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thesis entitled

INVESTIGATION OF METHODS OF INVIGORATING VEGETABLE AMARANTH (AMARANTHUS TRICOLOR L.) SEEDS

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in HORTICULTURE

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Date 10/11/96

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1996

ABSTRACT

INVESTIGATION OF METHODS OF INVIGORATING VEGETABLE AMARANTH (*AMARANTHUS TRICOLOR* L.) SEEDS

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In humid tropics, amaranth has long been consumed as a green vegetable, not only because of its robust growth, but as a source of nutrition. One of the major constraints to amaranth cropping is seedling establishment. In this study, *Amaranth tricolor* L. seeds were aged and their percentage germination tested against fresh seeds. Aged seeds exhibited lower germination rates than fresh seeds. Testing moisture content (MC) for priming, presoaking seeds at 35% MC was found appropriate for both seed types. In the greenhouse, seedling emergence rates were tested using different invigoration methods. Under crusted and non-crusted conditions of loam and sandy soils, priming of aged and fresh seeds at 35% MC gave the highest percentage emergence compared with the control, Polyethyl glycol-priming, or Solid-Matrix-Priming.

ACKNOWLEDGMENTS

I would like to convey my gratitude to my major Professor, Dr. John F. Kelly for his patience and support throughout my program. Even though things never seemed to work properly for me, Dr. Kelly remained supportive. His perseverance and his never-dying spirit for success served to auger my successful completion of this thesis.

I also would like to thank my family, especially my mother, Ciki and my sister Keke, who gave me special support, notably during my home-sickness moods. My friends Bernard, Thuli, Awonke, Thokoza, and all the folks in the Plant and Soil Sciences Building; thanks a lot.

I would also like to thank the participants who helped bring this thesis to completion: Dr. Alan Taylor of Cornell University, Dr. Larry Copeland of the MSU Crop and Soil Science Dept., Dr. Bob Herner of Horticulture and Dr. George Axinn of Resource Development. Special thanks to my sponsors, USIA through Fulbright, and the International Center.

Last but not least I would like to thank The Creator for being with me throughout my stay in the United States to acquire knowledge, which I hope, will help alleviate some of the conditions experienced by deprived rural farmers of my country, South Africa. Hopefully, this effort will join other contributions to help correct some of the

impoverishment introduced by the ruthless legacy of apartheid.

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INTRODUCTION

Poor and variable germination of amaranth (*Amaranthus* spp.) seeds may be associated with low vigor of the seeds. In turn, seeds with low vigor may not be able to tolerate adverse soil conditions, like crusting. Chate et al. (1987) reported that many disappointments occur in directly drilled crops in terms of rapid uniform emergence at a high level, because of soil crusting. In particular, Kauffman and Hass (1983) noted that among the difficulties experienced by farmers growing amaranth was crusting in fine-textured soils.

Although vegetable amaranth is an important nutritional crop (Grubben, 1976), its cropping is characterized by uneven emergence or lack of uniformity in rate of emergence. In turn, overall yields are reduced, because of variability in size, quality, and harvest dates, which are important in terms of demands of the market, grading, flowering and establishing a new crop in case of sequential cropping (Matthews and Powell, 1986).

This study was conducted following failure of field emergence of amaranth seedlings in a sequential cropping experiment, due to soil crusting. Since amaranth has very small seeds, soil crusting appeared to be the limiting factor for obtaining a good stand of amaranth. Even though direct-seeding offers the lowest cost method in most growing

situations, strict control of the soil environment is not always possible (Chate et al., 1987).

Some recommendations dealing with the soil crusting problem have been reported. Rodale (1989) recommended use of double-press wheels rather than single-press wheels on crusting soils to reduce crusting, because wheels provide good soil contact on both sides of the seed but maintain loose soil directly over the seed. Seeding at higher rates per unit area and mulching of soil are also some of the recommendations for alleviating the problem. The thinning and mulching recommendations involve laborious work, which is often not desirable.

Chate et al. (1987) found that it is possible to control the vigor of the seed to improve the probability of successful stand establishment. This study looked at the effect of conditioning aged and fresh amaranth seed, using different pre-plant seed enhancement treatments, on improving seed germination percentage, rate, vigor, and seedling emergence and uniformity under soil crusting conditions.

Amaranth (*Amaranthus tricolor* L.) was chosen for this study, because of its high nutritional content, its ability to thrive in hot, humid climates, and its relatively easy cultivation; therefore, making it an important crop in the tropics, where malnutrition is prevalent. Amaranth has a short growing season of 3-6 weeks, and can be subjected to

repeated cutting of edible tops. This attribute makes it useful in sequential intercropping, thus optimizing available land resources. However, despite all of these favorable attributes, vegetable amaranth remains an under-exploited crop, largely because of neglect by researchers and policy-makers (Makus, 1984).

This thesis presents topics that deal with the manipulation of water status of seeds to improve germination and seedling emergence. The first section is devoted to determining optimum germination for the seeds. In this section, optimum temperature, optimum days for germination, and germination rate of the seeds are investigated. The second section deals with the different approaches to hydration. Included is the determination of critical moisture content for presoaked seeds, the use of gels on pregerminated seeds, and solid matrix-priming.

This study was conducted to test the following hypotheses:

1. Aged and fresh amaranth seeds have different germination regimes for different temperatures.
2. Invigoration of amaranth seeds improves germination and seedling emergence.
3. Invigoration of aged seed differs from that of fresh seed.
4. In crusted soil, the extent of seed invigoration differs with the invigorating method.

Chapter 1

LITERATURE REVIEW

Taxonomy

There is considerable confusion regarding the taxonomy and nomenclature of amaranth used as a leafy vegetable. The confusion is attributed to the characteristics often applied in determining the species, these being unstable because of ecological or genetic influences within the same species (Grubben, 1976). Therefore, taxonomic distinction among species is difficult, and many synonyms exist (Schnetzler and Breene, 1994).

The collective name 'amaranths' for vegetable amaranth is comprised of all species and cultivars of the family Amaranthaceae, principally *Amaranthus cruentus* L., *A. dubius* Mart. ex Thell., *A. tricolor* L. and *Celosia argenea* L. The grain amaranths, still cultivated in tropical mountainous regions of South America and Asia (*A. caudatus* L., *A. hypochondriacus* L. and *A. cruentus* L.) also are used as leafy vegetables. The species most often grown as vegetable amaranth is *A. tricolor* L. (Makus and Davis, 1984).

History

Historical records of amaranth show that it was consumed both as a vegetable and as a cereal grain. Grain

amaranth, in which the seed is consumed, originated in South and Central America and was used by the Aztecs as a staple food and for ritual ceremonies before 1519 (Early, 1994). Most varieties of vegetable amaranth, in which the leaves and succulent stems are consumed, originated in Asia (Grubben, 1976). Amaranthaceae seeds have been found in prehistoric dwellings, even in Mediterranean Europe (Grubben, 1976).

Amaranth grows naturally in open habitats as a pioneer species of riverbanks, coastal marshes, and dunes. Amaranth also spreads as a weed of artificially disturbed habitats created by human activities. In prehistoric times, wild and weedy amaranths became common potherbs throughout the tropics and subtropics. In other parts of Africa and Asia, amaranth was domesticated as a potherb (Rodale, 1989).

Vegetable amaranth is now cultivated and consumed as a cooked vegetable or green in many of the countries of Africa, the Caribbean, and Asia (Singh and Whitehead, 1992). In the United States, *A. retroflexus* (red-root pigweed) is the common species, but it is mostly treated as a noxious weed (Rawate, 1983) because of its spiny leaf texture, making it less tender and therefore inedible. However, there have been some studies by Campbell and Abbott (1982), Abbott and Campbell (1982), Makus (1984), Makus and Davis (1984) and Rhoden and McKelly (1995) on the feasibility of

growing Asian cultivars of vegetable amaranth as a summer green in the southeastern United States.

Description

Amaranth (*Amaranthus* L.) is a herbaceous short-lived annual plant, monoecious or dioecious. The plant is upright, branching, and 0.5-2.5 m tall. Lea

axis or has small bundles throughout the length of the cotyledon. These provascular cells are small and elongated and have fewer reserves and more cellular organelles than the large

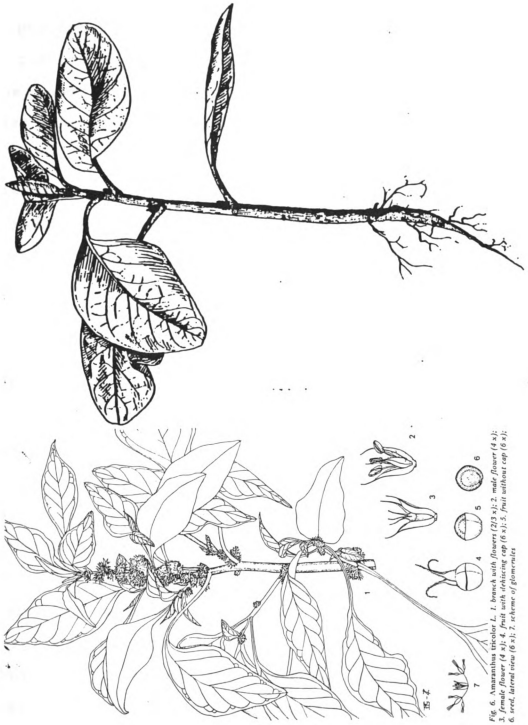

Figure 1. Morphology of an amaranth plant and its seed (*A. tricolor* L.) (Bittenbender, 1983; Grubben, 1976).

protoderm and ground meristem cells. The latter cells have more protein bodies and larger globoid crystal inclusions than the others. The perisperm is composed of starchy tissue, and its cells have thin walls and are full of angular starch grains (Coimbra and Salema, 1994).

