AN ABSTRACT OF THE THESIS OF

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Title: Characterization and Inheritance of Low Temperature Germination Dormancy of Cucumber (Cucumis sativus L.)

Abstract approved _____
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Freshly produced seeds of six cucumber (Cucumis sativus L.) cultivars found to be incapable of germination at sub-optimal temperatures of 15°C and 20°C germinated at an optimal temperature of 30°C. Following various durations of low moisture (approx. 6.5%) seed storage (after-ripening) seeds of the same seed lots were capable of high percentages of germination (PG) at 15°C and 20°C. The term low temperature germination dormancy (LTGD) was proposed to describe this phenomenon.

The objectives of this research were to determine the effect of several seed production, handling and evaluation treatments on the duration of after-ripening required to eliminate LTGD. Additionally the inheritance of duration of LTGD (after-ripening requirement) was studied among populations resulting from two inbred line crosses. Duration of LTGD was determined by testing seed lots for low temperature germination ability after several increasingly longer after-ripening periods.

A shorter duration of after-ripening was required to reach near maximal PG at 15°C when the blotters used for germination testing were soaked in a 0.2% solution of KNO_3 rather than de-ionized water. However, germination at 15°C with this KNO_3 treatment was still dependent on prior after-ripening. The required duration of after-ripening for germination at 15°C was approximately doubled when the

temperature of after-ripening was lowered from 24°C to 15°C. After-ripening did not take place when dry seeds were stored at -10°C. Excised embryos of some cultivars also expressed LTGD at 15°C, although much less after-ripening was required for radicle elongation of excised embryos compared to that required for germination of intact seeds. Seed maturity at harvest affected the capability of freshly harvested seeds to germinate at 15°C and 20°C. LTGD was not detected for very immature seeds (50% of mature seed dry weight). Seeds allowed to develop to just slightly less than maximum dry weight required longer durations of after-ripening than mature (maximum dry weight) or past mature seeds.

When seeds were produced, handled, and evaluated under a standardized procedure, the cultivars Mincu Extra Early (Mincu) and Wisconsin SMR-18 (SMR-18) required from 50 to 100 days of room temperature storage (DORTS) to eliminate the LTGD effect at 15°C, while the cultivar Sumter required 150 to 250 DORTS.

The inheritance of the duration of LTGD was studied by determining the PG, after several durations of after-ripening, of parental, reciprocal F_1, F_2, F_3, and backcross populations from the crosses 'Sumter' x 'Mincu' and 'Sumter' x 'SMR-18', in two years. Heritable differences for duration of LTGD were expressed by the embryo genotype in both crosses. The maternal genotype had an additional influence on the duration of LTGD in the 'Sumter' x 'SMR-18' cross, but had little or no influence in the 'Sumter' x 'Mincu' cross. The results suggested that a single embryo-expressed allelic difference may explain the majority of the genetic variability for duration of LTGD among these cultivars. This gene was linked to the fruit spine color locus. However, one or more additional modifying genes are likely involved in the expression of parental type short LTGD.

Following bi-directional between and within F_3-family selection, a realized heritability of over 95% was obtained for PG at durations of after-ripening where the parental differences were maximum.

Characterization and Inheritance of Low Temperature Germination Dormancy of Cucumber (Cucumis sativus L.)

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CHARACTERIZATION AND INHERITANCE OF LOW TEMPERATURE GERMINATION DORMANCY OF CUCUMBER (Cucumis sativus L.)

INTRODUCTION

Cucumber (Cucumis sativus L.) is a warm season crop, which will normally germinate at temperatures of 15°C to 35°C (Lorenz and Maynard, 1980). Uniform and vigorous stands are readily obtained when high quality cucumber seeds are planted in relatively warm soils (22°C to 35°C). Yet when the same seeds experience sub-optimum soil temperatures (below 20°C) poor or non-uniform stands may often result.

Cucumbers for processing (pickles) are harvested in two ways, multiple hand harvest or once-over machine harvest. Because of an unavailability of labor willing to hand harvest, especially in the midwest states, there has been a desire to shift more acreage to mechanical harvest. The development of predominantly female cultivars, which have a concentrated fruit set on low nodes, has made once-over mechanical harvest a possibility (Lower and Edwards 1986). Cucumber fruit for pickles are used in the immature state and rapidly pass through the marketable stage in two to three days. Also, a cucumber plant will only support one or two developing fruit at one time, other fruit will not develop until the first fruit to set, are either picked off or mature far beyond the marketable stage. Therefore, the economics are such that, for a grower to profit from a mechanical harvest system, high plant populations of predominantly female hybrid cultivars are used and it is crucial that emergence and subsequent growth is uniform. Non-uniform emergence in cool soils is a serious problem limiting the profitability of mechanical harvest.

Cucumbers are planted on a schedule designed to result in an uniform flow of the product though the processing plant. Therefore, to maximize the utilization of the plant it is desirable to plant as

early in the spring as possible. Currently this practice is risky due to the possibility of poor stand establishment in cool soils.

An attractive solution to the problem of poor low temperature seedling emergence is to develop cultivars with genetically superior low temperature emergence ability. Preliminary studies with this goal in mind have been reported. Wehner (1982) has shown the existence of useful genetic variability for percent and rate of low temperature germination. Nienhuis et al., (1983) reported a substantial increase in percent germination at 15°C after three cycles of recurrent selection in an adapted heterogeneous cucumber population. In a different broad based cucumber population, Wehner (1984) estimated the half sib heritability for percent germination and days to germination at 17°C to be 0.48 and 0.61 respectively. Based on the apparent existence of genetic variability and moderately high heritability for percent low temperature germination, it appears that genetic improvement for low temperature emergence may be possible.

Evaluation of low temperature germination ability in cucumber can be confounded by a fresh seed dormancy (Nienhuis, et al. 1983). In general freshly harvested cucumber seeds germinate satisfactorily at optimum temperatures (25°C to 35°C), although when germination is attempted at sub-optimal temperatures (15°C to 20°C) various degrees of dormancy may be encountered (Nienhuis and Lower, 1981). This "low temperature germination dormancy" (LTGD) is normally eliminated during typical dry (low moisture) seed storage.

Recently there has been a trend towards greater international seed production and marketing. Under this system seed may be produced in one hemisphere and shipped to the other for sale and planting with only a relatively short intervening storage duration. Under such a system, growers may plant seeds which have been stored for considerably shorter durations than under the strictly domestic system.

The general objective of these studies was to further characterize several aspects of cucumber LTGD, with special emphasis on characterization of genetic differences for length of LTGD once

they had been identified. The long term goal, which initiated the research, was to assess the feasibility of a genetic solution to the problems associated with LTGD. Two general problems have already been implied; specifically, a hindrance to selection for low temperature emergence and the need to store freshly harvested seed for several months before it can be planted by growers which demand uniform low temperature emergence.

Although cucumber LTGD is known to occur, very little information is available on its characteristics. Therefore, the objectives of the initial LTGD experiments, reported in Chapter 1, were to determine the effects of several diverse factors on the evaluation of LTGD. These factors included the effect of three seed treatments — seed storage temperature, seed maturity and seed coat removal — on LTGD. The expression of LTGD was also evaluated at two germination temperatures: 15°C and 20°C.

Imbibing dormant seeds of several species with a solution containing nitrate (or nitrite) has long been known to promote germination (Roberts and Smith, 1977). Preliminary experiments (1984, not reported) showed that soaking germination blotters with solutions of KNO_3, in place of de-ionized water, resulted in a dramatic increase in percentage of germination at 15°C in some relatively freshly harvested cucumber seed lots. Therefore, the effect of KNO_3 treatment on LTGD also was evaluated in the experiments reported here. The initial reason for pursuing the effect of KNO_3, was to evaluate its possible use as a technique to stimulate dormant seed germination in breeding programs, where LTGD is a general hindrance to rapid generation advance. However, for reasons which will be addressed, a desirable LTGD evaluation procedure resulted when KNO_3 was added to the blotters during laboratory germination testing at 15°C. Therefore, KNO_3 treatment in several of the experiments reported here was not only a treatment variable, but also was a useful addition to the LTGD evaluation procedure.

To determine the potential extent of genetic variability for the duration of LTGD, six genotypes (cultivars) were compared in the

experiments reported in Chapter 1. One of these cultivars, Sumter, appeared to have a much longer after-ripening requirement than the others before the LTGD effect was eliminated.

Based on this preliminary evidence for the existence of genetic variability for duration of LTGD, a study was initiated (Chapter 2) to confirm the existence of genetic variability for the relative duration of LTGD among 3 inbred cultivars. Additional objectives of this study were to provide information on the mode of inheritance and possible selection procedures to shorten the duration of LTGD. The cultivars used as parents in this study were chosen to address immediate concerns outlined below and to maximize the possibility of finding genetic differences.

Sumter is a monoecious pickling cucumber cultivar which is used as a pollinator in probably 95% of the hybrid seed sold in recent years. A pollinator is required in modern predominantly female hybrids because these cultivars lack or have very few male flowers. Good pollination is important for obtaining an adequate fruit set and desirable fruit shape. Also, poorly pollinated fruit often have a high degree of carpel separation and placental hollows which dramatically lower the quality of the pickle. In the past few years, a high frequency of carpel separation and placental hollows have been reported in several fields. The reason for these reports appears to be a lack of pollen during the crucial pollination period. A possible explanation for a lack of pollen may be that the pollinator ('Sumter') failed to germinate or was delayed in emergence more than the hybrid.

Therefore, 'Sumter' was crossed to two cultivars (Mincu and SMR-18) both with a hypothesized LTGD requirement much shorter than 'Sumter', to provide the various genetic populations for the inheritance study. It was hoped that the results of this study could help determine if there was a genetic basis for the apparently longer LTGD of 'Sumter' which may result in poor low temperature emergence ability in some field situations. Also, since 'Sumter' and 'SMR-18' represent, and probably share a considerable amount of germplasm with many important cucumber lines, determining the

inheritance of LTGD among populations derived from them, may be useful information for the improvement of several cultivars or breeding lines.

REVIEW OF LITERATURE

Cucumber Fresh Seed Dormancy

Watts (1938) reported near complete crop failure when freshly harvested cucumber seeds from breeding stocks were planted in the winter of 1937. To investigate the possibility that fresh cucumber seeds had a rest period (dormancy) seeds of six 'Black Diamond' fruits were divided into six lots each and tested for germination after 0, 10, 20, 30, 40, and 50 days of room temperature storage. Germination tests were conducted at 20°C in moist sand. The results obtained were not consistent between fruits. Two of the fruits had over 80 percent germination (PG) at all storage times. Three of the fruits had less than 20 PG at 0 days storage but gradually increased to greater than 85 percent germination after 40 days of storage. The final fruit had less than 10 percent germination at all storage times until the last one at 50 days when it had 96 percent germination. In similar experiments a correlation between length of rest period and fruit maturity at harvest was not found. However, high percent germination was obtained for fresh seeds by either seed coat removal or incubation at 30°C. The conclusion suggested from these experiments was that freshly harvested cucumber seed inconsistently exhibited a rest period which lasted from 40 to 50 days.

Shifriss and George (1965) report a seed dormancy in a plant introduction line from India known as 'Baroda'. Fresh seeds failed to germinate at 30°C until after 6 to 12 months of laboratory dry storage. The inbred cultivar Marketer exhibited no dormancy under the same conditions. Preliminary investigations showed that the dormancy of 'Baroda' was unaffected by seed coat removal, far-red light treatment, oxidizing agents, or gibberellic acid. Dormancy was rapidly eliminated when seeds were stored at 90% relative humidity and high temperatures. About 6, 11, 45, and 170 days of 50°C, 40°C, 30°C, and 20°C of 90% RH storage were required respectively to reach the non-dormant state. Under the high

relative humidity, high temperature storage the viability of the non-dormant 'Marketer' suffered significantly.

Shifriss and George (1965) also examined the inheritance of 'Baroda' dormancy by producing and testing 'Baroda' and 'Marketer' parental seed, the F_1 and F_2 seed produced by reciprocal crosses between them, and seed produced by backcrossing F_1 plants to 'Baroda'. The 'comparative speed of germination' (ie. cumulative % germination curve over 0 to 60 days) of the above populations was reported for incubation of fresh seeds at 30°C. Complete germination of the 'Marketer' and F_1 seeds occurred by 2 days and 11 days respectively. The reciprocal F_1 populations differed by about 2 days indicating a slight maternal effect. The cumulative percent germination curves for the F_2, backcross to 'Baroda' and 'Baroda' were distinct from one another with each reaching about 80 percent after 24, 36, and 48 days. The mode of inheritance for speed of germination could not be determined with certainly. They suggested that relatively few genes were involved, perhaps three, on the following basis. After 10 days of incubation the F_1, F_2, and backcross to 'Baroda' seed had 100, 41, and 10 percent germination respectively. The theoretical expectation of three interacting dominant genes for rapid germination would result in F_2 and backcross percentages of germination of 42.5 and 12.5 respectively. This approach to gene number estimation for percent germination data probably represents a desperation attempt to describe the inheritance and without supporting data ought to be seriously questioned. They disregard the fact that the F_1 seeds had a clearly slower 'speed of germination' than 'Marketer', indicating partial dominance or additive effects.

Nienhuis and Lower (1981) also noted a positive effect of dry seed storage on percent germination at 15°C. In a heterogeneous adapted cucumber population, percentage of germination of fresh seeds tested at 15°C increased from 1% to 18% after 30 weeks of uncontrolled laboratory storage. Germination at 25°C exceeded 90% regardless of storage time. Since seed moisture content decreased from 7.2 to 5.0% over the 30 week storage period they were not sure

whether the beneficial response to seed storage was due to simply a decreased moisture content prior to imbibition or to the dissipation of low temperature germination inhibitors with age, or some other phenomena.

