and vigor. Initial germination of fresh muskmelon seeds begins at 30 days after anthesis (DAA), while full germinability occurs between 35 and 45 DAA. Maximal seed vigor is attained when seeds are harvested from 60 DAA fruits (Welbaum and Bradford, 1988). In a previous study, the optimal harvest date for muskmelon cultivar Top Mark was determined to be 55 to 60 DAA using several indices of vigor (Welbaum and Bradford, 1989). There is limited information on how the vigor and viability of muskmelon seeds from different stages of development change during long-term storage. In an earlier study, optimal seed quality coincided with edible fruit maturity (Harrington, 1959). Immature 32-DAA seeds lost viability more rapidly after nine months of storage than those harvested at more advanced stages of development (Harrington, 1959). Harrington (1959) also showed that fermentation produced seed lots with greater viability than mechanical cleaning or rinsing in water. Bass (1973) reported that some muskmelon cultivars obtained from commercial sources maintained full viability during a 12-year storage experiment, while others declined after 7 years. Roos and Davidson (1992) also reported that some muskmelon cultivars maintained full viability during a 30-year storage period, while others lost ≥30% viability after 50 years. Although some commercial seed

companies wash their muskmelon seeds briefly after harvest and store them to increase vigor, the physiological basis for the enhancement caused by washing is poorly understood. Postharvest processing of muskmelon seed crops varies widely and may involve extensive or very little washing. Welbaum and Bradford (1989) observed that washed, dried, or washed and dried seeds gave improved seedling vigor compared to unwashed and undried seeds.

C) Viability and Vigor during Priming and Storage

Seed preservation is important for conserving plant genetic diversity and resources. An important indicator of seed vigor is the longevity of seeds in storage (Roberts, 1986). Loss of seed germination and early detection of deterioriation are major storage concerns. Seed deterioriation involves a series of irreversible degenerative changes that leads to delayed germination, slower seedling growth rates, abnormal growth, decreased storage potential, decreased tolerance to adverse conditions, and ultimately loss of germinability (Mathews, 1980; Abdul-Baki and Anderson, 1972; Delouche and Baskin, 1973). Deterioriation begins after physiological maturity when seeds are considered to be of optimal quality. The reduced germination rates of aged seeds may be due to impaired metabolic processes. Damage to seeds theoretically accumulates during storage at moisture contents too low for repair processes to occur (Priestley, 1986). There is considerable evidence that repair processes are initiated soon after imbibition of aged seeds (Davison et al., 1991; Elder et al., 1987; Rao et al., 1987). Ellis and Roberts (1981) developed a model to analyze the initial quality of a seedlot and the rate of subsequent aging during controlled deterioriation tests. The model proposed that, despite differences in initial seedlot quality as seeds age, the rate of viability loss should be constant within a species for a given seed storage

moisture content and temperature. The mean time to germination increases as viability declines during aging (Ellis and Roberts, 1981; Argerich et al., 1989).

Incubation at high relative humidity, soaking in water, or priming in osmotic solutions have been used to counteract deterioriative processes imposed by accelerated aging (Priestley, 1986). Repair of damage during priming may be one of the processes responsible for more rapid germination of primed seeds (Ellis and Butcher, 1988; Rao et al., 1987). After seed priming and dehydration, seeds are normally stored before use. In some studies, priming treatments did not adversely affect seed storage life. Primed carrot and leek seeds retained their enhanced vigor after storage for 450 days (Dearman et al., 1987b), while primed onion seeds maintained viability and vigor after 18 months of storage at 10°C. Primed spinach seed retained improved germination after 1 month of storage at 5°C (Atherton and Farooque, 1983). Akers et al. (1987) reported that parsley seeds primed in PEG 8000 did not lose their priming effect after 8 months of storage. Odell and Cantliffe (1986) found that final germination after 10 months of storage at 25°C was similar to germination without storage. A short hydration treatment applied to wheat seeds had little effect on germinability during storage, while longer hydration treatments increased the susceptibility to deterioration (Nath et al., 1991). Dearman et al. (1986) reported that primed and dried onion seed stored at 10°C and 9% moisture content (MC) maintained a faster rate of germination during 18 months in storage. Watermelon seeds primed in salt solutions maintained germination vigor after 20 weeks of dry storage (Sachs, 1977).

Other studies have shown that primed seeds have decreased storage life. Tomato (Alvarado and Bradford, 1988; Argerich et al., 1989; Owen and Pill, 1994) and wheat (Nath et al., 1991) seeds exhibited reduced storage life following priming. Prehydration treatments in water of less than 1 hour had little effect on the rate of germination or

storage life of lettuce seeds. However, increasing the duration of either priming or pregerminative hydration reduced the mean time to germination and seed longevity by as much as 61 and 84%, respectively, compared to nonprimed seeds (Tarquis and Bradford, 1992). Thanos et al. (1989) showed that pepper seeds stored for 3 years at 5°C maintained high germinability throughout the storage period but not when they were stored at 25°C.

Muskmelon seeds showed a differential response to priming that varied among seedlots (Bradford et al., 1988; Welbaum and Bradford, 1991a). The effects of priming were greater on newly matured seeds than on older seeds (Welbaum and Bradford, 1991a). Newly matured muskmelon seeds reached maximal vigor after a period of afterripening. Therefore, priming may enhance vigor by substituting for afterripening (Welbaum and Bradford, 1991a). Short-term aging treatments mimic afterripening and increase the vigor of newly matured seeds similar to priming treatments (Welbaum and Bradford, 1991b).

D) Role of the Endosperm and Enzymatic Activity during Germination

Endosperm or perisperm cells are constrained by tough polymeric walls that present a special problem to growing embryonic tissues/organs, which must penetrate the endosperm or perisperm cells for germination to occur. The interactive processes by which environmental and hormonal signals and endosperm integrity regulate germination are not fully understood.

In many species, the endosperm tissue completely envelops the embryo and restrains germination by presenting a physical barrier for radicle emergence (Weges, 1987; Bradford, 1990). In seeds of lettuce, tomato, *Datura* spp., pepper, and muskmelon, the

weakening of the endosperm or perisperm tissues adjacent to the radicle tip is required for germination to occur (Bradford, 1990; Groot and Karssen, 1987; Ni and Bradford, 1993; Sanchez et al., 1990; Welbaum and Bradford, 1990b). The physiological processes leading to endosperm or perisperm weakening are not completely understood. In seeds of lettuce (Psaras et al., 1981) and *Datura* (Sanchez et al., 1990), the cells of the endosperm tissue adjacent to the radicle tip undergo extensive morphological changes prior to radicle protrusion. In *Datura ferox* seeds incubated at alternating temperatures, increase in embryo growth potential, endosperm softening, and germination were found to be induced by phytochrome far-red (Pfr) (Sanchez and de Miguel, 1985; Sanchez et al., 1986), while low water potential inhibited Pfr action on endosperm softening and germination (de Miguel and Sanchez, 1992). It has been hypothesized that cell wall loosening enzymes modify the wall to allow turgor-driven extension (Cosgrove, 1993). The turgor pressure of the embryonic axes may increase through accumulation of osmotic solutes allowing the radicle to exceed the yield threshold of the tissues surrounding the radicle (Bradford, 1990, 1986; Thanos, 1984; Carpita et al., 1979; Takeba, 1980; Nabors and Lang, 1971). By increasing the growth potential of the embryo, the restriction to radicle emergence could be overcome (Nabors and Lang, 1971). However, in lettuce and watermelon, direct measurements of solute potential failed to completely account for the increases in growth potential observed, indicating that decreased yield threshold or increased cell wall extensibility may be partially responsible for radicle growth during germination (Bradford and Somasco, 1994; Carpita et al., 1979; Thanos, 1984).

The reduction in yield threshold in some germinating seeds involves enzymatic degradation of endosperm or perisperm tissues that form a physical barrier to radicle growth (Ni and Bradford, 1993). Endo-β-mannanase has been identified as the cell wall degrading enzyme responsible for the breakdown of endosperm cell walls during the

germination of tomato seeds (Groot et al., 1988) and pre- and post-germinative galactomannan hydrolysis in the endosperm cell walls of lettuce (Dutta et al., 1994; Dulson et al., 1988) and *Datura* spp. (Sanchez et al., 1990). The synthesis of mannanase in lettuce is regulated by a complex series of embryo-directed interactions, in which red light stimulus breaks dormancy and is transduced by the embryo into two promotive compounds, one of which acts on the endosperm to overcome an endogenous, diffusible inhibitor like ABA (Halmer and Bewley, 1979). The hydrolysis of mannans by endo-β-mannanase in lettuce endosperm cell walls has been detected only after germination suggesting that this enzyme is not involved in pregerminative endosperm wall weakening (Halmer et al., 1976). This supports the hypothesis that increased turgor may be necessary to rupture the endosperm in lettuce seeds (Tao and Khan, 1979). However, later studies showed no increase in the turgor of lettuce embryos prior to radicle emergence (Weges, 1987; Somasco and Bradford, 1993; Dutta et al., 1994). Instron measurements of mechanical strength of lettuce endosperm tissue declined during imbibition and were not well correlated with radicle emergence (Tao and Khan, 1979). Pavlista and Haber (1970) proposed that germination of lettuce seed requires a combined mechanical force of the growing embryo pushing against the endosperm and the chemical or enzymic weakening of the endosperm.

Endosperm weakening during tomato seed germination has been extensively studied (Groot et al., 1988; Haigh and Barlow, 1987; Liptay and Schopfer, 1983). Liptay and Schopfer (1983) concluded that differences in the extensive force exerted by the radicle were responsible for variations in tomato seed vigor. However, Haigh and Barlow (1987) observed no increase in hydrostatic pressure in the embryonic axis in tomato seeds prior to radicle emergence and concluded that the endosperm inhibits expansive growth but softens prior to radicle emergence. Instron analysis showed a decline in the force

required to penetrate tomato endosperm tissue during imbibition (Groot and Karssen, 1987). In pepper seeds, the mechanical resistance of the endosperm decreased from approximately 0.8 N at the start of imbibition to 0.3 N at radicle emergence (Watkins and Cantliffe, 1983).

It has been proposed that the softening of the endosperm also occurs during priming (Karssen et al., 1989). The cellular processes that occur during priming are similar to those in seeds imbibed in water (Bewley and Black, 1982). It may be hypothesized that endosperm weakening proceeds during priming, promoting the germination of seeds upon rehydration. Endo-β-mannanase and galactomannan hydrolyzing activities were greater in endosperm cell walls and whole seeds of primed tomato seeds than nonprimed seeds during germination (Karssen et al., 1989; Nonogaki et al., 1992).

Muskmelon embryos are enclosed in a single layer of endosperm and a two- to four- cell layered perisperm envelope (endosperm + perisperm) that represents a barrier to radicle elongation (Welbaum and Bradford, 1990a). An anatomical investigation of the perisperm envelope tissue adjacent to the radicle tip revealed an area of thin walled cells that must be penetrated for germination to occur (Welbaum and Bradford, 1990b). It has been proposed that weakening of the perisperm tissue surrounding the embryo initiates radicle growth (Welbaum and Bradford, 1990b). Turgor in the embryonic axis remained constant at 0.7 MPa for several hours prior to radicle emergence, suggesting that increased turgor is not solely the mechanism responsible for rupturing the perisperm envelope tissue during germination (Welbaum and Bradford, 1990b). A previous study showed that the force and energy required to penetrate muskmelon seed pieces declined during imbibition, demonstrating that the strength of muskmelon perisperm envelope declines during germination (Welbaum et al., 1995).

E) Effects of Priming on Nuclear Replication Activity during Germination

The maintenance of DNA integrity during cell division and differentiation is of critical importance to normal seed germinative processes. In dry seeds, degradative cellular changes occur and lesions accumulate in the structure of nuclear DNA during storage (Cheah and Osborne, 1978). Osborne (1982, 1983) showed that protein, RNA, and DNA synthesis are initiated within minutes of imbibing dry seeds or embryos in water, but DNA replication is a relatively late event. The cause of delay in the onset of DNA replication is not clear, but the delay may allow time for the repair of accumulated lesions that block replication (Thornton et al., 1993). In seeds of Brussels sprouts and cauliflower (*Brassica oleracea* var. *botrytis*), it has been proposed that the repair of accumulated DNA damage occurs upon hydration (Thornton et al., 1993). In rye (*Avena fatua*), Elder and Osborne (1993) reported that dormant imbibed embryos maintain the integrity of the genome by a continuous but slow replacement of DNA caused by a modified form of replicative DNA synthesis.

Protein synthesis is an essential metabolic process in the early stages of seed germination and is correlated with osmopriming techniques (Dell'Aquila, 1987). The rates of RNA and protein synthesis are higher in primed than in nonprimed seeds during germination (Bray et al., 1989). Khan et al. (1978) showed that RNA and protein metabolism are enhanced by priming. Replicative and repair-type DNA synthesis have been detected in the nuclei and mitochondria, respectively, of leek embryo tissue during osmopriming in the absence of any cell division (Ashraf and Bray, 1993). Burgass and Powell (1984) proposed that the onset or completion of DNA repair activity during osmopriming may be a significant factor accounting for the improvement in germination performance arising from osmopriming treatments. Priming stimulated DNA repair

mechanisms which reduced the incidence of chromosomal aberrations at first mitosis (Sivritepe and Dourado, 1995). Rao et al. (1987) previously concluded that post-storage priming treatments in lettuce seeds reduced the frequency of chromosomal aberrations, increased the rate of root growth, and decreased the frequency of morphologically abnormal seedlings. On the other hand, Rao (1986) proposed that the retention of the lag period between imbibition and DNA replication in aged seeds should enhance repair of DNA lesions induced during storage.

Previous reports on quantifying nuclear DNA content were estimated by microdensitometric measurements of the light absorbance of feulgen-stained nuclei. However, this method has several time-consuming steps, which may be a source of variation (Bennett and Smith, 1976). The introduction of flow cytometry has permited measurements of fluorescence signals from large numbers of stained nuclei within seconds. Flow cytometry has proved to be a powerful tool in cell cycle analysis (Galbraith et al., 1983) and estimation of nuclear DNA content (Galbraith et al. 1983; Rayburn et al., 1989, 1990; DeRocher et al., 1990). Proliferating cells pass through four cell cycles referred to as the G_1 (2C DNA content) or quiescent phase; S, the DNA synthesis phase; G_2 (4C DNA) or the second resting phase; and M (mitosis) or cell division phase (Bergounioux et al., 1992; Arumuganathan and Earle, 1991). In previous reports, the nuclei in embryos of fully matured dry pepper (Lanteri et al., 1993, 1994) and tomato (Lanteri et al., 1994) seeds revealed predominantly 2C signals. Bino et al. (1993) similarly showed that embryos of fully matured seeds from various plant species contained large amounts of 2C DNA signals. These results indicate that during seed maturation in some plant species, most cells had the nuclear replication activity arrested at the presynthetic G_1 phase of nuclear division, while in other species, more embryonic cells enter the G_2 phase during seed maturation. In experiments using autoradiography and feulgen staining techniques,

the embryos of *Pinus pinea*, *Allium cepa*, and *Lactuca sativa* revealed solely 2C nuclei, while embryos of *Triticum durum*, *Zea mays*, and *Vicia faba* contained both 2C and 4C nuclei (Bewley and Black, 1978; Deltour, 1985).

During priming in tomato and pepper seeds, induction of DNA synthesis occurs, and the percentage of 4C nuclear DNA signals in embryonic axis cells increases (Bino et al., 1992; Lanteri et al., 1993, 1994; Saracco et al., 1995). This indicates that when primed seeds are redried, the DNA in the embryonic axis cells cease cell cycle activity at the G_2 phase. It has been proposed that the beneficial effects of priming are correlated with DNA replication prior to germination (Bino et al., 1992). In pepper seeds, priming reduced the lag period of DNA synthesis during germination by 9 days, during which physiological processes that can sustain a rapid inception of nuclear replicative activity upon imbibition may have taken place (Lanteri et al., 1993). Osborne (1983) reported that in most seed species, the induction of DNA synthesis follows a time window in which repair mechanisms take place. Perhaps the changes in vigor that occur during priming and the later stages of development involve cell cycling, so when dried seeds are rehydrated, cell division may commence without the need for DNA replication. Flow cytometry may allow DNA replication activity in seeds to be used as an indicator of seed quality.

F) Purpose of Current Study

A fundamental understanding of the effects of priming and development on muskmelon seed vigor and storage would be useful in developing high quality seeds and improving postharvest storage. To achieve this objective, we sought to: 1) investigate the most effective priming treatments for improving muskmelon seed vigor; 2) examine the effects of post-storage priming, postharvest washing, and development on seed vigor and storage life; 3) determine the effect of pre-storage priming on germination performance of seeds

after long-term storage; 4) examine the effects of priming, development, and storage on the strength of the perisperm envelope tissue during germination; 5) evaluate the effect of priming on enzymatic activity during germination to determine how perisperm weakening occurs; 6) examine and compare the effect of priming and seed development on DNA replication activity in embryonic axis tissues during imbibition.

Chapter 2.

Osmotic and Matric Priming of Muskmelon (*Cucumis melo* L.) Seeds

Abstract. Muskmelon seeds were osmotically and matrically primed (controlled hydration followed by redrying) to determine the most effective priming treatment for improving germination. Seeds were primed for 6 days at 25°C in solutions of either 0.3 M KNO_3 or polyethylene glycol (PEG) 8000 at osmotic potentials ranging from –0.8 to –2.5 MPa. Seeds were also primed in solid carriers such as calcium silicate, vermiculite, and Hayter loam soil at water potentials ranging from –0.7 to –3.2 MPa, and durations of up to 264 hours (11 days) at 25°C. The optimal priming duration was between 96 to 168 hours (4 to 7 days). The mean time to germination (log $\bar{t}$) and standard deviation in germination time (σ) significantly decreased with priming duration to optimal range, then significantly increased at longer treatment durations. Generally, the most effective priming treatments for muskmelon seeds occurred at water potentials (Ψs) between –1.5 to –1.8 MPa. Reduction in log $\bar{t}$ was greater for osmotically primed seeds when compared to matric primed or nonprimed seeds. X-ray images and longitudinal planar measurements revealed an area between the periphery of the seedcoat and the embryo that was 13% greater in primed than in nonprimed seeds, indicating that the embryo shrank during the priming treatment. Priming with KNO_3 was the most effective treatment, reducing the log $\bar{t}$ by 56 and 41% for nonprimed seeds and seeds primed in water, respectively. Priming in KNO_3 solution also reduced log $\bar{t}$ by 13, 26, 31, and 31%, compared to the best priming treatments using PEG, vermiculite, calcium silicate, and Hayter loam soil, respectively.

Introduction

Production of early season muskmelons (*Cucumis melo* L.) is problematic because establishment of the crop is difficult due to low soil temperatures, seedling diseases, and soil crusting which reduce seedling emergence (Bradford et al., 1988). The minimal germination temperature for muskmelon seed is 15°C, while the optimal temperature is 32°C (Lorenz and Maynard, 1980). Improving muskmelon seed germination under supra- and sub-optimal temperatures is necessary to improve stand establishment under adverse conditions. Nerson et al. (1982) and Edelstein and Kigel (1990) showed that different melon cultivars vary greatly in their response to sub-optimal temperatures. It was determined that higher germination at low temperatures could be obtained in watermelons (*Citrullus lanatus* L.) by seed coat splitting (Nerson et al. 1985). Removal of the seed coat in melon seeds also improved germination at low water potential (Dunlap, 1988) and low temperature (Edelstein and Kigel, 1990; Edelstein et al., 1995). On the other hand, seed priming has been used extensively to improve low-temperature germination of many species (Bradford, 1986).

Seed priming is a controlled hydration treatment that allows pregerminative metabolic activity to proceed but prevents radicle emergence (Heydecker and Coolbear, 1977). The optimal duration of the priming process depends on the type of osmoticum, osmotic potential of the solution, temperature during the treatment, and species of the seed being primed (Parera and Cantliffe, 1994). Matric and osmotic priming are the two treatments most commonly used. Osmotic priming is a seed treatment in which seeds are incubated in osmotic solutions of inorganic salts such as KNO_3, K_2HPO_4, K_3PO_4, NaCl, or a high molecular weight inert osmoticum such as polyethylene glycol (Khan, 1992). Primed seeds can be dried and upon rehydration often exhibit faster rates of germination, greater tolerance to environmental stress, and reduced dormancy in many species

(Bradford, 1986; Khan, 1992; Welbaum and Bradford, 1991a). It has been reported that priming tomato (*Lycopersicon esculentum* Mill.), carrot (*Daucus carota* L.), and onion (*Allium cepa* L.) seeds in solutions of potassium salts reduced the time to 50% germination (Alvarado et al., 1987; Haigh and Barlow, 1987; Haigh et al., 1986). In muskmelon, priming in solutions of KNO_3 markedly improved the germination rates and final germination percentages, particularly at sub-optimal temperatures (Nerson and Govers, 1986; Bradford et al. 1988; Welbaum and Bradford, 1991a).

Matric priming is a seed treatment dependent upon the properties of moistened solid carriers such as calcium silicate, zonolite vermiculite, calcined clay, or leonardite shale, which have a high matric potential and little or no osmotic potential (Khan, 1992; Kubik et al., 1988; Harman and Taylor, 1988; Taylor et al., 1988). Matric priming treatments have successfully increased the germination rates of tomato, onion, carrot (*Daucus carota* L.) (Taylor et al., 1988), and broccoli (*Brassica oleracea* L. var. *italica*) (Jett et al., 1996) seeds. Wallace (1960) demonstrated that seed priming by pre-equilibration in unsaturated soil could also stimulate germination. However, a comparison of the germination performance of osmotic- and matric-primed muskmelon seeds has not been conducted.

The final germination percentages, mean time to germination, and spread in germination times of seeds subjected to several osmotic and matric priming treatments were compared in the laboratory to identify treatments for improving muskmelon seed vigor. The anatomy of primed and nonprimed seeds were characterized by X-ray images of longitudinal profiles of seeds, and planar areas were measured to determine physical changes due to priming.

Materials and Methods

Seed material and priming treatments - Muskmelon cv. Topmark (Asgrow Seed Co. Inc., Vineland, NJ) seeds harvested at 40 days after anthesis (DAA) were primed in a 0.3 M KNO_3 solution at a ratio of 5 ml of solution g^{-1} of seed. To determine the optimal water potential (Ψ) for a priming effect, seeds were primed in PEG 8000 (Carbowax, Fisher Scientific Co., Fair Lawn, N.J.) solutions at initial Ψs of −0.8, −1.0, −1.1, −1.3, −1.5, −1.6, and −2.5 MPa, prepared according to Michel (1983) and verified by vapor pressure osmometry (model 5500C, Wescor Inc., Logan, Utah). For osmotic priming, seeds were incubated in the dark for 6 days at 25°C in 9 x 9 x 1.5 cm transparent, covered, plastic boxes (Falcon 1012, Becton Dickinson and Co., Lincoln Park, NJ) on two thicknesses of germination blotter paper (Anchor Paper Co., Hudson, Wis.). After priming, seeds were rinsed and forced-air dried for 3 hours at room temperature before final drying over silica gel in a desiccator at 45% RH to a final moisture content of 4 to 6% (fresh wt. basis). The MC was determined by heating samples of 50 seeds for 1 hour at 130°C (ISTA, 1985). The Ψs of the blotters in each treatment were verified by osmometry (model 5500C, Wescor, Inc., Logan, Utah) from an average of four measurements taken from 4 to 5 days after the start of the experiment. The measured Ψ was 0.2 to 0.6 MPa lower than the initial Ψ due to the concentrating effect of imbibition and evaporation from the boxes during seed scoring. For convenience, the initial Ψs are shown. Some seed samples were also primed in distilled water for up to 12 hours as described above.

For matric priming treatments, calcium silicate (Micro-Cel E; Manville Corp., Denver, Colo.), vermiculite (horticultural grade no. 2), and Hayter loam (fine-loamy, mixed, mesic, ultic Hapludalf) soil were mixed thoroughly in varying ratios with water in

125 ml conical glass flasks sealed with wax film (Parafilm M, American National Can, Greenwich, Conn.) to prevent evaporation. Flasks were equilibrated for 24 hours at 20°C before the seeds were added. The seeds were uniformly mixed with the solid carrier in varying ratios of seed:carrier:water then incubated at 25°C for up to 11 days. The flasks were rotated every 12 hours to ensure uniform mixing. After priming, the moisture content of some seed samples were determined by oven drying at 130°C for 1 hour (ISTA, 1985), and the remaining seeds were thoroughly rinsed in distilled water for 30 min and then forced-air dried at 37°C for 3 hours. The seeds were then dried over silica gel in a desiccator before germination testing. The equilibrium and final Ψs of calcium silicate, vermiculite, and soil during priming were determined using a thermocouple psychrometer (model 85, J. R. D. Merrill, Logan, Utah) calibrated using NaCl solutions of known Ψ verified by osmometry (model 5500C, Wescor, Inc., Logan, Utah). Samples of carriers were equilibrated in psychrometer cups for 2 to 3 hours in a water bath maintained at 26 ± 1°C, and three readings were taken on each sample to ensure that equilibrium had been attained. Water potential measurements were replicates of eight samples for each treatment.

Seed physical characteristics - Longitudinal planar dimensions of nonprimed and primed (0.35 *M* KNO_3) seeds were analyzed by X-ray imaging (Swamithan and Kamra, 1961). Samples of 50 seeds of each treatment were exposed to X-rays for 2 min at 15 kV, using an X-ray System (Faxitron series, Hewlett Packard) (Argerich and Bradford, 1989). Images were recorded on film and enlarged five fold from the natural size on photographic paper (Fig. 2.3). A digitizer (Summasketch III-Professional, model MM 1812, Summagraphics, Seymour CT) was used to measure the dimensions of whole seed, cotyledons, and the embryonic axis.

Laboratory germination - Four replicates of 20 seeds each were placed in 9 x 9 x 1.5 cm transparent, covered, plastic boxes on two thicknesses of germination blotter paper saturated with 11 ml deionized water, after storage for 3 to 7 days after priming. Plastic boxes were placed in self-sealing plastic bags to reduce evaporation. The boxes containing seeds were placed in a dark incubator maintained at 25 ± 1°C, and germinated seeds were removed from boxes at 12 hour intervals for 21 days.

Statistical analysis - The log mean time (log $\bar{t}$) and the standard deviation (σ) of germination were determined by probit analysis (Finney, 1971). When germination percentages were plotted on a probit scale versus log time (t), straight lines of approximately equal slope were produced for different treatments, indicating a normal distribution of germination events with log time (Finney, 1971). The log mean time to germination (log $\bar{t}$) was determined graphically from the intersection of the least squares regression line of log time versus probit germination at 50% germination for treatments with greater than 20% germination. The slope of a probit plot is equal to the inverse σ (Finney, 1971). The significance of main effects of Ψs, seed treatment, and treatment duration for arcsin transformed germination percentages, log $\bar{t}$, and σ were compared by ANOVA (CoStat, CoHort Software, Minneapolis, Minn.) where appropriate. Mean separation was compared by LSD. Data for actual germination percentages are shown.

Results

Seed dimensions - Measurements of planar dimensions of whole, dry seeds revealed that osmotically primed (0.3 M KNO_3) seeds were 2% greater in area than nonprimed seeds. On the other hand, nonprimed seeds exhibited a 4% greater embryo and cotyledon area

than primed seeds (Figs. 2.1 and 2.2). The area occupied by the embryonic axis in nonprimed seeds was also greater than primed seeds by 3.4% (Fig. 2.1). However, the area between the periphery of the seedcoat and the embryo was 13% greater in primed seeds than in nonprimed seeds (Fig. 2.2). Analysis of X-ray images showed an apparent free space between the embryonic axis and cotyledons in 10% of nonprimed seeds analyzed (Figs. 2.3A and 2.4). No free space was observed in primed seeds, where the cotyledons and embryonic axis were tightly appressed (Figs. 2.3B and 2.4). The embryo of primed seeds also appeared distinctly darker than nonprimed seeds after X-ray analysis (Figs. 2.3 and 2.4).

Moisture retention curves of solid carriers - The water-holding capacity of calcium silicate was greater than soil and vermiculite (Fig. 2.5). Vermiculite had the lowest water holding capacity and showed the slowest change in moisture content with increasing Ψ compared to calcium silicate and soil. On the other hand, the soil exhibited the greatest change in moisture percentage with increasing Ψ (Fig. 2.5).

Germination after priming in water - Seeds pre-hydrated in water for up to 12 hours exhibited > 94% germination, which was not statistically different than nonprimed seeds (Fig. 2.6A). Pre-hydration in water for up to 8 hours markedly reduced log $\bar{t}$, but log $\bar{t}$ significantly increased when pre-hydration was extended to 12 hours (Fig. 2.6B). The σ in germination time was not affected or significantly increased by pre-hydration compared to nonprimed seeds (Fig. 2.6C).

Germination after osmotic priming - Germination percentages of seeds primed in PEG solutions decreased gradually from 100 to 88% as the priming Ψ was lowered from 0 to

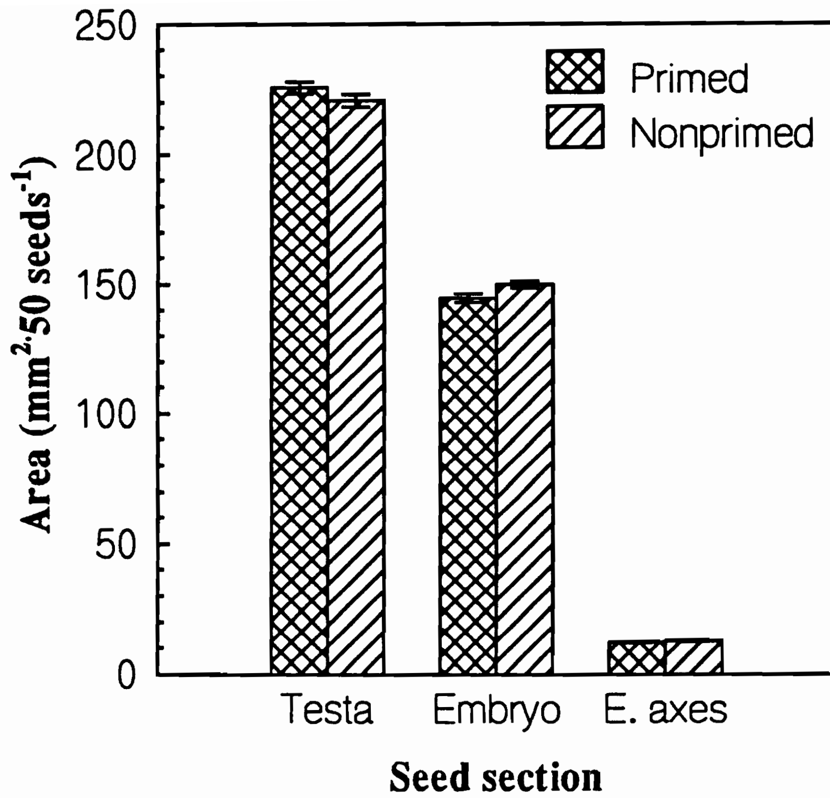

Fig. 2.1. Longitudinal planar area (mm^2) of magnified dry muskmelon seed testa, embryo, and embryonic axis of primed and nonprimed seeds. Vertical bars represent ±S.E. of the mean of 50 seed samples.