Nutrition

Amaranth contributes to the diet because of its high nutritional value (Table 1). The leaves are high in protein (17.4 - 38.3%, dry matter basis); the protein comprises 4.4% sulfur-containing amino acids and approximately 5% lysine (Rhoden and McKelly, 1995). Amaranth leaves also provide significant amounts of carbohydrates, vitamins, dietary fiber, and minerals, particularly calcium and iron. Dialyzable iron varies from 0.41 and 0.63 mg bioavailable Fe per 100 g fresh weight. In actual levels, a 100 g portion of amaranth could provide 40 to 60% of the daily Fe requirement for men and children (RDA of 1 mg/day), or 27 to 41% for women (RDA of 1.5 mg/day) (Rangarajan, 1995).

Amaranth contains more fiber, niacin, and ascorbic acid than spinach, cabbage, or lettuce, on a fresh weight basis (Grubben, 1976; Abbott and Campbell, 1982), and it is similar to spinach in flavor (Makus, 1984). Used as a vegetable, amaranth has about 3/4 as much Vitamin A as spinach, but the levels of protein, iron, and other minerals are similar. Nitrate and oxalate contents are comparable to

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those of other leafy garden vegetables (Abbott and Campbell, 1982). Amaranth greens can be prepared as a fresh salad or steamed, boiled, stir-fried, sauteed, baked, or pickled.

Table 1. Nutritional composition of amaranth leaves and other vegetables (Grubben, 1976).

Crop	Values for 100 g of dry matter							
	Proteins mg	CHO g	Fiber mg	Calcium mg	Fe mg	Niacin mg	Vit. C mg	Calories kcal
Amaranthus spp.	33	33	10	1,667	27	10	667	320
Brassica spp.	21	57	11	571	7	4	571	329
Lactuca sativa	23	50	8	583	17	7	250	317
Spinacia oleracea	25	6	-	1,625	37	7	313	162

Cultivation

Unlike most plants used for greens, amaranth grows best in hot, humid climates, or during summer months in temperate climates (Makus and Davis, 1984). Amaranth grows rapidly and succulently at day temperatures above 30C and night temperatures above 20C. Optimum vegetative development and market quality generally require large quantities of water. Watering regimes depend on soil type and the stage of growth, but irrigation should occur above the Pf=4.2 wilting point, to avoid yield reductions (Rodale,

1989). Amaranth grows best in loose, fertile, well drained soil (Makus, 1990). Since amaranth seeds are small, crop establishment needs special attention. Rodale (1989) warned that due to the small seed size, increased risk of poor plant establishment due to soil crusting occurs frequently. Amaranth can be seeded directly at a depth of 0.5 cm or transplanted at 21 days or 7-cm length. Shallow planting depth is important to maximize emergence (Rodale, 1989).

Spacing can range from 10-40 cm (Mortley et al., 1992) depending on the type of harvest to be followed. Plants are harvestable 3-9 weeks after transplanting by clipping them 20-25 cm above the soil line, and can be allowed to ratoon (growth of repeated harvests of the same plant) (Makus and Davis, 1984). Harvesting also occurs by clear-cutting (uprooting) and reseeding (Campbell and Abbott, 1982). Amaranth also can be grown in mixed cropping systems, and because of its vigorous growth, it is useful in 'intensive multiple cropping systems' (Grubben, 1976).

Market

In general, little has been done in terms of research on the cultivation, varietal improvement, or market potential of the crop, especially for developing countries (Makus, 1990). It has been documented (Grubben, 1976; Grubben, 1979; Oke, 1980) that in most tropical areas the crop is used in small-scale gardens for home consumption and

markets; therefore, it forms an important part of food security. There has been a potential acceptance of amaranth use as a mid-summer vegetable crop in the United States (Makus, 1992; Rhoden and McKelly, 1995), therefore its consumption and market potential are expected to increase in the future (Campbell and Abbott, 1982).

Background of Invigorating Seeds

Seed viability and low vigor affect the performance (i.e. percentage and rate of emergence) of seeds, which ultimately result in low yields. The slow rate of emergence associated with low-vigor seed results in small plants. Vigor also is associated with seed size; large seeds emerge faster small seeds (TeKrony and Egli, 1991).

Invigoration of seed involves primarily the use of presowing techniques for the improvement of seed germination, emergence, and stand establishment, which are essential for early and maximum yields. The aspect of invigoration addressed in this research is the enhancement of physiological and biochemical events in seeds during controlled hydration, and suspension of germination by low water potential of the imbibing medium (seed conditioning), either liquid or moist solid (Khan, 1992).

Rationale for Invigorating Seeds

The topic of seed hydration and invigoration has been reviewed by Khan (1992). The rationale for invigorating seeds is to mobilize the seeds' resources and to augment them with external resources to maximize improvement of stand establishment and yield (Khan, 1992). Physiological seed treatments used in this study to improve or enhance seed performance based on seed hydration include presoaking, pregermination, and matriconditioning. Prolonged seed hydration, especially at low water potential, influences the rapidity, synchrony, and the percentage of seeds that germinate (Khan, 1992). As a result, rate and uniformity of seedling emergence is improved. Vigorous plant stand is influenced by the interaction of seed quality, seed-soil water relations, and growing media environment (Taylor et al., 1992). Various environmental stresses, such as soil crusting encountered after sowing, may decrease or prevent seedling establishment (Taylor et al., 1992). Seeds which are too small or which have low germination and seedling vigor are limited first by the percent viability.

Invigorating seed may be used to upgrade seed quality before sowing, by eliminating non-viable or poor quality seeds. Bradford (1986) reviewed various methods of osmoconditioning to improve seed quality and vigor. Seed

enhancement techniques include seed hydration, biological seed treatments, and seed coatings.

Treatments which include seed hydration under controlled conditions before sowing have attracted interest over the years. Two techniques, namely, fluid drilling and seed priming have been studied for the improvement of the rate of germination (Taylor et al., 1992).

Moisture Control

Moisture control is a seed prehydration treatment in which the amount of water absorbed by the seed is controlled precisely to increase percentage and rate of germination and increase the uniformity of stand establishment (Bewley and Black, 1994).

Seed moisture content plays a major role in the life cycle of seeds (Taylor et al., 1992). Moisture content is associated with seed development, maturation, harvest, and storage. Water uptake, or imbibition in seeds, is the first key step toward germination; therefore, water is essential to change the status of seed development from quiescence to active growth (Taylor et al., 1992). The environmental forces that determine the rate of water imbibition by seeds are complex, but the ability to imbibe water is dependent on cell water potential and is a result of cell wall matric forces, cell osmotic concentration, and cell turgor pressure

(Copeland and McDonald, 1995; Vertucci, 1989; Bradford, 1986).

Germination of seeds is characterized by three phases (Figure 2) (Copeland and McDonald, 1995; Bewley and Black, 1994). The triphasic water uptake of seeds can be explained on the basis of water relations. In their resting stage, dry seeds are characterized by low moisture (5-15%) and relatively inactive metabolism (Copeland and McDonald, 1995; Bewley and Black, 1994). At this stage seeds have a lower water potential (-100 Mpa) than that of the surrounding moist substrate. Thus, a large water potential gradient initially exists between the seed and high water potential medium such as solution or water (Taylor et al., 1992, Vertucci, 1989). In this phase, imbibition is crucial in initiating seed germination. The events include controlled physical and physiological processes which direct flow of water into the dry seed and result in a large increase in seed moisture.

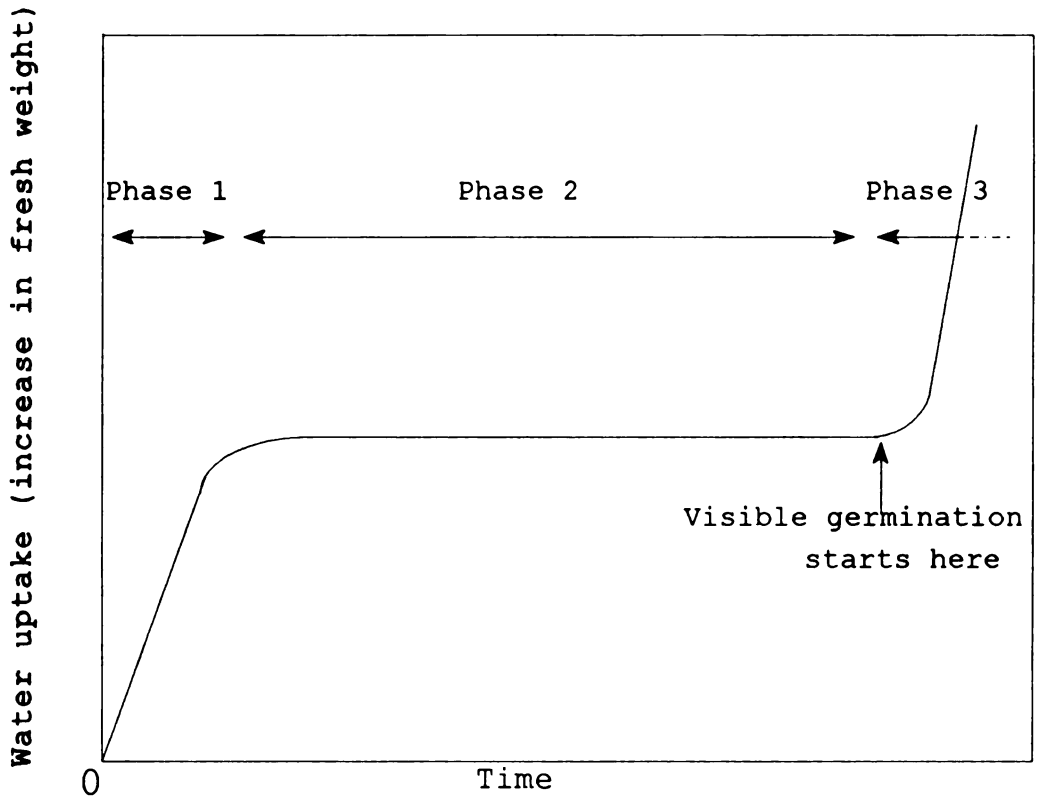

Figure 2. Triphasic pattern of water uptake by germinating seeds (Bewley and Black, 1994).