Edwards et al. (1986) tested the germination of freshly harvested seeds of three different cucumber populations at 15°C, 20°C and 25°C after one week and 6 months of storage. The seeds were open-pollinated and harvested from the field 3, 4, 5 and 6 weeks post pollination. Seeds of each maturity level were fermented for 0, 1, 4, 8, and 12 days. One population (WI cp/cp) was characterized by the presence of the homozygous recessive cp gene which confers compact plant habit. This population had seeds that were about 50% smaller and had a marked reduction for all germination parameters. In general, six months of storage had little impact on improving germination of the cp/cp population at any of the incubation temperatures.

Mature seeds of the two Cp/_ populations had zero percent germination at 15°C one week after harvest, but had greater than 50 percent germination when tested 6 months after harvest. A similar increase in percentage of germination was observed at the 20°C incubation temperature. The rate of germination at 20°C also greatly increased after 6 months of storage. Percent and rate of germination at 25°C were essentially maximal after one week of storage, and remained so after 6 months of seed storage.

Seed harvested only 3 weeks post pollination was only about 10 to 20 percent viable one week after harvest and nearly completely non-viable 6 months after harvest regardless of germination testing temperature. Seed lots harvested 4 weeks post pollination had greater than 80 percent germination at 25°C one week after harvest, indicating that about 4 weeks were required to develop seeds tolerant to seed drying. As would be expected the more mature seeds had in general more rapid rates of germination.

Seed fermentation durations of up to 4 days did not adversely affect percent or rate of germination. A general trend for lower percentages of germination with long durations of fermentation (8

and 12 days) was observed. They did, however, note an exception to the the adverse effect of long durations of fermentation on percent germination. Long fermentation treatments resulted in higher percentage of germination than short fermentation treatments, when mature seed lots, stored for one week, were tested at 20°C. Although after 6 months of storage the general relationship of lower percentages of germination with long fermentation was also observed for these conditions. From this evidence it was suggested that some factor involved in low temperature dormancy of fresh seeds was diminished by long fermentation, but fermentation also had a deleterious influence on seed storage ability, germination rate and germination percentage.

Seed Dormancies in Other Species

The literature pertaining to seed dormancy in general is much too broad to review adequately. Several reviews and books are available on seed dormancy (Bewley and Black 1982, 1986; Mayer and Poljakoff-Mayber, 1982). In the next few paragraphs an attempt is made to mention at least a sample of the literature relevant to the aspects of cucumber dormancy studied in this dissertation. Listed first are some examples of temperature dependent dormancy, and how the temperature of after-ripening effects these dormancies. Then some consideration is given to examples of seed coat-imposed dormancies and particularly to the point that some dormancies where seed coat removal promotes germination, the basis of the dormancy may still reside within the embryo tissue. A brief synopsis of nitrate promoted dormant seed germination is included, as well as some attempts to determine the mode of inheritance for a few seed dormancies.

Expression of seed dormancy in several species is dependent on the temperature at which germination is attempted (Vegis, 1964; Ross, 1980). Well known examples of this are found in cereal grains for example; wheat, barley, oats, (Harrington, 1923); triticale (Buraas and Skines, 1985); rice (Roberts, 1962); oats (Corbineau et al., 1986); and the weed wild oats (Naylor and Fedec, 1978).

Generally, fresh cereal grains will germinate at low temperatures (4°C to 10°C) but not at high temperatures (> 20°C). Following dry after-ripening (seed storage) grains will germinate under a much wider temperature range (approx. 1°C to 35°C; rice requires temperatures higher than those listed).

Dark germination of fresh redroot pigweed (Amaranthus retroflexus L.) is confined to occurring at only very high temperatures (approx. 40°C). However, after several months to years of dry laboratory storage, germination is possible over a much wider temperature range, 14°C to 40°C (Crocker, 1916; Schonbeck and Egley, 1980).

For dormancies in which dry after-ripening is affective, such as those listed above, increasing or decreasing the temperature of after-ripening decreases or increases the length of after-ripening required to reach the non-dormant state, respectively (Larson et al., 1936; Roberts, 1962; Buraas and Skinnes, 1985; Naylor and Fedec, 1978). Mares (1983) determined that fresh seed dormancy of wheat was preserved by freeze-storage (-15°C). Following several months of freeze-storage, seeds were moved to a normal after-ripening temperature and the rate of dormancy loss was essentially unchanged from non-freeze-stored seeds, as long as the moisture content of the seeds was below 12% when placed in the freezer.

Removal of the seed coat, fruit wall, subtending floral parts or endosperm of some dormant seeds alleviates the dormant state. This type of dormancy is often called "coat-imposed" (Bewley and Black, 1985). Removal of the seed envelopes could promote germination by several different mechanisms. In some seeds, particularly of the Leguminosae, the seed coat interferes with water uptake. In most other coat imposed dormancies the precise role of the seed coat is not clearly understood, but it is not due to water impermeability.

Evidence from work with Xanthium pennsylvanicum Wallr. (Esashi and Leopold, 1968), Lactuca sativa L. (Nabors and Lang, 1971) and Syringa sp. (Junttila, 1973) suggest the embryo coverings (particularly the endosperm) are primarily a mechanical barrier to

radical extrusion. Dormant embryos, for what ever reason, are unable to develop adequate growth potential to break through the seed coverings, while non-dormant seeds do have a sufficient growth potential. Thus the basis for these coat-imposed dormancies may actually be seated within the embryo.

Seed coats in some cases are known to interfere with gas exchange (rev. in Bewley and Black, 1985; 1982). Although dormant seeds are stimulated to germinate by supplying high oxygen tensions, in most cases the effect does not seem to be due to any need to supply oxygen for conventional respiration (ex. Katoh and Esashi, 1975; Porter and Wareing, 1974). For example the mechanism of coat imposed dormancy of charlock (Sinapis arvensis L.), appears to be due to the increased production of an inhibitor under low oxygen concentrations (Edwards, 1968a; 1968b), even though the concentration of oxygen reaching the embryo of an intact seed would be sufficient for germination if the inhibitor was not present (Edwards, 1969).

Imbibition of seeds in solutions of nitrite or nitrate salts has long been known to promote germination of dormant seeds of many species (see Roberts and Smith, 1977; for review and proposed mechanism). The mechanism by which nitrates affect dormancy has been studied in detail, and hypotheses have been proposed. Roberts and Smith (1977) suggest that it is utilized as an electron acceptor to preferentially drive the pentose phosphate pathway (PPP) under low oxygen conditions found in the seed. Bewley and Black (1982) point to the fact that involvement of the PPP in dormancy breaking has not been substantiated. In wild oats (Upadhyaya et al., 1981) the PPP appears not to be involved in the natural dormancy breaking mechanism, although nitrates significantly promote germination of partially after-ripened seeds. Adkins et al., (1984a) suggest involvement of alternate (CN resistant) respiration in dormancy breaking of wild oats, but in the next paper (Adkins et al., 1984b) they show that alternate respiration is not specifically required for nitrate promoted germination of dormant caryopsis. Although respiration in some form was required to produce sufficient ATP to

support germination induced by nitrate treatments. It seems clear that the mechanism of nitrate stimulated germination is still unclear, although it is generally recognized that it is the electron acceptor capacity of nitrate which seems to promote germination rather than it being a source of nitrogen.

Regardless of the mechanism by which it acts, KNO_3 is commonly suggested as a treatment to promote germination of fresh (dormant) seeds of a number of species (Association of Official Seed Analysts, 1981). Nitrate promotion in some cases is dependent on at least some prior dry after-ripening. The clearest example of this was recently reported by Adkins et al., (1984a) in wild oats. Several pure lines of wild oats have been developed, and subsequently intensely characterized for dormancy characteristics. In three dormant lines, imbibing seeds in nitrate, promoted germination relative to the water controls, but in each line promotion was only observed following a characteristic length of prior after-ripening. The length of after-ripening required was characteristic of the genetic depth of dormancy previously postulated on the basis of the length of after-ripening required for germination at various temperatures or the gibberellic acid concentration required to produce germination. A similar interaction between after-ripening and nitrate induced dormant seed germination was reported for red rice, Oryza sativa L. (Cohn et al., 1983).

Inheritance of Seed Dormancy

There are several reports in the literature of genetic variability for seed dormancy within a species, but in relatively few cases has there been an attempt to determine the mode of inheritance. The genetic variability for dormancy of weedy species, such as Avena fatua (wild oat) (Naylor and Jana, 1976) and Sinapsis arvensis (charlock) (Garbutt and Witcombe, 1986) has been studied to help understand the adaptive strategies these species have evolved which make them particularly troublesome weeds due to their seed dormancies.

Naylor and Jana (1976) collected seeds from individual wild oat

plants (< 1% outcrossing) at three different sites (generation 1). Differences for percentages of germination (tested 3 and 8 months after harvest at 20°C) among families collected at each site were observed. The range was from 0 to 100 percent. To determine if any of this variability was genetic, one progeny family was grown and tested from each generation 1 family. A genetic component of variability was postulated on the basis that, the average percentage of germination of the progenies originating from generation 1 families with 0 or 100 percent germination, were significantly different from each other. They report progeny - parent regressions (equal to heritability for inbred lines) for percent germination of

Subsequent research on inbred lines of wild oats showed that the duration of primary dormancy was dependent on germination test temperature (Naylor and Fedec, 1978) and on temperature, photoperiod, light quality, and water stress experienced by the mother plant during seed development (Sawhhey and Naylor, 1979; Adkins et al., 1987).

For the above reasons, Jana et al., (1979) studied the inheritance of seed dormancy in wild oats plants grown under defined growth chamber conditions. Parental (inbred), F_1, and F_2 seeds were used from all possible crosses between two lines selected for long dormancy (D) and one line selected for no dormancy (ND), although if did have a short dormancy. Dormancy was expressed as cumulative percent germination plotted over time in the imbibed state at 20°C. The ND parental seed reached 100 percent germination in 3 weeks, while both of the D parental populations had essentially zero percent germination for the 15 weeks of the experiment. They observed no reciprocal F_1 differences. F_1 seeds from one of the ND by D crosses gradually germinated over time until about 80 percent germination was obtained after 9 weeks and then no further germination took place. The other ND by D F_1 population also gradually germinated until 40 percent germination was reached after 5 weeks and no further germination took place. F_2 seeds from both these crosses showed a similar pattern of germination to their respective F_1 populations, only at slightly lower levels. The F_1

and F_2 seeds from the D by D cross had about 20 and 10 percent germination after 15 weeks of incubation. By classifying F_2 seeds as germinated or non-germinated after 2 weeks of incubation a satisfactory fit to a 1:3 ratio for germination (chi-square) was found for one of the D x ND F_2 populations. However at this time (2 weeks) the F_1 population also had 25 percent germination, which does not fit the hypothesis of mono-genetic segregation of a recessive allele for short dormancy. Since the rate of after-ripening (time to germination in the imbibed state at 20°C) was the important measurement of dormancy (in this case), they tried to interpret the data by constructing a genetic model based on the relative positions of the cumulative germination curves for the various genetic populations in each cross. By this criteria the data suggest that the ND parent carries one or more partially dominant alleles for after-ripening, and that the two D lines carry different alleles at at least 2 loci which can interact to cause the F_1 to have a shorter after-ripening requirement than either parent.

Witcombe and Wittington (1972) demonstrated genetic variability for dormancy in charlock (a cross pollinated Cruciferae) by establishing a line with consistently less dormancy (higher fresh seed percent germination and a lower GA requirement to overcome dormancy) by mass selection. They state that although it is useful to classify seeds of a population as either dormant (not germinated) or non-dormant (germinated), dormancy of charlock should not be considered discontinuous since some dormant seeds are more dormant than others, based on the concentration of GA required to cause germination.

Further, work on the mode of inheritance of dormancy in charlock was carried out by crossing two mass selected populations, one selected for short dormancy for 14 generations (ND) and one selected for long dormancy for 7 generations (Garbutt and Witcombe, 1986). Dormancy for the inheritance study was inferred simply by percentage of germination in a single germination test at 20°C. A significant reciprocal cross difference was obtained for the F_1 generations, indicating a maternal component of inheritance.

However, the embryo genotype also had an effect, because the ND parental seed had significantly higher percent germination than the F_1 seed produced on ND parental plants and likewise the D parental seed had a lower percent germination than F_1 seed produced on D parental plants. The mean germination of the F_2 seeds was just slightly (but significantly) higher than the F_1 seed produced on the D parental plants, thus indicating a general dominance for the maternal component of inheritance for long dormancy. They formulated a genetic model to explain the inheritance of dormancy between these two populations, consisting of a single major gene with maternal expression and complete dominance for long dormancy and one or more embryo expressed factors with less affect on dormancy. One hundred F_3 families (seeds from a single F_2 plant) were evaluated to test the model. There was a large discontinuity for percentage of germination of the F_3 families between 20% and 50%, thus families with less than 40 percent germination were classified as D and families with more than 40 percent germination were classified as ND. Following this classification, single gene segregation of the maternal component of inheritance was confirmed. The F_3 families were not helpful in providing additional information on the embryo component of inheritance.

Relatively few studies on the inheritance of dormancy have been reported in seed propagated crop species. Although it seems likely, that breeders of many crops are aware of genetic differences for dormancy in their germplasm. However, because it is difficult to measure dormancy in a precise and meaningful way, this information has largely probably not been published in major journals.