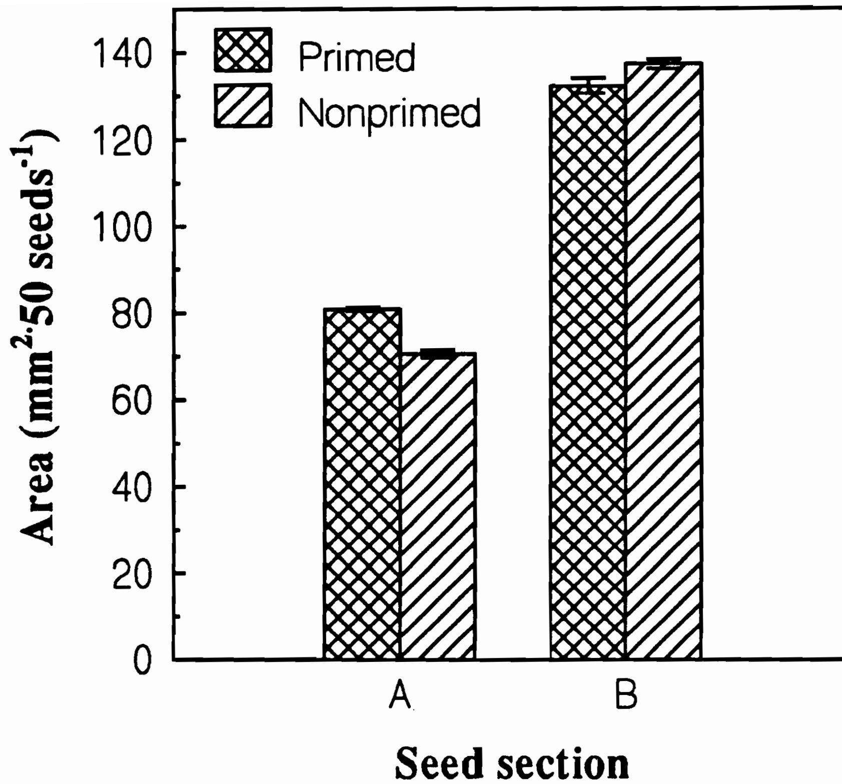

Fig. 2.2. Planar area (mm^2) between the periphery of the seedcoat and the embryo (A); and of the cotyledons (B), of magnified dry primed and nonprimed muskmelon seeds. Vertical bars represent ±S.E. of the mean of 50 seed samples.

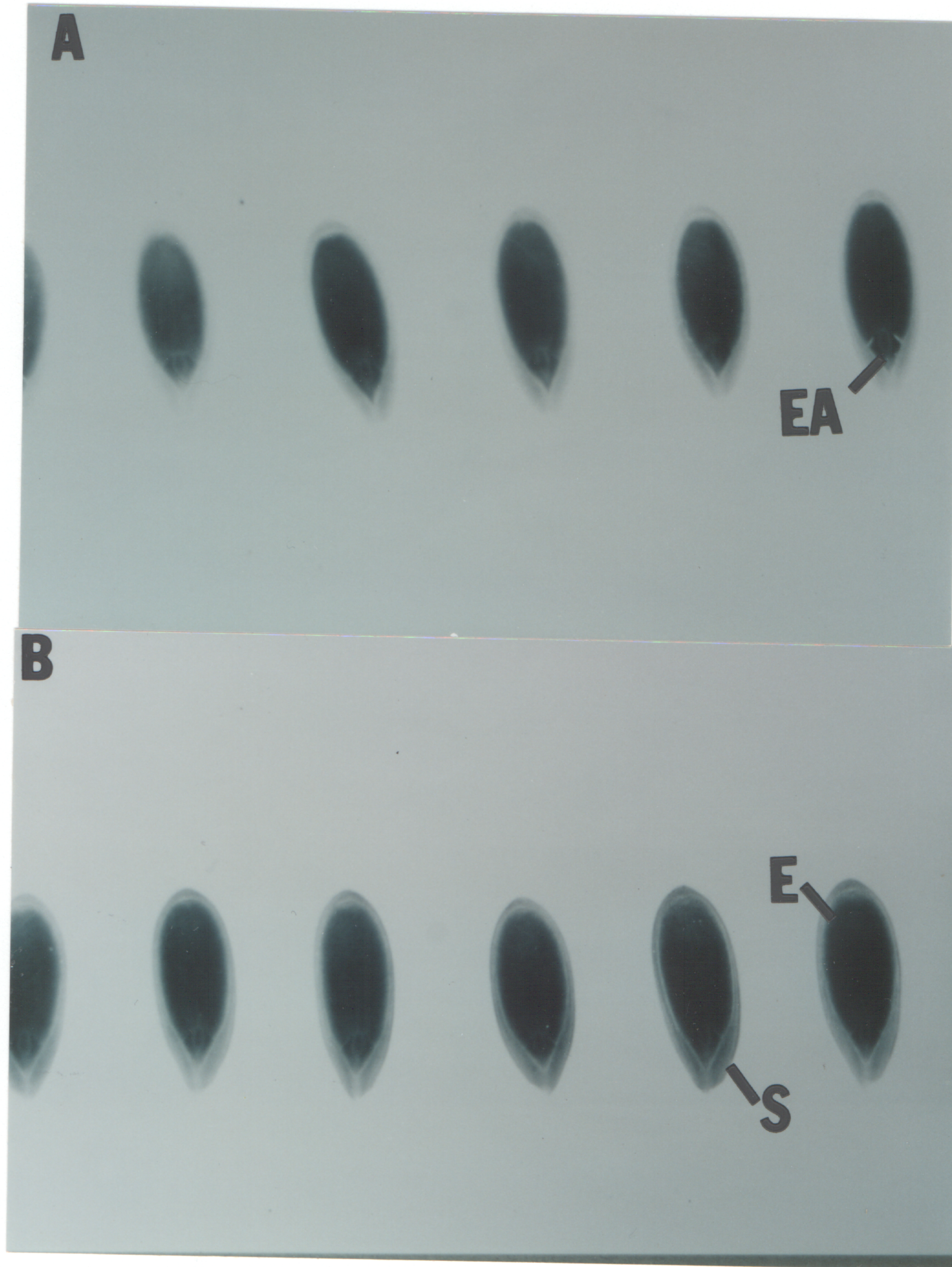

Fig. 2.3. Magnified X-ray images of nonprimed (A) and primed (B) seeds. Six representative seeds from the total sample of 50 seeds for each treatment are shown. S - seed coat; E - embryo; EA - embryonic axis. (Magnification = 6x)

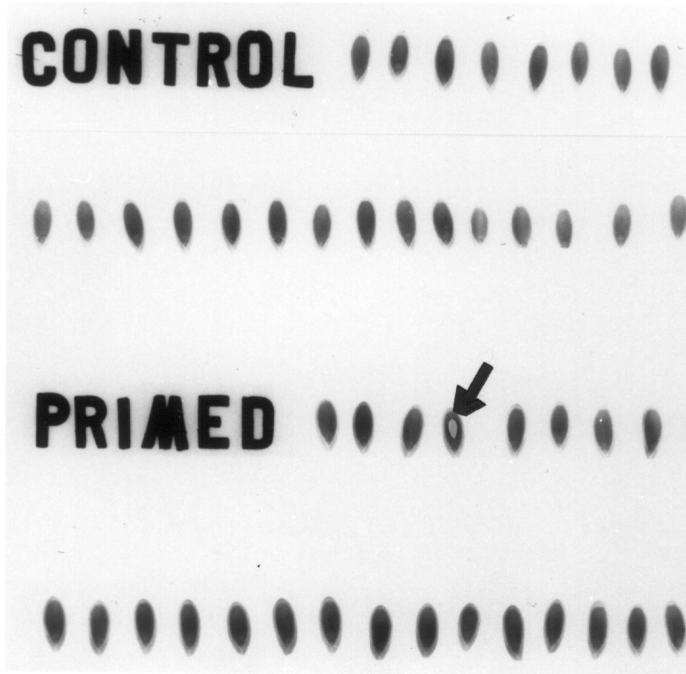

Fig. 2.4. Representative X-ray images illustrating longitudinal planar dimensions of magnified dry primed and nonprimed seeds. Arrow illustrates the planar size of an actual seed placed on the magnified image for comparison. (Magnification = 6x)

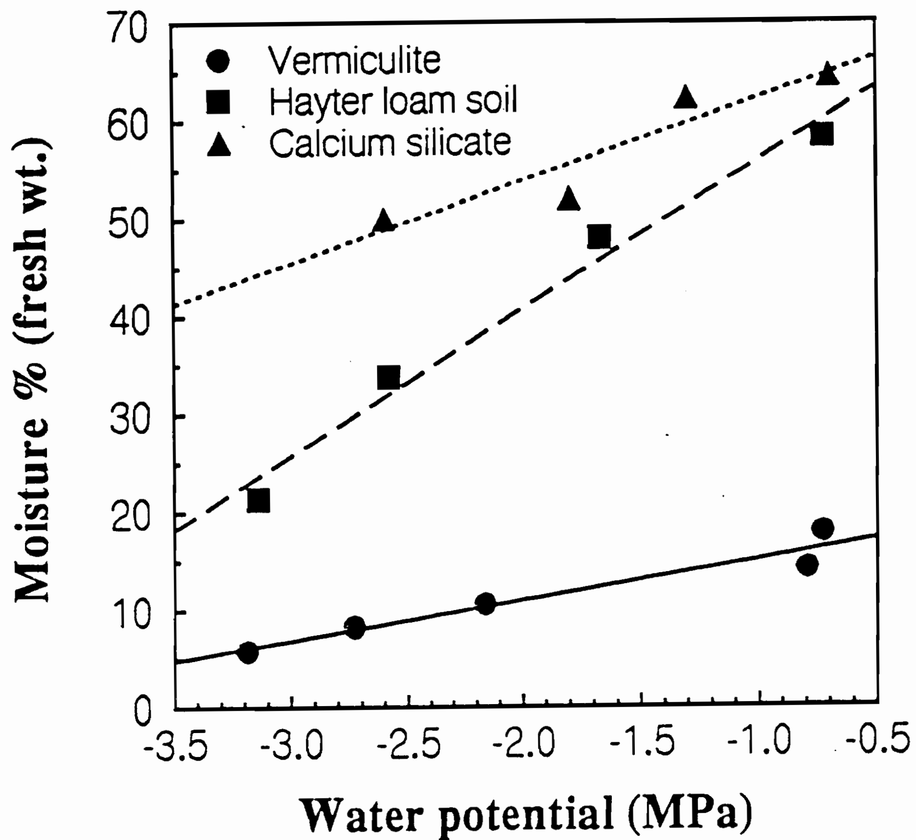

Fig. 2.5. Solid carrier moisture content versus water potential (Ψ) during the priming treatment. The regression equations are: *Vermiculite*, y = 19.11 + 4.08x (r^2 = 0.93); *Hayter loam soil*, y = 70.84 + 15.05x (r^2 = 0.98); *Calcium silicate*, y = 70.53 + 8.36x (r^2 = 0.87).

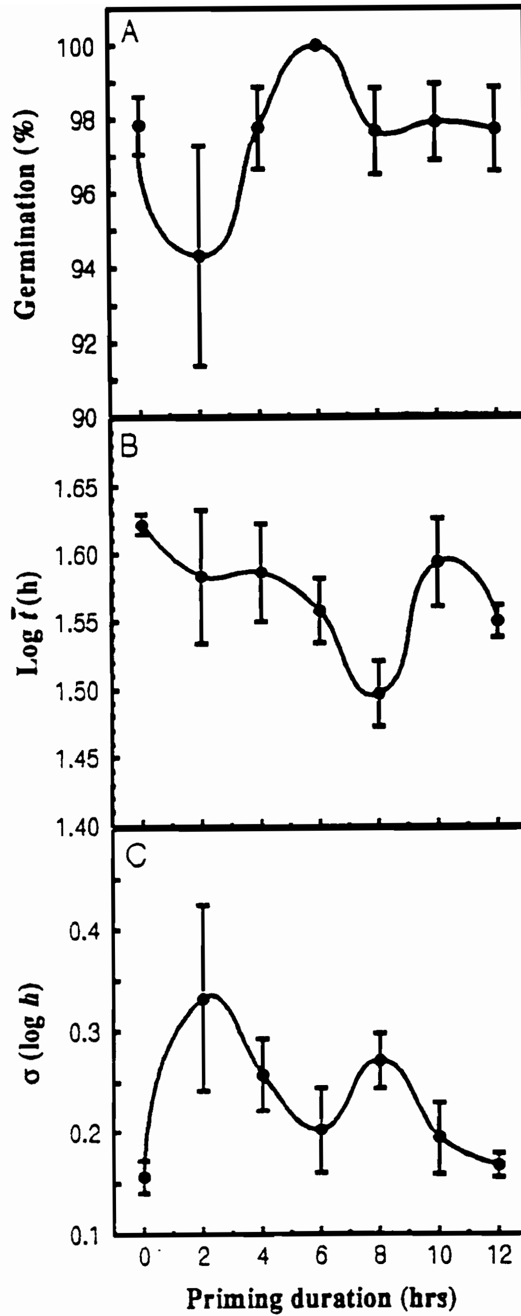

Fig. 2.6. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus duration of priming treatment (hrs) in distilled water at 25±1°C. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.

−2.5 MPa (Fig. 2.7A). A reduction in priming Ψ of PEG solutions from 0 to −1.6 MPa significantly reduced log $\bar{t}$, while lowering the priming Ψ to −2.5 MPa markedly increased log $\bar{t}$ (Fig. 2.7B). Seeds primed for 6 days in 0.3 *M* KNO_3, which has a Ψ of −1.5 MPa, germinated to 96% and produced the fastest mean time to germination of any osmotic or matric priming treatment tested (Table 2.1).

Germination after matric priming - Several ratios of seed:carrier:water were tested, and the ratio of 1g of seed:0.8g of calcium silicate:1.2 ml of water produced the highest germination percentage, the fastest germination, and the lowest σ at a priming duration of 144 hours (6 days) (Fig. 2.8A, B, C; Table 2.1). At the end of the treatment, the moisture content of calcium silicate, the seed water content, and the carrier Ψ at this ratio was 53%, 28%, and −1.2 MPa, respectively (Table 2.1). A reduction in carrier Ψ from −0.7 to −1.8 MPa significantly increased germination percentages (Fig. 2.8A). A similar reduction in carrier Ψ markedly reduced log $\bar{t}$ but did not affect σ (Fig. 2.8B, C). Seeds primed at Ψs < −0.7 MPa in calcium silicate gave significantly reduced log $\bar{t}$ and σ than seeds primed in water for 8 hours (Fig. 2.8B, C).

To test effects of priming duration, the ratio of 1.0 g of seed:0.8 g of calcium silicate:1.2 ml of water was tested at incubations ranging from 0 to 240 hours. A priming duration of 96 to 144 hours (6 days) produced the the smallest log $\bar{t}$, and the lowest σ (Fig. 2.9B, C). Priming durations from 0 to 144 hours produced near to maximum germination percentages. However, increasing the duration from 144 to 240 hours reduced germination percentages from 100 to 76%, increased log $\bar{t}$ from 1.43 to 1.54, and increased σ values from 0.05 to 0.22, respectively (Fig. 2.9).

The effect of Ψ on priming was tested by varying the amount of water while maintaining a ratio of 1g of seed:7.2g of soil at an incubation period of 168 hours (7 days).

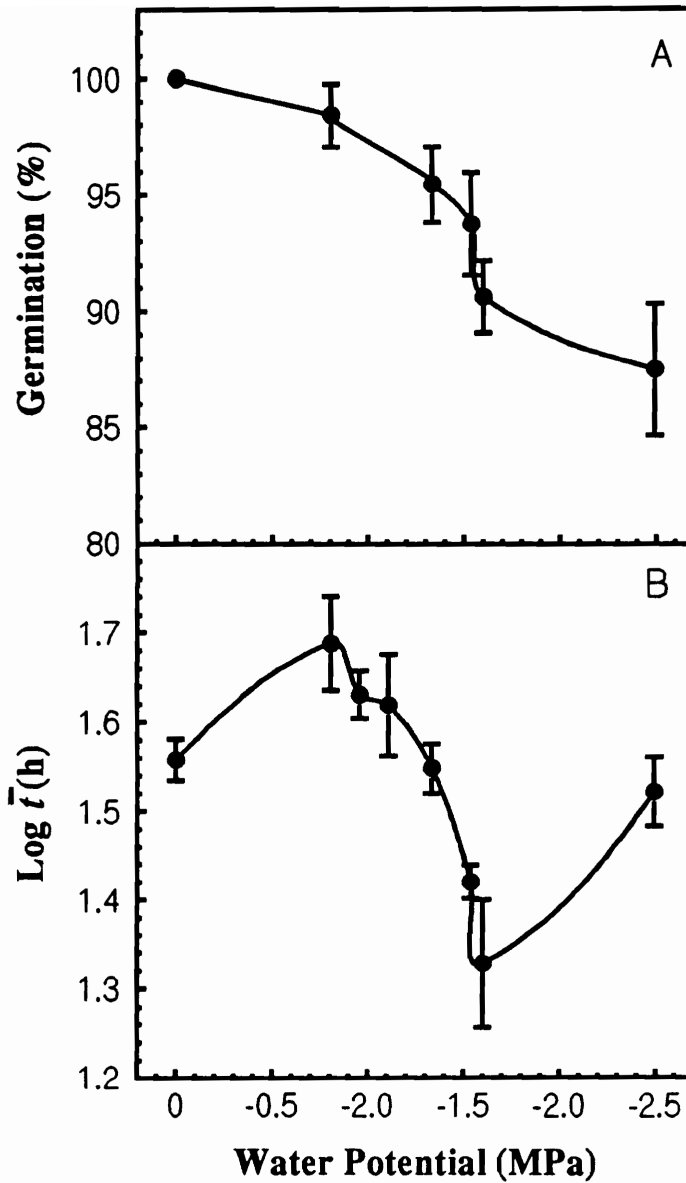

Fig. 2.7. Germination percentage (A) and mean log time to germination (log $\bar{t}$) (B) versus priming water potential (Ψ) using PEG 8000 solutions. Priming treatments were at 25±1°C for 6 days in the dark. The value at 0 MPa represent seeds primed in water for 8 hours. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E. when larger than the symbols.

Table 2.1. The best osmotic and matric priming treatments for improving muskmelon seed vigor. Seeds in all treatments were primed and germinated at 25°C.

Carrier	Treatment[y]	Priming duration (h)	Final MC[z] (%)		Final Ψ (MPa)	Equilibrium Ψ[x] (MPa)	Germination (%)***	Log $\bar{T}$ (*h*)***	σ (log *h*)**
			Carrier	Seed					
Nonprimed	—	—	—	—	—	—	98	1.62	0.16
KNO_3	0.30 M	144	—	53	−1.5	−1.4	96	1.27	0.18
PEG[w]	0.05 M	144	—	30	−3.1	−2.4	91	1.33	0.45
Water	0.00 M	8	100	46	0.0	0.0	98	1.50	0.21
Calcium silicate	1:0.8:1.2	144	53	28	−1.8	−1.2	100	1.43	0.05
Vermiculite no. 2	1:4.0:0.6	168	8	31	−1.7	−1.0	100	1.40	0.10
Hayter loam soil	1:7.2:0.8	120	11	34	−1.1	−0.8	100	1.43	0.06
$LSD_{0.05}$	—	—	—	—	—	—	*2.60*	*0.10*	*0.10*

, * Significant at $P = 0.01$ or 0.001, respectively.
[w]PEG was prepared as 413 $g \cdot kg^{-1}$ water.
[x]Determined from the average of initial and final Ψs.
[y]Molarity (M) and ratios of seed:carrier:water used.
[z]Initial moisture contents (fresh wt. basis) were: Seed (6.3%); Calcium silicate (3.1%); Vermiculite (1.2%); and Soil (0.8%).

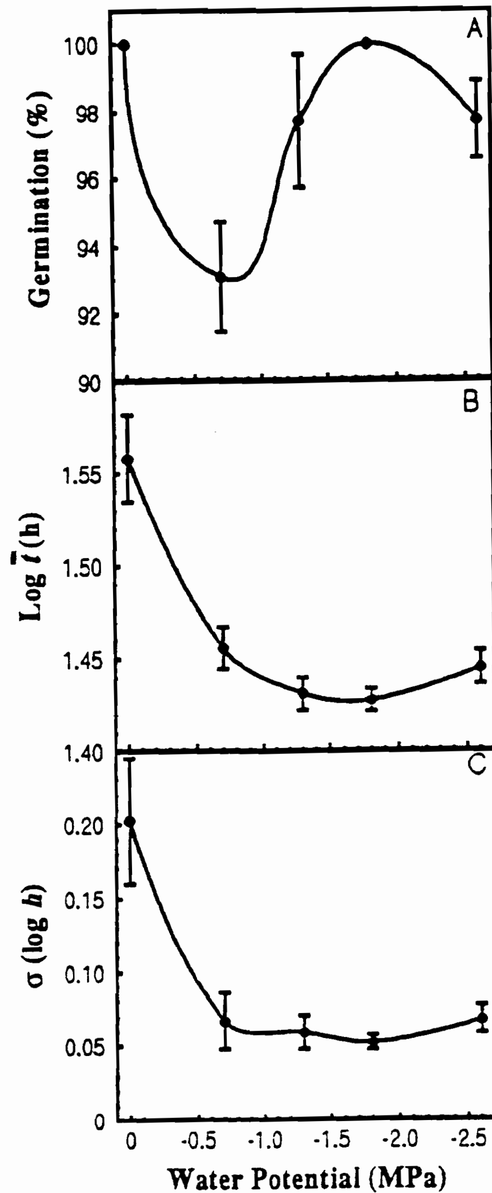

Fig. 2.8. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus decreasing Ψs for muskmelon seeds primed in calcium silicate at a ratio of 1 g of seed:0.8 g of calcium silicate. Priming treatments were at 25±1°C for 6 days in the dark. The values at 0 MPa represent seeds primed in water for 8 hours. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.when larger than the symbols.

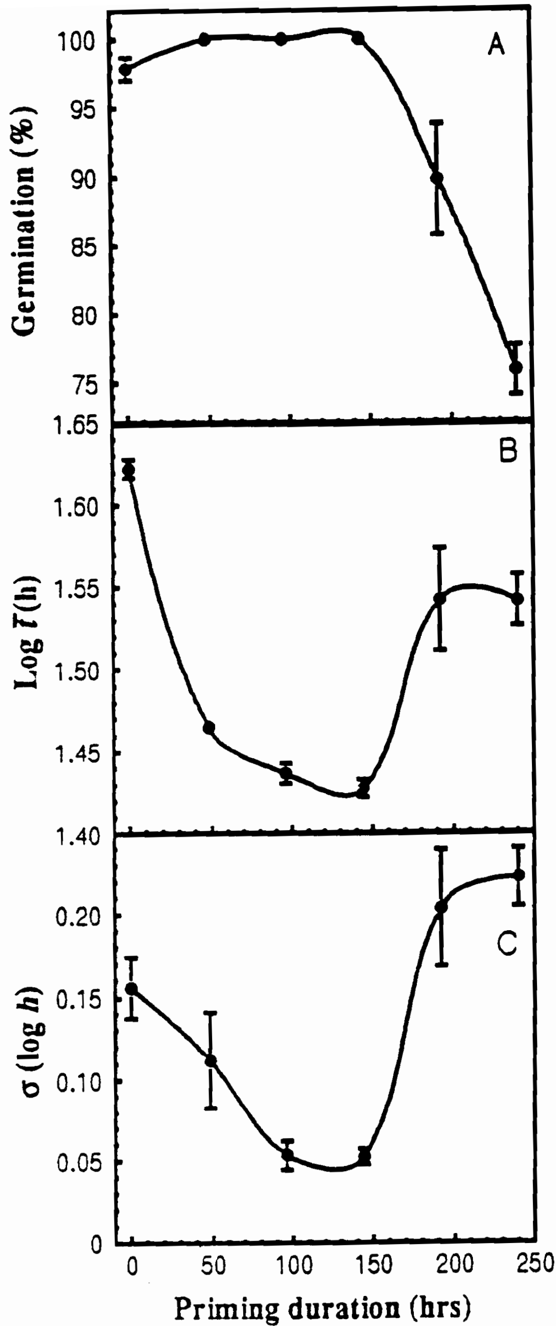

Fig. 2.9. Germination percentage (A), mean log time to germination (log $\bar{T}$) (B), and standard deviation (σ) in germination times (C) versus duration of priming treatment (hrs) for muskmelon seeds primed in calcium silicate at a ratio of 1 g of seed:0.8 g of calcium silicate:1.2 ml of distilled water. Priming treatments were at 25±1°C in the dark. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.when larger than the symbols.

A reduction in soil Ψ from –0.7 to –1.7 MPa significantly increased germination percentage from 87 to 100%, respectively. However, lowering the Ψ to –3.1 MPa markedly decreased germination percentages from 100 to 89% (Fig. 2.10A). Reducing the soil Ψ from –0.7 to –1.7 MPa decreased log $\bar{t}$ from 1.56 to 1.46 and reduced σ from 0.2 to 0.1, respectively (Fig. 2.10B, C). Lowering of the soil Ψ from –1.7 to –3.1 MPa significantly increased log $\bar{t}$ and σ (Fig. 2.10B, C). Priming treatments at Ψ higher than –0.7 MPa resulted in germination in the media within 25 to 30 hours of the start of priming treatment. Priming at a soil Ψ of –3.1 MPa had an adverse effect on seed performance (Figs. 2.10). At that Ψ, germination percentage was significantly lower than the control, while log $\bar{t}$ and σ were both higher than the control (Figs. 2.10).

Priming in soil at a ratio of 1 g of seed:7.2 g of soil:0.8 ml of water produced an optimum germination response at a duration of 120 hours (Figs. 2.11; Table 2.1). The equilibrium moisture content for soil, seed, and the soil Ψ at this ratio and priming duration was 11%, 34%, and –0.8 MPa, respectively (Table 2.1). Germination percentages remained near 100% for priming durations up to 120 hours, but longer durations gradually decreased germination to 23% at 264 hours (Fig. 2.11A). An increase in priming duration from 0 to 120 hours markedly reduced log $\bar{t}$ and σ, while longer durations increased log $\bar{t}$ and σ (Figs. 2.11B, C).

Seeds were primed at reduced Ψs and a constant ratio of 1g of seed:4 g of vermiculite at an incubation period of 168 hours (7 days). Lowering the priming Ψ from –0.7 to –1.7 MPa increased germination from 95 to 100%, decreased log $\bar{t}$ from 1.54 to 1.39, and reduced σ from 0.14 to 0.09, respectively (Figs. 2.12). Increasing the carrier Ψ from –1.2 to –0.8 MPa caused premature germination within 3 days of the start of the priming treatment, and thus could not be effectively compared with the germination responses in other treatments at similar Ψs (Figs. 2.12A, B, C).

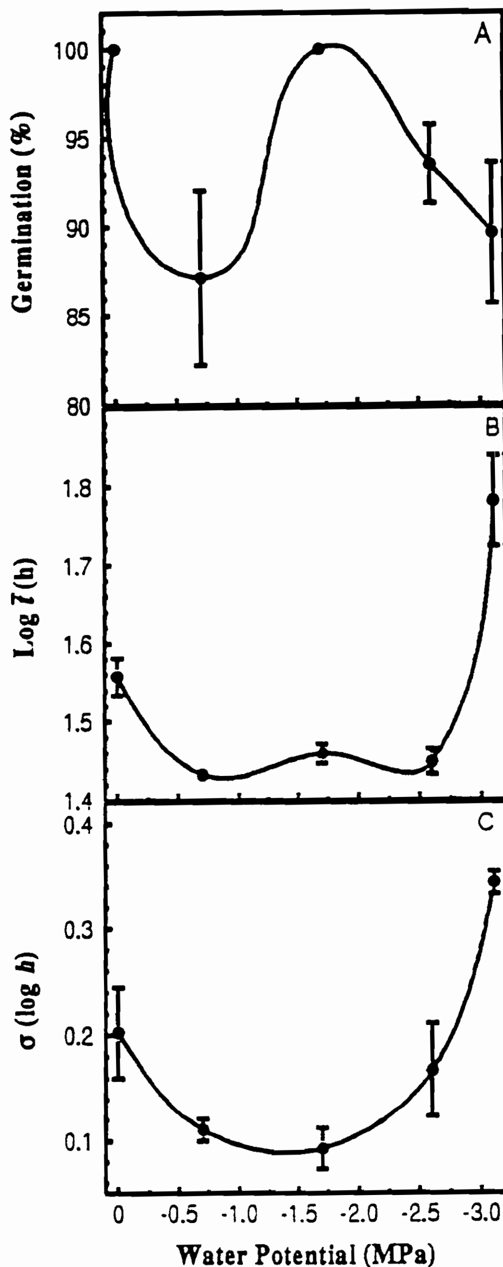

Fig. 2.10. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus decreasing Ψs for muskmelon seeds primed in Hayter loam soil at a ratio of 1 g of seed:7.2 g of soil. Priming treatments were at 25±1°C for 7 days in the dark. The values at 0 MPa represent seeds primed in water for 8 hours. Seeds pre-germinated within 30 hours of priming treatment at Ψs > –0.7 MPa and the data are thus not represented here. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.when larger than the symbols.