Phase two is the lag phase of water uptake; it occurs before the onset of visible germination or radicle emergence. In this phase, the seed's water potential plays a minor role. The osmotic potential and pressure potential create a static relationship between seed moisture and water uptake. During this phase, major metabolic events take place in preparation for radicle emergence (Bewley and Black, 1994). Enzymes are activated to break down stored tissue, to aid in the transfer of nutrients from storage areas in the cotyledons or endosperm to the growing points,

and to trigger chemical reactions that use breakdown products for the synthesis of new materials (Copeland and McDonald, 1995).

In phase three, another large increase in water uptake results, marking the transition from seed to seedling by the protrusion of the radicle. Cell elongation results in additional water uptake by the seedlings (Taylor et al., 1992). Water uptake is influenced by decreases in water potential, resulting from production of low-molecular-weight, osmotically active substances resulting from the postgerminative hydrolysis of stored reserves (Bewley and Black, 1994).

The water potential differential between the environment and the seed establishes the gradient, but not the rate of imbibition. The rate of imbibition is influenced by the permeability of the seed to water; therefore, imbibition rate can be regulated by the available water in the environment to the seeds. Components that influence the hydration rate are properties of the seed and the environment in which it is situated. Such components include seed morphology, structure, composition, moisture content, and temperature (Taylor et al., 1992; Bewley and Black, 1994).

Imbibition is accompanied by gains in weight and volume. However, the percent increase in weight is not necessarily proportional to the percent increase in volume.

Water volume does not displace its weight directly, because water affects the degree of swelling of seed polymers (Taylor et al., 1992).

Presoaking

Presoaking involves moisture control of hydrated seeds, which is enough to elevate their moisture content until the phase of imbibition or until moisture level is sufficient to initiate the early events of germination, but not sufficient to permit radicle protrusion (Figure 2) (Copeland and McDonald, 1995; Taylor et al., 1992; Tarquis and Bradford, 1992). The aim, therefore, is to find the percentage moisture at which the soaked seed will not germinate, but which will stimulate more rapid germination when taken out and placed under optimum germination conditions. Presoaking is a seed hydration approach involving soaking of seeds in a limited amount of water prior to planting, and to enhance germination and seedling growth by controlling the imbibition conditions and reducing the vagaries of adverse soil conditions (Bradford, 1986). Presoaking as a means of improving germination has been known for years. Studies that confirm the effectiveness of presoaking have been reviewed by Khan (1992).

Because the amount of water absorbed by the seed is controlled precisely to ensure that germination does not occur, presoaking is a simple method of accelerating the

germination of seed. This gives the same results as other methods, such a osmotic priming or solid-matrix-priming, since physiological mechanisms which result in greater seedling performance are considered the same (Copeland and McDonald, 1995; Khan, 1992). It also is considered a 'user-friendly' technology which can be adapted by the farmer (A.G. Taylor, Personal Communication).

Pregermination and Gel-seeding

Pregermination, or chitting, pertains to the seed hydration approach in which seeds are soaked under optimal hydration and temperature conditions to a point at which the radicle is just visible (Khan, 1992). Before planting, the pregerminated seeds are mixed with gels (fluid drilling). On other occasions, primed and pregerminated seeds are sown with gels. Seeds also can be germinated in gels and then sown (flow sowing). Seeds also may be allowed to imbibe (primed), but not to germinate, and then sown with gels. The methodology employed in fluid drilling or gel seeding has been reviewed by Gray (1981), Taylor and Harman (1990) and Khan (1992). Examples of gels include sodium alginate formulations, hydrolyzed starch-polyacrylonitiles and synthetic clays. Gels also can be used as a means of introducing hormones, starter fertilizers, or pesticides close to the seeds and developing seedlings (Gray, 1981).

Pregermination (gel-seeding) has proved effective for small, slow-germinating vegetable seeds such as celery, parsnip and carrot (Gray, 1981). The major advantage of the method is earlier, higher, and more uniform and predictable emergence, giving earlier growth, higher yields, and more uniform crop maturity. Permitting planting in cool soils and incorporation of nutrients and pesticides in gels are also advantages. The major disadvantage is the susceptibility of seeds to damage during handling, and loss of viability during storage of germinated seeds (Khan, 1992). Another technical, logistic, and economic disadvantage of using fluid-drilling, especially for commercial purposes, is the requirement of specialized planting equipment or modification of existing equipment to sow pregerminated seeds (Taylor and Harman, 1990).

Priming

Seed priming or osmoconditioning is a seed-hydration approach in which seeds are hydrated in a low-potential solution (Khan, 1992). Seeds are soaked in an aerated osmoticum of low water potential so that their moisture content is increased and pregermination metabolic activity is initiated, but the moisture level remains below that needed to initiate germination (Bewley and Black, 1994). The topic of priming was reviewed thoroughly by Bradford (1986) and Khan (1992).

In priming, physiological and biochemical events in seeds are enhanced during the suspension of germination by low osmotic potential and negligible matric potential of the imbibing medium. Salts or non-penetrating organic solutes in a liquid medium or matrix solution are used to establish equilibrium water potential between seed and the osmotic medium needed for conditioning.

Priming is effective in improving germination and seedling establishment, reduction of germination time, and synchronization of germination. Taylor et al. (1992) and Pill et al. (1994) found that primed seeds germinated more rapidly than non-primed seeds, especially under adverse field soil conditions. As a result, plants have more vigor, which leads to an advantage over weeds. Other field studies (Matthews and Powell, 1986) showed that poor seedling emergence is not associated with failure to germinate, but with low vigor, resulting in the failure to complete the post-germination, pre-emergence stage of growth. Therefore, vigor of sown seeds is the major physiological constraint on the field performance of most vegetable seeds (Matthews and Powell, 1986). Chate et al. (1987), however, found that even though germinated seeds could establish higher stands compared with non-germinated seeds in adverse conditions, soil contact with seed is still important. There is controversy as to whether priming invigorates low-vigor seeds more than it does high-vigor seeds. Clarke and James

(1991) found priming of little benefit to low-vigor leek seeds. With amaranth seeds, Pill et al. (1994) found that the invigorating effect of priming was more pronounced for low-vigor than for high-vigor seeds. Copeland and McDonald (1995) concluded that priming seems to be successful with small-seeded crops, but less so with large-seeded crops.

Although the benefit of solutions such as polyethylene glycol (PEG), NaCl, glycerol, and mannitol; help to supply seeds with nitrogen and other essential nutrients for protein synthesis during germination, their disadvantage is their occasional toxicity to the germinating seedling (Copeland and McDonald, 1995), or in the case of PEG, low oxygen solubility at higher concentrations. As a result, PEG, when used as an osmoticum, often requires aeration to ensure an adequate supply of oxygen to seeds. In addition, it is difficult to treat large quantities of seeds commercially using PEG.

Solid-Matrix-Priming

Solid-Matrix-Priming (SMP) or matriconditioning refers to seed conditioning by osmotic and/or matric component of the solid matrix water potential. It is another approach to controlled seed hydration, using solid carriers with low matric potentials (Copeland and McDonald, 1995). Khan (1992) reviewed work done on the use of solid carriers to

shorten time of germination and to improve the rate and synchrony of germination.

Seed-conditioning materials should have a high osmotic solute content or a high hydrophilic surface (Khan, 1992). Examples of solid carriers include natural substances such as vermiculite, peat moss and sand; or commercial substances such as diatomaceous silica products and vermiculite. Other substances include leonardite shale, bituminous soft coal, and expanded calcined clay (Khan, 1992; Copeland and McDonald, 1995).

Seed Aging

Seed aging usually is caused by a number of factors. One could be the loss of viability because of seed storage conditions (i.e. Relative Humidity and temperature). The other could be the natural low viability of a seed lot (low vigor lots), or it could be the natural deterioration of vigor in mature seeds (physiological aging), or the physical damage which causes structural damage (i.e. seed coat) (Matthews and Powell, 1986).

In experiments conducted using aged seeds, artificial deteriorating methods are used to speed the aging process. However, there has been growing discussion in the literature (Nath et al., 1991) about the assumptions that such aging-accelerating methods cause similar damage to that which would occur in natural aging. Nath et al. (1991) argued

that seed detoriation was a matrix of interrelated events, each susceptible to different environmental constraints, instead of a continuum of damage, resulting first in a loss of vigor and ultimately in loss of viability.

Chapter 2

MATERIALS AND METHODS

Commercial greenleaf vegetable amaranth (*Amaranthus tricolor* L.) seeds (Johnny's Select Seed, Albion, Maine) with a 76% labeled germination, were used to evaluate preplant seed treatments for seedling establishment. All seeds were treated with 20 ppm of Captan to prevent fungal growth during the aging process. To produce aged seeds with lower vigor than fresh seeds, the method of accelerated aging was used (Copeland and McDonald, 1995).

Accelerated Aging Method

Fresh amaranth seeds were aged by placing a small amount of seeds on a seed rack inside a plastic dish with a tight-fitting lid in an oven of 41C and 100% RH for three days.