There are, however, several reports on the inheritance of sprouting resistance in cereal grains. Sprouting resistance is a form of fresh seed dormancy, where dormancy is desired to prevent preharvest sprouting under damp conditions. In most studies percentage of germination, at 20°C, of partially dry after-ripened grains has been measured (e.g. Noll et al., 1982; Gfeller and Svejda, 1960). This measurement has been assumed to be an indication of sprouting resistance; although Derera et al., (1977)

has suggested selecting for sprouting resistance in wheat based on characteristics of alpha-amylase production under simulated damp preharvest conditions.

Equating laboratory determined percentage of germination, with sprouting sprouting resistance has been useful in classifying cultivars for field resistance (Mares 1983). Noll et al., (1982) concluded that both the embryo and maternal genotype of a seed were important in the inheritance of sprouting resistance, by comparing reciprocal F_1 populations and parental lines for percent germination after partial after-ripening. Also, by selecting non-germinating seeds, following partial after-ripening, a wheat cultivar with a high degrees of sprouting resistance has been developed (Campbell and Czarnecki, 1981; Noll et al., 1982).

Gfeller and Svejda (1960) reported a heritability estimate for "seed dormancy" of 0.73, among randomly derived F_7 lines which resulted from crossing two wheat cultivars differing in sprouting resistance. "Seed dormancy" was actually measured as percent germination after 13 days of seed storage. They did not discuss whether this measurement was actually an appropriate measure of "seed dormancy". They also found a complete association between white grain color and lack of dormancy, and suggested dormancy may be pleiotropic to some or all of the three genes for red grain color.

CHAPTER 1. CHARACTERIZATION OF LOW TEMPERATURE GERMINATION DORMANCY OF CUCUMBER.

INTRODUCTION

The objective of the experiments reported in this chapter was to further characterize the phenomenon of fresh seed low temperature germination dormancy (LTGD) in cucumber. The effects of seed storage time, seed storage temperature, KNO_3 treatment, seed maturity at harvest, seed coat removal, genotype, and seed production environment, on low temperature germination were studied.

LTGD in cucumber was assumed to normally disappear as a result of normal dry-seed storage. The duration of seed storage required before LTGD was no longer a problem was the parameter of practical concern. To address this concern, fresh seed of various cultivars were produced both in the field and the greenhouse under fairly repeatable conditions. The seeds were uniformly dried and placed into storage. During storage seed lots were repeatedly sampled, after sequentially longer seed storage durations, and tested for germination at 15°C and/or 20°C.

The effect of seed storage temperature was investigated because, should it have an effect on duration of LTGD, manipulation of storage temperature could be used to hasten or lengthen the storage requirement. In the present study, three storage temperatures were chosen, each for a specific reason. Room temperature (approx. 24°C) was chosen because it represents a standard temperature commonly encountered for research purposes. It also approximates uncontrolled commercial seed storage. To simulate commercial controlled temperature seed storage, 15°C was used. Finally -10°C was chosen because if LTGD could be retained essentially unchanged during freeze-storage, this would be advantageous in planning and executing subsequent experiments. Such a strategy has proven useful for screening large amounts of wheat

germplasm for sprouting resistance (Mares, 1983).

The effect of imbibing freshly harvested seeds in a 0.2% solution of KNO_3 on PG and rate of germination was investigated to determine the effectiveness of this treatment on reducing or eliminating the expression of LTGD.

Cucumber seeds harvested over a wide range of maturities are capable of germination. A knowledge of the effect of seed maturity on LTGD would be useful in the conduct of future experiments, especially those concerned with detecting genetic differences, since seed maturity may contribute significantly to non-genetic variability.

Determining whether cucumber LTGD was seed-coat imposed was investigated, because it would be a useful starting place in determining the mechanism of dormancy. It also could aid in the understanding of the genetic control of LTGD, since the seed coat is genetically maternal tissue.

To identify genetic variability for duration of LTGD, up to six different genotypes (cultivars) were used in the various experiments reported in Chapter 1. The inheritance of the duration of LTGD is the subject of Chapter 2.

MATERIALS AND METHODS

1985 Field Study

Inbred pickling cucumber cultivars Addis and Wisconsin SMR-18 (SMR-18) were used. Seeds of each cultivar were produced in two 3.6m x 7.2m field plots at the SUNSEEDS research farm in Brooks, Oregon in the summer of 1985. The plots were covered with insect proof screen cages during flowering. Pollination was accomplished by placing a hive of bees in each cage for five days, beginning when most plants had open female flowers on the 4th to 10th node. Seed thus produced was of near equal chronological maturity. Fruits were harvested 55 days after hive introduction and the seed was extracted and cleaned without fermentation. The seed was dried for 48 hours in a 30°C forced air drier, then held five days at 15°C and 37 percent relative humidity. This procedure resulted in a seed moisture content of 6.5 $\pm$0.5% on a wet weight basis. Seed samples were then sealed in 470ml jars and one jar of each cultivar was stored at each of the three storage temperatures to be investigated: -10°C, 15°C, and room temperature (approx. 24°C). An additional treatment was created by dividing the -10°C lots after 21 weeks of storage and subsequently storing part of the seeds at room temperature.

Germination tests were conducted at 15°C$\pm$1°C approximately every 4 weeks, spanning a storage time (STIME) of 0 to 45 weeks. Optimal germination was determined at 30°C$\pm$ 1°C after 4 storage times 0, 3, 7 and 45 weeks. Each germination test (excluding the 0 week) included 12 treatment combinations, derived from the 2x3x2 factorial set of 2 cultivars, 3 storage temperatures and 2 imbibitional treatments (H_2O or KNO_3). Germination tests were conducted by placing 50 seeds on top of an 11 X 11 cm blotter, fitted individually into covered acrylic boxes. Blotters were wetted with 13 ml of either de-ionized H_2O or a .2% solution of KNO_3. Small amounts of additional H_2O were added as needed during germination tests to keep the blotters damp. Treatment

combinations, replicated twice, were completely randomized within each storage time. Germinated seeds, (radicle length 3mm or more), were recorded and removed daily. Germination tests were terminated after 19 days incubation. Data analyzed included percent germination after 19 days incubation, and mean days to germination (MDG) calculated as follows (Edwards et al., 1986):

$$MDG = \sum_{i=1}^{19} (g_i \, t_i \, / \, T)$$

Where g_i = number of seeds germinating on day t_i and T = total number of seeds germinated by day 19. Tests with zero percent germination were set to 20 MDG. Percentage data were arcsin square root transformed (angular; Steel and Torrie, 1980. pg 236) and MDG was transformed to its reciprocal (1/MDG) for data analysis. Reciprocal transformation of MDG decreased non-homogeneity of variance between storage times. Linear regression analysis was performed on selected subset of the 1/MDG data.

1985 Greenhouse Study.

Fresh seeds of six monoecious cultivars, Addis, Beit-Alpha MR (B-A), Mincu Extra Early (Mincu), Poinsett 76SR (Poins.), Wisconsin SMR-18 (SMR-18) and Sumter were increased from ARCO Seed Co. stock seed reserves. Plants at the cotyledon stage were transplanted into SUNSEEDS, Brooks OR, greenhouse hydroponic troughs on 27 June 1985. Each cultivar was randomly planted in 3 blocks. The day-night temperature was set at 35-27°C. The troughs were filled with pea rock and flushed 3 times a day with nutrient solution containing 1kg Chem-Gro (8-16-36), cucumber formula (Hydro-Gardens inc., Colorado Springs, Colorado), 0.36kg Mg- sulfate and 1.0kg of Ca-nitrate per 1500 liters of water.

Different seed maturities, days after pollination (DAP), were obtained by hand pollinating one female flower per plant with pollen from 3 or 4 male flowers of the same cultivar on the dates shown in

Table 1. Fruits were harvested on two dates shown in Table 1. Seeds of 10 to 20 fruits (the number depending on successful pollinations) were bulked within each cultivar - maturity treatment combinations. The seeds were separated from the pulp without fermentation, dried and stored at room temperature as described in the 1985 field experiments.

Percent moisture and dry weight of freshly extracted seeds was obtained for the 'Addis', 'SMR-18' and 'Sumter' lots as follows:

1) Samples were separated into 4 replications of about 50 seeds each.
2) Excess water was blotted from freshly harvested and washed seeds.
3) Seeds were placed over $CaCO_3$ for 5 minutes.
4) Fresh weight determined.
5) Dried for 1.25 hours at 130°C.
6) Dry weight determined.
7) Percent moisture was calculated as (fresh wt. - dry wt.) / fresh wt..

Germination tests were conducted essentially the same as in the 1985 field experiments. Two samples (reps) of 50 seed each were used for each treatment combination, reps were blocked by shelf in the incubator. All lots were imbibed in de-ionized water and tested at 15°C and 20°C after 1, 5, 11, 18, 23 and 30 weeks of room temperature storage. Each lot was also tested an additional time at 15°C after 37 weeks of room temperature storage. The above tests were primarily designed for the seed maturity part of the study. For detecting cultivar differences, more seeds of the 55 DAP seed maturity class were produced, and they were tested at more frequent intervals than those mentioned above.

Because no germination had occurred in the 15°C germination tests over 13 weeks of storage, an additional treatment was added to the experiment. KNO_3 was used to imbibe seeds in the 55 DAP maturity class, beginning with the 15 week test.

Optimum germination was determined at 30°C after the 1 week seed drying period. Seed viability was determined after the

Table 1. Pollination and harvest dates used to produce seed of various maturities.

Seed Maturity DAP[z]	Pollination Date	Harvest Date
25	21 August	15 September
30	16 August	15 September
35	11 August	15 September
40	6 August	15 September
45	16 August	1 October
55	6 August	1 October

[z] Days after pollination.

completion of the low temperature germination tests (19 days) by moving the non-germinated seeds to 30°C.

Embryos were excised from 55 DAP seeds and tested for radical growth at 15°C after 1, 8, 13, 18 and 23 weeks of room temperature storage. Embryos were excised after soaking whole seeds in de-ionized water for about 1 hour at 15°C. After soaking, the seed coat was gently split at the radical end and removed with a forceps. The nucellar membrane was then cut at the cotyledon end and peeled away. Two replications of 5 embryos each were desired. From previous experience it was known that some embryos may be damaged and susceptible to pathogen attack, therefore 6 or 7 embryos were obtained per rep, but data was utilized only on 5 viable embryos determined strictly by location on the blotter (ie embryos distal to the fifth one were used only if one of the first five proved to be non-viable when moved to 30°C). However, in this study only 3 of the 60 first five embryos tested proved to be non-viable. Five soaked whole seeds and five soaked seeds with seed coat only removed were also included in each replication.

Data analyzed were percent germination after 19 days of low temperature incubation, and mean days to germination (MDG) of those seeds which germinated. When zero percent germination resulted, the MDG was set to 20 for the 15°C germination tests and to 15 for the 20°C germination tests. Percentage data was square root arcsin (angular) transformed, but the transformation had no effect on the interpretation of the results, so original data will be presented. MDG was reciprocally transformed to solve the problem of non-homogeneity of error variance between storage times and to facilitate its interpretation as a rate of germination.

RESULTS

1985 Field Study

Seed moisture. Seed moisture of all the lots, determined at 1, 20 and 45 weeks of storage remained within the range 6.5% ± 1.5%.

Germination at optimum temperature. Percentage of germination (PG) for 'Addis' increased while mean days to germination decreased in 'Addis' and 'SMR-18' between zero and 1 week of storage (Table 2). Storage time had no effect on the PG of 'SMR-18' lot. Except for the slight differences observed between wet seed (0 weeks) and dry seed (1 week), additional storage time at any of the three storage temperatures did not affect PG or rate of germination in either cultivar. Also, KNO_3 treatment did not differ from water alone.

Germination at 15°C. Percentage of germination for the lots stored at -10°C were always less than 4%, regardless of storage time or KNO_3 treatment. Inclusion of the -10°C storage treatment in the factorial analysis of variance had the effect of lowering the pooled error estimation, because the variation between replications was usually zero. Therefore, to provide a more conservative error estimation the -10°C storage treatment data were not included in the statistical analysis of the other factors. A separate analysis of variance was first performed on the data obtained at each storage time separately. Evidence for non-homogeneity of error variance between storage times was not found, $P > 0.5$ Bartletts test (Steel and Torrie, 1980. pg. 471). Therefore, a combined analysis of variance was performed with storage time included as a subplot factor (Gomez and Gomez, 1984. pgs. 256 to 262). All the main effects (cultivar, KNO_3, storage temperature and storage time) were significant sources of variation for percent germination and 1/MDG at 15°C (Table 3). Because of significant interactions, all

Table 2. Mean percentage and days to germination (MDG) of 'Addis' and 'SMR-18', after 4 storage times, imbibed in water or 0.2% KNO_3 at 30°C.

Storage Time (Weeks)	Addis				SMR-18			
	Water		KNO_3		Water		KNO_3	
	%	MDG	%	MDG	%	MDG	%	MDG
0	91a[z]	3.31a	93a	3.47a	98a	3.72a	99a	3.67a
1	99b	2.16b	97ab	1.94b	97a	2.44b	99a	2.54b
3	100b	2.17b	100b	2.25b	97a	2.26b	98a	2.35b
45	100b	2.10b	100b	2.08b	100a	2.28b	98a	2.19b

[z] Transformed means, averaged over storage temperature and replications, separated within columns by LSD (0.01 level with unequal replication).

Table 3. Combined analysis of variance for percentage (PG) and rate ($1/MDG^z$) of germination at 15°C, of 2 cultivars ('Addis' and 'SMR-18'), subjected to various treatments; imbibition in KNO_3, storage temperature (15°C and 24°C) and storage time (0 to 45 weeks).