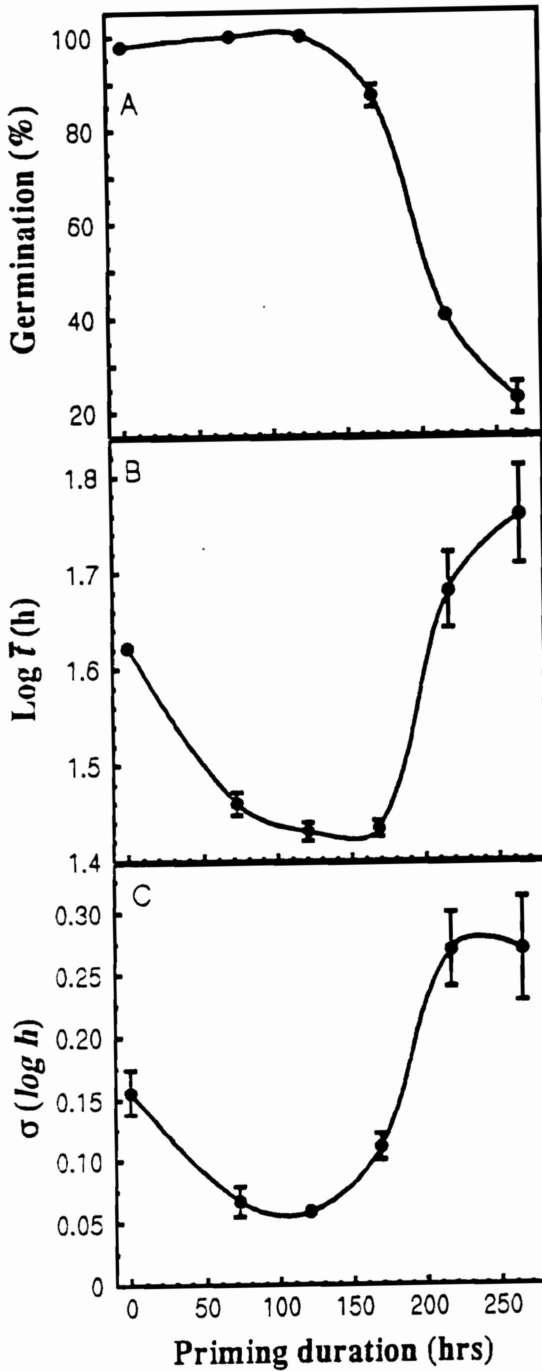

Fig. 2.11. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus duration of priming treatment (hrs) for muskmelon seeds primed in Hayter loam soil at a ratio of 1 g of seed:7.2 g of soil:0.8 ml of distilled water. Priming treatments were at 25±1°C in the dark. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.when larger than the symbols.

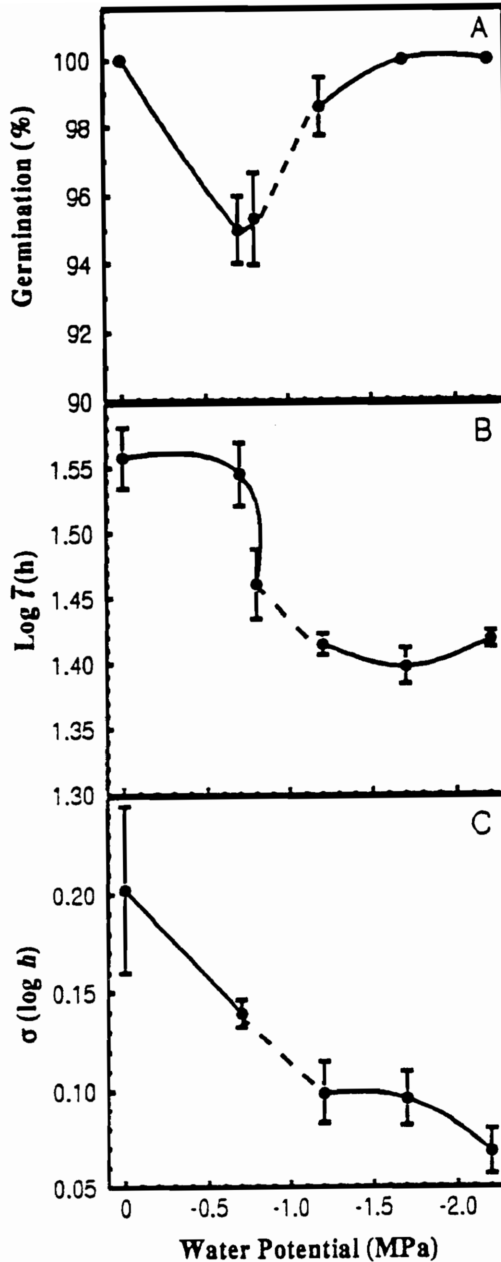

Fig. 2.12. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus decreasing Ψs for muskmelon seeds primed in vermiculite no. 2. at a ratio of 1 g of seed:4 g of vermiculite. Priming treatments were at 25±1°C for 7 days in the dark. The values at 0 MPa represent seeds primed in water for 8 hours. Seeds pre-germinated within 72 hours of priming treatment at Ψs > –0.7 MPa and are represented by dashed lines. The numbers at each point indicate corresponding Ψs (MPa) for each treatment. Each point is the mean of four samples of 20 seeds each, and error bars indicate ±S.E.when larger than the symbols.

To determine the best duration for priming with vermiculite, the ratio of 1 g of seed:4 g of vermiculite:0.4 ml of water was tested at incubation times ranging from 0 to 264 hours. Priming in vermiculite, regardless of treatment duration, decreased log $\bar{t}$ and σ compared to nonprimed seeds (Figs. 2.13A, B). The best priming treatments occurred at priming durations ranging from 72 to 168 hours (Figs. 2.13A, B). Germination percentages increased to 100% at 78 hours of priming and remained greater than 97% at durations up to 264 hours (Fig. 2.13A). Increasing the priming duration from 0 to 120 hours significantly reduced log $\bar{t}$ and σ to a minimum (Figs. 2.13B, C). On the other hand, increasing the priming duration from 120 to 264 hours increased log $\bar{t}$ and σ (Fig. 2.13B, C).

Discussion

Osmotic priming in salt solutions, in contrast to priming in polyethylene glycol, provides little enhancement of germination in some species because the high salt concentrations are toxic to embryos (Khan, 1992). Salt solution as a priming agent was toxic to sorghum seeds, while priming onion and asparagus seeds in salt solution was less beneficial than priming in PEG (Haigh and Barlow, 1987; Frett et al., 1991). On the other hand, priming tomato, carrot (Haigh and Barlow, 1987; Frett et al., 1991), and watermelon (Sachs, 1977) seeds in salt solutions enhanced germination better than priming in PEG.

Muskmelon seeds are not damaged by salt priming, because ions are excluded from the embryo by the perisperm envelope (Welbaum and Bradford, 1990). Osmotic priming of muskmelon seeds markedly improved the germination rates and final

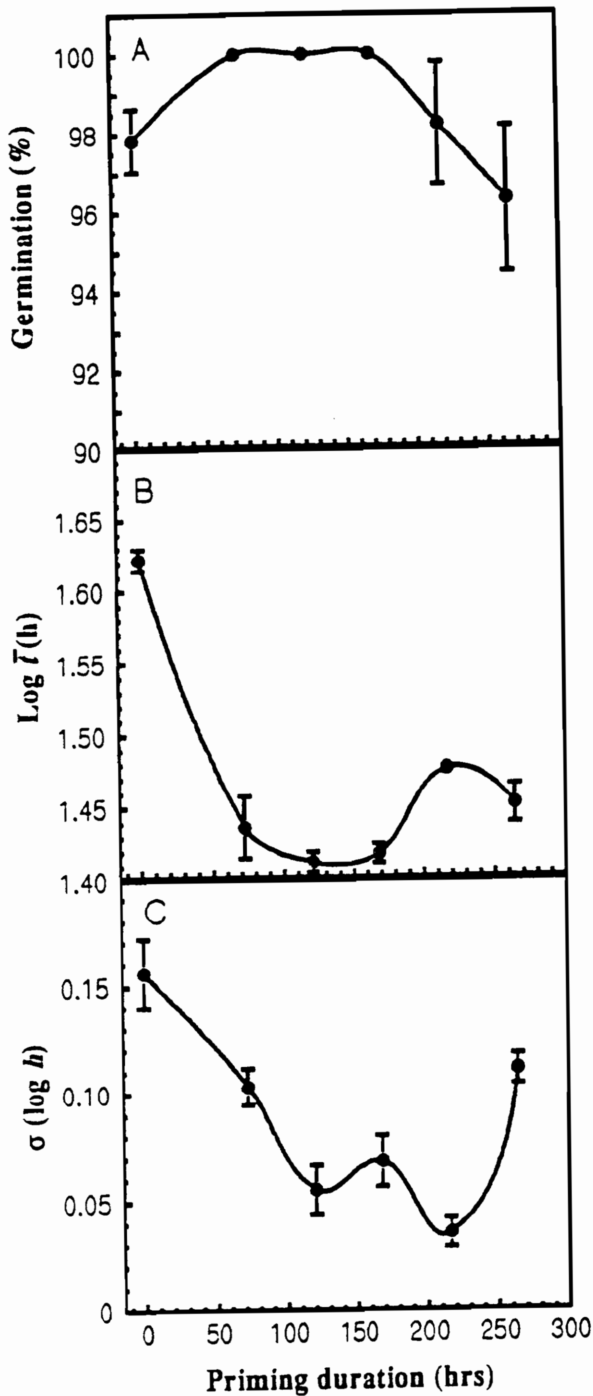

Fig. 2.13. Germination percentage (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination times (C) versus duration of priming treatment (hrs) for muskmelon seeds primed in vermiculite no. 2 at a ratio of 1 g of seed:4 g of vermiculite:0.4 ml of distilled water. Priming treatments were at 25±1°C in the dark. Each point is the mean of four samples of 20 seeds each, and error bars indicate ± S.E.when larger than the symbols.

germination percentages, particularly at low temperatures and water potential (Nerson and Govers, 1986; Bradford et al., 1988; Welbaum and Bradford, 1991a). In this study, the best priming treatment was observed with seeds primed in solutions of 0.3 M KNO_3 (Table 2.1). This treatment reduced the mean time to germination by 56, 41, 13, 31, 26, and 31%, when compared to control (nonprimed) seeds, and seeds primed in water, PEG 8000, calcium silicate, vermiculite, and soil, respectively (Table 2.1). Priming with PEG, water, calcium silicate, vermiculite, and Hayter loam soil as priming agents reduced the mean time to germination by 49, 25, 36, 40, and 38%, respectively, compared to nonprimed seeds (Table 2.1).

The presence of nitrate during priming may provide additional substrate for amino acid and protein synthesis causing enhanced germination following osmotic priming in solutions containing nitrogen (Khan et al., 1981). Haigh and Barlow (1987) showed that tomato and carrot seeds primed in solutions containing KNO_3 germinated more uniformly than seeds primed in K_2HPO_4, K_3PO_4, or PEG 6000 solutions.

The optimum priming treatment for seeds is determined by variable factors such as species, duration, temperature, and nature of the priming agent and its osmotic or matric potential (Parera and Cantliffe, 1994; Evans and Pill, 1989; Frett and Pill, 1989; Haigh and Barlow, 1987; Bradford, 1986). Studies with seeds of parsley (*Petroselinum crispum* L.) and tomato demonstrated detrimental priming effects at $\Psi s \leq -1.2$ MPa and long duration treatments of up to 10 weeks (Ely and Heydecker, 1981; Coolbear et al., 1980). Smith and Cobb (1991) showed that priming for longer than 12 days in 300 mM salt solutions partially reversed the beneficial effects of priming. In our study, priming at Ψs lower than -1.8 MPa and longer treatment durations with water, calcium silicate, soil, and vermiculite also reversed the beneficial effects of priming (cf. Figs. 2.6 to 2.13). For effective seed priming, the external Ψ should be sufficiently low to prevent radicle growth, or at higher

external Ψ, the priming duration should be shortened to be within the plateau phase of imbibition (Bradford, 1986). Hardegree and Emmerich (1992) showed data that indicated metabolic reactions resulting in positive priming effects may only occur at relatively high water potential. In muskmelon seeds, the greatest reduction in log t and σ occurred between −1.5 to −1.8 MPa Ψ (cf. Figs. 2.7; 2.8; 2.10; and 2.12). The minimum Ψ at which germination occurred during priming was ≈ −0.7 MPa (data not shown), which agrees with earlier reports by Welbaum and Bradford (1991a). Tarquis and Bradford (1992) indicated that the effectiveness of a given duration of priming is proportional to the difference between the actual seed Ψ and a minimum Ψ below which no priming effect would be observed.

Pre-hydration in water for short durations reduced the mean time to germination but was not as effective as priming in osmotic and matric agents (Fig. 2.6; Table 2.1). Bradford (1986) reported that the degree of seed hydration is correlated with the osmotic potential of the priming solution and determined the minimum effective Ψ for priming of lettuce (*Lactuca sativa* L.) seeds at between −2 and −3 MPa. The lower minimum Ψ required for metabolic advancement in lettuce seeds is ≈ −2.4 MPa (Tarquis and Bradford, 1992). It has been reported that pepper seeds pre-hydrated in water for 6 to 10 days and then dried, germinated faster than controls, but not as fast as seeds primed in solutions of 200 mM Na_2SO_4 (Smith and Cobb, 1991). Nath et al. (1991) also reported that a short hydration treatment applied to 'Karamu' wheat seeds had little effect on germinability. On the other hand, Tarquis and Bradford (1992) found that short pre-hydration treatments (1 hour) on lettuce seeds had little effect on germination rate but improved root growth rate. Damage often occurrs upon dehydration of seeds, particularly if expansive radicle growth has begun (Bradford, 1986; Heydecker and Coolbear, 1977). The loss in dessication tolerance may explain the aging effect of priming at high Ψs and long duration treatments,

as expansive growth may have occurred during priming, or compounds like raffinose or stachyose sugars, which stabilize membranes, may have been lost.

In our study, the poor priming effect at low Ψs was probably due to the lack of activation of metabolic repair processes. In muskmelon seeds held at Ψs below −2.5 MPa, aging continues at a faster rate than repair of damage can occur (Welbaum, 1993). At Ψs below ≈ −2.5 MPa, seeds were aged rather than primed. Low oxygen solubility may also limit oxidative processes during priming in PEG solutions.

The planar area between the periphery of the seed coat and the embryo was 13% greater in primed seeds than in nonprimed seeds, while the planar area of the embryo was 4% greater in nonprimed seeds than in primed seeds (Figs. 2.1 to 2.4). This phenomenon may be attributed to embryo shrinkage during the priming treatment possibly as a result of hydrolytic physiological processes or an expansion of the seed coat and thus an increase in seed volume. Although seed volume was not determined, measurements of the planar dimensions indicated that priming treatment influenced seed size. An increase in volume of tomato seeds of approximately 35% after seed priming has been reported (Argerich and Bradford, 1989).

In conclusion, our findings demonstrate that effective priming treatments for muskmelon seeds mostly depend on maintaining Ψs near equilibrium between −1.5 and −1.8 MPa. The duration of the priming treatment and the nature of the priming material were also important in determining the level of priming enhancement. Seeds primed for longer durations and at high Ψs exhibited poorer germination. The fastest germination occurred after priming in KNO_3 solutions.

Acknowledgements

We thank Paul Peterson (California State Seed Laboratory, Sacramento, California) for help in performing X-ray analysis.

Chapter 3.

Effect of Postharvest Washing and Post-storage Priming on Viability and Vigor of 6-year-old Muskmelon (*Cucumis melo* L.) Seeds from Eight Stages of Development

Abstract. Muskmelon (*Cucumis melo* L., 'TopMark') fruits were harvested at 5-day intervals from 30 to 65 days after anthesis (DAA). Seeds were removed and either dried without washing or washed vigorously for 3 hours and dried to a moisture content (MC) from 3.3 to 5.7% on a dry weight basis. Seeds were stored for 6 years (7 months at 20°C and 5 years and 5 months at 10°C) in tightly sealed containers before germination testing at 20, 25, and 30°C and from 0 to −1.0 MPa water potential (Ψ). Six-year-old seeds were aged using controlled deterioriation (21% MC, 45°C, up to 7 days) or primed (0.3 M KNO_3, 6 days, 25°C) and then aged prior to germination testing on blotter paper for 21 days. Results were compared with germination at harvest. Thirty- and 35-DAA seeds had less than 25% germination after storage. The germination of 40- and 45-DAA seeds exceeded 86% in water at all temperatures, but 4-day root lengths were shorter and viability was lost more rapidly than in older seeds during controlled deterioration. Fifty-, 55-, and 60-DAA seeds showed the greatest resistance to controlled deterioration, were the most tolerant of reduced Ψs, and had the greatest 4-day root lengths. Washed seeds had higher germination percentages, lower mean times to germination, and greater resistance to controlled deterioration than unwashed seeds. Priming reduced viability, decreased the mean time to germination, reduced the rate of viability loss during controlled deterioration of 40- and 45-DAA seeds but increased the rate for 55-DAA seeds. The highest quality seed was attained 50 to 60 DAA from fruit harvested after edible maturity but before the onset of severe decomposition.

Introduction

In many orthodox seeds, such as cereals or other grain crops, natural desiccation coincides with the attainment of maximum dry weight and physiological maturity (Rosenberg and Rinne, 1986). In many cases, drying coincides with discoloration of the plant and seed, allowing harvest dates to be easily determined based on appearance or seed moisture content (MC). Determining the optimum time of harvest for seeds that develop inside fleshy fruits is more complicated, because seed maturation may precede fruit maturity (Coombe, 1976). For example, muskmelon seeds are held inside fruits for several weeks after maximum dry weight accumulation at high MC before the seeds are released during fruit decomposition (Welbaum, 1993). In some cultivars, a high percentage of seeds are either dead or have very low vigor if harvest is delayed until fruits are severely decomposed, presumably because seed MC is high enough to cause rapid aging but too low to repair subcellular damage (Welbaum, 1993). Conversely, when fruits are harvested prematurely seeds must be afterripened or primed to increase vigor to the same level as older seeds, even though maximum seed dry weight has been obtained (Welbaum and Bradford, 1991a, b). Therefore, muskmelon seeds must be harvested at the correct stage of development to obtain maximum viability and vigor.

In a previous study, the optimum harvest date for muskmelon cultivar Top Mark was determined to be 55 to 60 days after anthesis (DAA) using several indices of vigor (Welbaum and Bradford, 1989). In another study of muskmelon seed development, optimum seed quality coincided with edible fruit maturity (Harrington, 1959). Immature 32-DAA seeds lost viability more rapidly after nine months of storage than more advanced stages of development (Harrington, 1959). Harrington (1959) also showed that fermentation produced seed lots with greater viability than mechanical cleaning or rinsing in water. However, the effects of these treatments on long-term storage and vigor were

not tested because seeds were stored for less than 1 year and germination was only tested under optimal conditions (Harrington, 1959). Muskmelon seeds require afterripening to develop maximum vigor (Welbaum and Bradford, 1991b). When the afterripening of muskmelon seeds was assessed for up to 12 months, the vigor of less mature seeds increased more than older seeds (Welbaum and Bradford, 1991b). There is limited information on how the vigor and viability of muskmelon seeds from different stages of development change during long-term storage. Bass (1973) reported that some muskmelon cultivars obtained from commercial sources maintained full viability during a 12-year storage experiment, while others declined after 7 years. Roos and Davidson (1992) also reported that some muskmelon cultivars maintained full viability during a 30 year storage period, while others lost ≥30% viability after 50 years. However, the effects of fruit maturity or postharvest washing treatments on seed longevity were not considered. Postharvest processing of muskmelon seed crops varies widely and may involve extensive or very little washing (unpublished observation). In this report, the germination of washed and dried or surface-cleaned and dried seed lots from eight stages of development was tested after 6 years of storage. Seed lots from the same stages of development were tested at harvest and the results published in a previous report (Welbaum and Bradford, 1989). Therefore, effects of postharvest washing and development on seed storage life can be directly compared with germination at harvest. Seeds from each stage of development were primed after storage and subjected to controlled deterioration to determine whether priming could repair putative damage incurred during storage.

Materials and methods

Seed production and storage conditions - Muskmelon cultivar TopMark (Asgrow Seed Co. Inc., Tracy, California, USA) was field grown in a silty clay loam (fine silty, mixed,

nonacidic, thermic Typic Xerorthents) soil in Davis, California, USA as previously described (Welbaum and Bradford, 1988). Hermaphroditic flowers were tagged at anthesis, and crown-set fruits containing from 400 to 500 seeds were harvested at 5- day intervals from 30 to 65 DAA. At harvest, seeds were removed from the fruit and either wiped with dry paper towels to remove the mucilaginous endocarp or washed vigorously in flowing tap water for 3 hours. Seeds from six to ten fruits from the same stage of development were harvested simultaneously and combined during drying to create a lot. Both washed and unwashed seeds were forced-air dried for 3 hours at room temperature, then final-dried over silica gel in a sealed container for 2 days at 30°C to a MC of $< 6.0\%$ (dry weight basis) determined by heating samples of 50 seeds for 1 hour at 130°C (ISTA, 1985). Dried seeds were stored for a total of 6 years (7 months at 20°C and 5 years 5 months at 10°C) in tightly sealed bottles. During 5 days in 1990, seeds were shipped from Davis, California to Blacksburg, Virginia, USA where they were stored at 10°C until germination testing began.

Germination experiments - Two replicates of 25 seeds each were placed in 9 x 9 x 1.5 cm transparent, covered plastic boxes (Falcon 1012, Becton Dickinson and Co., Lincoln Park, NJ, USA) on two thicknesses of germination blotter paper (Anchor Paper Co., St. Paul, Minnesota, USA) saturated with 12 ml of deionized water or polyethylene glycol 8000 solutions ranging in Ψ from 0 to -1.0 MPa in 0.2 MPa increments prepared according to Michel (1983). Boxes were incubated in the dark at 20, 25, and 30 $\pm$ 1°C inside self-sealing plastic bags (Ziploc, Dow Brands L. P., Midland, Michigan, USA) to reduce evaporation. The actual Ψs of the blotters were verified by osmometry (Model 5500, Wescor Inc., Logan, Utah, USA) 3 to 4 days after the start of incubation and were generally 0.1 to 0.4 MPa lower than initial values due to the concentrating effect of

imbibition and evaporation during seed scoring. Seeds were scored for radicle emergence at 12-hour intervals for 21 days and germinated seeds removed. The Ψs required to inhibit the maximum germination at each stage of development and treatment by 10%, 50% (Ψ_{50}), and 90% were determined graphically from a curve fitted to a plot of final germination percentage versus Ψ (Welbaum et al., 1990).

Two replications of 50 seeds each were placed on four layers of rolled germination paper towels (Anchor Paper Co.) and incubated in the dark at alternating 20°C (16 hours) and 30°C (8 hours). After 4 days, seedling root lengths were measured and averaged for all germinated seeds (Welbaum and Bradford, 1989).

Priming and controlled deterioration - Stored washed seeds were primed for 6 days in the dark at 25°C in aerated 0.3 M KNO_3 solution at a ratio of 5 ml of solution g^{-1} of seed. After priming, seeds were rinsed with tap water, dried in a desiccator for 24 hours to a MC of < 6%, and stored for 3 days prior to controlled deterioration (Mathews and Powell, 1987). One hundred seeds each of primed and nonprimed, washed, stored seeds were aged at 21% MC inside 7.5 x 1.0 cm sealed, plastic vials in a water bath at 45°C for up to 7 days. In a second experiment, 100 seeds each of 40- to 65-DAA washed and unwashed stored were aged at 21% MC for 72 hours at 45°C. After controlled deterioration, seeds were redried for 24 hours to a MC of < 6% in a desiccator, stored from 1 to 7 days at 20°C, and four replications of 25 seeds each were germinated at 25°C in plastic boxes for 21 days as described above.

Statistical analysis - Probit analysis was used to determine the log mean time and the variance of germination. When germination percentages were plotted on a probit scale versus log time (t), straight lines of approximately equal slope were produced for different treatments, indicating a normal distribution of germination events with log time (Finney,

1971). The log mean time to germination (log $\bar{t}$) was determined graphically from the intersection of the least squares regression line of log $\bar{t}$ versus probit germination percentage and 50% germination for treatments with greater than 20% germination. The slope of a probit plot is equal to the inverse standard deviation (σ) (Finney, 1971).

Linear regression was used to compare slopes of the plot of probit viability, log $\bar{t}$, and σ versus aging time to analyze the rate of deterioration of primed and nonprimed seeds at different stages of development (Ellis and Roberts, 1981). Differences among treatments were determined by comparing the standard error of the slopes. The regression lines were extrapolated to the intercept on the ordinate at 0 hours aging to obtain the initial probit viability (K_i). The mean time to viability loss (P_{50}) was taken as mean time to loss of half of the initial viability.

Analysis of variance (ANOVA) was used to test for washing, developmental, aging, and temperature effects as well as their interactions on arcsin transformed germination percentages, log $\bar{t}$, and σ for 45- to 65-DAA seeds (CoStat, CoHort Software, Berkeley, California, USA). The LSD values were determined from the pooled variances calculated from treatments where germination occurred. Untransformed germination percentages are shown in tables and figures.

Results

Germination percentages in water were significantly different among seedlots representing different stages of development (Table 3.1). In most cases, washed seeds had higher germination percentages than unwashed seeds (Table 3.1). Germination percentages did not differ among temperatures (Table 3.1). Germination percentages at 30°C for stored 30- and 35-DAA seeds were about 10-fold lower than at harvest (Table 3.1). Germination percentages of 30- and 35-DAA seeds were five-fold greater at –0.2 and –0.4 MPa Ψ

Table 3.1. Germination of washed (w) and unwashed (uw) muskmelon seeds at 20, 25, and 30°C and eight stages of development (days after anthesis) and after 6 years of storage. ANOVA was performed on arcsin transformed percentage data and actual percentages are shown.

		Temperature (°C)											
DAA	Storage MC (%)	20	25	25A*	30	20	25	25A*	30	20	25	25A*	30
		Germination (%)				log $\bar{t}$(*h*)				σ (log *h*)			
30w	3.9	0	12	NA**	2	—	—	—	—	—	—	—	—
35w	3.3	22	8	NA	6	3.36	—	—	—	0.38	—	—	—
40w	3.8	94	100	6	100	1.64	1.36	—	1.46	0.41	0.18	—	0.31
45w	3.9	86	98	20	98	1.92	1.59	—	1.48	0.20	0.16	—	0.34
45uw	3.5	70	86	24	98	1.96	1.80	3.05	1.63	0.24	0.38	0.86	0.24
50w	3.4	92	100	90	100	1.72	1.55	2.11	1.64	0.21	0.10	0.26	0.15
50uw	4.5	96	100	25	98	1.92	1.76	2.71	1.51	0.21	0.19	0.27	0.29
55w	5.4	100	100	92	100	1.72	1.54	2.21	1.40	0.05	0.08	0.28	0.22
55uw	4.6	80	88	58	98	2.05	1.96	2.36	1.81	0.26	0.24	0.29	0.34
60w	5.9	92	94	84	100	1.70	1.58	2.10	1.50	0.28	0.24	0.35	0.19
65w	5.7	84	90	80	74	1.82	1.61	1.86	1.47	0.34	0.34	0.36	0.12
65uw	4.6	94	78	NA	88	2.02	1.98	—	1.60	0.18	0.37	—	0.58

$LSD_{0.05}$ = Germination (%) = 12; log $\bar{t}$ = 0.16; σ = 0.20

*Aged at 21% MC and 45°C for 72 hours after storage

**Data not available

Treatments	Germination (%)	log $\bar{t}$	σ
Temperature (C)	NS	***	NS
Washing Treatment (W)	*	***	*
Maturity (M)	*	NS	NS
Aging (A)	***	***	***
C x M	NS	NS	NS
C x W	NS	*	NS
M x W	NS	***	NS
A x M	***	***	**
A x W	*	*	*
A x M x W	*	***	NS
C x M x W	NS	NS	NS

NS, *, **, ***Nonsignificant or significant at the 0.05, 0.01 and 0.001 probability levels, respectively, via F-test.

than in water at 30°C (data not shown). At 40 DAA, germination percentages were near 100% at all temperatures and higher than at harvest (Table 3.1). From 45- to 60-DAA, seeds were fully germinable at harvest and after storage (Table 3.1). Germination at 65 DAA was less than 90% at harvest and after storage (Table 3.1).

There was a significant interaction between seed maturity (DAA) and washing treatments, because washing decreased log $\bar{t}$ more in older seeds than in younger seeds (Table 3.1). An interaction between temperature and washing occurred, because washing decreased log $\bar{t}$ more at low temperature than at high temperature (Table 3.1). For all stages of development, germination was faster at higher temperatures than at lower temperatures (Table 3.1). Log $\bar{t}$ did not differ among seed maturities (Table 3.1). Germination of stored 40- and 45-DAA seeds was faster than at harvest, while in more mature seeds, log $\bar{t}$ values after storage and at harvest were similar (Table 3.1).

The σ did not differ with temperature or amongst stages of maturity (Table 3.1). Generally, the σ of washed seeds was less than unwashed seeds (Table 3.1). For most stages of development, σ was greater after storage than at harvest.

Less mature seeds lost viability more rapidly during controlled deterioration at 45° C for 72 hours than more mature seeds (Table 3.1). At 40 DAA, controlled deterioration killed most seeds, while at 50, 55, 60 and 65 DAA washed, aging did not significantly decrease germination (Table 3.1). Washed and unwashed 45-DAA seeds showed similar decreases in germination percentage, while at 50 and 55 DAA, washed seeds were significantly more tolerant of controlled deterioration than unwashed seeds. Controlled deterioration increased log $\bar{t}$ at most stages of development for both washed and unwashed seeds (Table 3.1). The only increase in σ occurred during the controlled deterioration of 45-DAA unwashed seeds (Table 3.1).