Germination Test Experiment

Optimum germination rates of aged and fresh seeds were tested in a laboratory, using a thermogradient table with five temperature ranges (10, 15, 20, 25, 30 and 35C)(Figure 3). A room temperature (25C$\pm$2) control also was included. Two

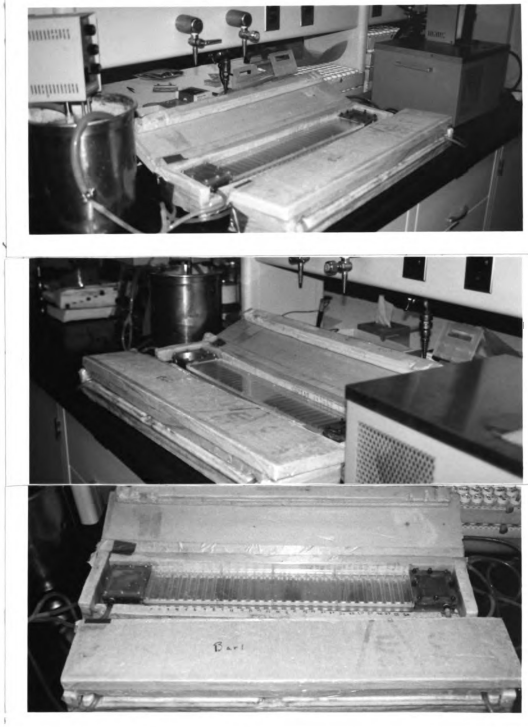

Figure 3. Thermogradient tables used to germinate *A. tricolor* seeds at different temperature regimes.

thermogradient tables with identical temperature ranges were used as two replications. Fifteen seeds of either fresh or aged seeds were placed on a layer of germination paper moistened with distilled water at each of the different temperatures. More distilled water was applied daily to maintain moisture of the seeds.

Germination (visible radicles) of the seeds was counted daily for eighteen days. The germination percentage and days to 50% (T-50) radicle emergence were calculated and analyzed using ANOVA and mean separation by F-test and LSD (P=0.05).

Initial Moisture Determination

The moisture contents (MC, fresh weight basis) of 1.5 g aged and fresh seeds were determined by the oven-drying method (3 h at 130C). Following oven-drying, seeds were allowed to cool for 1 h in a desiccator before reweighing. The loss in weight was calculated as percentage moisture content (Grabe, 1989). The procedure was repeated three times, and the average was used as the initial moisture content of the seeds.

Critical Moisture Experiment

The required moisture content for priming aged and fresh seeds was determined using an algebraic formula which increases the seed moisture content to a desired level.

Knowing the initial moisture content of a known quantity of seed (Initial Moisture Determination Experiment), the critical moisture can be determined by the following formula:

Critical moisture, fw basis = [(Desired % moisture content - Initial % moisture content) x (Initial weight of seed)]/(100 - Desired % moisture content) (A.G. Taylor, Personal Communication).

The critical moisture content value (f.w. basis) was converted into the amount of water to be mixed with the initial weight of the seed in order to increase the seed moisture content to a desired level. Desired moisture levels used ranged from 30 to 100% in 5% increments. For each desired moisture level, the procedure was repeated twice. The desired moisture level that gave the least germination rate was used as that critical moisture level that will allow seeds to imbibe water, but prevent radicle emergence (i.e. primed seeds). The seed and water mixture was held in air-tight glass tubes for seven to ten days. Then the seeds were dried for 12 h at room temperature and germinated under their optimal germination conditions (Germination Test Experiment), and their germination rates were recorded.

Pregermination and Gel-seeding Experiment

Fresh and aged seeds were germinated under optimum conditions (Germination Test Experiment) until radicle emergence. Pregerminated and non-pregerminated seeds were mixed with four gels; Natrosol 250 (hydroxyethyl cellulose) (Hercules Inc., Wilmington, Del.), Laponite 445 (magnesium silicate) (Laporte, Hackensack, N.J.), Liqua-gel (polyacrylamide) (Miller Chem. & Fert. Corp., Hanover, Pa.) and Agri-gel/Viterra hydrogel (water-soluble polymer) (Nepara Chem. Co., Harriman, N.Y.). Controls of aged and fresh seeds without treatments also were included.

Seedling emergence of gel-seeds and seeds without gels was investigated in the greenhouse. Five seeds per pot were planted 6 mm deep in a greenhouse medium (Baccto, Houston, Tex.), in pots of 10 cm diameter which randomly allocated in three blocks. Plants were watered as needed, and seedling growth was recorded daily for 3-4 weeks. Days to 50% (T-50) emergence and total percent emergence were analyzed by ANOVA using MSTAT (MSTAT, East Lansing, Mich.).

Solid-Matrix-Priming and Polyethyl Glycol-Priming Experiment

A sample of 0.5 g seeds was mixed with 10 ml distilled water and 10 g Celite and Aqua-mend or 50 g of silica sand. Celite (Aldrich Chem. Co., Inc., Milwaukee, Wis.) is a diatomaceous silica protective carrier, silica sand (Fisher

Scientific Co., Fair Lawn, N.J.) is a sand product, and AquaMend (Hydration Technology Corp., Santa Monica, Calif.) is a potassium polymer. The seed-protective carrier mixture was held at room temperature for seven to ten days.

Greenhouse seedling growth of the Solid-Matrix-Primed (SMP) seeds was compared with growth of control seedlings and Polyethyl Glycol (PEG)-primed seedlings. The PEG-primed seedlings were obtained by mixing a 25 ml solution of PEG (270 $g.L^{-1}$) providing -1.25 Mpa osmotic potential, with 0.5 g seeds for seven to ten days. After PEG-priming, the seeds were rinsed in distilled water while stirring for one minute. The seeds were drained and air-dried at room temperature for 1 h. The greenhouse seedling growth investigation methods and materials were the same as those in previous experiments.

Soil-Crusting Experiment

To test the best seed conditioning method under adverse conditions, all of the above treatments were tested under soil-crusting conditions in the greenhouse. The only exception was pregerminated seeds (Pregermination and Gel-seeding Experiment) because seeds (especially aged seeds) took long and variable time to germinate, causing large variation of radicle length.

Crusted and non-crusted soils of sandy (Marlette fine sandy loam) and loam (Glassoboric Hapludalf mixed mesic)

soil types (Michigan State University Horticulture Teaching and Research Center, East Lansing, Mich.) were used. The soil was steamed to eliminate weed seeds and finely seived. Crusted soil was obtained by irrigating soil (131 $ml.sec^{-1}$) at 0.5 m height. Seeds were planted in 10-cm diameter pots with holes underneath so that daily watering could be performed in trays, without interfering with the crusted or non-crusted top soil. Seedling growth was monitored using the same methods and materials as in other greenhouse experiments.

Chapter 3

RESULTS AND DISCUSSIONS

Experiment 1: Optimum Seed Germination Test

Fresh seeds exhibited mean germination of 78-80% at 20, 25, 30C and room temperature (25C±2) (RT) (Figure 4 & Table 2). There were no significant differences in germination response (P=0.05) at 20, 25, 30C, and RT. Aged seeds showed optimum mean germination of 52% at 30C (Table 2). Temperatures of 10 and 15C resulted in 0% germination for both fresh and aged seeds, as did 20 and 35C for aged seeds (Table 2, Figures 5a & 5d).

Table 2. Mean germination (%) of aged and fresh *A. tricolor* seeds at 10, 15, 20, 25, 30, 35C and room temperature (RT) after 18 days.

	Temperature						
	10	15	20	25	30	35	RT
	Mean Germination (%)						
Aged	0a≠	0a	0a	28b	52c	0a	14d
Fresh	0a	0a	78b	79b	80b	59c	79b

≠ Mean separation using LSD within horizontal rows by F test, P=0.05.

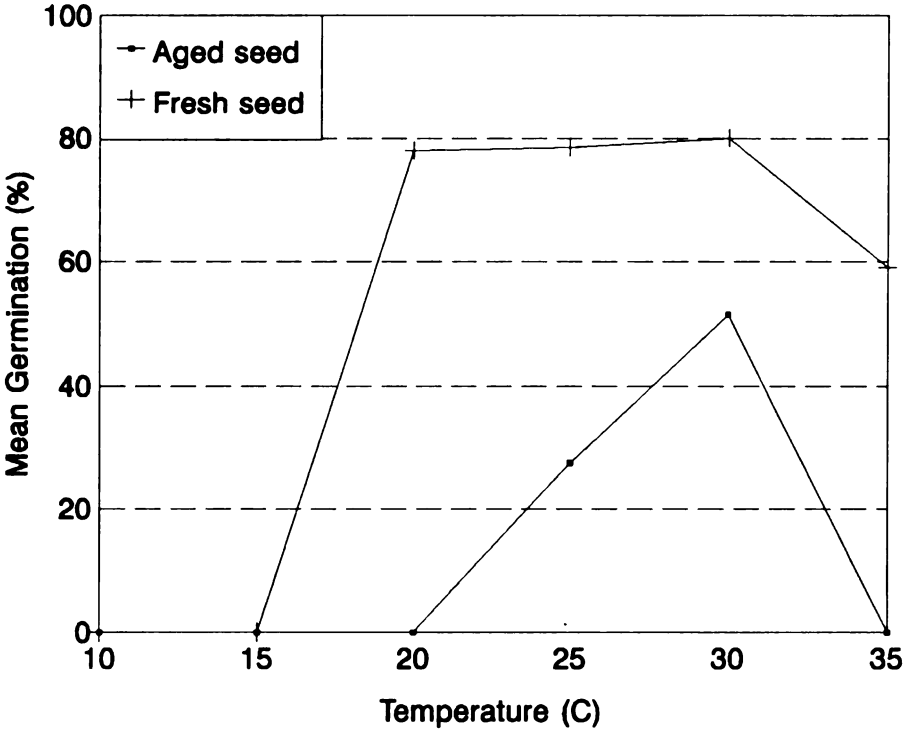

Figure 4. Mean percent germination of aged and fresh *A. tricolor* seeds at 10 to 35C after 18 days.