Source	df	Mean squares	
		PG^y	$1/MDG^x$
Cultivar	1	1644**	1.11*
KNO_3	1	11896**	2.25**
Cult x KNO_3	1	46ns	0.02ns
Storage temp (STemp)	1	30380**	100.88**
Cult x STemp	1	2ns	2.10**
KNO_3 x STemp	1	195ns	0.01ns
Cult x KNO_3 x STemp	1	201ns	0.14ns
Error A	8	46	0.12
Storage time (STime)	11	5908**	23.42**
Cult x STime	11	75**	0.52**
KNO_3 x STime	11	428**	0.19ns
STemp x STime	11	1026**	3.66**
Cult x KNO_3 x STime	11	22ns	0.19ns
Cult x STemp x STime	11	42ns	0.64**
KNO_3 x STemp x STime	11	205**	0.34ns
Cult x KNO_3 x STemp x STime	11	46*	0.23ns
Error B	88	23	0.20
Full model.	R^2 =	98%	96%
Model including main effects and 2-way interactions with STime only.	R^2 =	95%	92%

[z] Reciprocal mean days to germination.
[y] Angular transformed.
[x] Mean squares were multiplied by 1000 for presentation.
**, *, ns Significant at the 1% and 5% level and not significant respectively.

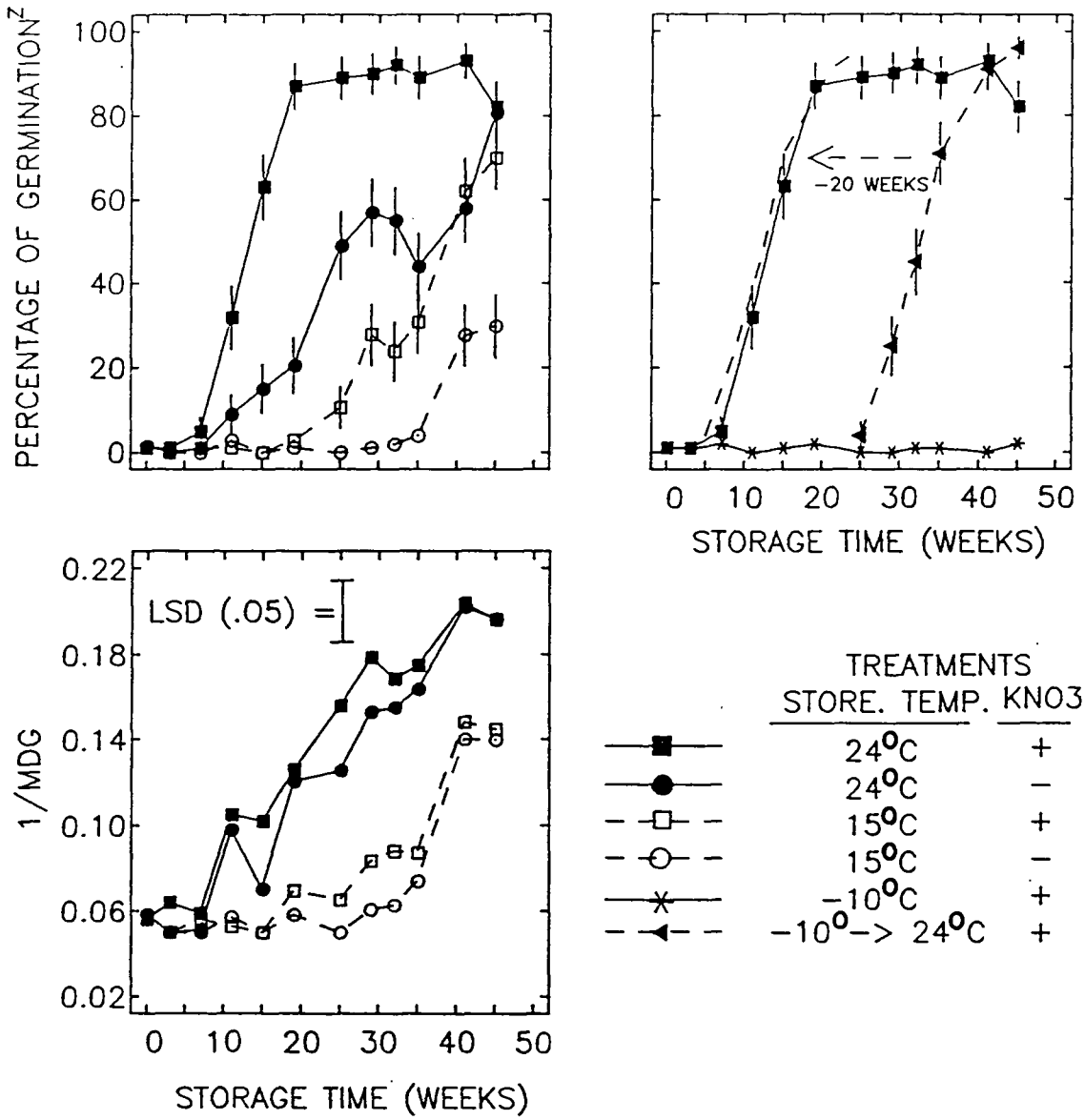

Figure 1. Percentage and rate (1/MDG) of germination at 15° for field grown seeds of 'Addis' after various durations of seed storage at -10°, 15° and 24°, with and without KNO_3 treatment.

[z] Bars represent LSD (.05 level).

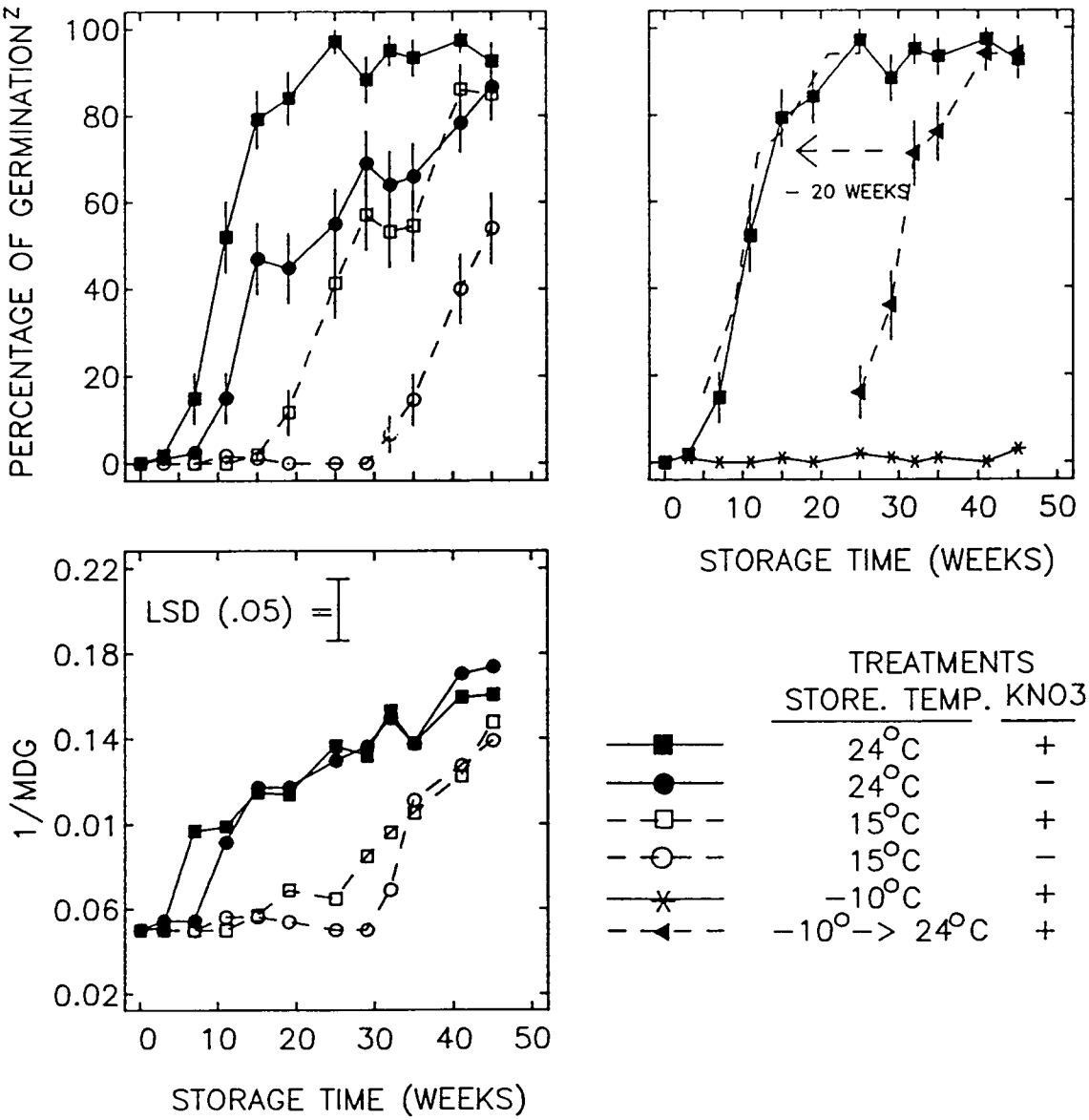

Figure 2. Percentage and rate (1/MDG) of germination at 15° for field grown seeds of 'SMR-18' after various durations of seed storage at -10°, 15° and 24°, with and without KNO_3 treatment.

[z] Bars represent LSD (.05 level).

individual treatment combinations are shown plotted with respect to storage time (Figs. 1 and 2).

The two cultivars differed slightly from one another but had similar responses to levels of the other factors (Table 3, non-significant interactions). 'SMR-18' had a higher PG than 'Addis' when significant difference occurred between them at various levels of the other factors.

Seeds of both cultivars failed to have significant germination following 0, 3 and 7 weeks of room temperature storage and when tested for germination at 15°C, imbibed in de-ionized water (Figs. 1 and 2). At 11 weeks 9% and 15% of the 'Addis' and 'SMR-18' lots germinated, indicating some of the seeds had satisfied their after-ripening requirement in 11 weeks. Maximal PG for the 24°C-H_2O treatment combination was attained in the 45 week test ('Addis'= 81%; 'SMR-18'= 86%). There was a significant increase in PG between 41 and 45 weeks of storage in the 'Addis' lot and between 35 and 45 weeks of storage in the 'SMR-18' lot, indicating some of the seeds in each lot were still dormant after 41 to 35 weeks of 24°C storage, respectively. The results of the successive germination tests clearly indicate that these lots expressed a LTGD effect to germination at 15°C, which disappeared during dry seed storage at 24°C. Individual seeds within in the seed lots investigated varied considerably for the duration of storage required before they could germinate at 15°C. Due to this variation the length of LTGD (after-ripening requirement) varied from a minimum of 11 weeks to a maximum of about 41 weeks in these lots. A possible explanation for this large variability may partially be attributed to a mixture of physiological seed maturities in these field grown lots. The results of the 1985 greenhouse study indicate that seed maturity has a significant effect on duration of LTGD (results presented in the next section). Although pollination was allowed to occur only over a 5 day period in this material, it is known that seed development proceeds at different rates depending on the number of pollinated fruit per plant and position of the fruit on the plant (McCollum, 1934; Tiedjens, 1928).

When seeds of these same lots, stored at 24°C, were imbibed in 0.2% KNO_3 instead of de-ionized water LTGD was eliminated by 7 to 19 weeks (Figs. 1 and 2). However, KNO_3 treatment alone was not sufficient to completely eliminate the dormancy effect (note PG for 24°C-KNO_3 in the 0 though 19 week tests).

Lowering the seed storage temperature to 15°C greatly increased the length of storage required to eliminate the LTGD effect. When seeds were imbibed in water significant germination was not observed until the 41 and 32 week tests for the 'Addis' and 'SMR-18' lots, respectively (Figs. 1 and 2). At the longest storage time investigated, 45 weeks, clearly a large percentage of the seeds stored at 15°C had not meet their storage requirement for germination at 15°C imbibed in water.

The KNO_3 treatment had essentially the same effect on the seeds stored at 15°C as it did on the seeds stored at 24°C. Specifically KNO_3 caused the seeds to germinate after less seed storage time than was required if the seeds were imbibed in water only, but KNO_3 promoted germination was also clearly dependent on prior dry seed storage.

Lowering the seed storage temperature from 24°C to 15°C appeared to effectively lengthen the duration of LTGD by 22 to 26 weeks. The evidence which suggested the above statement were as follows: For the KNO_3-treatment-combinations, near maximal PG of the lots stored at 15°C was first attained at 41 weeks ('SMR-18'= 86% ; 'Addis'= 62%), similar maximal and near maximal levels of germination were first attained for the lots stored at 24°C at 19 weeks (84%) for the 'SMR-18' lot and at 15 weeks (63%) for the 'Addis' lot. Thus, resulting in a 22 and 26 week difference in the duration of storage required to reach near maximal germination, when seeds of the two lots, were stored at 15°C compared to 24°C, respectively. A similar increase in the required duration of storage storage was also observed when seeds were imbibed in water. Comparable percentages of germination were obtained in the 19 week test for storage at 24°C and in the 41 week test for storage at 15°C ('SMR-18'= 44 and 40% ; 'Addis'= 21 and 27% respectively). Thus,

about 22 additional weeks of storage were required to attain approximately equivalent levels of germination at 15°C (imbibed in water) when the storage temperature was lowered from 24°C to 15°C.

Seed lots stored at -10°C (freeze-stored) failed to have greater than 3 PG at any storage time regardless of KNO_3 treatment or not (Figs. 1 and 2). To test whether freeze-storage maintains fresh seed dormancy essentially unchanged, the -10°C lots were divided at 21 weeks and part of each lot was subsequently stored at 24°C (-10°C - 24°C). The -10°C - 24°C lots were tested imbibed in KNO_3 only. The PG obtained for the -10°C - 24°C lots were nearly identical to the original 24°C lots when the storage time spent at -10°C was subtracted (20 weeks). This suggests that the dormancy breaking mechanism(s) either do not proceed or proceed very slowly when fresh dry seed is stored at -10°C.