In a separate experiment, 40- to 65-DAA seeds were aged at 45°C for up to 180 hours. All stages of development lost viability during the initial aging treatments except 50- and 55-DAA seeds which showed a lag phase of 72 and 48 hours, respectively, before viability declined (Fig. 3.1A). Values during the lag phase were not included in the graphs to improve the accuracy of the slopes of the viability loss time courses (Fig. 3.1). The lag period before the decline in viability resulted in high values for K_i of 50 and 55 DAA seeds (Table 3.2). The P_{50} for 50 and 55 DAA seeds was larger than for other stages of development (Fig. 3.1A; Table 3.2). In contrast, 40- and 45-DAA seeds had lower K_i values than either 50- or 55-DAA seeds (Table 3.2). The slope of the viability loss regression line is greater at 40 DAA than in more mature seeds (Table 3.2). At 45 DAA, both the slope of the viability regression line was less and the P_{50} greater than at 40 DAA (Fig. 3.1A; Table 3.2). Sixty-five-DAA seeds lost viability slowly during controlled deterioration, the K_i was lowest of any stage of development, and the P_{50} decreased to 163 hours (Fig. 3.1A; Table 3.2).

Before controlled deterioration, log $\bar{t}$ was essentially the same for all stages of development (Fig. 3.2A). There was an inverse relationship between loss of viability and increased speed of germination during controlled deterioration (cf., Fig. 3.1A, and 3.2A). Log $\bar{t}$ increased the fastest during controlled deterioration for 45-DAA seeds and the slowest for 55-DAA seeds, while 50 and 65 DAA were intermediate (Fig. 3.2A). Except for 0 hours aging, the σ for 45- and 65-DAA seeds was similar during controlled deterioration and higher than other stages of development (Fig. 3.3A). However, the rate of increase during aging was greater for 45-DAA seeds (Fig. 3.3A). Both 50- and 55-DAA seeds had the lowest initial σ, and values increased little during controlled deterioration (Fig. 3.3A).

Six-year-old 40- to 65-DAA seeds were primed prior to controlled deterioration.

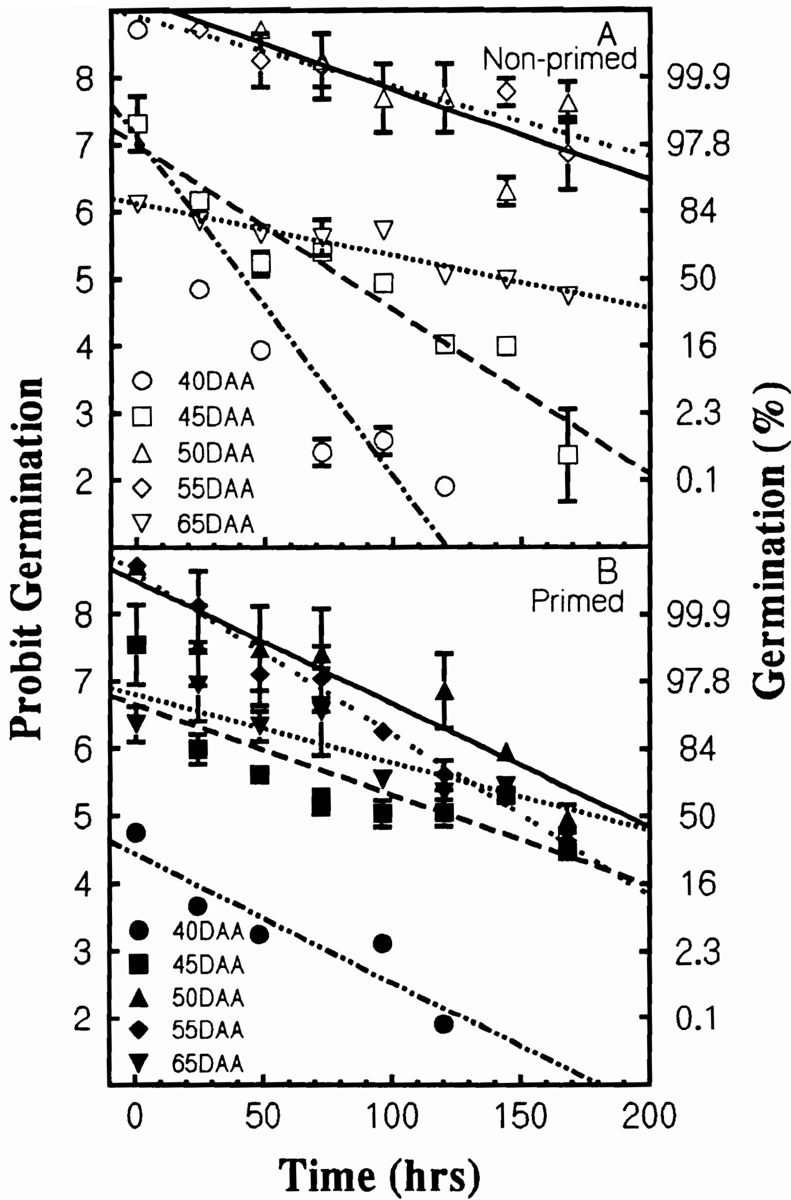

Fig. 3.1. Probit percentage germination versus hours of controlled deterioration (21% MC and 45°C) for non-primed (A) and primed (B; 0.3 M KNO_3, 6 days) 40-, 45-, 50-, 55-, and 65-DAA muskmelon seeds germinated at 25°C for 21 days after 6 years of storage. Slopes of regression equations, initial probit viability (K_i), and mean time to loss of viability (P_{50}) are summarized in Table 2. Values for 60 DAA were similar to 55 DAA and were not plotted. Each point is the mean of four samples of 25 seeds each, and error bars indicate ± S. E. when larger than the symbols.

Table 3.2. Effects of post-storage priming (6 days, 0.3 M KNO_3) and controlled deterioration (21% MC and 45°C for upto 172 hours) of washed muskmelon seeds at six stages of development (days after anthesis) on mean time to loss of viability (P_{50}), the initial probit viability determined by extrapolation from the viability loss regression line (K_i); and the slope of the probit viability loss regression line ($1/\sigma$) determined from Figure 1.

DAA	Treatment	Moisture Content (%)^Y	P_{50} (h)	K_i (probits)	$-1/\sigma$ (probits h^{-1} ±s.e.)
40	Non-primed	20.4	42	7.1	0.050 ± 0.012
	Primed	20.8	14	4.4	0.019 ± 0.003
45	Non-primed	20.5	101	7.0	0.020 ± 0.002
	Primed	20.5	131	6.6	0.013 ± 0.002
50	Non-primed	20.0	299	9.2	0.014 ± 0.005
	Primed	21.0	194	8.4	0.018 ± 0.005
55	Non-primed	22.0	391	8.9	0.010 ± 0.005
	Primed	20.9	180	8.6	0.020 ± 0.002
60	Non-primed	22.1	272	7.8	0.011 ± 0.002
	Primed	21.4	276	7.5	0.010 ± 0.003
65	Non-primed	22.3	163	6.1	0.008 ± 0.001
	Primed	20.3	195	6.8	0.010 ± 0.002

^{Y}controlled deterioration moisture content (dry weight basis).

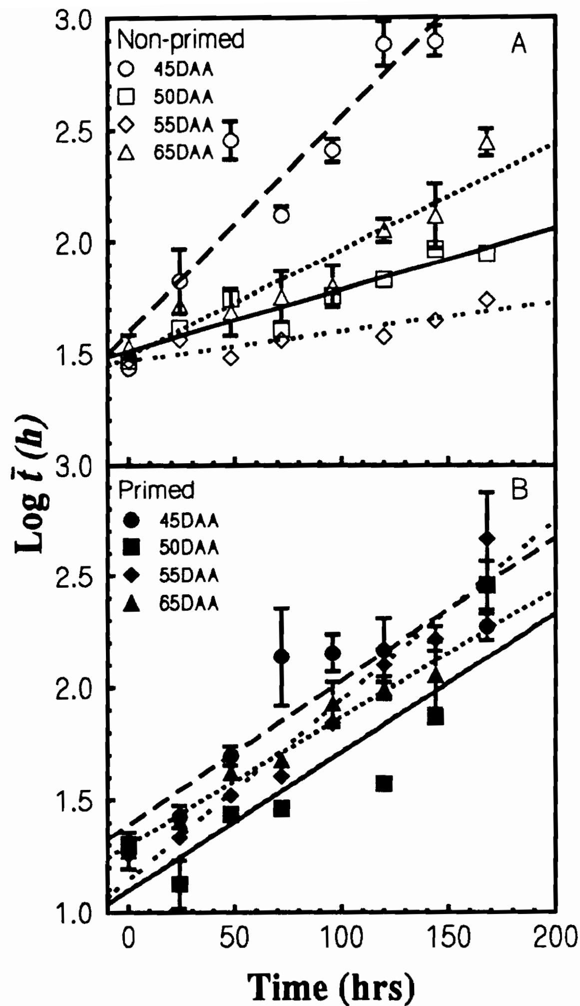

Fig. 3.2. The mean log time of germination (log $\bar{t}$) versus hours of controlled deterioration (21% MC and 45°C) for nonprimed (A) and primed (B; 0.3 M KNO_3, 6 days) 45-, 50-, 55-, and 65-DAA muskmelon seeds germinated at 25°C for 21 days after 6 years of storage. Slopes of regression equations (± S. E. for the slopes) for non-primed seeds are: 45 DAA, y = 1.6 + –0.01x (±0.002), r^2 = 0.86; 50 DAA, y = 1.51 + 0.003x (±4.44^{-4}), r^2 = 0.86; 55 DAA; y = 1.47 + 0.001x (±3.79^{-4}), r^2 = 0.78; 65 DAA, y = 1.49 + 0.005x (±4.47^{-4}), r^2 = 0.89; and primed seeds: 45 DAA, y = 1.39 + 0.006x (±0.001), r^2 = 0.85; 50 DAA, y = 1.098 + –0.006x (±0.001), r^2 = 0.78; 55 DAA, y = 1.15 + 0.008x (±6.98^{-4}), r^2 = 0.96; 65 DAA; y = 1.301 + –0.006x (±6.93^{-4}), r^2 = 0.97. Values for 60 DAA were similar to 55 DAA and were not plotted. Each point is the mean of four samples of 25 seeds each, and error bars indicate ± S. E. when larger than the symbols.

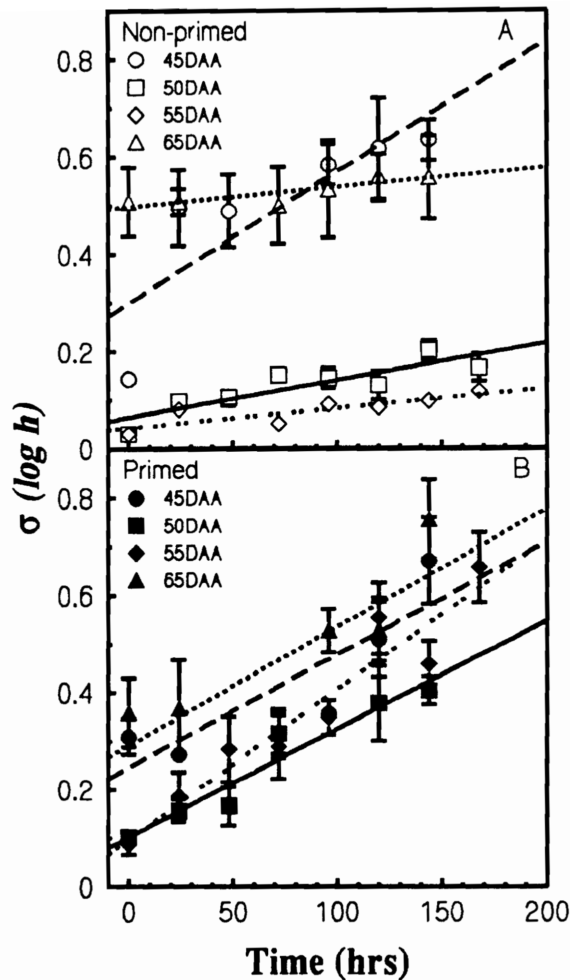

Fig. 3.3. Radicle emergence times (standard deviation, σ) versus hours of controlled deterioration (21% MC and 45°C) for nonprimed (A) and primed (B; 0.3 M KNO_3, 6 days) 45-, 50-, 55-, and 65-DAA muskmelon seeds germinated at 25°C for 21 days after 6 years of storage. Slopes of regression equations (± S. E. for the slopes) for non-primed seeds are: 45 DAA, $y = 0.3 + -0.003x$ ($\pm 6.86^{-4}$), $r^2 = 0.71$; 50 DAA, $y = 0.065 + 7.66x$ ($\pm 1.45^{-4}$), $r^2 = 0.75$; 55 DAA, $y = 0.04 + 3.99x$ ($\pm 1.17^{-4}$), $r^2 = 0.70$; 65 DAA, $y = 0.50 + 3.98x$ ($\pm 1.27^{-4}$), $r^2 = 0.72$; and primed seeds: 45 DAA, $y = 0.25 + 0.002x$ ($\pm 7.5^{-4}$), $r^2 = 0.76$; 50 DAA, $y = 0.10 + -0.002x$ ($\pm 1.51^{-4}$), $r^2 = 0.94$; 55 DAA, $y = 0.098 + 0.003x$ ($\pm 3.78^{-4}$), $r^2 = 0.92$; 65 DAA, $y = 0.29 + 0.002x$ (± 0.001), $r^2 = 0.68$. Values for 60 DAA were similar to 55 DAA and were not plotted. Each point is the mean of four samples of 25 seeds each, and error bars indicate ± S. E. when larger than the symbols.

Priming decreased K_i compared to non-primed seeds at all stages of development except 65 DAA (Fig. 3.1B; Table 3.2). At 40 and 45 DAA, the slope of the viability loss regression line decreased after priming, while at 55 DAA priming increased the slope (Fig. 3.1B; Table 3.2). Priming decreased P_{50} at 40, 50, and 55 DAA but increased values at 45 and 65 DAA (Fig. 3.1B; Table 3.2).

Priming decreased log $\bar{t}$ compared to non-primed seeds at all stages of development (Fig. 3.2B). Priming decreased the slope of the log $\bar{t}$ versus hours of controlled deterioration plot at 45 DAA but consistently increased the slope compared to non-primed seeds at other stages of development (cf., Fig. 3.2A and B). The slope of the plot of σ versus hours of controlled deterioration was greater for primed seeds than non-primed seeds at all stages of development except 45 DAA (cf., Fig. 3.3A and B). Even though all primed seeds had similar slopes, primed 50- and 65-DAA seeds had the lowest and highest σ, respectively, at most aging times (Fig. 3.3B).

The water stress tolerance of stored seeds increased during development and was highest from 50 to 65 DAA (Fig. 3.4). All Ψ_{50} values for stored seeds were approximately 0.2 MPa lower than the values reported for newly harvested seeds in an earlier study (Fig. 3.4). Insufficient seed prevented comparison of washed and unwashed seeds at all stages of development. Washed and unwashed seeds had similar water stress tolerance at 45 DAA, but unwashed seeds were more sensitive to water stress at 65 DAA (Fig. 3.4).

The seedling root lengths of washed stored seeds increased linearly between 40 and 60 DAA (Fig. 3.5). Unwashed stored seeds produced shorter roots than washed stored seeds from 45- and 55-DAA (Fig. 3.5). Root lengths after storage and at harvest were similar for 45- to 65-DAA washed seeds. After storage, root lengths of unwashed seeds were longer than at harvest (Fig. 3.5).

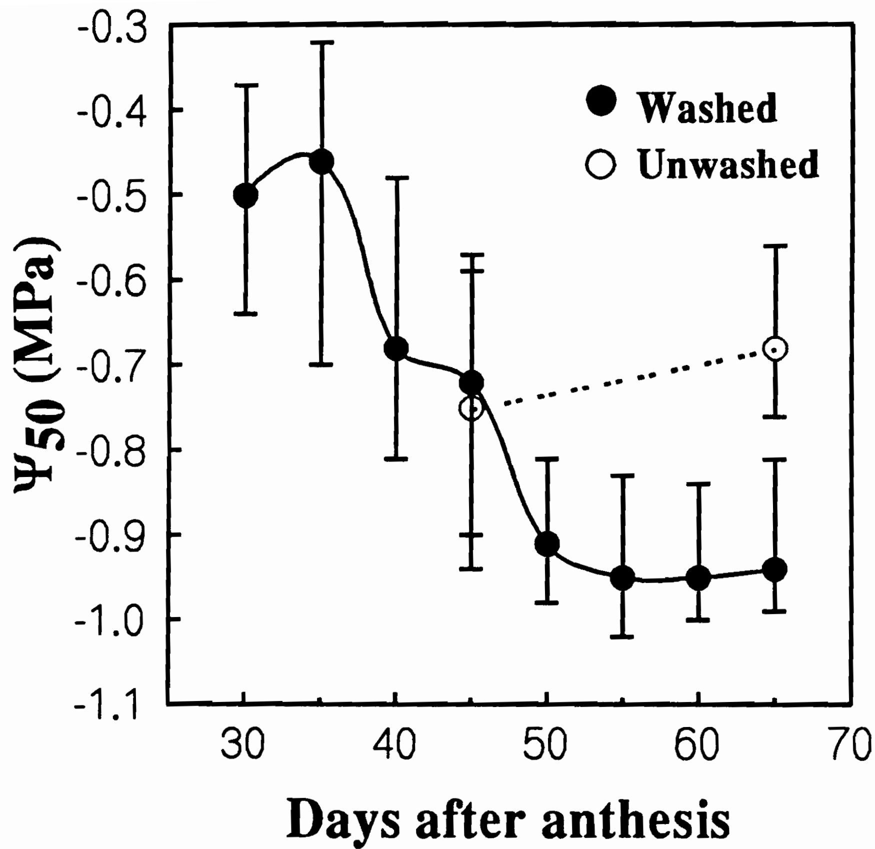

Fig. 3.4. The Ψ required to inhibit maximum germination by 50% (Ψ_{50}) at 30°C for washed (•) or unwashed (○) 30-, 35-, 40-, 45-, 50-, 55-, 60-, and 65-DAA muskmelon seeds after six years of storage. The values were derived from plots of final germination percentage versus Ψs of the polyethylene glycol imbibition solution. The error bars on the Ψ_{50} values indicate the range of Ψs allowing 10 to 90% germination.

Discussion

Fresh 30- and 35-DAA seeds germinated poorly at harvest, but drying and afterripening for 2 months increased germination to greater than 90% in water (Welbaum et al., 1990; Welbaum and Bradford, 1991b). This viability was short-lived, because both stages of development germinated poorly after 6 years of storage (Table 3.1). Muskmelon seeds do not achieve maximum dry weight until 35 DAA, so 30- and 35-DAA seeds lost viability during storage because they were not fully developed (Welbaum and Bradford, 1989). At harvest, 30 and 35 DAA germinated faster and to higher percentages at –0.2 and –0.4 MPa Ψs than in water (Welbaum et al., 1990). After storage, approximately 25% of 30- and 35-DAA seeds germinated at –0.2 and –0.4 MPa Ψs, so the germination was still stimulated by reduced Ψs, but percentages were much lower than at harvest (Welbaum et al., 1990). The physiological basis of muskmelon seed dormancy at high Ψs is poorly understood, but in this study the phenomenon was only detected in 30- and 35-DAA seeds at harvest and after storage (Dunlap, 1988; Welbaum et al., 1990).

At harvest, germination percentages for 40-DAA washed and dried seeds were at least 87%, but 40-DAA seeds lacked vigor by other criteria such as germination rate, germination at reduced Ψs, and 4-day root lengths (Welbaum and Bradford, 1989; Welbaum et al., 1990). During the 6 year storage period, the 4-day root lengths of 40-DAA washed seeds increased dramatically, log $\bar{t}$ at 30°C decreased, and Ψ_{50} decreased compared to values at harvest possibly due to afterripening (Table 3.1; Figs. 3.4 and 3.5). However, 40- and 45-DAA washed seeds had shorter 4-day root lengths and higher Ψ_{50} values compared to 50- to 60-DAA seeds (Figs. 3.4 and 3.5). The slope of the viability loss curve was greater for 40-DAA seeds, and both 40- and 45-DAA seeds had lower P_{50} and K_i values than more mature seeds (Table 3.2). Despite similar germination

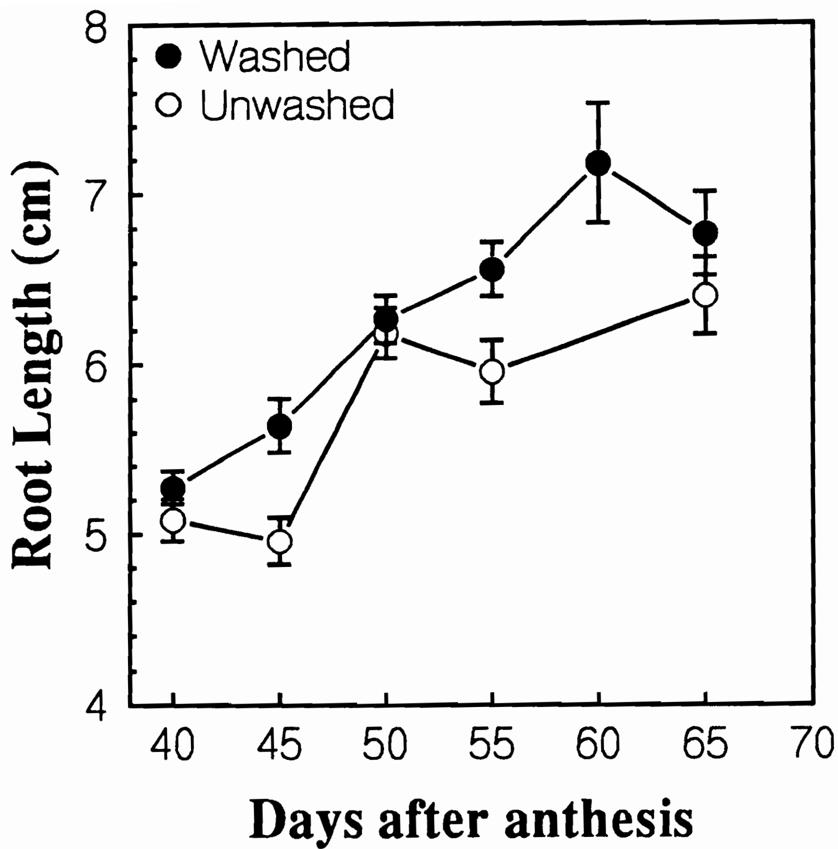

Fig. 3.5. Comparison of washed (●) and unwashed (O) postharvest seed treatments on muskmelon seedling vigor for six stages of development after 6 years of storage as indicated by seedling root lengths 4 days after imbibition on paper towels. Error bars indicate ± S. E. for two replications of 50 seeds each.

percentages for 40- to 60-DAA seeds (Table 3.1), the lower initial germination (smaller K_i) and the more rapid deterioration of 40- and 45-DAA seeds during controlled deterioriation indicates inferior quality compared to the more mature seeds (Table 3.2; Fig. 3.1A). During controlled deterioration, 65-DAA seeds lost viability more slowly than other stages of development, but also had the lowest K_i (Fig. 3.1A). At harvest, the viability of 65-DAA seeds was reduced because aging inside the fruit prior to harvest killed a fraction of the seeds (Welbaum, 1993). Since aging effects are cumulative, controlled deterioration further reduced the viability of some 65-DAA seeds, while the remaining fraction of viable seeds was highly vigorous by most criteria (Fig. 3.1A; Table 3.2).

Maximum 'Top Mark' seed quality at harvest was obtained from 55- to 60-DAA (Welbaum and Bradford, 1989; Welbaum et al., 1990). Six years later, the vigor of 50-, 55-, and 60-DAA washed seeds was similar by most criteria and exceeded other stages of development (Table 3.1; Figs. 3.1-3.5). Although 50-DAA seeds were not among the most vigorous at harvest, afterripening increased germination performance to the same level as 55- and 60-DAA seeds. An earlier study concluded that the optimum time for 'Hale's Best No. 45' muskmelon seed harvest was at edible fruit maturity (47 DAA), but more advanced stages of maturity were not considered (Harrington, 1959).

Ellis and Roberts (1980) proposed that in the absence of dormancy, the slope of controlled deterioration plots should be constant within species following storage at a given temperature and moisture content. The slope of the controlled deterioration plots for 40- and 65-DAA seeds differed from other stages of development even though all seeds had attained maximum dry weight, were aged under similar conditions, and had no apparent dormancy. Previous studies with tomato (*Lycopersicon esculentum* L.) seeds showed that the time to germination increased logarithmically as viability declined during

aging (Argerich et al., 1989). Muskmelon seeds showed a similar relationship (cf., Figs. 3.1 and 3.2). In addition, σ generally increased as viability declined during controlled deterioration for most stages of development, although the relationship was not as strong as between the decline in probit viability and the increase in log $\bar{t}$ (cf., Figs. 3.1 and 3.3). Thus, changes in the viability and speed (log $\bar{t}$) of muskmelon seed germination are interrelated and excellent indicators of seed quality.

At harvest, washed and dried muskmelon seeds exhibited superior viability, faster germination, less sensitivity to exogenous ABA, faster root growth, and greater tolerance of germination at reduced Ψs than fresh seeds or seeds dried without washing (Welbaum and Bradford, 1989; Welbaum et al., 1990). The greater viability and vigor attributed to washing was maintained after 6 years of storage (Tables 3.1 and 3.2; Figs. 3.4 and 3.5). The washing treatment may have leached inhibitors from the embryo. However, after 40 DAA the perisperm envelope behaves as a semipermeable membrane blocking the movement of macromolecules, so washing should not leach inhibitory compounds from the embryo in mature seeds (Welbaum and Bradford, 1990). The 3-hour washing treatment at harvest may have acted as a priming treatment. In lettuce (*Lactuca sativa* L.), priming treatments in water for as little as 3-hours increased vigor but caused more rapid aging compared to priming in osmoticum at reduced Ψs (Tarquis and Bradford, 1992). In muskmelon, controlled deterioriation decreased viability of 50- and 55-DAA unwashed seeds more than washed, indicating that postharvest washing not only increased vigor but also improved storability of some stages of development as well (Table 3.1).

One of the reported benefits of priming is to repair damage incurred during storage (Ward and Powell, 1983; Dearman et al., 1986; Rao et al., 1987). To test this hypothesis, muskmelon seeds were primed after storage. Priming reduced K_i at all stages of development except 65 DAA, so priming caused further aging and did not improve the

viability of stored seeds (Fig. 3.1; Table 3.2). However, priming increased the speed of germination for all stages of development, although during controlled deterioration log $\bar{t}$ and σ increased more rapidly than for nonprimed seeds at all stages of development except 45 DAA (Figs. 3.2 and 3.3). Priming decreased the slope of the controlled deterioration versus probit viability plot at 40 and 45 DAA, but increased the slope at 55 DAA, illustrating that the effects of priming varied with the stage of development (Fig. 3.1B; Table 3.2). Overall, priming equalized the slopes of the controlled deterioration plots for probit viability, log $\bar{t}$, and σ at all stages of development (Figs. 3.1 and 3.3; Table 3.2). The reduced rate of viability loss during controlled deterioration of primed 40- and 45-DAA seeds is consistent with earlier reports that priming extends seed longevity (Burgass and Powell, 1984). However, the faster rate of decline in viability for 55-DAA primed seeds during controlled deterioration agrees with previous results showing that 9-year-old primed 'Top Mark' muskmelon seeds from a commercial seedlot lost viability more rapidly, germinated more slowly, and lost uniformity more rapidly than non-primed seeds during controlled deterioration (Oluoch and Welbaum, 1996). Studies of tomato (Alvarado and Bradford, 1988; Argerich et al., 1989; Owen and Pill, 1994), wheat (Nath et al., 1991), and lettuce (Tarquis and Bradford, 1992) also showed that priming was deleterious to seed longevity.

After 6 years of storage, seeds washed at harvest were more vigorous and resistant to accelerated aging than unwashed seeds at most stages of development (Table 3.1). Afterripening during storage improved vigor at all stages of development, but did not raise all seeds to the same level of vigor (Welbaum and Bradford, 1991b). Priming increased the storage life of newly matured 40- and 45-DAA stored seeds but decreased the storage life of 55-DAA seeds, showing that priming affects seeds differently depending on their stage of development (Fig. 3.1). Combining seeds from a wide range of developmental

stages at harvest may produce seedlots of variable quality. For example, 35- and 40-DAA seeds have lower viability and vigor than more mature seeds but cannot be easily separated from seedlots because all stages of development past 35 DAA have the same dry weight (Welbaum and Bradford, 1988). Seed maturation inside the fruit after maximum dry weight accumulation is necessary for highest muskmelon seed vigor, so maximum dry weight accumulation is not the best indicator of muskmelon seed quality. Based on the cultivar Top Mark, the highest seed quality is attained from 50 to 60 DAA or from muskmelon fruits harvested just after edible maturity but before the onset of extensive fruit decomposition.

Acknowledgements

We thank Kent J. Bradford for storing seeds during the initial years of this experiment.

Chapter 4.

Viability and Vigor of Osmotically Primed Muskmelon Seeds After 9 Years of Storage

Abstract. The viability and vigor of osmotically primed (0.3 M KNO_3, 6 days, 25°C) and nonprimed muskmelon (*Cucumis melo* L., cv. PMR 45) seeds were compared after storage for 9 years at less than 20°C and 6% moisture content (MC, dwt. basis). Viability was compared at 20, 25, and 30°C at water potentials of 0, –0.2, –0.4, –0.6, and –0.8 MPa, and in soil. Additionally, stored primed and nonprimed seeds were either primed, aged (15% MC and 45°C) for up to 8 days, or aged for 72 hours and primed. The force required to puncture 5-mm-long, micropylar seed pieces was measured using an Instron Universal Testing Machine. Less force was required to puncture primed seed pieces at 0, 5, 15, 20, and 25 hours of imbibition, demonstrating that osmotic priming weakens the perisperm envelope tissue which the radicle must penetrate for germination to occur. In an earlier report, germination rate and final germination percentages were higher for osmotically primed seeds both in laboratory tests and field emergence studies conducted immediately after priming. After 9 years in storage, nonprimed seeds germinated to higher percentages in water at 30°C and reduced water potential at all temperatures, while primed seeds germinated to higher percentages in water at 20°C and 25°C and exhibited a higher percentage of seedling emergence at a soil MC of 17%. Priming durations of 5 days or less had no effect on the viability, while longer durations decreased the viability of both stored primed and nonprimed seeds. Priming generally decreased the log mean time to germination of stored nonprimed seeds but increased values for stored primed seeds. Controlled deterioration increased the log mean time to germination and decreased the

viability of primed seeds faster than nonprimed seeds. Priming following controlled deterioration had no effect on nonprimed seeds and reduced the percent viability of primed seeds by 20%. Osmotic priming has a deleterious effect on the seed storage life of muskmelon seeds.