Aged seeds showed low percentage germination compared to fresh seeds (Figure 4). At very low (10, 15 and 20C) or high (35C) temperatures, aged seeds did not germinate (Table 2, Figure 5a & 4d). Fresh seeds exhibited a wide range of temperatures (20, 25, 30C and RT) for optimum percent germination; only 10 and 15C temperatures had the lowest percentage germination (0%).

The shortest times for seeds to germinate were two and four days for fresh and aged seeds, respectively, both at 30C (Figure 5c). For aged seeds, the shortest average time

to T-50 was 7.5 days at 30C (Table 3). The highest T-50 for fresh seeds occurred at room temperature (Table 3 & Figure 5e), whereas RT and 25C (Figure 5b & 5c) resulted in the highest T-50 for aged seeds. Fresh seeds exhibited a wide range of temperatures (20, 25, 30 and 35C) for low T-50, whereas aged seed germination was confined to one specific temperature (30C) for rapid germination (Table 3). Even though RT (Table 2) had significantly similar percentage germination (P=0.05) as other temperatures (20, 25, 30, 35C) for fresh seeds, it took longer for the seeds to germinate (Table 3). Part of the reason for this difference could be that the environment at RT was not controlled as strictly as in the thermogradient tables; e.g. light conditions.

Table 3. Mean time to 50% germination (T-50%) of aged and fresh *A. tricolor* seeds for 18 days at 10, 15, 20, 25, 30, 35C and room temperature (RT).

	Temperature						
	10	15	20	25	30	35	RT
	T-50% (days)						
Aged	- a*	- a	- a	13.0c	7.5b	- a	14.5c
Fresh	- a	- a	5.0b	4.0b	4.0b	4.0b	9.0c

*Mean separation using LSD at P=0.05.

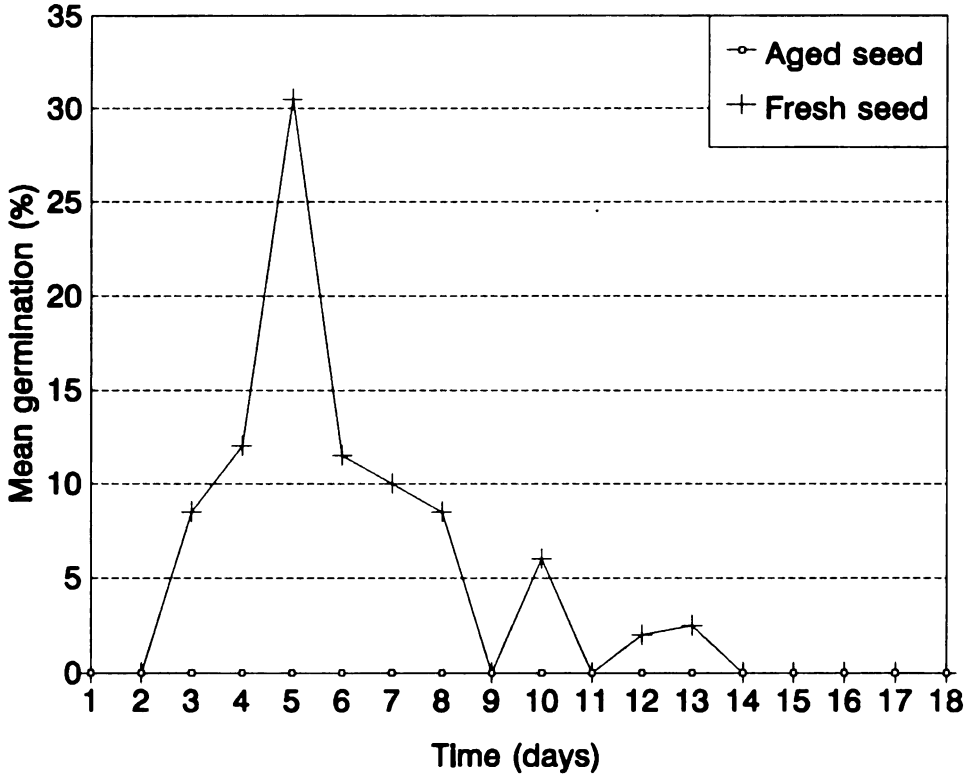

Figure 5a. Daily mean germination (%) of aged and fresh *A. tricolor* seeds for 18 days at 20C.

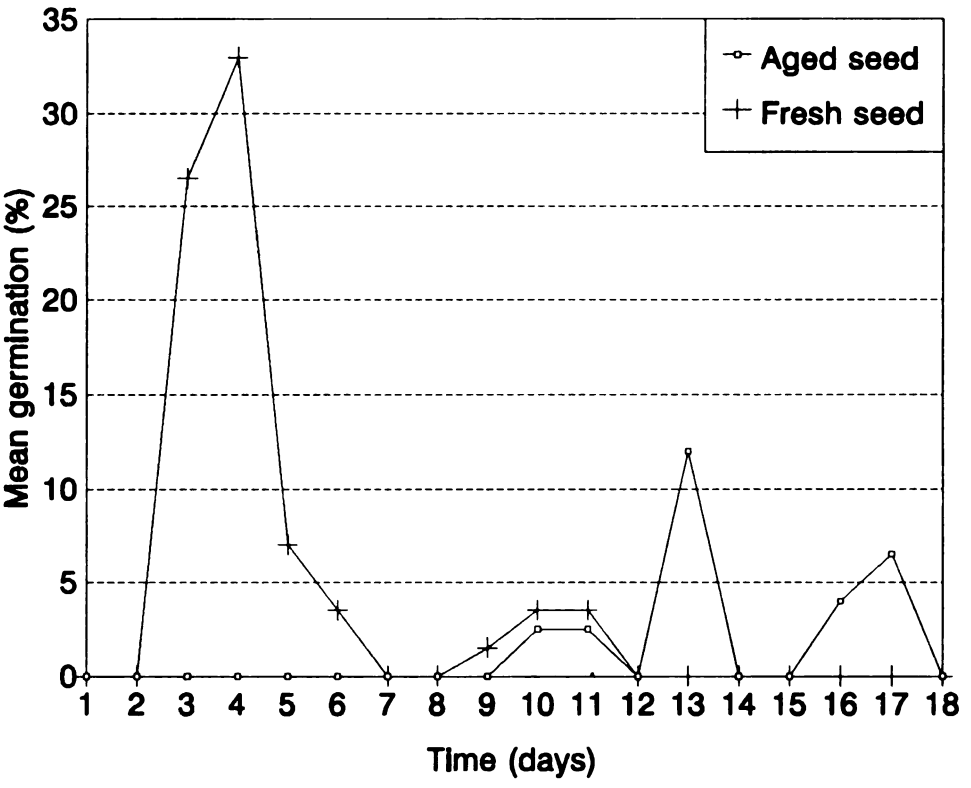

Figure 5b. Daily mean germination (%) of aged and fresh *A. tricolor* seeds for 18 days at 25C.

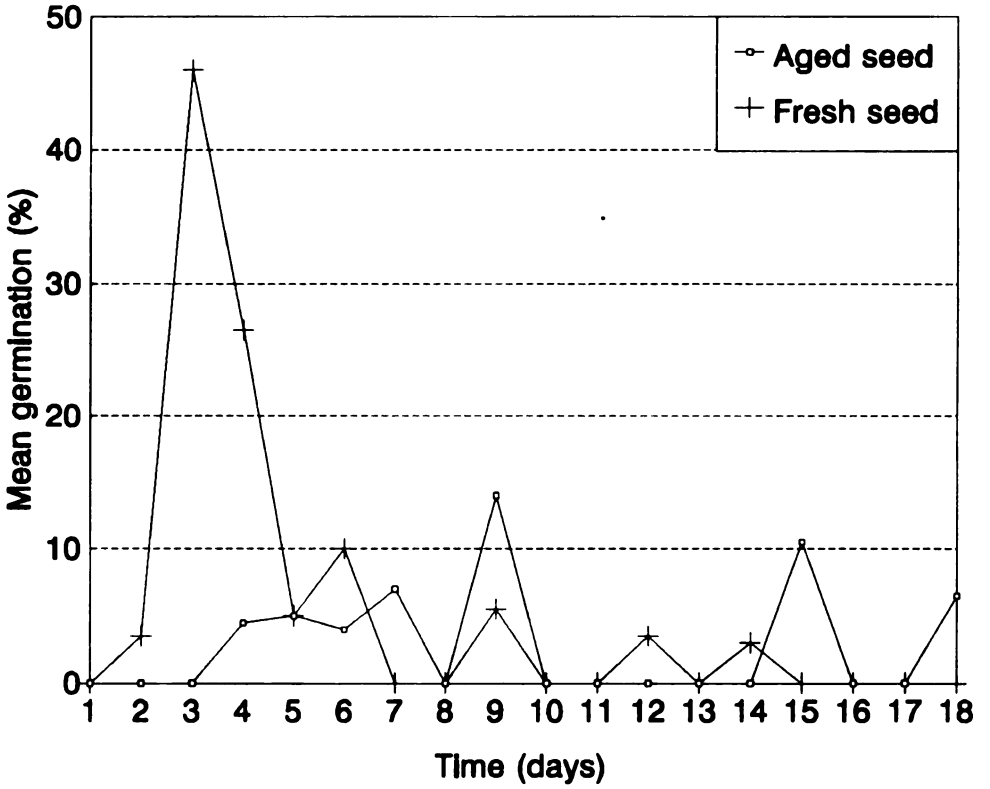

Figure 5c. Daily mean germination (%) of aged and fresh *A. tricolor* seeds at 18 days at 30C.