Rate of germination. All treatment main effects (cultivar, KNO_3, storage temperature and storage time) and the 2-way interaction involving cultivar and storage temperature and several of the interactions involving storage time were significant sources of variation for 1/MDG (Table 3). Due to the presence of significant interactions individual treatment combinations were plotted (Figs. 1 and 2).

Clearly there was a trend for an increased rate of germination with additional storage time. The trend appeared linear for the 24°C storage temperature lots. Therefore 1/MDG was regressed on storage time, although storage times with less than 6 PG were not included to avoid treatment combinations with zero PG where 1/MDG could not be determined. The slopes of all the regression lines were positive and significantly greater than zero (Table 4).

In marked contrast to the effect of KNO_3 on PG, KNO_3 treatment did not affect the rate of germination in any of the lots where PG was greater than 6% (Figs. 1 and 2). The significant mean square for KNO_3 in the factorial analysis (Table 3) appears to be an artifact of assigning 20 MDG to experimental units with zero PG in order to balance the experimental design (there were several more 0 PGs

Table 4. Linear regression equations for rate of germination (1/MDG) at 15°C on storage time.

Cultivar (Storage Time[z])	Equation[y]	R^2	P-value[x]
	Y = a + b(x)		
Addis H_2O (11 to 45)	Y = 0.042 + .0035(x)	.91	< 0.001
Addis KNO_3 (7 to 45)	Y = 0.055 + .0035(x)	.92	< 0.001
SMR-18 H_2O (11 to 45)	Y = 0.075 + .0022(x)	.93	< 0.001
SMR-18 KNO_3 (7 to 45)	Y = 0.084 + .0018(x)	.93	< 0.001

[z] Range of 24°C storage times included in the analyses, in weeks, after omitting treatment combinations with less than 6 percent germination.
[y] Y = 1/MDG at x weeks; a = Y intercept; b = slope.
[x] Probability level of t-test (H_o: b = 0).

obtained for H_2O compared to KNO_3).

The rate of germination increased as storage time increased in both cultivars. In general the two cultivars did not differ for rate of germination except for treatment combinations where 'Addis' had less than 6 PG (in these cases 'Addis' had a lower 1/MDG) and after long storage times at 24°C where 'Addis' had relatively higher 1/MDG. Thus, it appears that for these particular lots, the 'Addis' lot ultimately had a more rapid rate of germination at 15°C as soon as LTGD was not a problem, but at shorter storage times its rate of germination appeared to be more suppressed by the dormancy phenomenon that those of the 'SMR-18' lot.

1985 Greenhouse Study

Fresh seed dry weight and moisture content. Dry weight per 100 seeds at least doubled between the 25 and 40 DAP seed maturity classes (Fig. 3). At 40 DAP seed dry weight reached a maximum in all three cultivars evaluated and remained relatively constant through 55 DAP. Percent moisture of freshly harvested seeds dropped rapidly between the 25 and 30 DAP followed by a more gradual decrease through the 45 DAP maturity (Fig. 3). From this limited data it appears that physiological maturity (maximum dry weight) occurred at about 40 DAP in these seed lots.

Germination at optimum temperature. The mean PG obtained when the 36 seed lots were tested at 30°C is shown in Table 5. All lots with a seed maturity of 35 DAP or older had above 95 PG after 1 week of storage, indicating that dormancy was not detected at an optimum temperature for germination in these lots. All the 25-DAP-maturity lots had poor germination (less than 53%). Some of the 30-DAP-maturity lots, especially 'Beit-Alpha', also had low PG. These same lots also had low viability even after 37 weeks of room temperature storage. Therefore it appears that many of the seeds in these immature seed lots were either not desiccation tolerant or too

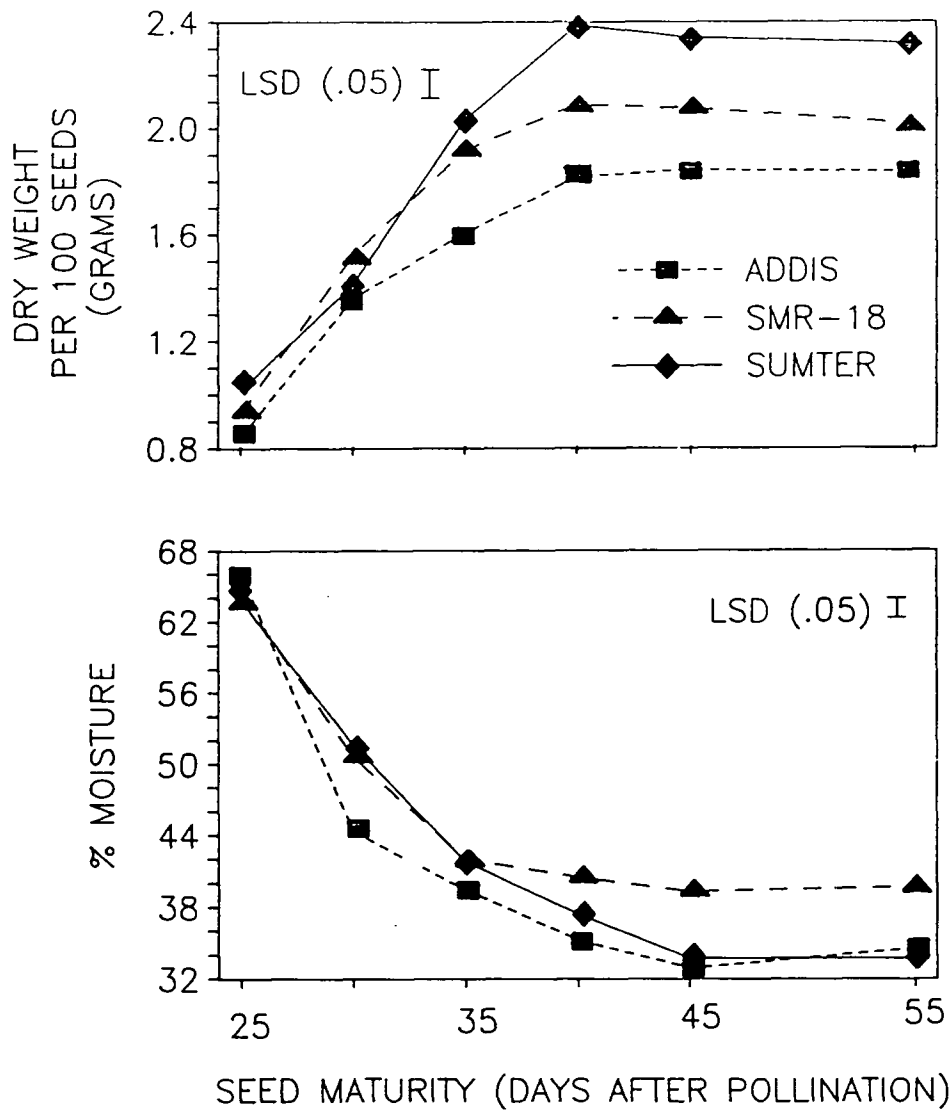

Figure 3. Dry weight and percent moisture of freshly harvested, greenhouse grown seeds of three cultivars harvested at six seed maturities.

Table 5. Percentage of germination at 30°C of six cultivars at six seed maturities, tested after a 1 week seed drying period.

	Percentage of germination					
	Seed maturity at harvest (DAP[z])					
Cultivar	25	30	35	40	45	55
Addis	23	92	95	96	100	100
B-A	0	65	100	100	100	100
Mincu	53	84	100	100	100	100
Poins.	0	86	100	100	100	100
SMR-18	51	94	98	99	100	100
Sumter	5	99	100	100	100	100

LSD (0.05) = 7.3

[z] Days after pollination.

immature to germinate, and were not dormant to germination at 30°C.

Germination at 15°C. All of the mature seed lots (40 through 55 DAP) had very low PG throughout the whole experiment when imbibed in water. Thirty percent was the highest PG obtained in any of the mature lots even after 37 weeks of room temperature storage. The mean PG for the 55 DAP lots of each cultivar are plotted in Figure 4. Because of a large non-homogeneity of error variance between storage times, an analysis of variance including blocks and cultivars was performed at each storage time. Significant variation due to cultivar (F-test, .01 level) was indicated only in the 35 and 37 week tests. In these two tests the mean germination of 'SMR-18' was greater than any of the other 5 lots (LSD, .05)

The 25-DAP-maturity lots of 'Mincu' and 'SMR-18' and the 30-DAP-maturity lots of 'Mincu', 'Poinsett' and 'SMR-18' had 10 to 70 PG (which was often up to 100% of the viable seeds) at 15°C imbibed in water, at most of the storage time tests (data not shown). Germination of these immature lots was rather erratic, and difficult to interpret since germination usually followed colonization of the seeds by an unidentified fungus. Additional information on the effect of seed maturity on LTGD was obtained for germination at 20°C, therefore it was analyzed in greater detail at that temperature.

Significant PG was obtained at 15°C when the 55 DAP lots of 'Mincu', 'Poinsett' and 'SMR-18' were imbibed in KNO_3 (Fig. 5). Cultivar differences for PG (with KNO_3 treatment) were indicated in all of the tests conducted (15 through 37 weeks) by a significant F-test on cultivar when the data for each storage time was analyzed separately. The cultivars fell into two distinct groups. 'Mincu', 'Poinsett' and 'SMR-18' had greater than 70 PG in the 20 through 37 week tests while 'Addis', 'Beit-Alpha' and 'Sumter' had less than 30 PG in the same tests. Cultivars of one group were different from cultivars of the other group but cultivars within a group were not different from each other (mean separation by LSD at .05 level).

Dormancy at 15°C was expressed by the 'Mincu', 'Poinsett' and

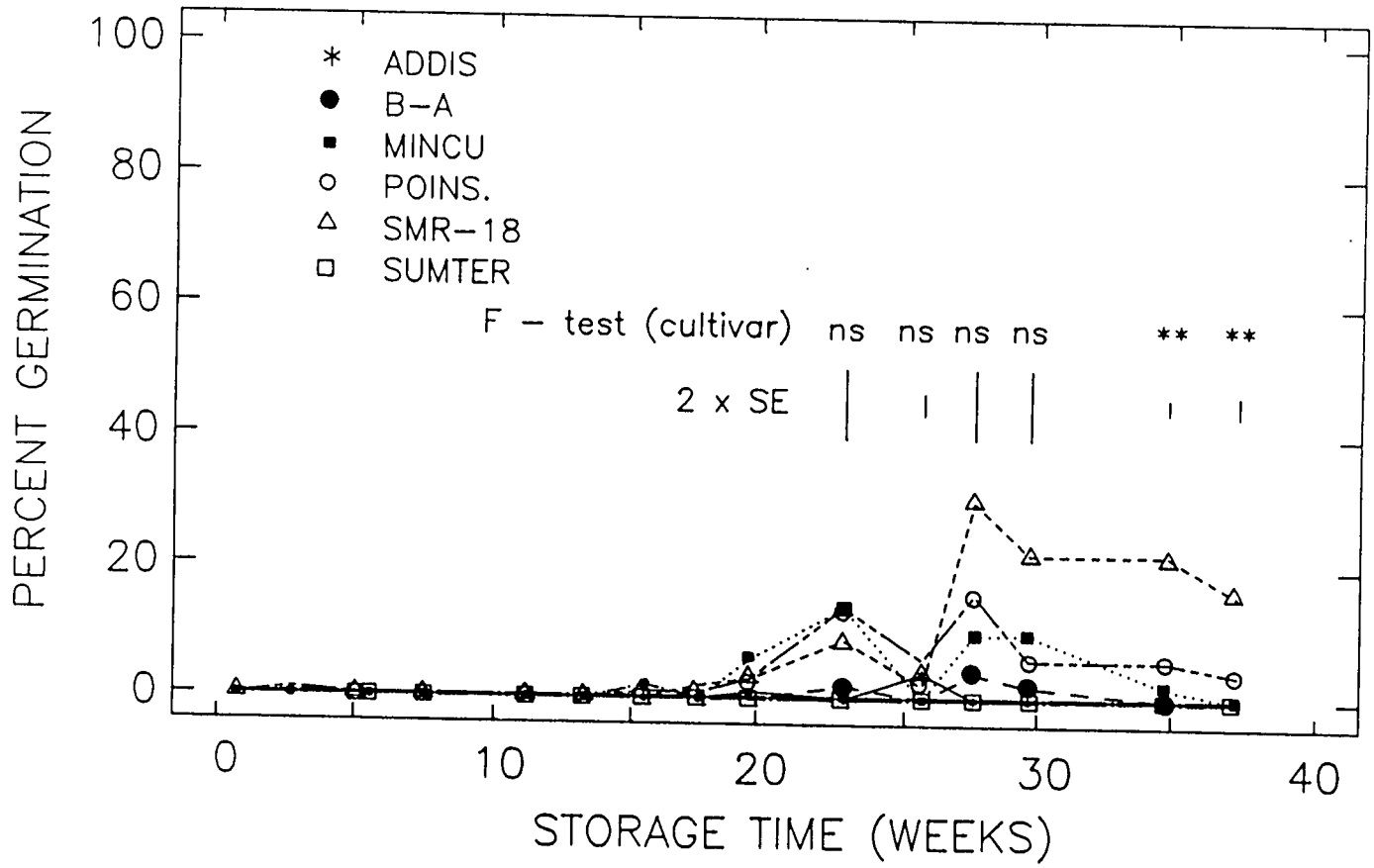

Figure 4. Percent germination at 15° (without KNO_3 treatment), after several durations of seed storage at 24°, for greenhouse grown seeds harvested 55 days after pollination.