Introduction

Seed priming or osmotic conditioning is a controlled hydration treatment that allows pregerminative metabolic activity to proceed but prevents radicle emergence. Primed seeds can be dried and upon rehydration may exhibit faster rates of germination, greater tolerance to environmental stress, and reduced dormancy (Bradford, 1986; Khan, 1992). However, the effect of priming on the storage life of seeds remains unclear.

In some studies, priming treatments did not adversely affect the storage life of seeds. Primed carrot (*Daucus carota* L.) and leek (*Allium porrum* L.) seeds retained their enhanced vigor after storage for 450 days (Dearman et al., 1987b). The improved germination performance of primed spinach (*Spinacia oleracea* L.) seeds was retained after 30 days of storage at 5°C (Atherton and Farooque, 1983). A short hydration treatment applied to wheat (*Triticum aestivium* L.) seeds had little effect on germinability during storage, while longer hydration treatments increased the susceptibility to deterioration (Nath et al., 1991). Dearman et al. (1986) reported that primed and dried onion (*Allium cepa* L.) seeds stored at 10°C and 9% moisture content (MC) maintained a faster rate of germination after 18 months in storage.

Other studies have shown that primed seeds have decreased storage life. Tomato (*Lycopersicon esculentum* L.) (Alvarado and Bradford, 1988; Argerich et al., 1989; Owen and Pill, 1994) and wheat (Nath et al., 1991) seeds exhibited reduced storage life

following priming. Prehydration treatments in water for less than 1 hour had little effect on the rate of germination or storage life of lettuce seeds, but increasing the duration of either priming or prehydration reduced the mean time to germination and seed longevity by as much as 61 and 84%, respectively, compared to nonprimed seeds (Tarquis and Bradford, 1992).

Muskmelon seeds showed a differential response to osmotic priming among seedlots (Bradford et al., 1988; Welbaum and Bradford, 1991a). The effects of priming were greater on newly matured seeds than on older seeds (Welbaum and Bradford, 1991a). Since newly matured muskmelon seeds reach maximum vigor after a period of dry storage called "afterripening", priming may enhance vigor by substituting for afterripening (Welbaum and Bradford, 1991a). Short-term aging treatments mimic afterripening and increase the vigor of newly matured seeds similar to priming treatments (Welbaum and Bradford, 1991b). Therefore, priming may also age muskmelon seeds decreasing their storage life compared to nonprimed seeds. To test this hypothesis, we examined the performance of primed and nonprimed muskmelon seed lots stored for 9 years at less than 20°C and 6% MC. Germination performance after storage was compared with germination and field emergence results in an earlier study conducted immediately after priming (Bradford et al., 1988). In addition, the strength of the perisperm envelope tissue surrounding the embryonic axis was tested at six time intervals during imbibition using an Instron Universal Testing Machine to determine whether osmotic priming decreases the resistance of the perisperm envelope to radicle emergence.

Materials and Methods

Priming treatments and storage conditions - Muskmelon (*Cucumis melo* L. cv. PMR 45,

Petoseed Co.) seeds were primed in February 1984 for 6 days in the dark at 25°C in an aerated 0.3 M KNO_3 solution (Bradford et al., 1988). Seeds were dried to a MC of 6.6 and 6.0% for primed and nonprimed seeds, respectively, and stored in tightly sealed plastic bottles at 20°C for the first 4 years and then at 5°C for the final 5 years. Primed and nonprimed seeds were primed at 25°C in an aerated 0.3 M KNO_3 solution as described above at the end of the storage period. Seeds were redried in a desiccator to a MC of 6%. Seed MC was expressed as a percentage of the dry wt. after heating in an oven at 130°C for 1 hour (ISTA, 1985).

Laboratory Germination - Three replicates of 25 seeds each were placed in 9 x 9 x 1.5 cm transparent, covered, plastic boxes (Falcon 1012, Becton Dickinson and Co., Lincoln Park, N.J.) on two thicknesses of germination blotter paper (Anchor Paper Co., Hudson, Wis.). The blotters were saturated with 12 ml deionized water or solutions of PEG 8000 with Ψs ranging from 0 to –0.8 MPa in 0.2 MPa increments prepared according to Michel (1983). Plastic boxes were placed in self-sealing plastic bags (Ziploc, Dow Brands L. P.) to reduce evaporation. The Ψs of the blotters were verified by osmometry (Model 5500, Wescor Inc., Logan, Utah) 3 to 4 days after the start of incubation and were uniformly 0.2 to 0.4 MPa lower than the initial Ψs due to the concentrating effect of imbibition and evaporation from the boxes during seed scoring. For convenience, the initial Ψs are given. The boxes containing seeds were placed in dark incubators maintained at 20, 25, and 30 ± 1°C, and germinated seeds were removed from boxes at 12 hour intervals for 21 days.

Controlled deterioration - One hundred seeds were incubated in 7.5 x 1.0 cm plastic vials for 24 hour with the precise amount of water to raise the MC to 15%. Vials were uniformly submerged in a water bath at 45°C for up to 8 days. The seeds were removed

from each vial and slowly dried in a desiccator maintained at 45% RH for 7 days at 20°C prior to germination testing in blotters saturated with 11 ml of deionized water. Some samples of primed and nonprimed seeds were aged for 72 hours and immediately primed for 6 days, redried to a MC of 6%, and stored for 24 hour prior to germination testing. Germination testing of aged and reprimed seeds was conducted at 25°C using four replicates of 25 seeds each as described above.

Seedling Emergence - Metal flats (50 x 35 x 8 cm) were filled with Hayter Loam (fine-loamy, mixed, mesic, Ultic Hapludalf) soil, hydrated to field capacity (21% MC) and sealed in plastic bags to prevent moisture loss. Some flats were dried to 19 and 17% MC. Soil MC was determined by heating samples in an oven for 2 hour at 105°C. Muskmelon seeds were planted by hand 2.5 cm deep with 25 seeds of each treatment to a flat. There were three replications (flats) for each soil MC. Seedling emergence was scored at 24 hour intervals for 21 days. The experiment was conducted in a greenhouse with air temperatures ranging from 13 to 23°C with a mean of 19°C.

Instron Analysis - The mechanical resistance of the micropylar perisperm envelope tissue surrounding the radicle was measured on decoated seed pieces cut 5 mm above the tip of the embryonic axis. A seed tip (embryonic axis and perisperm envelope) was mounted in a wooden holder placed on a 20 N load cell of an Instron Universal Testing Machine (model, 1123, Instron Engineering Corporation, Canton, Mass.). The crosshead of the Instron was fitted with a tapered steel needle with a tip diameter of 0.24 mm that approximated the size and shape of the radicle. The needle was lowered through the sample at a rate of 5 mm min^{-1} and advanced without resistance through a hole in the center of the sample holder (Welbaum et al., 1995). The penetration force for the

perisperm envelope was determined from the peak of the load-deflection curve. At least 20 individual primed, nonprimed, and nonprimed boiled (control) seeds were measured at each time interval and expressed as means ± SE.

Statistical Analysis - Probit analysis was used to determine the log mean time and the variance of germination. When germination percentages were plotted on a probit scale versus log time (t), straight lines of approximately equal slope were produced for different treatments, indicating a normal distribution of germination events with log time (Finney, 1970). The log mean time to germination (log $\bar{t}$) was determined graphically from the intersection of the least squares regression line of log $\bar{t}$ versus probit germination percentage and 50% germination for treatments with greater than 20% germination. The slope of a probit plot is equal to the inverse standard deviation (σ) (Finney, 1970). Linear regression was used to compare slopes of the plot of probit viability versus aging time to analyze the rate of deterioration of primed and nonprimed seeds (Ellis and Roberts, 1981). The significance of main effects of Ψs, seed treatment, and temperature for arcsin transformed germination percentages, log $\bar{t}$, and σ were compared by ANOVA (CoStat, CoHort Software, Minneapolis, Minn.). Data for actual germination percentages are shown.

Results

Germination at different temperatures and Ψs - Germination percentages of nonprimed seeds were highest in water at 30°C and declined by 20% at both 25°C and 20°C (Table 4.1). The germination percentages of primed seeds were highest in water at 25°C and lowest at 30°C (Table 4.1). Primed seeds germinated at higher percentages in water compared to nonprimed seeds at 20°C and 25°C but not at 30°C. Log $\bar{t}$ was essentially

Table 4.1. Germination of primed and nonprimed seeds at five water potentials (Ψs) and three temperatures after 9 years of storage. Means were calculated from laboratory germination tests conducted for 21 days using three replications of 25 seeds each. The mean log time to germination (log $\bar{t}$) was calculated using probit analysis for treatments with greater than 20% germination. ANOVA was performed on arcsine transformed percentage data, and actual percentages are shown.

	Temperature														
	20°C					25°C					30°C				
	Ψs (MPa)					Ψs (MPa)					Ψs (MPa)				
Seed Treatment	0	−0.2	−0.4	−0.6	−0.8	0	−0.2	−0.4	−0.6	−0.8	0	−0.2	−0.4	−0.6	−0.8
	Germination (%)														
Primed	81	32	3	0	0	87	75	20	5	0	77	92	26	20	11
Nonprimed	73	29	20	0	0	72	60	45	20	9	91	84	40	38	20
	log $\bar{t}$ (h)														
Primed	2.33	2.99	–	–	–	2.37	2.68	–	–	–	2.35	2.25	3.29	–	–
Nonprimed	2.43	2.97	–	–	–	2.55	2.57	2.88	–	–	2.40	2.30	3.41	3.54	–

Mean separation: $LSD_{0.05}$ Germination % = 7; log $\bar{t}$ = 0.26

Treatments	Germination %[z]	log $\bar{t}$
Water Potential (Ψ)	***	***
Seed Treatment (P)	*	NS
Temperature (T)	***	NS
Ψ x P	**	NS
T x Ψ	***	**
T x P	NS	NS
T x Ψ x P	*	NS

[z]F-test results

NS,*,**,***nonsignificant or significant at P = 0.05, 0.01, and 0.001 levels, respectively.

the same for nonprimed and primed seeds in water at all temperatures (Table 4.1).

Germination percentages for nonprimed seeds declined with decreasing Ψs (Table 4.1). Seeds were more sensitive to reduced Ψs at low temperature than at high temperature. Reduced Ψs had a similar effect on the germination percentage of primed seeds, but at most Ψs and temperatures the germination percentage of primed seeds was lower than nonprimed seeds (Table 4.1). The Ψs needed to inhibit germination by 50% for primed and nonprimed seeds were –0.25 and –0.33 MPa, respectively, at 30°C and were not significantly different (data not shown). Log $\bar{t}$ also declined with Ψs, and there were no differences between the values for primed and nonprimed seeds (Table 4.1). After 4 days at 25°C, mean root lengths for nonprimed and primed seeds were 53 mm and 62 mm respectively, and not significantly different (data not shown).

Seedling Emergence - In a greenhouse, seedlings from stored primed or nonprimed seeds failed to emerge in soils saturated to field capacity (21% MC) (Table 4.2). As soil MC declined, emergence percentages increased. At 17% soil MC, seedlings from primed seeds emerged at a higher percentage than from nonprimed seeds. Seedling emergence was fastest at 17% soil MC and there was no difference in log $\bar{t}$ for primed and nonprimed seeds (Table 4.2).

Germination after controlled deterioration - The germination percentages of primed and nonprimed seeds were similar before aging treatments were applied (Fig. 4.1A). During controlled deterioration, primed seeds lost viability more rapidly than nonprimed seeds. The time required to reduce the germination percentage of primed seeds to 50% was 180 hours compared to 290 hours for nonprimed seeds.

Before controlled deterioration, primed seeds had a shorter log $\bar{t}$ than nonprimed

Table 4.2. Seedling emergence percentage and mean log time to emergence (log $\bar{t}$) of primed and nonprimed muskmelon seeds at three soil moisture contents in a greenhouse after 9 years of storage.

	Soil Moisture Content (%)					
Seed Treatment	17	19	21	17	19	21
	Seedling Emergence %[z]			**log $\bar{t}$ (*h*)**		
Primed	73	24	0	2.31	3.14	–
Nonprimed	56	15	0	2.36	3.30	–

$LSD_{0.05}$ Germination % = 14; log $\bar{t}$ = 0.4

[z]Seedling emergence percentages were arcsine transformed prior to analysis, but actual percentages are shown. Means were calculated from three replications of 25 seeds each.

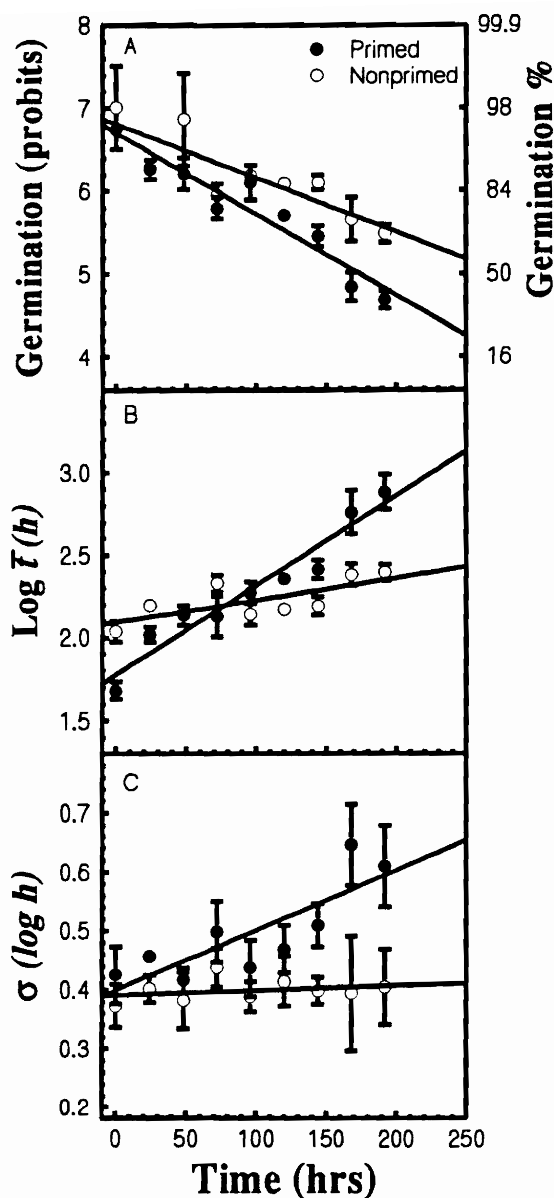

Fig. 4.1. Effects of controlled deterioration on primed (●) and nonprimed (O) seed germination percentage plotted on a probit scale (A); mean log time to germination, log $\bar{t}$, (B); and the standard deviation of germination, σ, (C). The regression equations and ± SE of the slopes are: A: Primed, y = 6.70 + –0.010x (± 0.001), $r^2 = 0.91$; Nonprimed, y = 6.81 + –0.007x (± 0.001), $r^2 = 0.74$; B: Primed, y = 1.78 + 0.005x (± 0.001), $r^2 = 0.94$); Nonprimed, y = 2.09 + 0.001x (± 4.8^{-4}), $r^2 = 0.53$; C: Primed, y = 0.40 + 0.001x (± 2.7^{-4}), $r^2 = 0.68$); Nonprimed, y = 0.39 + 8.06^{-5}x (± 1.1^{-4}), $r^2 = 0.76$. Each point is the mean of four replicates and the error bars indicate ± SE.

seeds (Fig. 4.1B). However, log $\bar{t}$ increased during controlled deterioration at a faster rate for primed seeds; and after 200 hours of aging, log $\bar{t}$ of primed seeds was significantly greater than for nonprimed seeds.

The σ of germination for both primed and nonprimed seeds were similar prior to controlled deterioration treatments (Fig. 4.1C). However, as the duration of the controlled deterioration treatment increased, the σ of primed seeds also increased, while the σ of nonprimed seeds remained constant.

Priming after storage - Stored primed and nonprimed seed lots were primed in an attempt to restore lost vigor and viability. Germination percentages of nonprimed seeds remained constant for priming durations of up to 8 days and then declined (Table 4.3). Repriming primed seeds reduced viability at all durations except 5 days (Table 4.3).

Log $\bar{t}$ for primed seeds remained the same or increased after repriming (Table 4.3). However, priming nonprimed seeds for 5 to 8 days decreased log $\bar{t}$ (Table 4.3). The σ was not consistently altered by priming stored primed and nonprimed seeds (Table. 4.3).

Priming after controlled deterioration - Some nonprimed and primed stored seeds were subjected to controlled deterioration tests for 3 days before priming. Accelerated aging decreased germination percentages, increased log $\bar{t}$, and had little effect on σ for both primed and nonprimed seeds, although accelerated aging reduced the performance of primed seeds more substantially than nonprimed seeds (Table 4.4). Priming had no effect on the germination percentage of aged nonprimed seeds (Table 4.4). However, repriming primed seeds following controlled deterioration decreased the germination percentage from 80 to 69%. Priming did not affect log $\bar{t}$ for either treatment. Priming after controlled deterioration had no effect on the σ of stored primed and nonprimed seeds

Table 4.3. The effect of priming (0.3 M KNO_3) for up to 12 days on germination percentage, mean log time to germination (log $\bar{t}$), and standard deviation (σ) of primed and nonprimed seeds after 9 years of storage. Germination was tested at 25°C for 21 days.

	Duration of Priming (days)									
Prestorage Treatment	0	4	5	6	7	8	9	10	11	12
					Germination %**					
Primed	100[z]	89	95	81	88	88	73	68	79	52
Nonprimed	96	100	96	84	99	96	83	89	79	72
					log $\bar{t}$ (h)***					
Primed	1.58	1.72	1.60	1.80	1.70	1.73	1.88	2.13	2.03	2.18
Nonprimed	1.90	1.76	1.58	1.63	1.62	1.55	1.79	1.75	1.89	1.90
					σ (log h)^{NS}					
Primed	0.79	0.47	0.33	0.42	0.37	0.43	0.33	0.36	0.46	0.56
Nonprimed	0.46	0.38	0.16	0.45	0.41	0.38	0.41	0.44	0.37	0.40

$LSD_{0.05}$ Germination % = 11; log $\bar{t}$ = 0.17

NS,**,***Nonsignificant or significant at p = 0.01 or 0.001 level, respectively, for the F-test of stored primed versus nonprimed seeds across all priming durations.

zANOVA was performed on arcsine transformed percentage data, but actual percentages are shown. Means were calculated from three replications of 25 seeds each.

Table 4.4. The effect of priming (6 days, 0.3 M KNO_3) after controlled deterioration (15% MC and 45°C for 72 hourrs) on germination percentage, mean log time to germination (log $\bar{t}$), and standard deviation (σ) of primed and nonprimed seeds after 9 years of storage. Germination was tested at 25°C for 21 days.

	Treatments		
Prestorage Treatment	Control	Aged	Aged + Primed
		Germination %* **	
Primed	96[z]	80	69
Nonprimed	94	86	89
		log $\bar{t}$ (h)* **	
Primed	1.68	2.12	2.17
Nonprimed	2.04	2.26	2.34
		σ(log h)^{NS}	
Primed	0.43	0.41	0.57
Nonprimed	0.37	0.39	0.40

$LSD_{0.05}$ Germination % = 5; log $\bar{t}$ = 0.12

^{NS},***Nonsignificant or significant at p = 0.001, for F-test of prestorage primed versus nonprimed seeds across all treatments.

[z]ANOVA was performed on arcsine transformed percentage data and actual percentages are shown. Means were calculated from four replications of 25 seeds each.

(Table 4.4).

Instron analysis - The penetration force was highest in dry seeds and declined sharply during the first few hours of hydration (Fig. 4.2). The force needed to penetrate nonprimed seed was consistently higher than primed seed throughout the imbibition time course. Primed seeds first germinated at 25 hours, and the force required to puncture primed seed piece was 0.97 N compared to 1.27 N for nonprimed seed which initially germinated at 28 hours (Fig. 4.2).

Discussion

Osmotic priming in salt solutions provides little enhancement of germination in some species (Khan, 1992). Salt solutions are generally less effective than other priming agents, because the high salt concentrations can damage embryos (Khan, 1992). In muskmelon seeds, osmotic priming in salt solutions significantly improves germination rate and germination percentages at low temperature and water potential (Nerson and Govers, 1986; Bradford et al., 1988; Welbaum and Bradford, 1991a). The endosperm and perisperm tissue in muskmelon seeds form a protective envelope around the embryo (Welbaum and Bradford, 1990). The small pore size of the perisperm cell wall acts as a molecular sieve allowing water to pass but not K^+ or other ions of similar size (Welbaum and Bradford, 1990). Muskmelon seeds are not damaged by priming in salt solutions, because ions are excluded from the embryo by the perisperm envelope. The osmotic potential inside developing muskmelon fruits approximates an osmotic priming solution (Welbaum and Bradford, 1991a). Osmotically primed, immature seeds germinate with the same vigor as fully mature seeds because developmental processes continue during priming (Welbaum and Bradford, 1991a). Therefore, the more rapid loss of vigor and

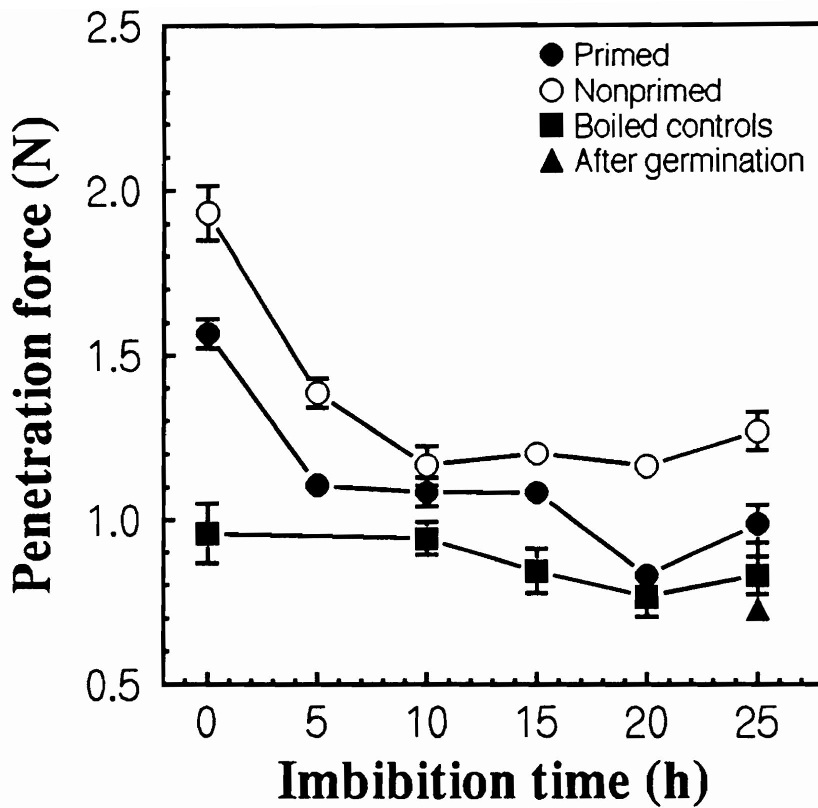

Fig. 4.2. The force required to penetrate primed (●), nonprimed (O), and boiled (■) muskmelon seed pieces during imbibition before and after (▲) germination measured using an Instron Universal Testing Machine. First radicle emergence began 25 hours after the start of imbibition for primed seeds and at 28 hours for nonprimed seeds. Error bars are ± SE when larger than the symbols.

viability by osmotically primed seeds during the 9 years of storage and controlled deterioration experiments was not likely due to embryo damage from salts in the priming solution.

In laboratory germination tests conducted at 18°C immediately after priming, germination rate and final germination percentages were higher for primed than for nonprimed seeds (Bradford et al., 1988). Seedling emergence from sterilized soil in flats under ambient outdoor temperatures (7 to 23°C) was also improved by priming (Bradford et al., 1988). Primed seeds emerged more rapidly or showed increased final emergence percentages in five of seven field trials at two locations (Bradford et al., 1988).

After storage for 9 years at < 6.6% MC and < 20°C, primed seeds exhibited higher final seedling emergence percentages in soil at 17% MC but not at other soil moisture contents (Table 4.2). Log $\bar{t}$ for seedling emergence in soil did not differ between treatments. At reduced Ψ or at higher temperature, there was either no difference between treatments or nonprimed seeds outperformed primed seeds (Table 4.1). During controlled deterioration, primed seeds lost viability faster and σ and log $\bar{t}$ increased more rapidly than for nonprimed seeds (Fig. 4.1A, B, C). When results of germination tests conducted after 9 years of storage were compared to those in the original study where osmotically primed seeds were clearly superior by most criteria, it was apparent that primed muskmelon seeds lost viabilty and vigor more rapidly than nonprimed seeds during long-term storage (Table 4.1, 4.2, 4.3, 4.4). Additionally, when compared to nonprimed seeds, primed muskmelon seeds also lost viability more rapidly, germinated more slowly, and lost uniformity more quickly following controlled deterioration experiments (Fig. 4.1). Our results along with studies of tomato (Alvarado and Bradford, 1988; Argerich et al., 1989; Owen and Pill, 1994), wheat (Nath et al., 1991), and lettuce (Tarquis and Bradford, 1992) indicate that priming before storage is deleterious to seed longevity in some species.

Differences between the seed testing procedures may explain some of the conflicting results among previous studies on the effect of priming on seed storage life. When the performance of primed and nonprimed stored seeds was compared at several temperatures and Ψs, using germination rate, or using accelerated aging tests, it has been concluded that primed seeds deteriorated more rapidly (Alvarado and Bradford, 1988; Argerich et al., 1989; Dearman et al., 1987a; Tarquis and Bradford, 1992). However, when comparisons were made after short-term storage or based on germination percentages from tests conducted under optimum conditions, priming did not appear to adversely affect seed storage life (Atherton and Farooque, 1983; Burgass and Powell, 1984; Dearman et al., 1987b). In this study, standard laboratory germination tests, soil emergence, and controlled deterioration tests indicate that osmotically primed muskmelon seeds have reduced storage life compared to nonprimed seeds.

Previous studies with tomato seeds showed that the mean time to germination increases logarithmically as viability declines during controlled deterioration (Argerich et al., 1989). Muskmelon seeds show a similar relationship (Fig. 4.1A, B). Thus, changes in germination rate, seed viability, and seed deterioration during storage are closely correlated. The delay in germination that occurs as seeds deteriorate is presumably related to the damage accumulated during storage. One of the reported benefits of priming is to repair damage incurred during storage, which would also improve the vigor of aged seeds (Dearman et al., 1986; Rao et al., 1987; Ward and Powell, 1983). However, in this study repriming stored primed seeds resulted in either no effect or further losses in viability, which is inconsistent with the repair hypothesis (Table 4.3). Similarly, repriming stored primed seeds following controlled deterioration decreased viability (Table 4.4). The maximum enhancement of germination was obtained from a single priming treatment, and additional priming treatments either had no effect or decreased viability and vigor (Table

4.3, 4.4).

Some muskmelon seedlots germinate better under mild water stress than in water (Dunlap, 1988; Welbaum et al., 1990). At 30°C, primed muskmelon seeds germinated to a higher percentage at –0.2 MPa Ψ than in water, while nonprimed seeds did not, suggesting that the combined effects of priming and storage increased the sensitivity of muskmelon seeds to germination in water at high temperatures (Table 4.1). Muskmelon seeds are not as sensitive to high Ψ at lower temperatures, explaining why the phenomena was only observed at 30°C (Dunlap, 1988). The physiological basis of sensitivity to high water potential remains poorly understood, but priming apparently contributes to the development of the phenomenon during long-term storage.

Muskmelon seeds germinate when the turgor in the embryonic axis exceeds the yield threshold for radicle growth (Welbaum et al., 1995). The threshold for germination is a combination of the yield threshold of the perisperm envelope, which surrounds the embryo, plus the yield threshold of the radicle tissue itself (Welbaum et al., 1995). Since priming weakened the perisperm envelope tissue (Fig. 4.2), primed seeds germinated faster, because they reached the yield threshold earlier during imbibition than nonprimed seeds. Therefore, priming increases the rate of muskmelon seed germination by weakening the perisperm envelope. After the initial priming treatment, additional priming caused no further enhancement because further weakening of the perisperm was not possible (Table 4.3).

Seeds germinate best immediately after osmotic priming. If long-term storage is necessary before planting, seeds should be primed as close to the planting date as possible. The results of both long-term storage experiments and controlled deterioration tests show that osmotically primed muskmelon seeds exhibit decreased storage life compared to nonprimed seeds.

Chapter 5.

Changes in Perisperm Strength and Enzymatic Activity during Development, Afterripening, Germination, and Priming

Abstract - Muskmelon embryos are enclosed in a single layer of endosperm and a two- to four-cell layered perisperm envelope (endosperm + perisperm) that forms a barrier to radicle elongation. The force and energy required to penetrate the perisperm envelope tissue were measured after 0, 5, 10, 15, 20, 25, and 30 hours of imbibition at 25°C using an Instron Universal Testing Machine at a crosshead speed of 5 mm min^{-1}. Seeds harvested at 40 days after anthesis (DAA) were primed (controlled hydration in a 3% (w/v) KNO_3 solution for 6 days followed by redrying) then tested for mannanase activity. Water potentials (Ψ) of excised cotyledons and embryonic axes were also measured. Results were compared with nonprimed seeds. The germination percentage and the force and energy required to puncture the perisperm envelope increased steadily between 30 to 55 DAA then decreased from 55 to 65 DAA. Afterripening, which increases germination vigor, also increased the penetration force and energy. The perisperm tissue of 50- to 65-DAA seeds was stiffer than 45-DAA seeds. Endo-β-mannanase-like activity increased during the first 10 hours of imbibition, then declined steadily to 40 hours. There was greater mannanase activity in nonprimed seeds as compared to primed seeds during imbibition. During imbibition, the Ψ values for cotyledons and embryonic axes isolated from primed seeds were markedly lower than those for nonprimed seeds.