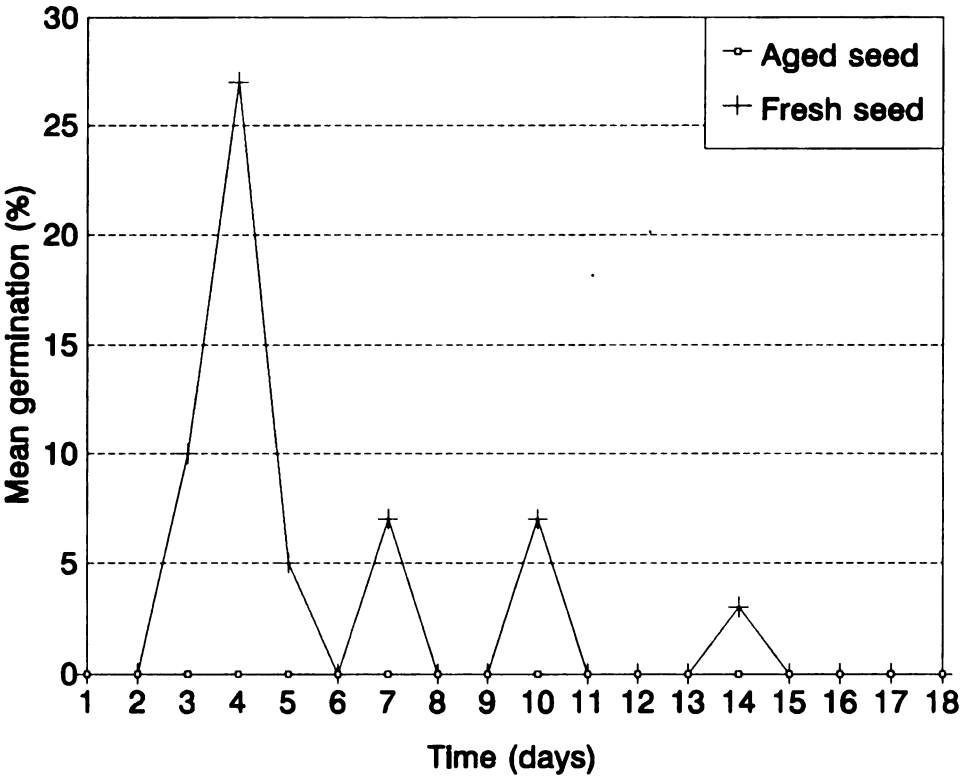

Figure 5d. Daily mean germination (%) of aged and fresh *A. tricolor* seeds for 18 days for 35C.

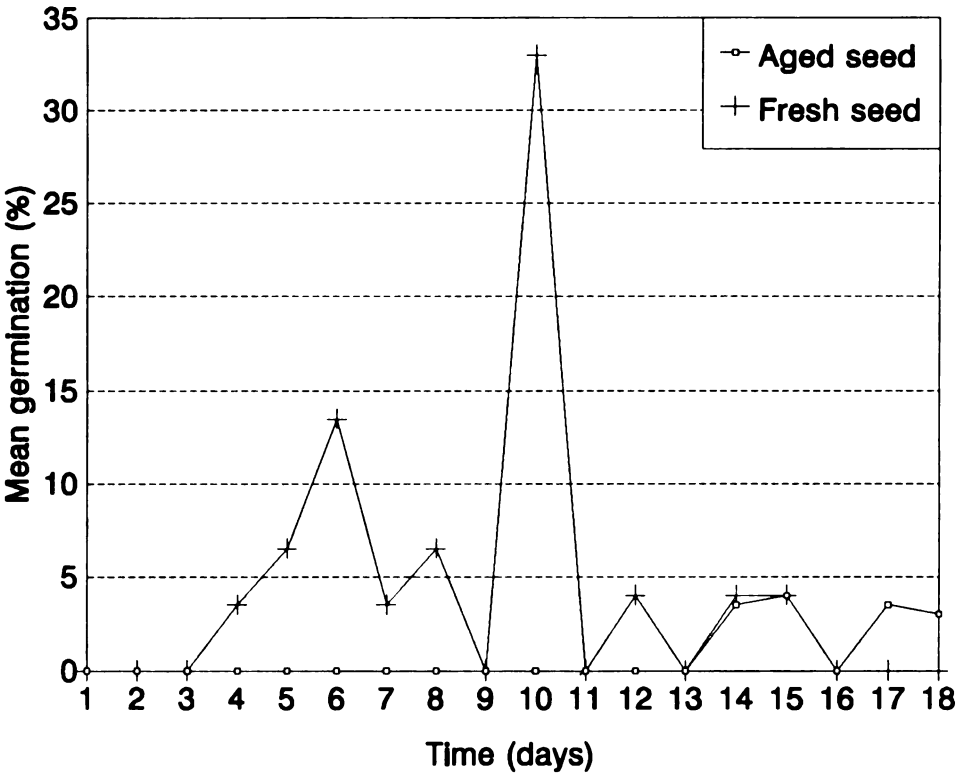

Figure 5e. Daily mean germination (%) of aged and fresh *A. tricolor* seeds for 18 days at room temperature.

This experiment supports one of the original hypotheses; that aged seeds' germination rate is much lower and slower than that of fresh seeds. It also showed the wide temperature germination ability of fresh seeds compared with aged seeds. These results were similar to those found by Clarke and James (1991) and Powell et al. (1991) in their study of germination performance of leek and brassica seeds, respectively. Aged seeds' germination rates were slower, and the time required for germination was greater than for

fresh seeds. The reason for the slower germination is due to reduced seed vigor. Reduced vigor involves the accumulation of degenerative changes until eventually the ability to germinate is lost (Naylor and Gurmu, 1990).

Experiment 2: Pregermination and Gel-seeding

There was a significant interaction (Table 4) between seed type, germination pattern and gel type on mean percent emergence. Overall, fresh seeds which were non-pregerminated, with all four gels; or pregerminated without a gel, significantly had better percentage emergence than all aged seeds (Table 5).

Table 4. The effect of germination pattern, gel type and seed condition of *A. tricolor* seeds expressed as percent seedling emergence.

Source of variance	DF	S.S.	M.S.	F
Blocks	2	280	140	
Seed type	1	16667	16667	37.31*
Error (a)	2	893	447	
Germination	1	1307	1307	7.0 ns
Seed type X Germination	1	4507	4507	24.14**
Error (b)	4	747	187	
Gel type	4	4507	1127	6.5**
Seed type X Gel type	4	2400	600	3.46**
Germination X Gel type	4	14426	3607	20.81**
Seed X Germination X Gel	4	3360	840	4.85**
Error ©	32	5547	173	
Total	59	54640		

ns, *, **:*Nonsignificant or significant at P=0.05 or 0.01 respectively.*

©or fresh seeds, there was a significant difference in percentage emergence between germination pattern and gel types. Non-pregerminated fresh seeds with Laponite, Natrosol, Agri-gel; or pregerminated fresh seeds without a gel, emerged significantly better than all other fresh seeds treatments. With all four gels, non-pregerminated fresh seeds gels gave significantly higher percentage emergence than pregerminated seeds with the four gels. When no gel was included, pregerminated fresh seeds gave significantly

higher percentage emergence than non-pregerminated seeds (Table 5).

For aged seeds, no significant difference in percentage emergence occurred with pregerminated or non-pregerminated seeds with all four gels; without a gel, pregerminated seeds obtained higher percentage emergence than non-pregerminated seeds and all other treatments with gels (Table 5).

Table 5. Effects of seed treatments on mean percent emergence of aged and fresh *A. tricolor* seeds.

		Seed Type	
		Aged	Fresh
Gel Type	Germination Pattern	Emergence (%)	
Laponite	No Pregermination	6.67cd	86.67a#
	Pregermination	6.67cd	26.67bc
Liqua-gel	No Pregermination	- d	20.67bcd
	Pregermination	6.67cd	6.67cd
Natrosol	No Pregermination	13.33cd	66.67a
	Pregermination	6.67cd	13.33cd
Agri-gel	No Pregermination	6.67cd	83.33a
	Pregermination	6.67cd	20.00bcd
No gel	No Pregermination	- d	20.00bcd
	Pregermination	40.00b	80.00a

Mean separation using LSD P=0.05

For fresh seeds, no significant difference in days to T-50 occurred, either with gel type and/or germination pattern. For aged seeds, non-pregerminated seeds with

Laponite and Agri-gel, and pregerminated seeds without a gel; had a significantly lower T-50 than all other treatments (Table 6). The highest percentage emergence as well as the shortest time to T-50 for fresh seeds were; non-pregerminated seeds with Laponite or Agri-gel gels, or pregerminated seeds without a gel. The best percentage emergence treatment in aged seeds, giving the shortest time to T-50 was pregermination without a gel.

The significant response of non-pregerminated fresh seeds to gels might be supported by the claims that the role of gels is to provide water for growth of germinated or dry seeds (Gray, 1981). Therefore, Laponite and Agri-gel gels were the best in providing water for the growth of non-pregerminated seeds.

Table 6. Effects of gel types and germination pattern on mean time to 50% germination (T-50%) (days) for aged and fresh *A. tricolor* seeds.