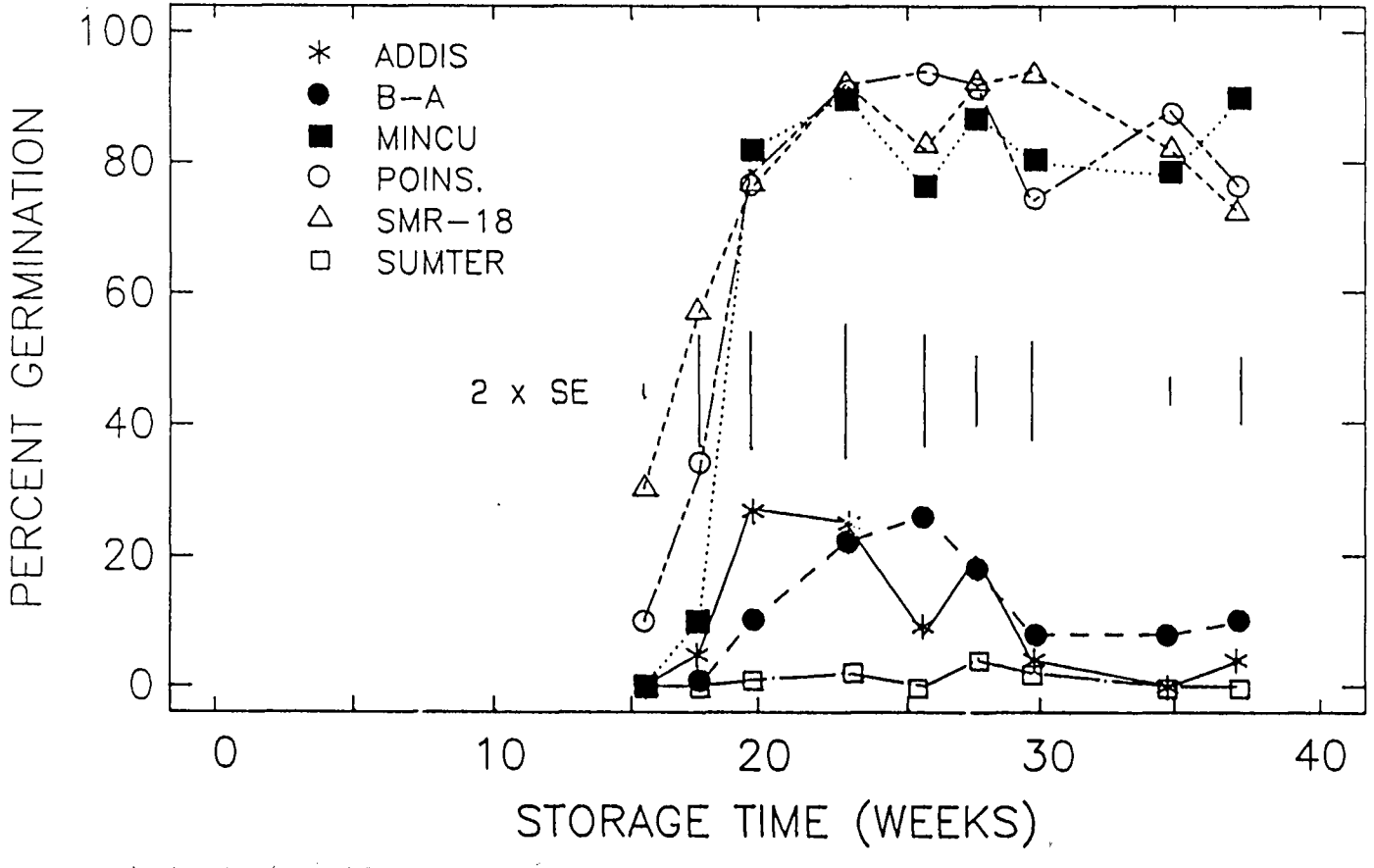

Figure 5. Percent germination at 15° with KNO_3 treatment, after several durations of seed storage at 24°, for greenhouse grown seeds harvested 55 days after pollination.

'SMR-18' lots when the seeds were imbibed in KNO_3 (Fig. 5). The increases in PG, and the termination of dormancy, occurred approximately at the same time for lots of all three cultivars (between 15 and 20 weeks of room temperature storage). The 'Addis' and 'Beit-Alpha' lots tended to have an increased PG in the 20, 23, 26 and 28 week tests, but these higher PG were due to germination in only one of the two blocks (shelves in the incubator). The means of the 'Addis' and 'Beit-Alpha' lots in the 20, 23, 26 and 28 week tests were not significantly different from zero (T-test 0.05 level). Therefore, an estimation of the room temperature storage duration required to eliminate the dormancy at 15°C was not obtained for these particular 'Addis', 'Beit-Alpha', and 'Sumter' lots. This failure was due to either a after-ripening requirement longer than 37 weeks, or to the possibility that these lots lack the ability to germinate at 15°C per se regardless of LTGD effect.

Germination at 20°C. Seeds of the 25-DAP-maturity class were largely non-viable, although, apparently all viable seeds in these lots germinated at 20°C at all storage times, because no further germination occurred when these lots were moved to 30°C after 19 days incubation at 20°C (the normal test for viability). The PG obtained for the 25-DAP-seed maturities as shown in Figure 6 (pg. 42) have not been corrected for seed viability.

Substantial percentages of non-viable seeds were also detected in the lots of the 30-DAP-seed maturity class. The percent viable seeds in each of the six 30 DAP lots averaged over all storage times were as follows; 'Addis'= 87% ; 'Beit-Alpha'= 55% ; 'Mincu'= 85% ; 'Poinsett'= 85% ; 'SMR-18'= 88% ; 'Sumter'= 94%. The average viability of the 35 DAP lots was 98%. Seed maturities of 40-DAP or older were more than 99% viable. In order to compare the effect of seed maturity on LTGD without the added complication of seed viability, the PG obtained for the 30-DAP-lots were corrected for seed viability by the following formula: PG = cumulative number of seeds germinated during 19 days incubation at 20°C / cumulative number of seeds germinated following 4 additional days at 30°C.

Percentages of germination obtained for the six cultivars, five seed maturities 30, 35, 40, 45 and 55 DAP) and six storage times (1, 5, 11, 18, 23 and 27 weeks of room temperature storage) were analyzed as a randomized block, three factor, split plot experiment. Cultivar and seed maturity were whole plot treatments and storage time was a sub-plot treatment. The blocking factor (shelves in the incubator) was non-significant (the block sums of squares were added to error A). The analysis was performed on angular transformed data, but the results were not perceptibly altered by the transformation so the non-transformed analysis is shown (Table 6). All the treatment interactions were highly significant, therefore each cultivar - seed maturity - storage time treatment combination was plotted with respect to storage time (Fig. 6).

Examination of Figure 6 clearly indicates that the cultivar Sumter consistently had lower PG than the lots of the other cultivars. A single degree of freedom comparison on the main effect of 'Sumter' verses the mean of the other cultivars was highly significant, accounting for over 95% of the sums of squares due to the main effect of cultivar. Further partitioning of cultivar Addis versus the average of 'Beit-Alpha', 'Mincu', 'Poinsett' and 'SMR-18' was also highly significant. The residual 3 df F-test was significant at the 5% level only, indicating that the differences in mean percentages of germination among the remaining 4 cultivars were not highly significant.

A second analysis was then performed on a reduced data set, omitting 'Addis' and 'Sumter' (Table 7). In the reduced data set all main effects and interactions except the cultivar x storage time interaction were significant. The magnitude of the mean squares for cultivar and interactions involving cultivar were small compared to the seed maturity and storage time mean squares. Therefore, to examine the average effect of seed maturity on PG at the different storage times the maturity-storage time treatment combination means averaged over cultivars were plotted (Fig. 7). The LSD's shown in Figure 7 were calculated after pooling the interaction sums of squares involving cultivar with the error term.

Table 6. Analysis of variance for percentage of germination at 20°C for 1985 greenhouse produced seed lots of six cultivars, harvested at 5 seed maturities and tested after 6 seed storage times.

Source	df	Mean Square	F
Cultivar (C)[z]	5	17811	313.5 **
S -vs- others	1	85202	1500.0 **
A -vs- (B+M+R+P)/4	1	3300	58.1 **
residual	3	184	3.2 *
Maturity (M)	4	10563	184.4 **
C x M	20	567	10.0 **
Error A	30	56.8	
Storage time (STime)	5	49708	930.8 **
STime x C	25	677	12.7 **
STime x M	20	2244	42.0 **
STime x C x M	100	300	5.6 **
Error B	150	53.4	

[z] Cultivar abbreviations are 'Sumter'(S), 'Addis'(A), 'Beit-Alpha'(B), 'Mincu'(M), 'SMR-18'(R), 'Poinsett'(P).
**,* Significant at 1% and 5% level.

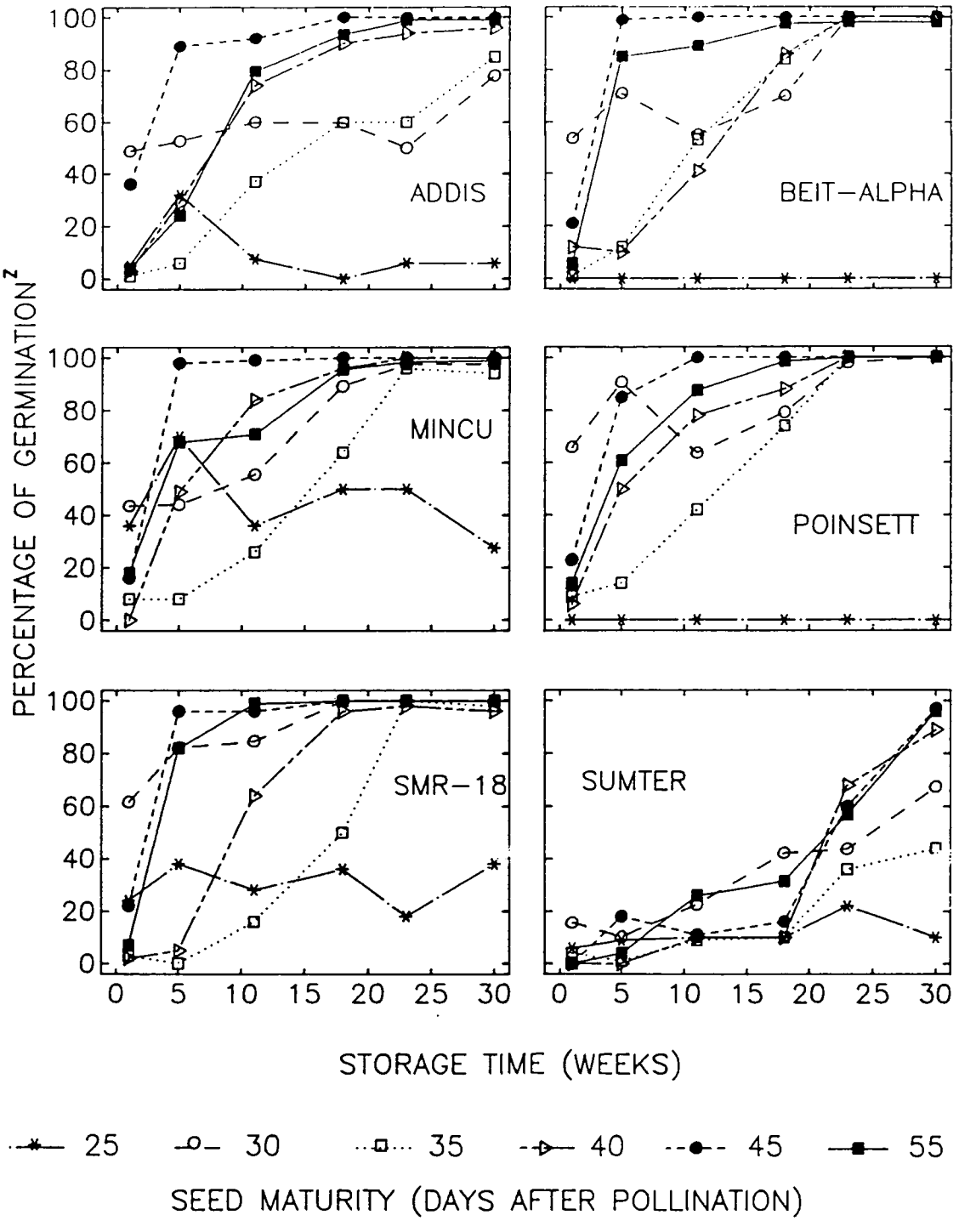

Figure 6. Percentage of germination at 20° after 6 seed storage durations of greenhouse grown seeds of 6 cultivars harvested at 6 seed maturities.

[z] LSD (.05 level) 14.5%

Table 7. Analysis of variance for percentage of germination (PG) and rate of germination (1/MDG) at 20°C for 1985 greenhouse produced seed of the cultivars 'Beit-Alpha', 'Mincu', 'Poinsett' and 'SMR-18', harvested at 5 seed maturities and tested after 6 seed storage times.