Introduction

In many species, the endosperm tissue completely envelops the embryo and restrains the germination process by presenting a physical barrier which restricts radicle emergence (Weges, 1987; Bradford, 1990). In seeds of lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.), *Datura* spp., pepper (*Capsicum annuum* L.), and muskmelon, the weakening of the endosperm or perisperm tissues adjacent to the radicle tip is required for germination to occur (Bradford, 1990; Groot and Karssen, 1987; Ni and Bradford, 1993; Sanchez et al., 1990; Welbaum and Bradford, 1990b). The physiological processes leading to endosperm or perisperm weakening are not completely understood. The turgor pressure of the embryonic axes may increase through accumulation of osmotic solutes allowing the radicle to exceed the yield threshold of the tissues surrounding the radicle (Bradford, 1990, 1986; Thanos, 1984; Carpita et al., 1979; Takeba, 1980; Nabors and Lang, 1971). However, in lettuce and watermelon (*Citrullus lanatus*), direct measurements of solute potential failed to completely account for the increases in growth potential observed, indicating that decreased yield threshold or increased cell wall extensibility may be partially responsible for radicle growth during germination (Bradford and Somasco, 1994; Carpita et al., 1979; Thanos, 1984).

The reduction in yield threshold in some germinating seeds involves enzymatic degradation of endosperm or perisperm tissues that form a physical barrier to radicle growth (Ni and Bradford, 1993). Endo-β-mannanase has been identified as a key enzyme responsible for the hydrolysis of endosperm tissue during the germination of tomato seeds (Groot et al., 1988) and pre- and post-germinative galactomannan hydrolysis in the endosperm cell walls of lettuce (Dutta et al., 1994; Dulson et al., 1988) and *Datura* spp. (Sanchez et al., 1990). The hydrolysis of mannans by endo-β-mannanase in lettuce

endosperm cell walls has been detected only after germination had occurred, suggesting that this enzyme is not involved in pre-germinative endosperm wall weakening (Halmer et al., 1976). This supports the hypothesis that increased turgor may be necessary to rupture the endosperm in lettuce seeds (Tao and Khan, 1979). However, later studies show no increase in the turgor of lettuce embryos prior to radicle emergence (Weges, 1987; Somasco and Bradford, 1993; Dutta et al., 1994).

Endosperm weakening during tomato seed germination has been extensively studied (Groot et al., 1988; Haigh and Barlow, 1987; Liptay and Schopfer, 1983). Liptay and Schopfer (1983) concluded that differences in the extensive force exerted by the radicle were responsible for variations in tomato seed vigor. However, Haigh and Barlow (1987) observed no increase in hydrostatic pressure in the embryonic axis in tomato seeds prior to radicle emergence, and they concluded that the endosperm inhibits expansive growth but softens prior to radicle emergence. Instron analysis showed a decline in the force required to penetrate tomato endosperm tissue from 0.6 N at 4 hours of imbibition to 0.2 N at 32 hours when radicle emergence occurred (Groot and Karssen, 1987). In pepper seeds, the mechanical resistance of the endosperm decreased from approximately 0.8 N at the start of imbibition to 0.3 N at radicle emergence (Watkins and Cantliffe, 1983). In lettuce, Instron measurements of mechanical strength of the endosperm tissue declined during imbibition, and were not well correlated with radicle emergence (Tao and Khan, 1979).

Seed priming or osmotic conditioning is a controlled hydration process that permits pre-germinative metabolic activity to proceed but prevents radicle emergence, such that priming results in more uniform and faster rates of germination (Bradford, 1986; Khan, 1992). It has been proposed that softening of the endosperm occurs during priming (Karssen et al., 1989). Endo-β-mannanase and galactomannan hydrolyzing activity

developed more in the endosperm cell walls and whole seeds of primed tomato seeds than nonprimed seeds during germination (Karssen et al., 1989; Nonogaki et al., 1992).

Muskmelon embryos are enclosed in a single layer of endosperm and a two- to four- cell layered perisperm envelope (endosperm + perisperm) that forms a barrier to radicle elongation (Welbaum and Bradford, 1990a). An anatomical investigation of the perisperm envelope tissue adjacent to the radicle tip revealed an area of thin-walled cells that must be penetrated for germination to occur (Welbaum and Bradford, 1990b). It has been proposed that weakening of the perisperm tissue surrounding the embryo initiates radicle growth (Welbaum and Bradford, 1990b). Turgor in the embryonic axis was observed at a constant 0.7 MPa for several hours prior to radicle emergence, suggesting that increased turgor is not the mechanism responsible for rupture of the perisperm envelope tissue prior to germination (Welbaum and Bradford, 1990b). A previous study showed that the force and energy required to penetrate muskmelon seed pieces declined during imbibition, demonstrating that the strength of muskmelon perisperm envelope declines during germination (Welbaum et al., 1995, Chapter 3).

Muskmelon seeds mature inside fruits several weeks after maximum seed dry weight has been attained and before fruit maturity. When fruits are prematurely harvested, seeds require an afterripening period or priming to develop maximum viability and vigor (Welbaum and Bradford, 1991a, b). Less force was required to puncture primed seed tissues compared to nonprimed during imbibition, indicating that osmotic priming weakens the perisperm envelope tissue prior to radicle emergence (Chapter 3). Previous studies with muskmelon cultivar TopMark showed 50- to 60-days after anthesis (DAA) seeds to be more viable and vigorous than earlier maturity stages (Welbaum and Bradford, 1989; Chapter 4). However, the changing strength of muskmelon perisperm tissue during seed development was never quantified. The strength of the perisperm envelope tissue

surrounding the radicle was tested at eight different stages of development during imbibition and after 5 months of storage using an Instron Universal Testing Machine to determine whether seed development and afterripening increases or decreases the resistance of the perisperm envelope tissue to radicle growth. Galactomannan hydrolyzing activity from leachate of the perisperm tissue adjacent to the radicle tip of primed and nonprimed seeds during germination were also examined to determine whether galactomannan hydrolyzing enzyme activity, which is believed to be responsible for the perisperm weakening process, is increased by priming.

Materials and Methods

Plant material - Muskmelon cultivar Topmark (Asgrow Seed Co. Inc., Vineland, New Jersey) was field grown in a Hayter loam (fine loamy, mixed, mesic, Ultic Hapludalf) soil in Blacksburg, Virginia as previously described (Welbaum and Bradford, 1988). Hermaphroditic flowers were tagged at anthesis, and crown-set fruits containing from 400 to 500 seeds were harvested at 5-day intervals from 30 to 65 DAA. At harvest, seeds were removed from the fruit and washed vigorously in flowing tap water for 3 hours. Seeds from five to ten fruits from the same stage of development were harvested simultaneously and combined during drying to create a seed lot. Seeds were forced-air-dried for 3 hours at room temperature, then finally dried in a desiccator to a MC of $< 6.0\%$ (dry weight basis) determined by heating samples of 50 seeds for 1 hour at 130°C (ISTA, 1985). Dried seeds were stored for 5-months at room temperature in tightly sealed screw-top plastic bottles before testing for germination. In a second experiment, dried 40-DAA seeds were primed (6 days in the dark at 25 °C in aerated 0.3 M KNO_3 solution at a ratio of 5 ml of solution g^{-1} of seed) 21 days after harvest, then rinsed and

redried in the laboratory bench. Dried primed and nonprimed seeds were stored at a MC of 6.6% and 5.5%, respectively, for 12 months at room temperature prior to testing for enzymatic activity and water potential determination of excised cotyledons and embryonic axes during germination.

Germination experiments - Four replicates of 25 seeds each were placed in 9 x 9 x 1.5 cm transparent, covered plastic boxes (Falcon 1012, Becton Dickinson and Co., Lincoln Park, New Jersey) on two thicknesses of germination blotter paper (Anchor Paper Co., St. Paul, Minnesota) saturated with 11 ml deionized water. Boxes were incubated in the dark at 25 ± 1°C inside self sealing plastic bags (Ziploc, Dow Brands L. P., Midland, Michigan) to reduce evaporation. Seeds were scored for radicle emergence at 12-hour intervals for 21 days, and germinated seeds were removed.

Instron analysis - Three replicates of 15 seeds each were incubated at 25 ± 1°C for 10, 15, 20, 25, and 30 hours in 9 x 9 x 1.5 cm transparent covered plastic boxes on two thicknesses of germination blotter paper moistened with 11 ml of distilled water. At the end of each incubation period, seeds were immediately removed from the solution, decoated, and cut approximately 5 mm above the radicle tip (Fig. 5.1A). The tip was mounted in a wooden test fixture, and a puncture test was conducted on individual seeds using an Instron Universal Testing Machine (model 1123, Instron Engineering Corporation, Canton, MA) (Fig. 5.1B). A tapered steel needle, with a tip diameter of 0.24 mm that approximated the size and shape of the radicle was attached to the crosshead of the Instron (Fig. 5.1C). The needle and seed piece were carefully aligned and the needle lowered through the seed sample at a loading rate of 5 mm min^{-1} (Fig. 5.1). The needle

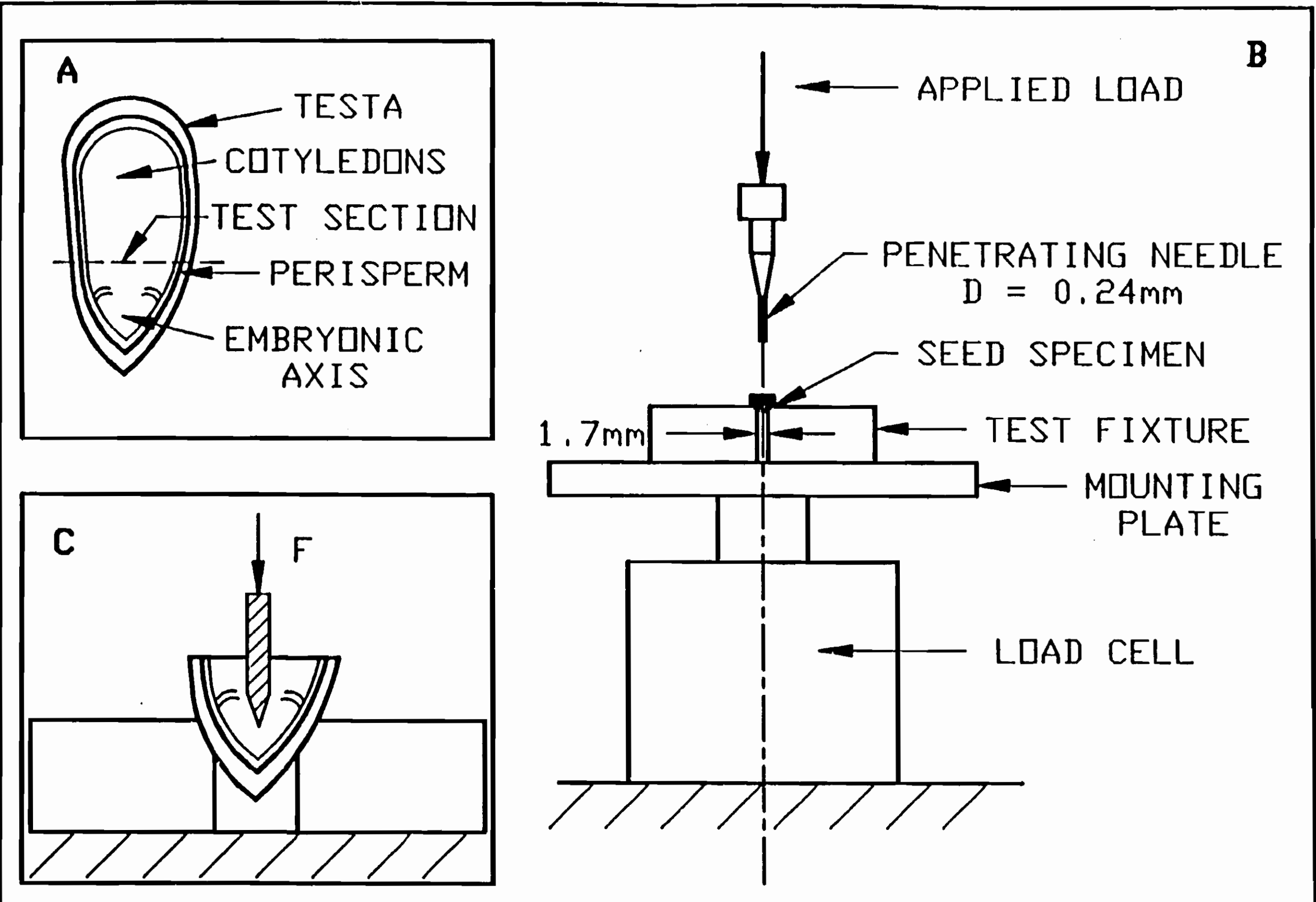

Fig. 5.1. Cross-sectional diagram of a muskmelon seed (A). Test apparatus with specimen in place (B). Close-up of a puncture test showing the needle penetrating a seed piece immobilized in a wooden sample holder (C).

advanced without resistance through a hole in the center of the sample holder. The force required to penetrate the perisperm envelope tissue was determined from the peak of the load-deflection curve, and the fracture energy was calculated from the area under the curve up to the point of fracture (Fig. 5.2; Welbaum et al., 1995). The stiffness was calculated from the slope of the force-deflection curve.

Water potential measurements - Four replicates of 25 seeds each were incubated as described for Instron analysis. At the end of each incubation period, seeds were immediately removed from the solution, decoated, and the embryonic axes and the cotyledons excised in a humidified box. The water potential (Ψ) of excised embryonic axes and cotyledons were measured using a thermocouple psychrometer (model 85, J. R. D. Merrill, Logan, Utah) as previously described (Welbaum and Bradford, 1988). The psychrometer was calibrated with NaCl standards of known Ψs that were verified by vapor pressure osmometry (model 5500, Wescor Inc., Logan, Utah). Pooled samples from 25 seeds each were equilibrated in psychrometer cups for 2 to 3 hours in a water bath maintained at 26 ± 1°C and two to three readings were taken on each sample to ensure that equilibrium had been attained. Water potential measurements were replicates of four to eight samples for each imbibition time.

Gel diffusion assay - In preparation for analyzing mannanase activity, four replicates of eight seeds each were incubated at 25 ± 1°C for 0.5, 5, 10, 15, 20, 25, 30, 35, or 40 hours in 9 x 9 x 1.5 cm transparent covered plastic boxes on two thicknesses of germination blotter paper moistened with 11 ml of distilled water. At the end of each incubation period, seeds were immediately removed from the solution, dissected, and the perisperm envelope separated from the embryo by cutting at the radicle tip. The perisperm envelope

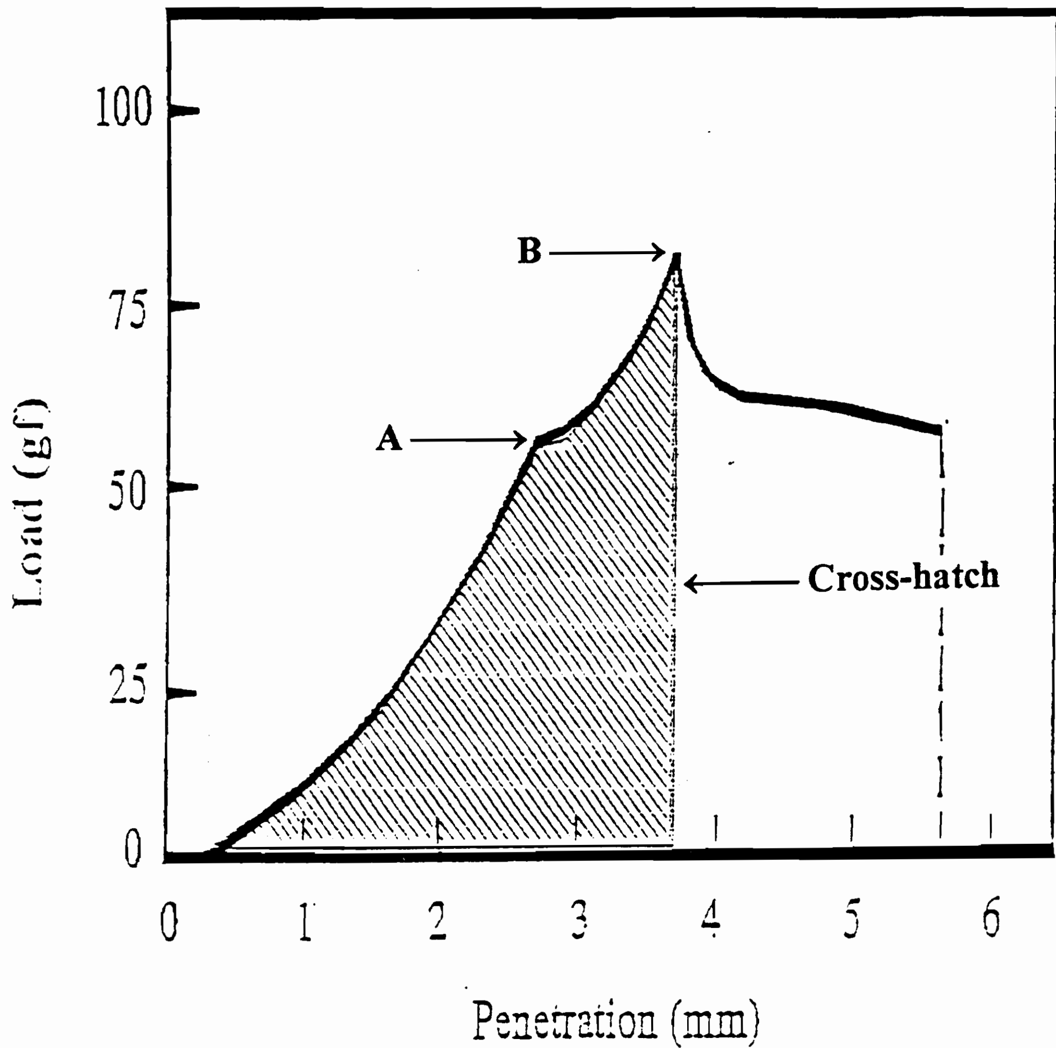

Fig. 5.2. Changes in pressure of the muskmelon seed tissues at 22 hours during penetration; showing the penetration of the embryonic axis (A), and the peak (maximum load) of the load-deflection curve when the needle ruptures the perisperm tissue (B). The cross-hatch shows the total energy from an Instron test of a seed piece.

could not be separated from dried embryos. Pooled samples of perisperm cone tips from eight seeds were carefully weighed, and 1 mg of tissue was placed in microtitre plate in 40 µl 0.1 M citric acid monohydrate/0.2 M sodium phosphate buffer (1:4.7 ratio, pH 7). The plate was incubated at 25°C in a dark incubator for 1.5 to 2 hours inside a self-sealing plastic bag to reduce evaporation. Leachate released into buffer from perisperm cone tip tissue was assayed for galactomannan hydrolyzing activity by a gel diffusion assay.

Locust bean galactomannan substrate (0.1% w/v) (Sigma Chemical Co., St. Louis, Missouri) from seeds of *Ceratonia siliqua* was dissolved in 0.1 M citric acid monohydrate/0.2 M sodium phosphate buffer (1:1.1 ratio, pH 5) by heating to 80°C while stirring for 20 min. The mixture was centrifuged for 15 min. at 11,000 g and the supernatant collected. Agarose (0.8%) was dissolved in the mixture by heating in a microwave oven, and 5 ml pipetted into 15 x 100 mm plastic petri plates and left to set for 1 hour. Wells were made in the gel with a 3 mm diameter cork-borer. Four µl of enzyme leachate was pipetted in each well and plates were covered, placed in self-sealing plastic bags, and incubated at 25°C for 20 hours in the dark.

After incubation, the gels were stained with 10 ml of 0.5% Congo red dye (in water solution) for 20 min at 80 rpm in a shaker. The gels were rinsed in 0.1 M citrate/0.2 M phosphate buffer (1:4.7 ratio, pH 7) and shaken gently until the hydrolyzed zones around each well turned red and the background completely turned black (Fig. 5.3). The diameters of the hydrolyzed zones were measured in two directions with electronic calipers (Digimatic, Mitutoyo Corporation, Japan) and averaged. The diameter of the well was substracted from the value. Enzyme activity in pkatals was calculated from the standard curve for mannanase.

Commercially prepared endo-β-D-mannanase enzyme from *Aspergillus niger* was purchased from Megazyme (North Rocks, Sydney, Australia), stored at 4°C, and used as a

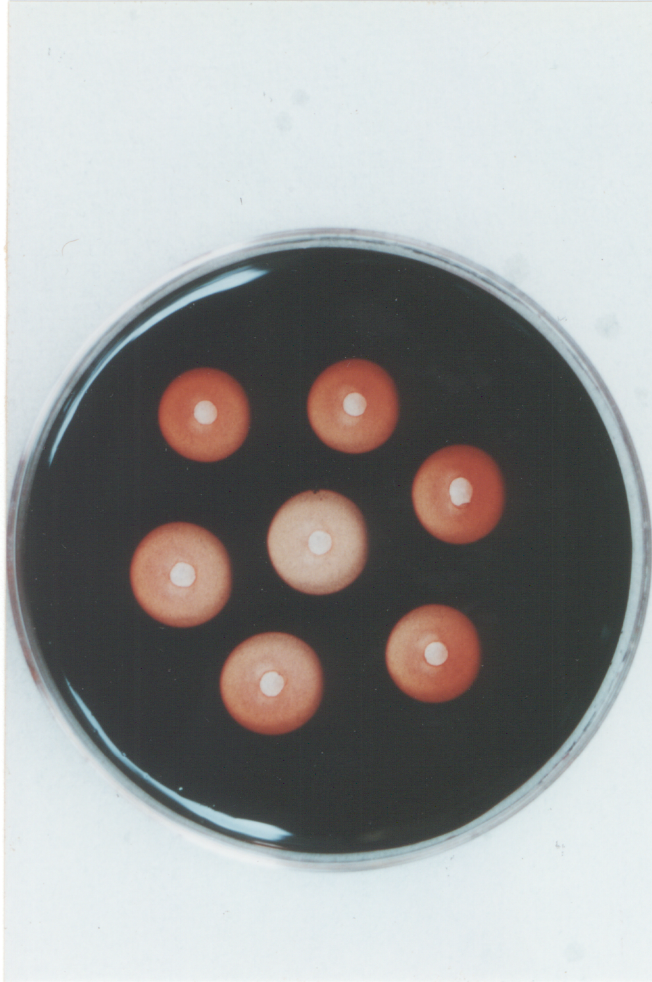

Fig. 5.3. Gel diffusion assay showing endo-β-mannanase-like activity on plates with locust bean galactomannan substrate in agarose gel using Congo red dye. The wells contain 4 μl of crude leachate from perisperm tissue adjacent to the radicle tip.

control for each gel diffusion assay. The enzyme was repeatedly diluted from 0.099 to 0.00495 pkatal to develop a standard curve to quantify enzyme activity.

Statistical analysis - Probit analysis was used to determine the log mean time to germination. When germination percentages were plotted on a probit scale versus log time (t), straight lines of approximately equal slope were produced for different treatments, indicating a normal distribution of germination events with log time (Finney, 1971). The log mean time to germination (log $\bar{t}$) was determined graphically from the intersection of the least squares regression line of log time versus probit germination at 50% germination for treatments with greater than 20% germination. The significance of the main effects of imbibition time and seed treatments for arcsin transformed germination percentages and log $\bar{t}$ were compared by ANOVA (CoStat, CoHort Software, Minneapolis, Minn.). Data for actual germination percentages are shown.

Results

Germination after storage - Germination percentages in water significantly increased with seed maturity until seeds reached maximum germinability at 55 DAA (Fig. 5.4A). The mean log time to germination (log $\bar{t}$) of newly harvested seeds decreased with maturation to a minimum at 60 DAA and then increased slightly at 65 DAA (Fig. 5.4B).

Dry storage (afterripening) for 5 months increased germination percentages from 30 to 50 DAA (Fig. 5.4A), while log $\bar{t}$ decreased with maturity from 30 DAA to minimum at 40 to 60 DAA and then increased slightly to 65 DAA (Fig. 5.4B). Germination of afterripened seeds was markedly faster than newly harvested from 30 to 50 DAA and at

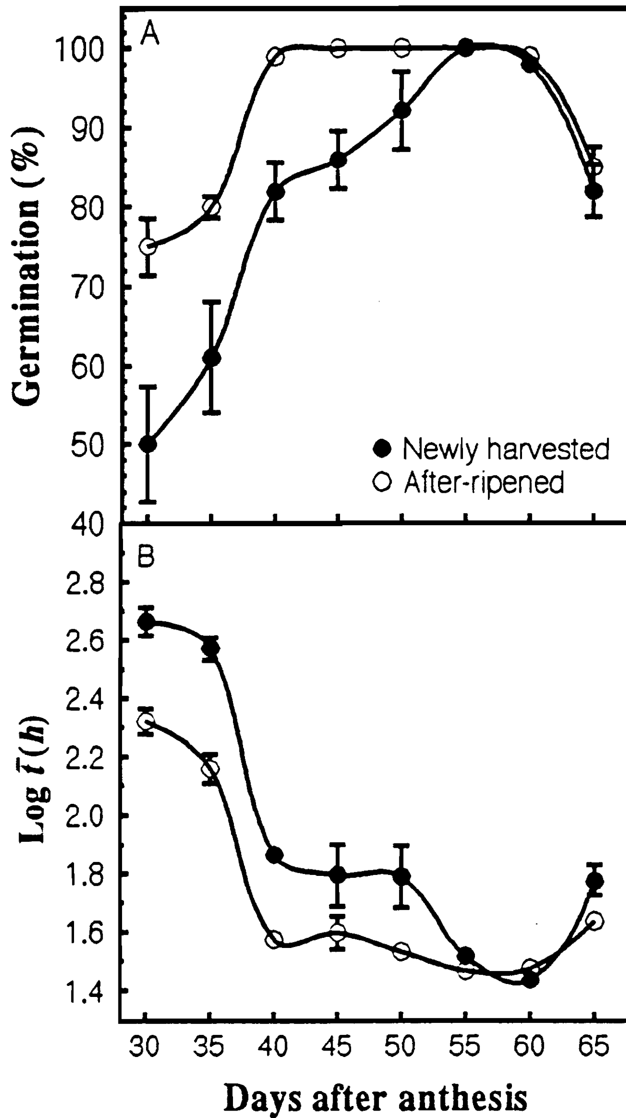

Fig. 5.4. Germination percentages (A) and mean log time to germination (log $\bar{t}$) (B) versus stage of seed development for newly harvested and afterripened seeds germinated at 25°C for 21 days. Each point is the mean of four samples of 25 seeds each, and error bars indicate ± S.E. when larger than the symbols.

65 DAA (Fig. 5.4B). Generally, germination was faster in older seeds than in younger seeds (Fig. 5.4B).

Changes in perisperm strength during development and after storage - The force required to penetrate cone tips of newly harvested seeds at 15 hours of imbibition was lowest at 30 DAA, then increased rapidly with seed maturity to a maximum at 50 and 55 DAA, before slowly declining to 65 DAA (Fig. 5.5A). Afterripened seeds showed a similar trend, but values were higher than newly harvested at all stages of development (Fig. 5.5A). The energy required to penetrate the perisperm tissue followed the same trend as the penetration force for both treatments and all stages of seed development (Fig. 5.5B).

Changes in perisperm strength during imbibition - The penetration force at all stages of seed development dropped rapidly during the initial 10 hours of hydration then decreased gradually to a minimum at 30 hours (Fig. 5.6A). Initial germination occurred at 25 hours after the start of imbibition. At 30 hours of hydration, 75% of 50 to 65 DAA seeds had germinated compared to 40% for 40- to 45-DAA seeds (data not shown). The force required to penetrate the perisperm tissue of 40- to 45-DAA seeds was consistently lower than 50- to 65-DAA seeds throughout imbibition (Fig. 5.6A). Energy required to penetrate the tissues of younger and older mature seeds exhibited the same trend as the penetration force (Fig. 5.6B). The stiffness of the perisperm envelope tissue was highest in dried samples then gradually declined to a minimum after 30 hours of imbibition (Fig. 5.7). Fifty- to 65-DAA seeds had a stiffer perisperm tissue than 45 DAA seeds at all times during imbibition (Fig. 5.7).

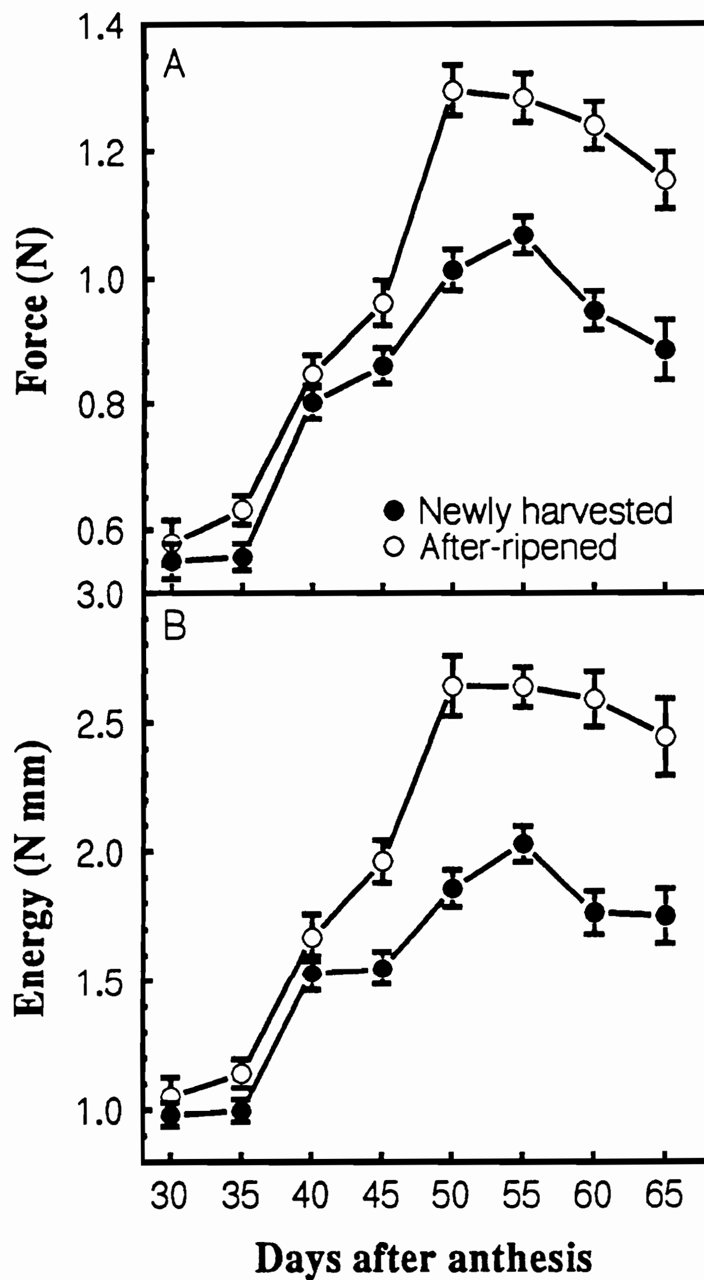

Fig. 5.5. The force (A) and energy (B) required to penetrate muskmelon seed pieces at 15 hours of imbibition during seed development at harvest (●) and after five months of storage (O). Each point is the mean of three samples of eight seeds each, and error bars are ± S.E. when larger than the symbols.