Gel Type	Germination Pattern	Aged	Fresh
		T-50% (days)	
Laponite	No Pregermination	10.0c	5.3a≠
	Pregermination	15.0d	7.0ab
Liqua-gel	No Pregermination	- e	8.5b
	Pregermination	15.0d	9.0bc
Natrosol	No Pregermination	10.0c	7.0ab
	Pregermination	11.0c	7.0ab
Agri-gel	No Pregermination	9.0bc	6.0a
	Pregermination	15.0d	9.0bc
No gel	No Pregermination	- e	7.0ab
	Pregermination	7.0ab	7.0ab

≠ *Mean separation using LSD P=0.05*

In contrast with results found in this experiment, studies by Odell et al. (1992), Pill (1986) and Ghate and Phatak (1982) on different crops found that pregerminated seeds with gels hastened seedling emergence and improved seedling emergence. The probable explanation for such differences may be the vulnerability of pregerminated seeds to physical damage during handling, and the variability of germination (even at optimal germination conditions) which force storage of seeds, resulting in reduced viability (Gray, 1981). Another reason for variations in crop

response to fluid-drilling noted by Pill (1986); was the variability of radicle length at the time of sowing a batch of seeds with variable germination rates. Taylor and Harman (1990) offered another reason for variation of pregerminated seeds. They suggested that reduction in plant stands of pregerminated seeds might be due to desiccation intolerance, and therefore death of seeds because of insufficient moisture for continued growth. Following that reasoning, with results obtained in this experiment, the possible explanation might be the inability of the gels to supply sufficient moisture to the pregerminated seeds to continue growth, or the failure of gels in protecting the vulnerable radicle. Another possibility for low performance of pregerminated seeds could be the desiccation intolerance of already germinated seeds during the germination process.

As a solution to the variability of pregerminated seeds, Pill (1986) suggested seed osmoconditioning before pregermination.

Experiment 3: SMP and PEG-priming

Since there were significant interactions among priming methods and seed type (Table 7), discussion for main effects was ignored. Emergence of fresh seeds did not significantly differ as a result of the type of priming method used. Within aged seeds, priming with PEG and Celite resulted in the best percentage seed emergence (Table 8).

Priming fresh seeds with silica sand and Celite gave the shortest time to T-50, whereas priming with PEG gave the longest time to T-50. Combining the highest percentage emergence with the shortest time to T-50, Celite and PEG gave the best responses for aged seeds (Table 8). If a choice between priming with PEG and Celite was given, Celite would be more favorable, because it is easier to handle than PEG (Taylor and Harman, 1990).

Table 7. Analysis of variance for mean percent emergence of Solid-Matrix-Primed and PEG-primed aged and fresh *A. tricolor* seeds.

Source of variance	D.F.	S.S.	M.S.	F
Block	3	110	36	
Seed type	1	15210	15210	51.00**
Error (a)	3	830	277	
Priming method	4	2460	615	2.65 ns
Seed type X Priming	4	4940	1235	5.33**
Error (b)	24	5560	232	
Total	39	29110		

ns, **: No significance and significance P=0.05, respectively.

Table 8. Effects of priming treatment on mean percent emergence, mean days to 50% emergence (T-50) of aged and fresh *A. tricolor* seeds.

	Seed Type			
	Aged		Fresh	
Priming Method	Emergence (%)	T-50% (days)	Emergence (%)	T-50% (days)
Control	49.00abc*	15.00e	53.00a	4.50ab
Celite	53.00a	9.00d	51.00ab	3.00a
Silica sand	30.26bc	7.00cd	71.00a	3.30a
AquaMend	28.72c	9.00d	52.50a	5.80bc
PEG	57.50a	8.30d	60.00a	9.00d

* Mean separation by LSD at P=0.05.

In their priming experiment with leek seeds, Clarke and James (1991) noticed the same trend as in this experiment; that, even though on the overall, priming can increase germination performance of aged seeds, their viability will still be lower than that of fresh seeds. The authors cited part of the reason for this as that aging the seeds had some effect on the nucleic acids of the whole seeds prior to germination. Even though subsequent priming increased germination percent, this was not enough to replace all the loss of nucleic acids. In conclusion to their study, Clarke and James (1991) suggested that the relationship between priming and aging was not simple, and therefore proposed that as seeds move down a viability curve, the beneficial effects of priming are reduced.

Performance of PEG-primed aged and fresh seeds was the same (Table 8). In their experiment, Pill and Evans (1994) found that PEG-priming invigorated mechanically harvested (low-vigor) amaranth seeds more than hand-harvested (high-vigor) seeds. They also showed that mechanically injured seeds can activate their nucleic acids during the priming process, whereas aged seeds cannot.

In their review on seed vigor, Matthews and Powell (1986) suggested that in controlling field conditions such as crusting, the economic benefits of presowing treatments such as priming and pregermination can be seriously undermined when seeds are of low-vigor. They argued that such expensive treatments can be of little benefit to low-vigor seed. They suggested that the major benefits of the many seed treatments lie in the indirect influence they have in ensuring that only highly germinable, vigorous seed lots are given expensive treatments. This leads to the conclusion that, in this experiment, even though overall response to priming was higher for aged than fresh seeds, priming fresh seeds would be more economical than priming aged seeds. Supporting this conclusion, are the arguments noted from prior statements that, the overall percentage emergence of primed aged seeds would not surpass that of fresh seeds due to viability problems.

Experiment 4: Presoaking

Using the gravimetric technique (see Materials and Methods: Initial Moisture Determination) in determining the initial moisture content (fresh weight basis), the average was 13% for both aged and fresh seeds.

The critical moisture content to prime seeds are shown in Table 9. However, the critical seed moisture content selected should prevent germination, because seeds become desiccation-intolerant after visible germination (A.G. Taylor, Personal Communication). Based on the results obtained, seeds started to germinate at 40% MC, therefore, 35% desired moisture content would be selected as the critical moisture content to prime seeds.

Table 9. The effect of moisture content (MC) (%) on germination of aged and fresh *A. tricolor* seeds when presoaked at room temperature for 7 to 10 days.

	Desired MC (%)									
	30	35	40	45	50	55	60	65	75	99
	Germination (%)									
Aged	0	0	1	3	5	50	70	80	85	90
Fresh	0	0	3	7	40	67	82	85	90	96

Table 10 showed that the mean percentage germination for 30, 35 and 40% desired MC was not significantly different in aged versus fresh seeds. 50% desired MC had

significantly the highest mean germination, but since 5% of the seeds (Table 9) had already germinated, these MCs would be undesirable for priming seeds. With fresh seeds, 30 and 35% desired MC gave the least mean percentage germination; whereas 40, 45 and 50% desired MC gave 59.5, 68.0 and 84.9% mean germination, respectively (Table 10). Even though 40, 45 and 50% desired MC gave higher percentage germination than 30 and 35% desired MC, they cannot be used as critical moisture contents, because the seeds were already visibly germinating (Table 9).

Table 10. Mean germination (%) of aged and fresh *A. tricolor* seeds at 30 to 50% desired moisture content at 30C.

	Desired Moisture Content (%)				
	30	35	40	45	50
	Mean Germination (%)				
Aged	49.90c	51.45c	52.65c	58.50b	66.50a*
Fresh	50.00d	51.00d	59.50c	68.00b	84.90a

*Mean separation using LSD within horizontal rows by F test, P=0.05.

Although mean percentage germination of 30 and 35% desired moisture contents did not surpass that of control aged and fresh seeds at 30C (Table 2 & 10), time to 50% germination was reduced (Table 3 & 11) by two days for aged seeds and one day for fresh seeds. In the case of aged seeds, these results were similar to those found by Nath et al. (1991). In their experiment with wheat seeds, seed

hydration treatments applied after aging did not allow recovery of germination percentage. The authors attributed their findings to the fact that aged seeds might have lost viability prior to treatment application. The hydration treatments did, however, result in marginal decrease in T-50 of the remaining viable seeds, as well as T-50 of fresh or non-aged seeds.

Contradicting the results found in this experiment and those found by Nath et al. (1991) are reports by Tarquis and Bradford (1992) quoting prehydration studies which improved the vigor of aged seeds. One factor which could be attributed to these contradictions is that duration and temperature of the presoaking or prehydration treatments differed in each of the experiments. Another factor could probably be the difference in crop species that were under investigation.

Table 11. Mean time to 50% germination (T-50%) of aged and fresh *A. tricolor* seeds at 30 to 50% desired moisture content at 30C.

	Desired Moisture Content (%)				
	30	35	40	45	50
	T-50% (days)				
Aged	6	6	6	4	4
Fresh	3	3	3	2	2

Although the initial moisture content (f.w.) of aged and fresh seeds was the same (13%), fresh seeds seemed to imbibe faster than aged seeds, since the latter obtained higher percentage germination rates (especially at 45 & 50% desired MC) than the former (Table 9). The reason for this scenario is that seeds (aged or fresh) will have the same moisture content as long as they equilibrate to the same Relative Humidity (A.G. Taylor, Personal Communication).

Vertucci (1989) used seeds of different chemical composition but identical water potentials. It was found that the permeability of the seeds' tissue to water was more important than initial moisture potentials in determining imbibition rates. In this experiment, the aged seeds' coats appeared to be less permeable than those of fresh seeds. Nonetheless, Vertucci (1989) concluded that seed permeability was a complex function of seed morphology, structure, composition, moisture, and temperature that could not be elucidated easily.

Experiment 5: Soil Crusting

There was a significant interaction between seed type, soil type, soil condition, and treatments[1] (Table 12).

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[1] Treatments = Seed Desired Moisture Contents (30, 35, 40, 45, 50, 55 & 60%), Non-pregerminated gel-seeding (Laponite, Liqua-gel, Natrosol, Agri-gel & AquaMend gels), Solid Matrix Priming (Celite & Silica sand), Polyethylene glycol Priming, and Control.

Compared with the control, some seed-invigorating treatments of aged seed in crusted loam soil gave higher significant mean percentage emergence (Table 13). Those that did were, 35, 50, 60% desired MC, non-pregermination seeds in Laponite and Agri-gel gels, SMP seeds with silica sand, and PEG-primed seeds.

With fresh seeds under crusted conditions on loam, treatments that produced significantly higher mean percentage emergence than the control were; 30, 35, 40 and 45% desired MC, non-pregerminated seeds in Liqua-gel, Natrosol, Agri-gel and AquaMend gels, and SMP seeds with Celite.