		Mean Squares	
Source	df	PG	1/MDG[z]
Cultivar (C)	3	184 **	36.0 **
Maturity (M)	4	8426 **	379.9 **
C x M	12	167 *	8.0 **
Error A	20	53.6	0.7
Storage time (STime)	5	38177 **	201.8 **
STime x C	15	58 ns	1.7 ns
STime x M	20	2353 **	12.5 **
STime x C x M	60	195 **	2.9 **
Error B	100	44.1	1.0

**, *, ns; F-test significant at 1%, 5% or not significant.
[z] Mean squares were multiplied by 1000 for presentation.

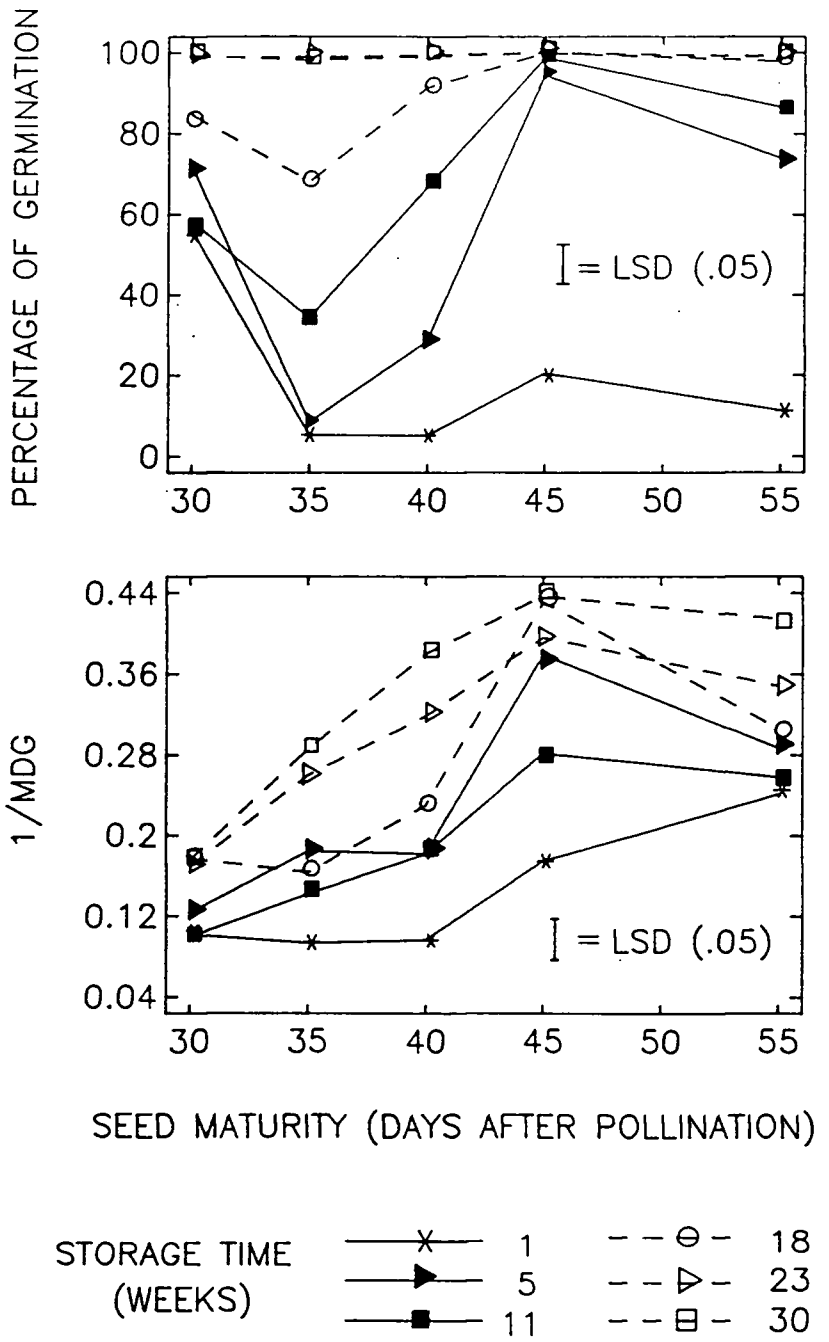

Figure 7. Percentage and rate (1/MDG) of germination at 20° after 6 seed storage durations of greenhouse grown seeds harvested at 5 seed maturities. Points are means of 4 cultivars and 2 replications.

The nature of the seed maturity-storage time interaction for PG was complex. After long durations of seed storage (23 and 30 weeks) all seed maturities had 100 PG. After one week of storage, seed maturities of 35 DAP or more mature had less than 20 PG, but nearly 60 PG was obtained for the 30-DAP-maturity. This data suggests that very immature seeds are less affected by (or possibly by-pass) the dormancy phenomenon. This conclusion is supported by the 25-DAP-seed maturity, where an effect of storage time on PG was not found (F-test; not shown).

The 35-DAP-maturity class had the longest average storage requirement. The difference in PG between the 30 and 35 DAP seed maturities was very large in the 1,5, and 11 week tests, but clearly PG was similar in the 23 and 30 week tests. Thus it appears that a important change takes place rather abruptly during seed development at about 30 DAP which substantially effects fresh seed low temperature germination ability. It appeared that this change actually happened quite close to 30 DAP since part of the 30 DAP seeds have a long dormancy and part had no detectable dormancy at all, and essentially all 35-DAP-seeds had a relatively long dormancy.

Considering the relatively mature seeds (40 to 55 DAP), the 45-DAP-maturity showed the most rapid and complete transformation from the largely dormant state at one week (>20 PG) to non-dormancy (> 95 PG) in the 5 week test. The 55-DAP-class on average had a slightly lower PG than the 45-DAP-class. However, before any biological significance can be placed on this observation, of a decrease in PG between the 45 and 55 DAP-seed maturities, the phenomenon would have to be shown to be repeatable (this has not been attempted). The average duration of dormancy for the 40-DAP-class was intermediate between the 35 and 45 DAP-classes.

The effect of seed maturity on the length of LTGD in the 'Addis' lots followed approximately the same pattern as discussed above (Fig. 6). The effect of seed maturity on length of LTGD for 'Sumter' lots was less defined than in the other cultivars (Fig. 6). The 'Sumter' 30-DAP-class did not avoid LTGD by nearly the same

magnitude as the 30-DAP-class of the other cultivars. The 35-DAP-class was apparently the most dormant, similar to that observed in the other cultivars. All three mature seed classes reacted similarly to storage time.

The rate of germination significantly increased with increasing seed maturity between 30 and 35 DAP and 35 and 40 DAP when tested after long seed storage times (23 and 30 weeks; Fig. 7). The rate of germination of all seed maturities also increased between 1 and 30 weeks of seed storage, although, in this experiment, the increase was not necessarily linear over the whole range of storage times. In fact there was little convincing evidence suggesting a significant increase in the rate of germination occurred with additional storage time after maximum percentages of germination had been realized.

Genetic variability for the duration of seed storage required to eliminate the dormancy effect was clearly indicated in the 45 and 55-DAP-lots (Table 8). As a reference point note that mature seed lots of all six cultivars had greater than 95 PG after 30 weeks of room temperature storage. Thus there were no significant cultivar differences for PG at 20°C after the dormancy requirement was satisfied. The cultivars could be unambiguously placed into two groups based on the relative duration of seed storage required to reach near maximal PG at 20°C. For the 45-DAP-seed maturity, the two group classification was readily apparent, all cultivars except 'Sumter' reached near maximal PG in the 5 week test, where as 'Sumter' had only 18 PG in the 5 week test and did not reach maximal germination until after 23 weeks of storage. The 55-DAP-lots did not fall quite so neatly into just two groups. Differences in PG at 5 and 11 weeks indicate up to four statistically different groups. However, the distinction between 'Sumter' and the other cultivars still remained as the major division. The relatively finer distinctions in PG among the other cultivars are difficult to unambiguously ascribe to genetic differences for LTGD, on the basis of one experiment.

Excised embryos. Seed coat and nucellar membrane (NM) removal was greatly eased by soaking the seeds in water at 15°C for about

Table 8. Percentage of germination obtained at 20°C for mature seed lots of six cultivars tested after six storage times.

	Percentage of germination (45 DAP[z])					
	Storage Time (weeks)					
Cultivar	1	5	11	18	23	30
Addis	36a[y]	89a	92a	100a	100a	100a
B-A	21b	99a	100a	100a	100a	100a
Mincu	16b	98a	100a	100a	100a	100a
Poins.	23ab	85a	100a	100a	100a	100a
SMR-18	22ab	96a	100a	100a	100a	100a
Sumter	0c	18b	11b	16b	60b	97a

	Percentage of germination (55 DAP[z])					
	Storage Time (weeks)					
Cultivar	1	5	11	18	23	30
Addis	4ab	24c	79bc	93a	99a	99a
B-A	6ab	85a	89ab	97a	98a	98a
Mincu	18a	68b	71c	96a	98a	99a
Poins.	14ab	61b	87ab	98a	100a	100a
SMR-18	7ab	82a	99a	100a	100a	100a
Sumter	0b	4d	26d	31b	57b	96a

[z] Days after pollination.
[y] Means separated by LSD 5% level within columns.

one hour. The soaked seeds with intact seed coats and soaked seeds with only the hard seed coat removed all failed to germinate at 15°C, on blotters moistened with de-ionized water, at all storage times investigated. Complete non-germination of whole seeds agrees well with the results obtained with these same seed lots (55 DAP) in the regular germination tests at 15°C (Fig. 4).

The percentage of excised embryos in which radicle elongation was recorded (out of 10 viable embryos) when incubated at 15°C on blotters moistened with de-ionized water are shown in Table 9. Following one week of seed drying radicle elongation was not observed for a significant percentage of the 'Beit-Alpha', 'Mincu', 'SMR-18' and 'Sumter' embryos, although 9 out of 10 of the 'Addis' and 'Poinsett' embryos expressed radicle elongation. In the 8-week test none of the 'Sumter' embryos showed radicle elongation at 15°C, however, at least nine of ten of the embryos of the other cultivars had radicle elongation. After 13, 18 and 23 weeks of room temperature storage essentially all excised embryos were capable of radicle elongation at 15°C.

Storage time significantly affected the reciprocal of the average time in the incubator before radicle elongation was recorded (1/MDG; Table 10). The average time before radicle elongation was first observed (MDG) significantly decreased between tests conducted at 1 and 8 weeks of storage for all cultivars except 'Sumter', in which a measurement was not possible (Table 9).

These results suggest that excised embryos also are affected by the LTGD phenomenon, especially those of the cultivar Sumter. However, a comparison of Table 9 with Figure 4 clearly illustrates that radicle elongation of excised embryos was possible after much shorter seed storage durations than those required for whole seed germination.

Table 9. Percentage and average time (MDG) before radicle elongation was observed for excised embryos of the 1985 greenhouse produced 55 DAP[z] lots, when incubated on water moistened blotters at 15°C.

	Percentage				
	Storage Time (weeks)				
Cultivar	1	8	13	18	23
Addis	90	100	100	100	100
B-A	40	100	100	100	100
Mincu	20	100	90	100	100
Poins.	90	90	100	100	100
SMR-18	30	100	100	100	100
Sumter	0	0	100	100	100
LSD (.05)	24.5	14.1	-	-	-

	Average time (MDG)[y]				
	Storage Time (weeks)				
Cultivar	1	8	13	18	23
Addis	10.2a[x]	6.9ab	6.2b	5.2b	5.2b
B-A	9.1a	7.2b	4.9bc	4.4c	3.8c
Mincu	12.0a	6.9b	6.0bc	5.1bc	4.8c
Poins.	10.5a	8.7ab	7.0abc	5.5c	5.6c
SMR-18	12.6a	7.8ab	5.6bc	4.7c	4.6c
Sumter	-	-	6.0a	4.8a	4.8a

[z] Days after pollination.
[y] Back transformed means from analysis on 1/MDG.
[x] Transformed means separated within rows by LSD (.05).

Table 10. Analysis of variance for 1/MDG for excised embryos of the 1985 greenhouse production of 55-DAP[z] lots, when incubated on water moistened blotters at 15°C at 5 seed storage times.

Source	df	Mean Square	F
Cultivar	5	0.00252	3.2 *
Storage Time	4	0.02405	30.9 **
Interaction	20	0.00028	0.4 ns
Residual	26[y]	0.00078	

[z] Days after pollination.
[y] Data obtained at 1 and 8 weeks for 'Sumter' treated as missing values.
**, *, ns; F-test significant at 1%, 5% or not significant.

DISCUSSION

Several annual plant species express seed dormancies which are temperature dependent, or have so-called "relative" seed dormancy (Bewley and Black, 1985). In other words, expression of dormancy is dependent on the temperature of the imbibed seed's environment. In the present study, freshly harvested cucumber seeds were tested for germination at 15°C, 20°C and 30°C. The results obtained agree with those previously reported (Watts, 1938; Nienhuis and Lower, 1981; Edwards et al., 1986), which suggested that expression of cucumber dormancy only occurs at temperatures below optimum for cucumber germination (15°C and 20°C). Accordingly, the term "low temperature germination dormancy" (LTGD) will be used to refer to this particular dormancy phenomenon.

Cucumber LTGD was found to disappear during typical dry (low moisture) seed storage. Such a phenomenon is typical for relative seed dormancies of other annual plant species (Bewley and Black, 1985). The processes occurring during seed storage are commonly referred to as after-ripening. The physiological mechanism involved in after-ripening remains essentially unknown.

In the present study, an attempt has been made to separate the phenomenon of LTGD from low temperature germination ability per se. Thus, seeds failing to germinate at low temperatures were assumed to be dormant only if fewer seeds from the same lot failed to germinate after longer durations of after-ripening.

Recently much consideration has been placed on improving the low temperature germination ability of cucumber seeds (Nienhuis et al., 1983; Wehner, 1984; Staub et al., 1986). The focus their research is to assure more timely and uniform emergence of crops destined to be mechanically harvested. The LTGD condition of cucumber seeds is an important consideration when evaluating them for low temperature germination ability. The present study addresses several characteristics of LTGD of cucumber seeds which have not been previously described. This increased understanding

should be helpful in further research devoted to the improvement of low temperature germination ability of cucumber seeds, and also as a guide to avoid serious crop loss due to the planting of dormant seeds.