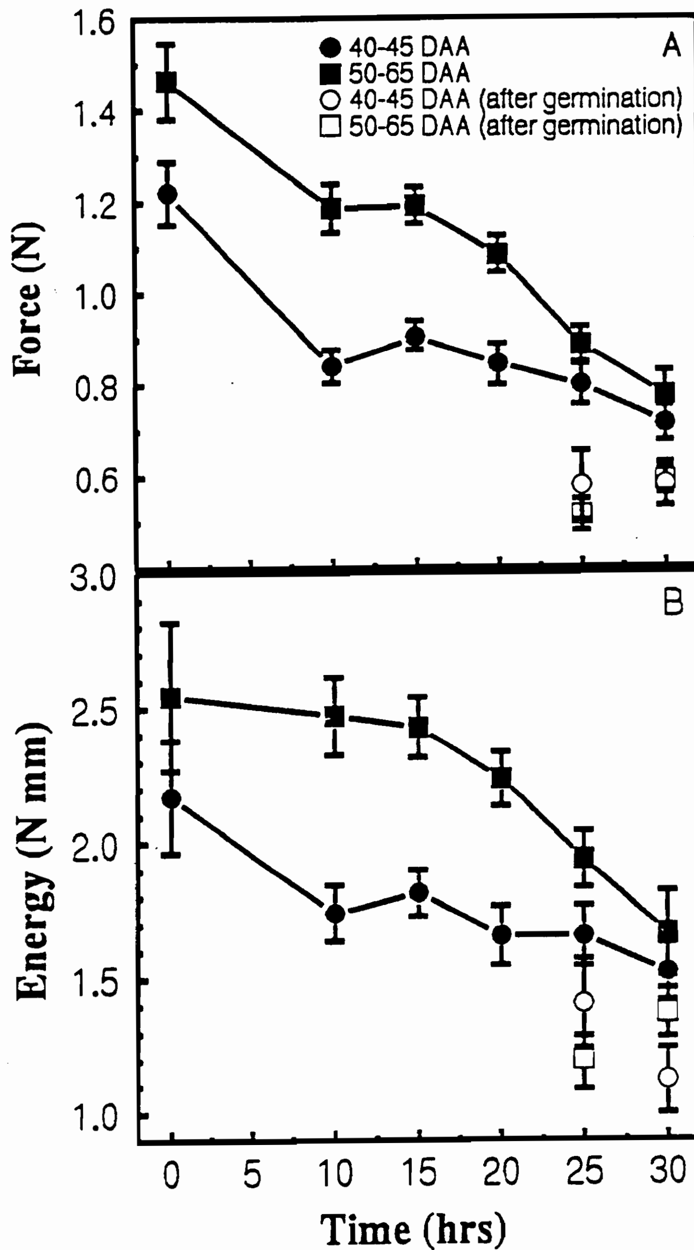

Fig. 5.6. The force (A) and energy (B) required to penetrate muskmelon seed pieces during imbibition and development before (■, •) and after (O, □) germination. Radicle emergence began 25 hours after the start of imbibition. Each point is the mean of three samples of eight seeds each, and error bars are ± S.E. when larger than the symbols.

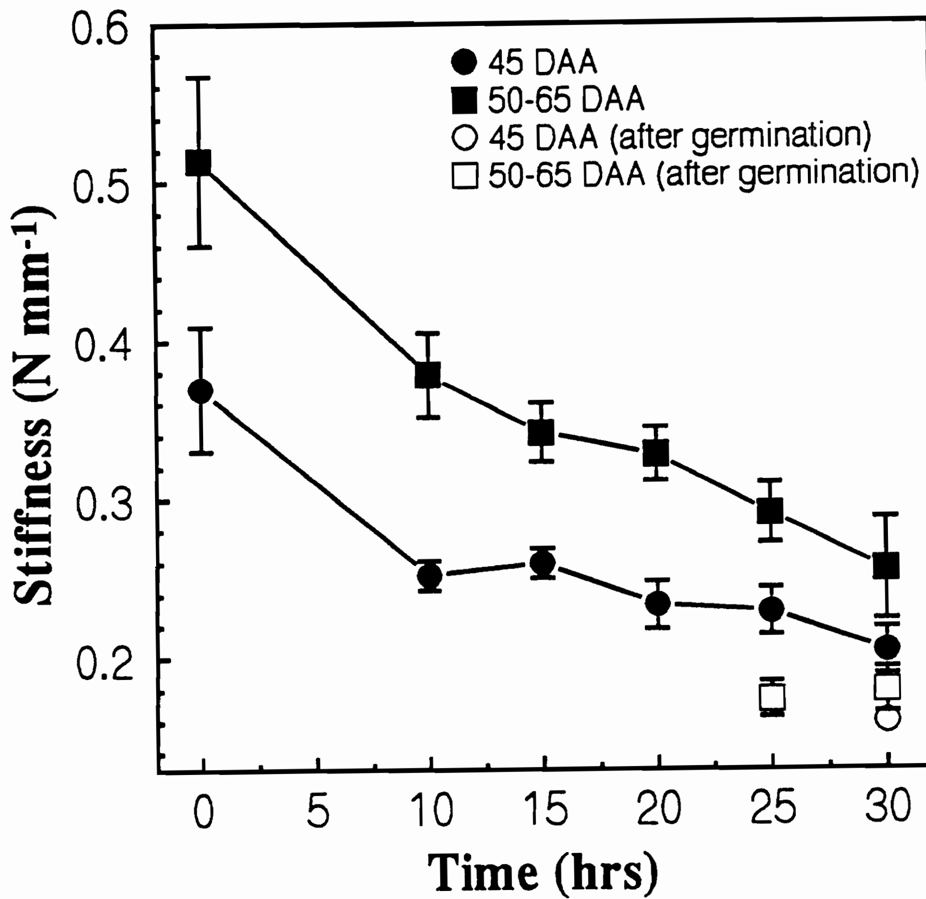

Fig. 5.7. The mechanical stiffness of muskmelon seed pieces during imbibition and development as determined by Instron analysis before (■,●) and after germination (O, □). The stiffness was calculated from the slope of the force-deflection curve. Each point is the mean of three samples of eight seeds each, and error bars indicate ± S.E. when larger than the symbols.

Changes in water potential during imbibition - The Ψ of cotyledons and embryonic axes isolated from imbibed intact seeds were measured during imbibition for both primed and nonprimed seeds. The Ψ of cotyledons increased slightly from 10 to 30 hours of imbibition, while the Ψ of embryonic axes decreased slightly (Fig. 5.8). Cotyledons and embryonic axes isolated from nonprimed seeds exhibited markedly higher Ψs than primed seeds during imbibition (Fig. 5.8). Water potentials of cotyledons isolated from nonprimed seeds increased from –0.65 MPa at 10 hours to –0.45 MPa at 30 hours of hydration, while Ψ for primed seeds increased over the same period (Fig. 5.8). The Ψ values measured for embryonic axes isolated from nonprimed seeds were not different than for primed seeds at most points during imbibition (Fig. 5.8).

Enzymatic activity - Using Congo Red gel-diffusion assay, endo-β-mannanase-like activity for nonprimed seeds was significantly higher than primed seeds at 0.5, 5, and 35 hours of imbibition (Fig. 5.9). Generally, nonprimed seeds consistently exhibited a higher enzymatic activity than primed during imbibition (Fig. 5.9). Germinating primed seeds exhibited a dramatic increase in mannanase-like activity from 0.5 to 10 hours of imbibition, decreased to 15 hours, then slowly declined to the least activity after 35 and 40 hours of hydration (Fig. 5.9). Maximum enzymatic activity was obtained at 5 and 10 hours of imbibition for nonprimed and primed seeds, respectively (Fig. 5.9).

DISCUSSION

Germination percentages increased steadily with seed maturity from 30 DAA to near maximum at 45 to 60 DAA, while log $\bar{t}$ decreased with maturation to minimum values at 55 and 60 DAA (Welbaum and Bradford, 1989; Figs. 5.4A, B). Freshly harvested 30- and

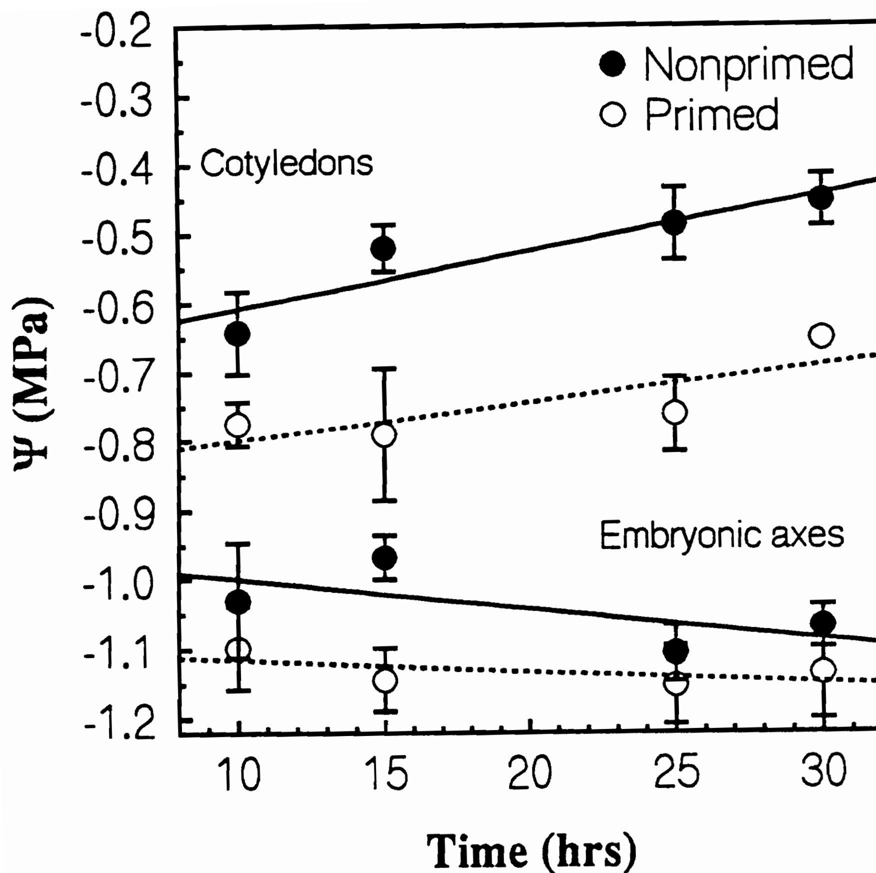

Fig. 5.8. Water potentials (Ψ) measured for cotyledons and embryonic axes for primed (O) and nonprimed (●) seeds during imbibition. Measurements of Ψ were made by thermocouple psychrometry on tissues isolated from intact seeds imbibed on water. Slopes of the regression equations for the nonprimed seeds are: Embryonic axes; $y = -0.96 + -0.004x$, $r^2 = 0.65$; Cotyledons; $y = -0.69 + 0.008x$, $r^2 = 0.83$; and primed seeds are: Embryonic axes; $y = -1.1 + -0.002x$, $r^2 = 0.69$; Cotyledons; $y = -0.85 + 0.005x$, $r^2 = 0.63$. Error bars are means ± S.E. of 4 to 8 samples of 25 seeds each.

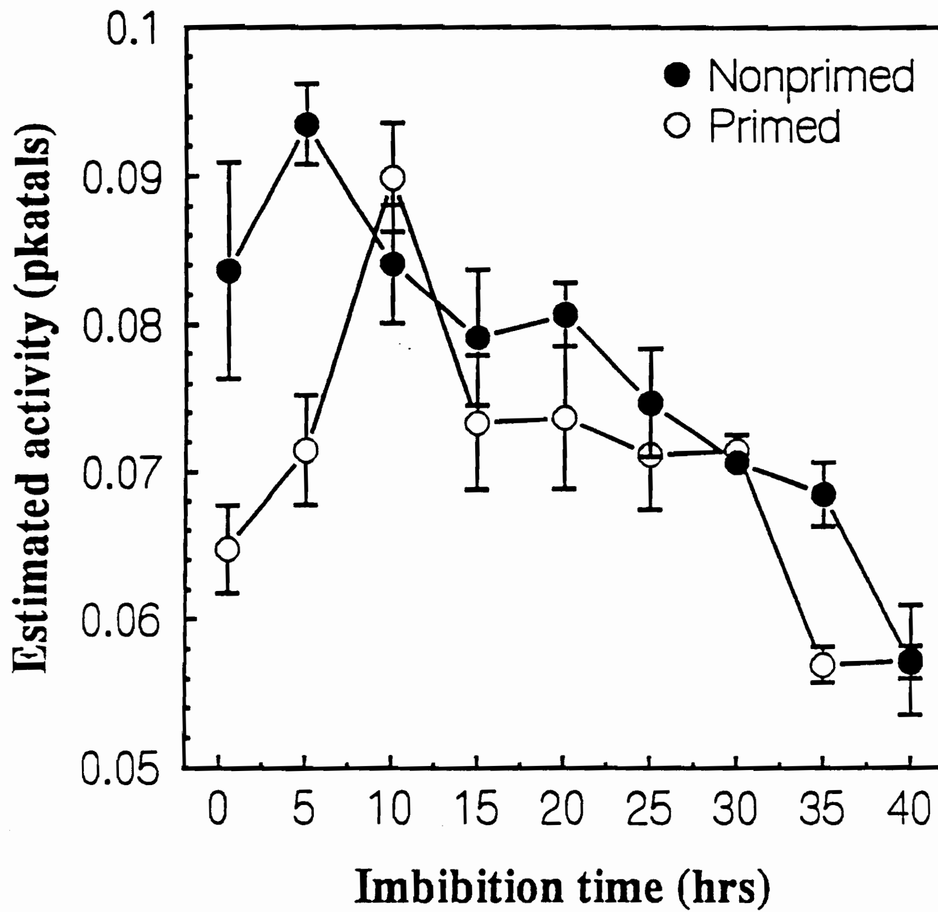

Fig. 5.9. Endo-β-mannanase-like activity of primed and nonprimed seeds from 0.5 to 40 hours of imbibition. The enzyme was leached from the perisperm tissue adjacent to the radicle tip. The error bars are means ± S.E. of four samples of eight seeds each.

35-DAA seeds exhibited poor germination at harvest, but drying and afterripening for two months increased germination to greater than 50 and 90%, respectively, in water (Welbaum et al., 1990; Welbaum and Bradford, 1991b). In this study, five months storage period increased germination of immature seeds to greater than 75% in water, with storage having greater effect on the germination of immature than mature seeds (Figs. 5.4A, B)

Muskmelon seeds do not accumulate maximum dry weight until after 35 DAA (Welbaum and Bradford, 1989), so the perisperm tissue strength of 40- to 65-DAA seeds could be expected to remain unchanged during seed development. However, the force and energy required to puncture the perisperm envelope tissue increased steadily with seed maturity, and values were higher in mature than immature seeds (Fig. 5.4A, B). The force and energy required to penetrate the perisperm envelope tissue during seed development was negatively correlated with seed vigor (cf. Figs. 5.4 and 5.5). This is indicative that the perisperm cell walls is possibly strengthened when seeds are developing and during afterripening, with the more mature seeds exhibiting stronger cell walls than the immature seeds. Analysis of the perisperm envelope tissue showed no evidence of suberization at 20 DAA, but at 50 DAA, the perisperm consisted of dark staining dense material with no distinctive cellular structure (Welbaum and Bradford, 1990a).

The puncture force and the energy required to rupture the endosperm or perisperm envelope tissue of lettuce, pepper, tomato, and *Datura* declined steadily during imbibition (Tao and Khan, 1979; Watkins and Cantliffe, 1983; Sanchez et al., 1986; Groot and Karssen, 1987). However, no study has previously shown that perisperm or endosperm strength increased during development. The net force required to puncture the perisperm envelope of 40- to 45-DAA seeds at radicle emergence was 0.22 N and 0.13 N at 25 and 30 hours, respectively, compared to 0.37 N and 0.18 N, respectively, for 50- to 65-DAA

seeds (Fig. 5.6A). These values are in agreement with earlier measurements in muskmelon (Welbaum et al., 1995; cf. Fig. 3.2) and conforms with measurements of the force required to rupture endosperm tissue at radicle emergence in tomato, pepper, and lettuce seeds (Groot and Karssen, 1987; Watkins and Cantliffe, 1983; Tao and Khan, 1979).

Structural modifications in lettuce, *Datura* spp., and tomato seeds have been observed in the micropylar region of the endosperm tissue adjacent to the radicle tip and prior to radicle emergence (Psaras and Georghiou., 1983; Psaras et al., 1981; Sanchez et al., 1990; Haigh, 1988; Karssen et al., 1989). In muskmelon, structural changes were observed in the perisperm envelope during imbibition and prior to radicle emergence. Cracking was less severe in the lateral regions, indicating that the micropylar end of the perisperm envelope adjacent to the radicle tip was broken down more rapidly (Welbaum et al., 1995). The degradation of cells during imbibition supports our findings that perisperm weakening precedes radicle emergence. The negative correlation between perisperm strength and seed vigor during development suggests that older seeds have greater hydrolyzing enzymatic activity or turgor is higher in the embryonic axis of mature seeds (cf. Figs. 5.4 to 5.7).

Tissue stiffness calculated from the slope of the force-deflection curve (Fig. 5.2) also increased following the same trend as the force and energy required to puncture the perisperm tissue (cf. Figs. 5.6 and 5.7). Stiffness of the perisperm tissue continued to increase with seed age even though seed development had apparently ended at 35 DAA when seeds attained maximum dry weight (Fig. 5.7; Welbaum and Bradford, 1989). A previous study showed that the stress necessary to fracture muskmelon perisperm tissue during imbibition is directly proportional to the perisperm thickness. It was proposed that the variability in perisperm thickness could account for variations in the time to radicle

emergence among seeds (Welbaum et al., 1995). If this is true, then the differences in stiffness between less and more mature seeds may be attributed to differences in perisperm tissue thickness (cf. Figs. 5.5 and 5.7). We could thus hypothesize that the stress necessary to fracture the perisperm tissue during germination is correlated to the stiffness of the tissue.

Endo-β-mannanase has been implicated in the hydrolysis of mannans and endosperm weakening in tomato and lettuce seeds (Halmer et al., 1976; Groot and Karssen, 1987, 1992; Groot et al., 1988). Weakening of the endosperm or perisperm by enzymatic hydrolysis of cell wall polysaccharides is correlated to the release of mannose and appears to precede radicle growth in tomato, *Datura*, pepper, and muskmelon seeds (Groot et al., 1988; Watkins et al., 1985; Sanchez et al., 1990; Dutta et al., 1994; Welbaum et al., 1995). However, in lettuce, mannanase activity in the endosperm tissue is not well correlated with radicle emergence, but is involved with storage reserve mobilization after germination (Halmer et al., 1976; Halmer and Bewley, 1979). A gel diffusion assay for the quantification of endo-β-mannanase activity has been developed using galactomannan substrate (McCleary, 1978; Downie et al., 1994). In tomato, a high endo-β-mannanase- related activity in the micropylar part of the endosperm preceded germination (Toorop et al., 1994). A faster germinating genotype developed less mannanase activity prior to radicle emergence than a slower germinating genotype, but the activity in endosperm caps did not correlate well with germination timing (Dahal et al., 1994).

Seed priming improves the germination performance and uniformity of germination in many species. The response to priming could be correlated with specific physiological changes within the seeds. Karssen et al. (1989) proposed that softening of the endosperm occurs during priming and promotes the germination of primed seeds. In tomato, higher

endo-β-mannanase activity has been observed in the endosperm cell walls of germinating primed seeds than in germinating nonprimed seeds (Karssen et al., 1989; Nonogaki et al., 1992). However, in muskmelon, primed seeds developed less endo-β-mannanase-like activity than nonprimed seeds during imbibition (Fig. 5.9). Mannanase-like activity of nonprimed and primed seeds did not correlate well with germination timing and seed vigor but conforms to a previous observation in tomato (Dahal et al., 1994). In this study, endo-β-mannanase-like activity may have been underestimated since locust bean galactomannan was used as a substrate rather than mannan from muskmelon cell walls. Hatfield and Nevins (1986) concluded that the activity of cell wall-hydrolyzing enzymes may be highly specific for structural features of native polymers. The endo-β-mannanase may hydrolyze cell wall galactomannans during the priming treatment thus softening the perisperm tissue (Chapter 3, Fig. 3.2). Therefore, when dried primed seeds are re-imbibed, they develop less mannanase activity than nonprimed seeds as the initial perisperm weakening had occurred during priming.

Embryo turgor in excess of a threshold is directly correlated to the rate of germination in lettuce and tomato seed (Bradford, 1990; Ni and Bradford, 1993). Direct psychrometric measurements of cotyledons and embryonic axis Ψs in muskmelon seeds during imbibition gave lower values in primed seeds than nonprimed seeds (Fig. 5.8). This may be indicative that primed seeds accumulate osmotic solutes during the priming treatment which allowed expanding radicles to attain a yield threshold higher than for nonprimed seeds during germination.

In conclusion, the penetration force and energy required to puncture the perisperm envelope tissue increased during seed development and after-ripening but was negatively correlated with seed vigor (cf. Figs. 5.4 to 5.7). In general, nonprimed seeds developed

more endo-β-mannanase-like activity than primed seeds during imbibition (Fig. 5.9), showing that priming affects enzymatic activity.

Acknowledgements

We thank Dr. James Wilson and Donnie Wingo (Dept. of Biological Systems Engineering) for help in performing Instron Analysis.

Chapter 6.

Effects of Priming and Seed Development on Nuclear Replication Activity in Muskmelon Seeds.

Abstract - In tomato and pepper, priming induces replicative DNA synthesis which correlates with seed vigor. Flow cytometry was used to evaluate the relative DNA replication activity of nuclei isolated from embryonic axes cells of imbibed muskmelon seeds at 30, 40, 50, and 65 days after anthesis (DAA). The nuclear replication activity of 40-DAA osmotically primed (0.3 M KNO_3, 6 days, 25°C) and matric primed (1g seed:4g vermiculite:0.6 ml water, 7 days, 25°C) seeds were also evaluated during imbibition. In mature seeds, the embryonic axis cells of dry and imbibed seeds of all treatments revealed predominantly 2C DNA signals. This indicates that during seed maturation, most embryonic axes cells had arrested cell cycle activity at the presynthetic G_1 phase (quiescent phase). Seed maturity and priming treatments significantly enhanced the mean time to germination but did not change the percentage of total DNA nuclei exhibiting 2C, 4C, and 8C signals. The 4C/2C ratio of 30- and 40-DAA seeds markedly increased up to 15 hours during imbibition then sharply declined at 20 hours. Imbibition had slight variable effects on percentage of total DNA nuclei from embryonic axes cells of 40-, 50-, and 65-DAA seeds. In 30-DAA seeds, the induction of 4C DNA signal increased by 20% after 20 hours of hydration. DNA synthesis in muskmelon embryonic axis tissues probably does not occur before radicle emergence during priming, or the nuclear replication activity was so fast during imbibition that the accumulation of 4C nuclei was not detected. In muskmelon, the beneficial effects of priming and seed maturity do not correlate with DNA

replication activity, so flow cytometry cannot be reliably used to detect differences in seed vigor.

Introduction

The maintenance of DNA integrity during cell division and differentiation is of critical importance to normal seed germinative processes. In dry seeds, lesions in the structure of nuclear DNA occur during storage (Cheah and Osborne, 1978). Osborne (1982, 1983) showed that protein, RNA, and DNA synthesis are initiated within minutes of imbibing dry seeds or embryos in water, but DNA replication is a relatively late event. The cause for delay in the onset of DNA replication is not clear, but may allow time for the repair of lesions that block replication (Thornton et al., 1993). During hydration of seeds of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) and cauliflower (*Brassica oleracea* var. *botrytis*), a recovery process occur which repairs DNA damage (Thornton et al., 1993). In rye (*Avena fatua*), Elder and Osborne (1993) reported that in dormant imbibed embryos, a continuous but slow repair of DNA occurs by a modified form of replicative DNA synthesis.

Seed priming is a controlled hydration process that permits pre-germinative metabolic activity to proceed, but prevents radicle emergence, such that priming results in more uniform and faster rates of germination (Bradford, 1986; Khan, 1992). Osmotic priming in inorganic salts has successfully improved the germination rates and uniformity of germination of tomato (*Lycopersicon esculentum* Mill.; Alvarado et al., 1987), onion (*Allium cepa* L.), carrot (*Daucus carota* L.; Haigh and Barlow, 1987; Haigh et al., 1986), lettuce (*Lactuca sativa* L.; Bradford and Somasco, 1994), and leek (*Allium porrum* L.; Ashraf and Bray, 1993) seeds. Similarly, matric priming in moistened solid carriers has

improved the germination rates of tomato, onion, and carrot seeds (Taylor et al., 1988). In muskmelon, osmotic priming in solutions of KNO_3 markedly improved the germination rates and final germination, particularly at sub-optimal temperatures (Nerson and Govers, 1986; Bradford et al., 1988; Welbaum and Bradford, 1991).

Physiological factors responsible for the beneficial effects of priming are still not fully understood. Protein synthesis occurs in imbibed seeds and during most osmopriming treatments (Dell'Aquila, 1987). The rates of RNA and protein synthesis are higher in primed than in nonprimed seeds during germination (Bray et al., 1989). DNA synthesis has been detected in the nuclei and mitochondria of leek embryo tissue during the osmopriming treatment in the absence of cell division (Ashraf and Bray, 1993). Burgass and Powell (1984) proposed that the onset or completion of DNA repair activity during osmopriming may be a significant factor accounting for the improvement in germination performance arising from osmopriming treatments.

Flow cytometry has proved to be a powerful tool in cell cycle analysis (Galbraith et al., 1983) and for the estimation of nuclear DNA content (Galbraith et al. 1983; Rayburn et al., 1989, 1990; DeRocher et al., 1990). With flow cytometry, the relative DNA amounts, expressed as C-values, of large numbers of individual nuclei in embryos can be easily determined. Proliferating cells pass through four cell cycles referred to as the G_1 (2C DNA content) or quiescent phase; S, the DNA synthesis phase; G_2 (4C DNA) or the second resting phase; and M (mitosis) or cell division phase (Bergounioux et al., 1992; Arumuganathan and Earle, 1991). In previous reports, the nuclei in embryos of fully matured dry pepper (Lanteri et al., 1993, 1994) and tomato (Lanteri et al., 1994) seeds predominantly revealed 2C signals. Bino et al. (1993) similarly showed that embryos of fully matured seeds of various plant species revealed largely 2C DNA signals. These

results indicate that during seed maturation in these species, most cells had the nuclear replication activity arrested at the presynthetic G_1 phase of nuclear division.

During priming in tomato and pepper seeds, induction of DNA synthesis and the percentage of 4C nuclear DNA signals in embryonic axis cells increase (Bino et al., 1992; Lanteri et al., 1993, 1994; Saracco et al., 1995). This indicates that when primed seeds are redried, the DNA in the embryonic axes cells cease cell cycle activity at the G2 phase. It has been proposed that the beneficial effects of priming are correlated with DNA replication prior to germination (Bino et al., 1992). However, more species need to be tested to determine whether DNA replication activity is a common response to priming.

Determining the optimal time of harvest for seeds that develop inside fleshy fruits is complicated because seed maturation may precede fruit maturity (Coombe, 1976). For muskmelon, maximal seed dry weight is obtained several weeks before fruit maturity (Welbaum and Bradford, 1988). When fruits are prematurely harvested, seeds require an afterripening period or priming to develop maximum germinability (Welbaum and Bradford, 1991a, b). Studies on muskmelon cultivar Top Mark showed 50- to 60-days after anthesis (DAA) seeds to be more vigorous than earlier maturity stages (Welbaum and Bradford, 1989; Welbaum et al., 1990; Chapter 4). Perhaps changes in vigor during development involve cell cycling, so when dried seeds are rehydrated, cell division may commence without the need for DNA replication. Relative DNA contents in various plant tissues and cells at specific developmental stages have been determined with accuracy using DNA-specific fluorescent dyes and flow cytometry (Galbraith et al., 1983; Bino et al., 1990; Michaelson et al., 1991). Flow cytometry measures DNA replication activity of seeds which may serve as a reliable indicator of seed quality. In the current study, a flow cytometer was used to quantitate DNA replication activity in embryonic axes of

muskmelon seeds during germination, and to determine whether priming increased the percentage of 4C cells.

Materials and Methods

Plant material - Muskmelon cultivar Topmark (Asgrow Seed Co. Inc., Vineland, New Jersey) was field grown in a Hayter loam (fine loamy, mixed, mesic, Ultic Hapludaf) soil in Blacksburg, Virginia as previously described (Welbaum and Bradford, 1988). Hermaphroditic flowers were tagged at anthesis, and crown-set fruits containing from 400 to 500 seeds were harvested at five-day intervals from 30 to 65 DAA. At harvest, seeds were removed from the fruit and washed vigorously in flowing tap water for three hours. Seeds from five to ten fruits from the same stage of development were harvested simultaneously and combined during drying to create a lot. Seeds were forced-air dried for three hours at room temperature, then finally dried in a desiccator to a MC of $< 6.0\%$ (dry wt. basis) determined by heating samples of 50 seeds for one hour at 130°C (ISTA, 1985). Dried seeds were stored for 10 months at room temperature in tightly sealed screw-top plastic bottles before testing.