With aged seeds under crusted sandy soil, the control treatment's mean percentage seedling emergence was surpassed by the following treatments; 35 and 60% desired MC, non-pregerminated seeds with Laponite, Agri-gel and AquaMend gels, and SMP seeds with silica sand.

Table 12. Analysis of variance for aged and fresh seed types (seed), clay and sandy soil types (soil), crusted and non-crusted soil conditions (condition), administered with different treatments (trts).

SOURCE OF VARIANCE	DF	S.S.	M.S.	F
Blocks	1	76.6	76.6	
Seed	1	8326.6	8326.6	44.0^{ns}
Error a	1	189.1	189.1	
Soil	1	27639.1	27639.1	242.3^{**}
Seed X Soil	1	1.6	1.6	0.01^{ns}
Error b	2	228.1	114.1	
Condition	1	4726.6	4726.6	22.4^{**}
Seed X Condition	1	1314.6	1314.6	6.23^{ns}
Soil X Condition	1	2139.6	2139.6	10.1^{*}
Seed X Soil X Condition	1	4064.1	4064.1	19.3^{*}
Error c	4	843.8	210.9	
Trts	15	53198.4	3546.6	17.9^{**}
Seed X Trts	15	30098.4	2006.6	10.2^{**}
Soil X Trts	15	11685.9	779.1	3.95^{**}
Seed X Soil X Trts	15	10623.4	708.2	3.59^{**}
Condition X Trts	15	18398.4	1226.6	6.22^{**}
Seed X Condition X Trts	15	14510.9	967.4	4.91^{**}
Soil X Condition X Trts	15	15585.9	1039.1	5.27^{**}
Seed X Soil X Condition X Trts	15	14360.9	957.4	4.86^{**}
Error d	120	23662.5	197.2	
Total	255	241673.4		

$^{ns, *, **}$ No significance, significance at 5% and 1% , respectively.

Table 13. Mean emergence (%) of aged and fresh *A. tricolor* seeds treatments under crusted and non-crusted loam and sand soil types.

Treatment	Crusted				Non-Crusted			
	Loam		Sand		Loam		Sand	
	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh
	Mean Emergence (%)							
30% MC	30fgh	40efg	60cde	100a	30fgh	40efg	100a	30fgh
35% MC	80abc	80abc	100a	100a	30fgh	50def	90ab	70bcd
40% MC	20ghi	40efg	20ghi	20ghi	60cde	20ghi	100a	90ab
45% MC	20ghi	40efg	10hi	80abc	20ghi	0i	30fgh	60cde
50% MC	50def	30fgh	50def	40efg	20ghi	40efg	100a	20ghi
55% MC	10hi	10hi	40efg	90ab	30fgh	30fgh	100a	90ab
60% MC	40efg	20ghi	70bcd	10hi	40efg	70bcd	80abc	80abc
Laponite	60cde	20ghi	100a	30fgh	100a	40efg	60cde	80abc
Liqua-gel	30fgh	40efg	40efg	40efg	80abc	40efg	100a	20ghi
Natrosol	20ghi	50def	50def	50def	40efg	100a	100a	80abc
Agri-gel	100a	50def	100a	100a	80abc	100a	100a	50def
AquaMend	30fgh	40efg	100a	60cde	60cde	70bcd	100a	40efg
Celite	30fgh	40efg	60cde	30fgh	60cde	60cde	80abc	50def
Sand	80abc	30fgh	80abc	60cde	40efg	40efg	70bcd	20ghi
PEG	50def	20ghi	40efg	80abc	80abc	80abc	100a	100a

With fresh seeds under crusted sand, treatments 30, 35, 45 and 55% desired MC, non-pregerminated seeds with Agri-gel and PEG-primed seeds; produced significantly higher mean emergence than the control.

With aged seeds under non-crusted loam soil conditions, mean percentage emergence for the control was significantly lower than for seeds primed at 40% desired MC, non-pregerminated seeds in Laponite, Liqua-gel, Agri-gel and AquaMend gels, SMP seeds with Celite and PEG-primed seeds.

Mean percentage emergence of 35 and 60% desired MC, non-pregerminated seeds in Natrosol, Agri-gel and AquaMend gels, SMP seeds with Celite, and PEG-primed seeds with fresh seeds under non-crusted clay soil conditions was significantly higher than that of the control.

With aged seeds under non-crusted sandy soil conditions, all seed-invigorating treatments except priming at 45% desired MC produced significantly higher mean percentage emergence than the control.

Comparing mean percentage emergence of the control with all other seed-invigorating treatments with fresh seeds under non-crusted sandy soil conditions; 35, 40, 45, 55 and 60% desired MC, non-pregerminated seeds in Laponite and Natrosol gels and PEG-primed seeds, significantly surpassed the control (Table 13).

Due to the large number of treatments, and the significant four-way interaction, data were difficult to

interpret (Tables 12 & 13). To facilitate simpler presentation of data while testing the hypothesis that in crusted soil, the extent of seed invigoration differs with the invigoration method, promising treatments (from Table 13) were contrasted with each other or with a control under crusted and non-crusted soil conditions. The selected major treatments (priming by presoaking at 35% desired MC, gel-seeding non-pregerminated seeds with Agri-gel, SMP with Celite and silica sand, PEG-priming and control), were contrasted using SAS (SAS Institute, Inc., Cary, NC).

Contrasting treatments in crusted soil, there were significant differences (P=0.05) for; 35% MC vs. Agri-gel, 35% MC vs. control, Agri-gel vs. control, Celite plus sand vs. control and PEG vs. control (Table 14).

Contrasting treatments in non-crusted soil conditions, there were no significant differences between 35% MC and Agri-gel, but there were significant differences in percentage emergence between 35% MC and control, Agri-gel and control, Celite plus sand and control, and PEG and control (Table 14).

Table 14. Mean emergence (%) and contrasts of control, selected treatments of presoaking, gel-seeding, SMP and PEG under crusted and non-crusted soil conditions.

Treatment	Soil Condition	
	Crusted	Non-crusted
	Mean Emergence (%)	
35% MC	75.0	75.0
Agri-gel	95.0	75.0
Celite	57.5	45.0
Silica Sand	67.5	37.5
PEG	67.5	70.0
Control	25.0	25.0
	Contrasts	
35% MC vs Agri-gel	**	ns
35% MC vs control	**	**
Agri-gel vs control	**	**
Celite + Sand vs control	**	**
PEG vs control	**	**

**,ns: Significance by F-test at P=0.05 or non-significance, respectively.

Finally, the hypothesis was found true in that, in crusted soil, all invigorating methods differed in extent of invigoration. All selected treatments performed better than the control under both crusted and non-crusted soil conditions. Furthermore, all selected treatments resulted in equal or better percentage emergence under crusted than non-crusted soil conditions except for PEG-priming treatment (Table 14). These results also prove the hypothesis that

invigoration of amaranth seeds improves germination and seedling emergence.

Under crusted conditions, using Agri-gel for seeding non-pregerminated seeds was a better invigorating method than 35% MC, but under non-crusted conditions, both treatments performed the same (Table 14). Therefore, under crusted conditions, using Agri-gel to seed non-pregerminated seeds would be preferred over 35% MC. Comparing priming techniques in crusted soil, presoaking at 35% MC showed better potential for improving percentage emergence of aged and fresh seeds than did PEG-priming or SMP.

CONCLUSIONS

Makus (1984) stated that future commercialization of amaranth as a green vegetable depended upon solving problems associated with seedling establishment. The hypothesis that invigoration of seeds can improve emergence of seeds has been proven correct. Furthermore, aged seeds, with lower germination rates than fresh seeds at 20, 25, 30, 35C and room temperature; exhibited different emergence rates than fresh seeds for different invigoration methods. In gel-seeding, non-pregerminated fresh seeds emerged better than aged seeds. After PEG-priming and SMP, aged seeds responded better to priming than fresh seeds, but the overall percentage emergence of aged seeds was lower than that of fresh seeds. When presoaking was used for priming, aged and fresh seeds did not differ in percentage germination (in the lab), except at higher than 45% desired moisture contents.

In sub-optimal conditions (soil crusting) of loam and sandy soil types, this study showed that priming aged and fresh seeds by presoaking them in 35% desired moisture content (f.w.) can increase their vigor over control seeds, PEG-primed or Solid-Matrix-Primed seeds. Priming or invigoration method to be commercialized depends on the development of a reliable, economical, environmentally friendly, large-scale aerobic method that suppresses pathogen growth on the seed (Parera and Cantliffe, 1994;

Taylor and Harman, 1990). The presoaking method for seed invigoration is less expensive than SMP or PEG-priming, and, therefore, could be affordable even for small-holder farmers of lesser developed countries. Based on the preceding reasons and from results of this study, it would be recommended that the presoaking method (at 35% desired MC) be used to invigorate seeds under crusting conditions. For instance, 1.5 g seeds would be primed by soaking in 0.5 ml of distilled water at room temperature for 7 to 10 days.

Further research is needed for the success of the presoaking priming method. Since invigorating methods are influenced by a complex interaction of factors, including plant species, osmoticum, water potential of the priming agent, duration of priming, temperature, seed vigor, and dehydration and storage conditions following priming or invigoration (Parera and Cantliffe, 1994); more study is needed, especially regarding the optimum duration of presoaking and the interaction of presoaking duration at different temperatures.

In addition, to achieve consistent and beneficial results from priming or seed invigoration, it is advisable that only seed of high quality be used. To further refine invigoration methods and obtain greater and more consistent benefits from the process requires an understanding of the physiological and molecular effects of priming, which remains a largely unelucidated topic, regardless of the

quality and quantity of studies that have been conducted (Parera and Cantliffe, 1994).

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