Priming treatments - Stored 40-DAA seeds were osmotically primed in 0.3 M KNO_3 solution at a ratio of 5 ml of solution g^{-1} of seed for 6 days in the dark at 25°C then rinsed and redried on a laboratory bench. For matric priming, vermiculite was mixed thoroughly with water in 125 ml conical flasks sealed with wax film (Parafilm M, American National Can., Greenwich, CN) to prevent evaporation then equilibrated for 24 hours at 20°C before adding the seeds. Stored 40 DAA seeds were uniformly added and mixed to obtain a ratio of 1g seed:4 g vermiculite:0.6 ml water before incubation at 25°C for 7 days. After the priming treatment, the seeds were thoroughly rinsed in distilled water then forced-air

dried at 37°C for 3 hours. Primed seeds were then dried over silica gel in a desiccator at 45% RH to a final moisture content of <6% (dry wt. basis) prior to germination testing and analysis by flow cytometry.

Laboratory germination - Four replicates of 20 seeds each were placed in 9 x 9 x 1.5 cm transparent, covered plastic boxes (Falcon 1012, Becton Dickinson and Co., Lincoln Park, New Jersey) on two thicknesses of germination blotter paper (Anchor Paper Co., St. Paul, Minnesota) saturated with 11 ml solutions of deionized water. Boxes were incubated in the dark at 25 ± 1°C inside self-sealing plastic bags (Ziploc, Dow Brands L. P., Midland, Michigan) to reduce evaporation. Seeds were scored for radicle emergence at 12 hour intervals for 21 days and germinated seeds were removed.

Nuclear isolation and flow cytometry - Subsamples of the same seed lots used for germination testing were analyzed by flow cytometry at the Mann Laboratory, University of California-Davis. Embryonic axes were excised from dry seeds and seeds imbibed for 5, 15, and 20 hours in water at 23 ± 2°C for each stage of development and priming treatment. Twenty five embryonic axes were pooled for each imbibition treatment then chopped with a razor blade in 1.2 ml of nuclear isolation buffer (50 mM Tris-HCL, 1 mM $MgCl_2$, 0.1% Triton X-100) containing 4', 6-diamidino-2-phenylindole (DAPI), a stain specific for DNA. The sample was filtered through a 15 μm nylon filter, and the DAPI-stained nuclei were immediately analyzed with a Partec CA II flow cytometer (Partec GmbH, Münster, Germany). Data were electronically processed as fluorescent signals and displayed as pulse amplitude frequency distribution histograms over 510 channels, starting from channel 28. Histograms were stored on a floppy disk and processed off-line using Multicycle software (version 2.53, P. S. Robinovitch, U.C. Washington, WA.) for DNA

content and cell cycle analysis. The amount of DNA in the nucleus is proportional to the fluorescent signal and is expressed as C values in which the 1C value comprises the DNA content of the unreplicated haploid chromosome complement. The percentages of nuclei in the G_1, S, and G_2 phases of the cell cycle were determined from the frequency histograms. Each histogram was derived from the analysis of approximately 15,000 nuclei. The treatments were not replicated for lack of seed but there was reasonable confidençe in the data because of the large number of nuclei counted per sample.

Statistical analysis - Probit analysis was used to determine the log mean time to germination. When germination percentages were plotted on a probit scale versus log time (t), straight lines of approximately equal slope were produced for different treatments, indicating a normal distribution of germination events with log time (Finney, 1971). The log mean time to germination (log $\bar{t}$) was determined graphically from the intersection of the least squares regression line of log time versus probit germination percentage and 50% germination for treatments with greater than 20% germination. The slope of a probit plot is equal to the inverse standard deviation (σ) (Finney, 1971). The significance of the main effects of seed treatments for arcsin transformed germination percentages, log $\bar{t}$, and σ, were compared by ANOVA using CoStat (CoHort Software, Minneapolis, Minn.). DNA replication activity were compared by Multivariate analysis of variance using SAS. Means were compared by DMRT or LSD where appropriate. Data for actual germination percentages are shown.

Results

The effect of priming and seed development on germination - Osmotic and matric priming treatments had no effect on final germination, but significantly reduced the log mean time to germination (log $\bar{t}$) compared with untreated seeds (Table 6.1). Osmotically primed seeds gave the fastest log $\bar{t}$ compared to control and matric primed seeds (Table 6.1). Germination percentages in water significantly increased with seed maturity from 75% at 30 DAA to maximum germinability at 50 DAA, then declined to 85% at 65 DAA (Fig. 6.1A). Log $\bar{t}$ and standard deviation (σ) in germination time significantly decreased with seed maturity to a minimum at 50 DAA then increased at 65 DAA (Figs. 6.1 A, B).

Effects of priming on nuclear replication activity during imbibition - Using multicycle flow cytometric analysis, the 4C/2C ratio of 40 DAA nonprimed seeds increased from 1.99 in dry seeds to 2.27 after 15 hours hydration then declined to a ratio of 2 after 20 hours of hydration (Table 6.2). The 4C/2C ratio of osmotic primed seeds sharply increased to a value of 2.17 after 5 hours of imbibition then declined with increasing hydration (Table 6.2).

Analysis of DNA amount in embryonic axes of 40 DAA muskmelon seeds at all times during imbibition revealed predominantly 2C signals (Table 6.3). Variations were observed in the percentage of 2C nuclei during imbibition of osmotic and matric primed seeds (Table 6.3). However, the percentage of 2C nuclei was significantly higher than 4C, 8C, and synthesis fraction (S) for all seed treatments at all times during imbibition (Table 6.3). At 5 hours of hydration, the percentage of 4C nuclei for nonprimed seeds was markedly higher than osmotic and matric primed seeds (Table 6.3). The percentage of 4C nuclei for nonprimed seeds increased at 15 hours of hydration then declined slightly at 20

Table 6.1. Germination percentages (%), mean time to germination (log $\bar{t}$) and standard deviation (σ) in germination time (log h) of nonprimed, osmotic primed (0.3M KNO_3, 6d, 25°C), and matric primed (1g seed:4g vermiculite:0.6ml water, 7d, 25°C) 40 DAA muskmelon seeds. Each data point is the mean of four replicates of 20 seeds each.

	Treatment		
Seed treatment	**Germination (%)**	**log $\bar{t}$ (h)**	σ (log h)
Nonprimed	98[z]	1.63	0.14
Osmotic primed	93	1.29	0.19
Matric primed	100	1.42	0.11
$LSD_{.05}$	4.9*	0.1***	NS

NS, *, *** Nonsignificant or significant at p = 0.05 or 0.001 level, respectively.

[z]ANOVA was performed on arcsine transformed percentage data and actual percentages are shown.

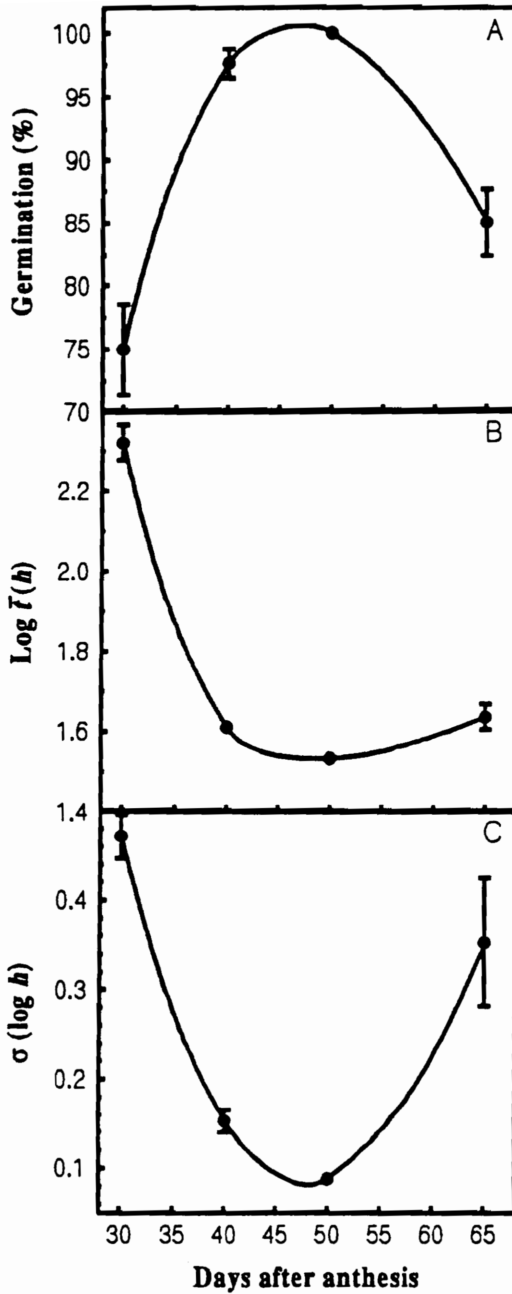

Fig. 6.1. Germination percentages (A), mean log time to germination (log $\bar{t}$) (B), and standard deviation (σ) in germination time (log *h*) (C) versus stage of seed development for muskmelon seeds germinated in water at 25°C for 21 days. Each point is the mean of four samples of 25 seeds each, and error bars indicate ±S.E. when larger than the symbols. ANOVA revealed significant differences for germination percentages, log $\bar{t}$, and σ.

Table 6.2. A cell cycle analysis of nuclear DNA replication activity showing the ratio of 4C/2C nuclear signals of cells in the embryonic axes of 40 DAA nonprimed, osmotic primed (0.3M KNO_3, 6d, 25°C) and matric primed (1g seed:4g vermiculite:0.6ml water, 7d, 25°C) muskmelon seeds during imbibition. Twenty-five seeds were analysed at each imbibition time for each treatment.

Imbibition time (h)	4C/2C ratio		
	Seed Treatment		
	Non-primed	Osmotic primed	Matric primed
0	1.99	1.85	1.88
5	2.15	2.17	2.16
15	2.27	1.88	2.16
20	2.00	1.99	1.88

Table 6.3. Cell cycle stage (C values) analysis showing nuclear DNA replication activity expressed as percentages of total nuclei in embryonic axes cells of 40 DAA nonprimed, osmotic primed (0.3M KNO_3, 6d, 25°C), and matric primed (1g seed:4g vermiculite:0.6ml water, 7d, 25°C) muskmelon seeds during imbibition. The C signals in each sample were derived from ≈ 15,000 nuclei. Twenty five seeds were analysed at each imbibition time for each treatment. Multivariate ANOVA revealed highly significant and nonsignificant differences for percentage of total nuclei during cell cycling and imbibition times, respectively, for all seed treatments.

	Cell Cycle Stage (% of total nuclei)											
	2C			4C			8C			S		
	Seed Treatments											
Imbibition time (h)	Non-primed	Osmotic Primed	Matric primed	Non-primed	Osmotic Primed	Matric primed	Non-primed	Osmotic Primed	Matric primed	Non-primed	Osmotic Primed	Matric primed
0	58	54	64	19	16	20	3	3	3	20	27	13
5	61	64	55	23	14	12	3	3	2	14	19	32
15	59	56	64	27	21	25	4	5	7	10	18	4
20	58	63	66	22	19	29	5	3	5	15	15	0.3

hours of hydration (Table 6.3). Imbibition had no significant effect on DNA replication activity in the embryonic axes cells of all seed treatments, but increased the 4C and 8C signals for matric primed seeds between 5 and 15 hours (Table 6.3). DNA synthesis (S fraction) for imbibed matric primed seeds increased with hydration to 5 hours then markedly decreased with further imbibition (Table 6.3).

The effect of seed development on nuclear replication activity - Thirty DAA seeds generally exhibited a higher 4C/2C ratio during imbibition than other developmental stages (Fig. 6.2). The ratio of 4C/2C for dry 30 DAA seeds was markedly higher than 40 DAA seeds, but was essentially similar with increasing hydration. The 4C/2C ratio for the embryonic axes tissues of 30- and 40-DAA seeds increased during imbibition up to 15 hours then sharply declined at 20 hours (Fig. 6.2). For 50-DAA seeds, five hours of imbibition lowered the 4C/2C ratio which sharply increased at 15 hours then markedly declined at 20 hours after imbibition (Fig. 6.2).

In the embryonic axes cells of dry seeds and seeds imbibed for up to 20 hours during development, the determination of DNA amounts largely revealed 2C signals (Fig. 6.3). The percentage of total nuclei exhibiting 2C signals was significantly higher than S, 4C, and 8C signals at all stages of seed development (Fig. 6.3). The percentage of 4C signals slightly increased to 40 DAA then significantly declined to a minimum at 65 DAA (Fig. 6.3). On the other hand, the percentage of nuclei in the synthesis fraction slightly decreased to a minimum at 50 DAA then markedly increased to 65 DAA (Fig. 6.3).

Imbibition had no effect on the percentage of 2C nuclei for 30-, 40-, and 65-DAA seeds but significantly decreased the 2C nuclei after 5-hours in 50-DAA seeds (cf. Figs. 6.4). The percentage of 4C nuclei for 30 DAA seeds gradually increased with imbibition, while the percentage of nuclei in the synthesis fraction gradually decreased with imbibition

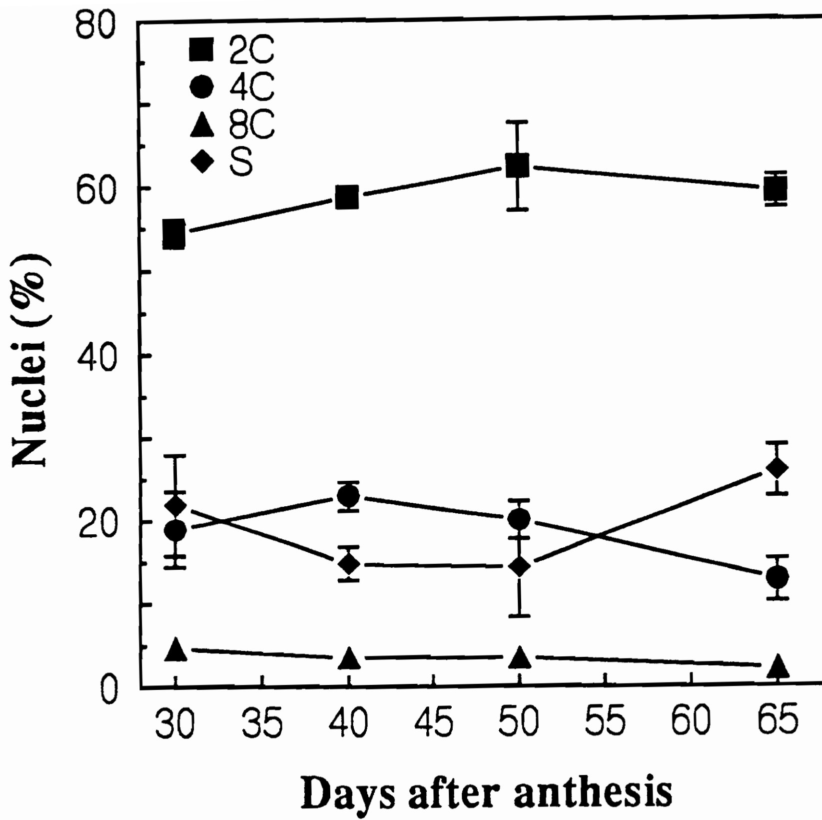

Fig. 6.3. Percentage of nuclei in 2C, 4C, 8C, and S fraction for embryonic axes cells from 30-, 40-, 50-, and 65-DAA seeds averaged across imbibition treatments of 0, 5, 15, and 20 hours. Twenty-five seeds were tested at each imbibition time for each stage of development. Error bars are ±S.E. when larger than symbols.

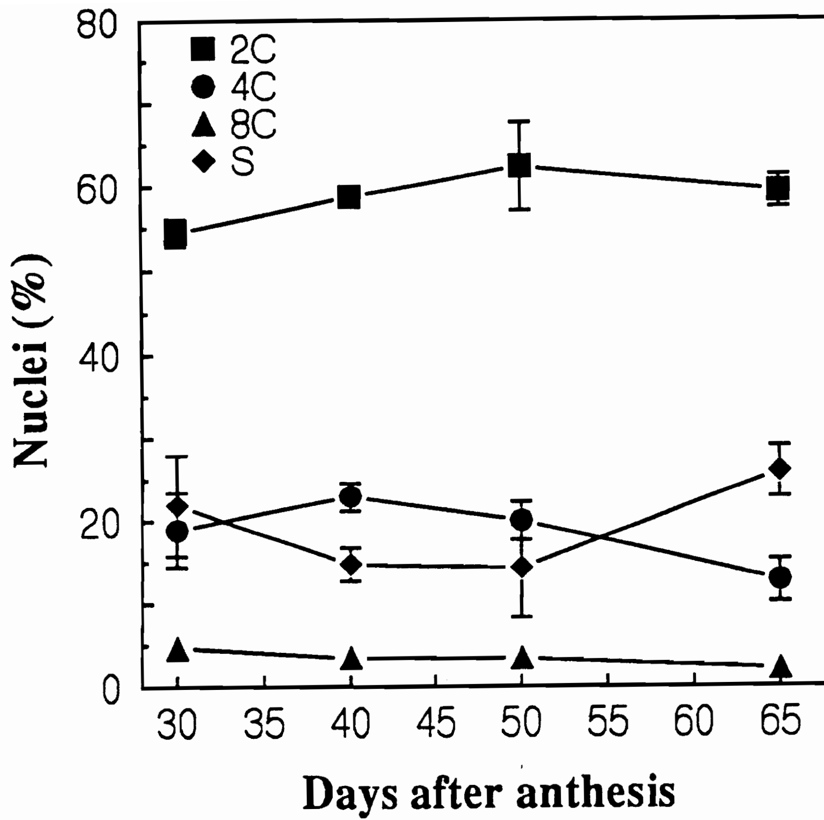

Fig. 6.3. Percentage of nuclei in 2C, 4C, 8C, and S fraction for embryonic axes cells from 30-, 40-, 50-, and 65-DAA seeds averaged across imbibition treatments of 0, 5, 15, and 20 hours. Twenty-five seeds were tested at each imbibition time for each stage of development. Error bars are ±S.E. when larger than symbols.

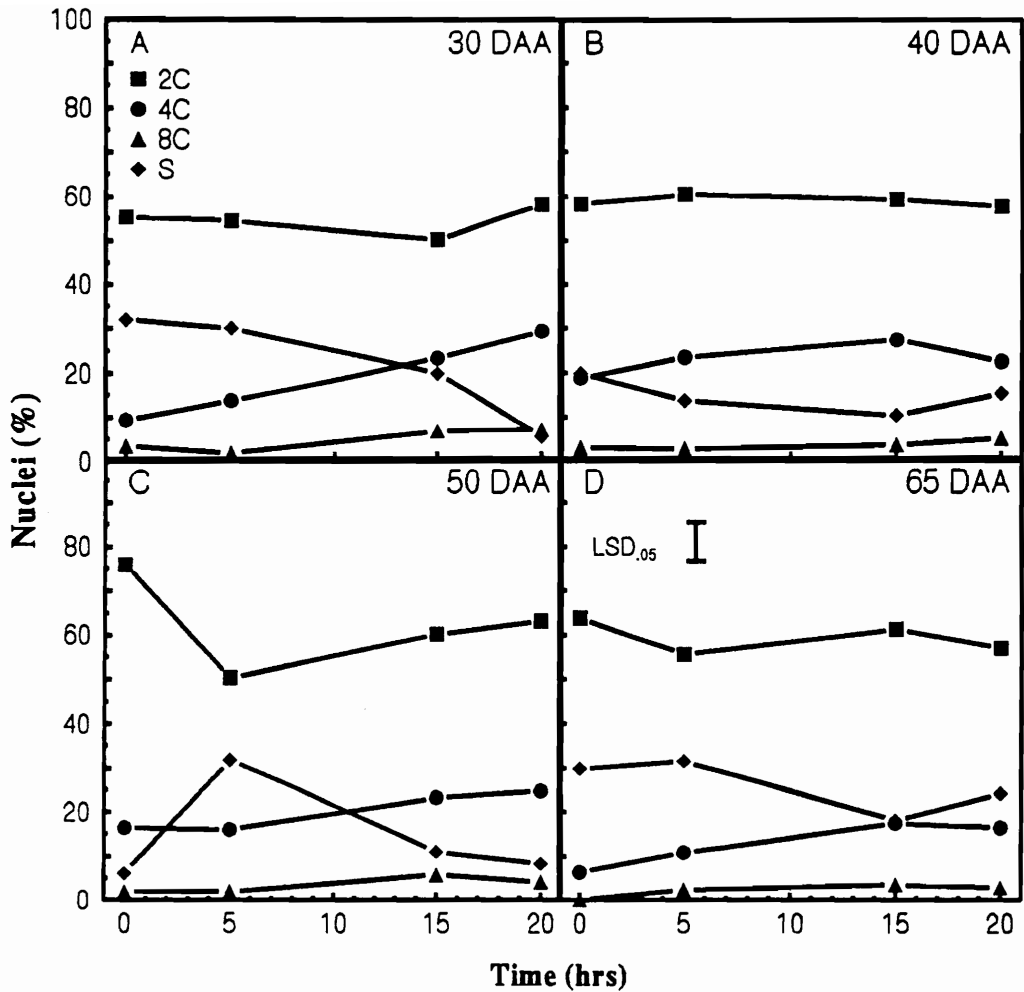

Fig. 6.4. Percent nuclei versus hours of imbibition for (A) 30-, (B) 40-, (C) 50-, and (D) 65-DAA seeds. Twenty five seeds were analysed for each imbibition time and stage of development. LSD was calculated from pooled data across all treatments and all imbibition times to test the main effects.

(Figs. 6.4A). At 50 DAA, the synthesis fraction sharply increased after five hours of imbibition then gradually declined to a minimum at 20 hours (Fig. 6.4C). Imbibition had no effect on 8C DNA levels at all stages of development (cf. Figs. 6.4).

Discussion

Flow cytometric analysis of DNA replication activity in the embryonic axes of dry and imbibed muskmelon seeds at different developmental stages and priming treatments predominantly revealed 2C signals (Table 6.3; Figs. 6.3; 6.4). This indicates that during seed maturation, most nuclei in the embryonic axes cells had arrested cell cycle activity at the presynthetic G_1 phase. These results are in agreement with those obtained previously in tomato (Bino et al., 1993; Lanteri et al., 1993) and pepper seeds (Lanteri et al., 1993, 1994). In dry tomato seeds, 89% of the cells of embryo radicle tip exhibited 2C signals (Bino et al., 1993). In experiments using autoradiography and feulgen staining techniques, it was reported that embryos of *Pinus pinea*, *Allium cepa*, and *Lactuca sativa* solely revealed 2C nuclei (Bewley and Black, 1978; Deltour, 1985). On the other hand, embryos of *Triticum durum*, *Zea mays*, and *Vicia faba* contained both 2C and 4C nuclei (Bewley and Black, 1978; Deltour, 1985). These findings show that in some species, the quiescent embryo arrests cell cycle activity in the G_1 phase, while in other species more embryonic cells enter the G_2 phase during seed maturation.

In this study, cell cycle activity had no apparent correlation with seed vigor during seed development and after priming (Table 6.1; Fig. 6.1). Seed development affected 4C/2C ratios (Figs. 6.2), but osmotic or matric priming treatments had no marked effect on the 4C/2C ratio of embryonic axes cells of dry or imbibed seeds (Table 6.2). In tomato seeds, the 4C signal of embryonic axes cells started to increase after 3 days incubation in

PEG, while the ratio of 4C/2C was markedly enhanced by priming although the enhancement did not reach the level obtained after hydration in water (Bino et al., 1992). It was proposed that the beneficial effects of priming are associated with the action of replicative DNA synthesis prior to germination. In pepper seeds, a positive correlation was found between the efficiency of the priming treatment and the induction of DNA synthesis (Lanteri et al., 1993). When primed seeds were imbibed in water, the induction of DNA synthesis started about 12 hours earlier than in untreated seeds. It was observed that priming reduced the lag period of DNA synthesis by 9 days, during which physiological processes that can sustain a rapid inception of nuclear replicative activity upon imbibition may have taken place (Lanteri et al., 1993). On the other hand, during priming of tomato seeds, the lag period prior to DNA replication lasted about 2 days (Bino et al., 1992). The optimal effects of priming were found after 14 days imbibition in PEG.

In pepper seeds, the beneficial effects of priming are not only correlated with completion of DNA repair mechanisms, increased protein synthesis (Davison and Bray, 1991), and reduced ribosomal RNA degradation (Davison et al., 1991), but also with the initiation of replicative DNA synthesis (Lanteri et al., 1993). In muskmelon, 4C/2C ratios did not change during priming and were not correlated with seed vigor (Tables 6.1 & 6.2). DNA synthesis in muskmelon embryonic axes tissues probably does not occur before radicle emergence during priming, or the nuclear replication activity was so fast during imbibition that the accumulation of 4C nuclei was not detected.

The percentage of total DNA nuclei exhibiting 2C, 4C, and S signals did not show any marked change during seed development and imbibition (cf. Figs. 6.3; 6.4). However, all developmental stages predominantly exhibited 2C signals, which shows that during seed maturation, nuclear replication activity become suppressed early in development. The

higher ploidy levels detected in the embryonic axes cells may have come from adjacent perisperm and endosperm tissues during sample preparation (Table 6.3; Fig. 6.3), or may be attributed to cellular endoreduplication.

Measurements of the amount of DNA replication that occurs during priming are positively correlated with increases in seed vigor in tomato and pepper seeds. However, in muskmelon seeds, there was no corrrelation between the enhancement obtained from priming and an increase in 4C nuclei. Flow cytometry can not be reliably used to assess the quality of muskmelon seed lots.

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Conclusions

A fundamental understanding of the effects of priming and development on muskmelon seed vigor could improve the production of high quality seeds and seed storage life. The most effective priming treatments for germination enhancement of muskmelon seeds occurred mainly near equilibrium Ψs between −1.5 and −1.8 MPa and at priming durations of 4 to 7 days. The magnitude of reduction in mean time to germination was significantly higher for seeds osmotically primed in KNO_3 and PEG solutions than for nonprimed seeds and seeds matrically primed in calcium silicate, vermiculite, and hayter loam soil.

The production of good quality seed requires timely harvest for maximum seed vigor. However, determining the optimal time of harvest for seeds that develop inside fleshy fruits is more complicated, because seed maturation may precede fruit maturity (Coombe, 1976). Muskmelon seeds must be harvested at the correct stage of development and washed to obtain maximum viability and vigor. After 6 years of storage, seeds washed at harvest were more vigorous and resistant to accelerated aging than unwashed seeds at most stages of development. Priming increased the storage life of newly matured 40- and 45-DAA stored seeds but decreased the storage life of 55-DAA seeds, showing that priming affects seeds differently depending on their stage of development. Fifty-, 55-, and 60-DAA seeds showed the greatest resistance to controlled deterioriation, were the most tolerant of reduced Ψs, and had the greatest 4-day root lengths. The highest quality seed was attained 50 to 60 DAA from fruit harvested after edible maturity but before the onset of severe decomposition. Seed maturation inside the fruit after maximum dry weight accumulation is necessary for highest muskmelon seed vigor.

Longevity of seeds in storage is an important indicator of seed vigor (Roberts, 1986). Loss of seed germination and early detection of deterioriation during storage are

major concerns of the seed industry. In laboratory germination tests conducted at 18°C immediateley after priming, germination rate and final germination percentages were higher for primed muskmelon seeds (Bradford et al., 1988). However, when results of germination tests conducted after 9 years of storage in this study are compared to those in the original study (Bradford et al., 1988), it is apparent that primed muskmelon seeds lost viability and vigor more rapidly than non-primed seeds after long-term storage. Additionally, when compared to nonprimed seeds, primed muskmelon seeds also lost viability more rapidly, germinated more slowly, and lost uniformity more quickly following controlled deterioriation experiments. Muskmelon seeds germinate best immediately after osmotic priming. If long-term storage is necessary before planting, seeds should be primed as close to the planting date as possible.

In seeds of lettuce, tomato, *Datura* spp., pepper, and muskmelon, the weakening of the endosperm or perisperm tissues adjacent to the radicle tip is required for germination to occur (Bradford, 1990; Groot and Karssen, 1987; Ni and Bradford, 1993; Sanchez et al., 1990; Welbaum and Bradford, 1990b). The penetration force and energy required to puncture muskmelon perisperm envelope tissue increased steadily during seed development and short-term storage and was negatively correlated with seed vigor. Less force was required to puncture primed seed pieces compared to nonprimed, indicating that osmotic priming weakens the perisperm envelope tissue prior to radicle emergence. The force and energy required to penetrate the perisperm tissue decreased gradually with imbibition, but the penetration force of 40- to 45-DAA seeds was consistently lower than 50- to 65-DAA seeds throughout imbibition. Direct psychrometric measurements of the Ψ of cotyledon and embryonic axis tissue in muskmelon seeds during imbibition gave markedly lower values in primed seeds than nonprimed seeds. The lower Ψ values suggest that primed seeds accumulated osmotic solutes during the priming treatment

which allowed expanding radicles to attain a yield threshold higher than for nonprimed seeds during germination.

Endo-β-mannanase has been identified as the cell-wall-degrading enzyme responsible for the breakdown of endosperm cell walls during the germination of tomato seeds (Groot et al., 1988), and pre- and post-germinative galactomannan hydrolysis in the endosperm cell walls of lettuce (Dutta et al., 1994; Dulson et al., 1988) and *Datura* spp. (Sanchez et al., 1990). The endo-β-mannanase-like activity in muskmelon perisperm tissue increased during the first 10 hours of imbibition, then declined steadily to 40 hours. In general, nonprimed seeds developed more endo-β-mannanase-like activity than primed seeds during imbibition, which shows that priming affects enzymatic activity. However, the results did not correlate well with germination timing and seed vigor.

Flow cytometry may allow the use of DNA replication activity in seeds as an indicator of seed vigor. In tomato and pepper, priming induces replicative DNA synthesis which correlates with seed vigor (Bino et al., 1992; Lanteri et al., 1993, 1994; Saracco et al., 1995). In muskmelon, the beneficial effects of priming and seed maturity do not correlate with DNA replication activity. DNA replication in muskmelon embryonic axis tissues probably does not occur before radicle emergence, or the nuclear replication activity is so fast during imbibition that the accumulation of 4C nuclei can hardly be detected in growing embryonic axes. Flow cytometry cannot be reliably used to detect differences in muskmelon seed vigor.

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