Germination of partially after-ripened seeds was clearly promoted by allowing them to imbibe in a 0.2% solution of KNO_3 in germination tests at 15°C (Figs. 1 and 2). KNO_3 treatment alone was not sufficient to completely eliminate LTGD, but had the affect of shortening the duration of after-ripening required before germination was possible at 15°C. Similar interactions between after-ripening and nitrate treatment have been reported for the relative seed dormancies of Avena fatua (Adkins et al., 1984a) and red rice (Cohn et al., 1983). In these species, although the after-ripening requirement was shortened by nitrate treatment, the ranking among lines for characteristic genetic depth of dormancy was not altered. Evidence to date suggest that such a relationship also exists for cucumber LTGD (Figs. 1 and 2; and non-reported research)

Possibly KNO_3 could be utilized in germination tests designed to select for superior low temperature germination ability or short dormancy genotypes. The advantage of such a system would be that the screening could take place after a considerably shorter seed storage (after-ripening) time, thus accelerating the whole breeding process.

Cucumber LTGD is especially troublesome to a breeding program designed to select for low temperature germination ability per se, but it also can present considerable problems for cucumber breeding programs in general. It is often desirable to establish plants from freshly harvested (dormant) seeds in order to rapidly advance breeding materials. To accomplish dormant seed germination, breeders often incubate the seeds at high temperatures (under heat lamps or pads). If the plants are to be grown in the field, seeds may be germinated in the greenhouse and seedlings transplanted, or the breeder may try to delay planting until warm soil temperatures are more likely to occur (which may defeat the purpose of rapid generation advance). KNO_3 treatment seems to offer an alternative

or addition to methods now commonly used to facilitate the germination of freshly harvested seeds. In fact, the percent and uniformity of field and greenhouse planted dormant seeds has been increased by drenching the freshly planted seeds with a KNO_3 solution (personal experience).

The duration of seed storage (after-ripening) required to overcome fresh seed dormancy at 15°C was approximately doubled by lowering the storage temperature from 24°C to 15°C in the present study. This result suggests that when freshly harvested cucumber seeds are to be planted soon after harvest the dormancy effect can be diminished more rapidly by higher seed storage temperatures. However, relatively high seed storage temperatures are also known to adversely affect the longevity and vigor of most orthodox seeds, including cucumber (Bewley and Black, 1985; chapter 3). The seriousness of LTGD in commercially produced lots probably would not warrant intentionally increasing seed storage temperatures above 24°C, although it now seems reasonable to try to avoid low temperature storage whenever LTGD may be a potential problem.

LTGD was maintained virtually unchanged by storing freshly harvested (and dried) cucumber seeds at -10°C for at least 21 weeks (Figs. 1 and 2). It is probably safe to assume that freeze-storage will maintain fresh seed dormancy for several years, although these experiments have not been completed. The ability to maintain fresh seed dormancy could be (and has been) useful as a research tool. Seeds may be stored until it is convenient to test them for dormancy. Also, a seed lot's ability to germinate may be determined after several durations of after-ripening in the same physical germination test, by sequentially sampling a freeze-stored lot.

The effect of seed maturity on the length of LTGD was significant (Fig. 7). Very immature seeds (25 and 30 DAP), apparently on the verge of desiccation tolerance, seemed to largely by-pass the LTGD effect. Slightly more mature seeds (35 DAP), but not yet at maximum dry weight (physiological maturity), had the longest dormancy. At physiological maturity, the dormancy was partially decreased. Lots harvested just past maturity (45 DAP)

were usually even less dormant, although lots harvested 10 days later (55 DAP) were no less dormant and may have actually been slightly more dormant than the 45 DAP lots. These results illustrate the importance of controlling seed maturity when selecting for dormancy characteristics and low temperature germination ability in a breeding program, since failing to do so will certainly cause a great increase in non-genetic variation for LTGD. Also, the existence of a maturity x genotype interaction suggests that selection should be avoided on immature seeds, since only mature seeds are important in commerce.

Potential genetic variability for duration of LTGD was indicated. Two cultivars were compared in the field study. These cultivars 'Addis' and 'SMR-18' differed significantly for PG at 15°C when averaged over 12 storage times at 2 storage temperatures and KNO_3 treatment (Table 2). After plotting out all the individual treatment combinations, it was clear that these specific lots of these cultivars did not differ in PG after the LTGD requirement was met. But the 'Addis' lot always lagged behind the 'SMR-18' lot, in that slightly more storage time was required to attain comparable levels of germination. This suggests the potential for genetic variation for length of LTGD, since these cultivars were grown in a replicated uniform environment. The difference between these cultivars in the field study was admittedly small and could easily have been due to some factor not closely related to LTGD per se; therefore the adjective "potential" genetic variability must be stressed.

Potential genetic variability for duration of LTGD was more evident among the six cultivars compared in the greenhouse study (Fig. 6). Mature seed lots of the cultivar Sumter clearly required at least 12 additional weeks of room temperature storage before they were non-dormant at 20°C, compared to similarly treated lots of 'Addis', 'Beit-Alpha', 'Mincu', 'Poinsett 76' or 'SMR-18' (Table 8). Also the 'SMR-18' greenhouse-produced lot harvested 55 DAP but not at 45 DAP had a significantly higher PG at 20°C than the 'Addis' lot after 5 and 11 weeks of room temperature storage.

The attempt to compare the six greenhouse grown cultivars at the 15°C germination temperature (without KNO_3 treatment) failed because the mature seeds failed to have significant levels of germination, even after 37 weeks of room temperature storage (at which time insufficient reserves of seeds terminated the experiment). However, when the KNO_3 treatment was used, dormancy at 15°C was found to have lasted about 15 to 20 weeks for the 'Mincu', 'Poinsett 76' and ' SMR-18' 55-DAP lots (Fig. 5). The 'Addis', 'Beit-Alpha' and 'Sumter' lots failed to have a PG statistically different than zero in any of the germination tests at 15°C. Therefore, their relative duration of LTGD could not be estimated for germination at 15°C, nor can a comparison be made between them and the lots for which an estimate of LTGD was obtained.

The nucellar membrane (NM), which is genetically maternal tissue, has a role in determining the duration of after-ripening required before seeds are capable of germination at 15°C. Radicle elongation usually took place when the NM was removed from seeds which were unable to germinate at 15°C due to LTGD. However, radicles of 'Sumter' embryos, with the NM removed, failed to elongate when 1 to 8 weeks of after-ripening had taken place. Also, radicle elongation was more rapid in the other five cultivars following 8 or more weeks of after-ripening in comparison to 1 week of after-ripening. Therefore, it appears that the physiological mechanism of LTGD is a characteristic of the embryo itself, since excised embryos also expressed LTGD, although to a much lesser degree than whole seeds. The NM may act as a mechanical barrier to radicle elongation similar to the suggested role of the endosperm in photo-dormant lettuce seeds (Nabors and Lang, 1971), and/or the NM may alter the internal gaseous environment or prevent the leaching of inhibitors. Clearly, more definitive experimental approaches must be taken to further elucidate the physiological mechanism of LTGD in cucumber. However, the present study points to an underlying mechanism within the embryo, although the NM is certainly involved, in some way, in the final expression of germination.

CHAPTER 2.
INHERITANCE OF LOW TEMPERATURE GERMINATION DORMANCY AMONG THREE INBRED LINES OF CUCUMBERS

INTRODUCTION

The results presented in Chapter 1 suggested that genetic differences exist among cucumber cultivars for duration of LTGD. During the development of new cultivars it may be desirable to select for relatively short LTGD requirement as a means of avoiding or minimizing the problems associated with LTGD. Also, identification of genetic differences among lines and a knowledge of how these differences are inherited could be a valuable tool in further basic study on the mechanism of LTGD in cucumber.

The objectives of this phase of the study were to:

1. Confirm the existence of genetic variability for duration of LTGD.
2. Determine if heritable differences for duration LTGD are due to the expression of the maternal and/or the embryo genotype.
3. Determine appropriate selection procedures for the manipulation of LTGD.

To accomplish these objectives, the parental, F_1, F_2, F_3, and backcross populations resulting from two different crosses between 3 inbred lines were evaluated for low temperature germination ability after several durations of after-ripening. The inbred lines used as parents were chosen to address specific concerns outlined in the general introduction. They were also chosen to potentially maximize the possibility of finding genetic differences.

To obtain additional information on the heritability of duration of LTGD and to evaluate the appropriateness of a selection procedure, F_4-lines were obtained from bi-directional selection between and within F_3-families at the duration of after-ripening when the parental difference for PG was maximum.

During the course of the study it appeared that fruit spine color (a simply inherited trait) and duration of dormancy did not segregate independently. Therefore an additional objective was to further explore this relationship.

The relative duration of LTGD is dependent on a number of non-genetic factors. The experiments reported in chapter 1 showed that seed storage temperature, seed maturity, germination testing temperature and KNO_3 imbibitional treatment all affected the duration of LTGD. Therefore, in this preliminary inheritance study, as many potential non-genetic sources of variation as possible were controlled. Among the genetic populations which were to be compared, all seeds were simultaneously produced, after-ripened and tested in as uniform manner as possible. Most populations, however, were grown and tested in two years to asses the repeatability of the results.

Ultimately only a single germination testing procedure was utilized. It was a laboratory germination testing procedure where seeds were imbibed in a 0.2% solution of KNO_3 and incubated at 15°C for 18 days. The decision to use a 15°C germination testing temperature with KNO_3 treatment was made on the basis of available facilities and the proven ability of this procedure to reveal dormancy patterns. The major drawback of this method is the unknown extent to which a genotype by KNO_3 treatment interaction might occur. Evidence for such an interaction has not been found (results of 1985 studies and several non-reported studies), although an exhaustive search for one has not been conducted. Actually, a genotype by KNO_3 treatment interaction should not invalidate the results of the present study to any great extent, as nitrates and nitrites occur, possibly at active levels, in field soil.

A 15°C germination temperature without KNO_3 treatment was ruled out as a practical procedure for LTGD determination, because at least some genotypes were expected to be dormant for possibly up to one year of after-ripening under these conditions.

MATERIALS AND METHODS

Genetic Populations

Parental, F_1, F_2, backcross, F_3 (F_3 families) and F_4 (selected F_4-lines) seed populations were produced and tested for LTGD from the crosses 'Sumter' x 'Mincu' and 'Sumter' x 'SMR-18'. A summary of the populations tested within each cross, including the expected maternal and embryo genotypes is shown in Table 11. The nomenclature describes the embryo genotype of the seeds being tested. Percentage of germination (PG) can not be a measurement of a single seed, so PG refers to a population or family. Therefore PG of the F_2 population refers to seeds with a F_2 embryo genotype, while PG of a F_3 family refers to the F_3 seeds obtained from a single F_2 plant, and indicates the genotypic character of the F_2 seed from which it was derived.

A brief description of the parental lines follows: All the parents had a monoecious flowering habit.

Mincu Extra Early ('Mincu') is a white spine pickling or slicing cultivar with no disease resistances. It was developed many years ago and currently has little commercial value. It appeared to have a relatively short LTGD requirement in the 1985 studies.

Wisconsin SMR-18 ('SMR-18') is a black spine pickling cultivar with resistance to scab (Cladosporium cucunerinum Ellis & Arthur) and Cucumber mosaic virus (CMV). It appeared to have a short LTGD requirement in the 1985 studies. It currently is used in home gardens and as the male parent in a few hybrids used in northern growing regions, where good low temperature emergence ability is required. It also has been used as a source of disease resistance and fruit quality in the development of many of the currently important university released and proprietary inbreds.

Sumter is a white spine pickling cultivar resistant to scab, CMV, anthracnose (Colletotrichum lagenarium Arx.), powdery mildew [Sphaerotheca fuliginea (Schlecht. ex Fr.)Poll.], downy mildew [Pseudoperonospora cubensis (Berk. & Curt.)Rostow] and angular leaf spot [Pseudomonas lachrymans (E.F. Smith and Bryan)Carsner]. It is a male line used in making hybrid cultivar(s) as well as a source of pollen (pollinator) when blended with predominantly female hybrid cultivars. It appeared to have a long LTGD in the 1985 experiments.

Cucumbers are highly cross pollinated in the field, but since they do not suffer from inbreeding depression most "open-pollinated" cucumber cultivars including Mincu, SMR-18, and Sumter were developed as inbreds. However, following release, cultivars are normally maintained by open (sib)-pollination in isolation. Known homozygous (recently self pollinated) lines of the cultivars used were not available at the initiation of the inheritance study, although carefully maintained stock seed reserves of the cultivars were available. To partially address the uncertainty of the homozygosity of the parents, a single plant, derived from stock seed, of each cultivar was selfed, from the seed obtained, one seed was randomly chosen to produce a "founder plant" .

Founder plants were multiplied by cuttings in the fall of 1985. Self- and cross-pollinations to the other parents were made at this time on the founder plants. The resulting parental and F_1 seeds were used to produce the various populations evaluated in this study. F_1 plants were grown and selfed in the spring of 1986 to provide F_2 seed for early summer planting.

Seed Production

All seeds were produced in poly covered greenhouses at the SUNSEEDS research station in Brooks, Oregon. Seedlings at the 1^{st} true leaf stage were transplanted into 21.1 x 21.1 cm black plastic pots. Table 12 indicates the planting dates and greenhouse temperature settings for the various experiments conducted. The

Table 11. Maternal and embryo genotypes of populations used in the inheritance experiments.

Population	Maternal genotype	Embryo genotype	Notation[z]
Parental			
Parent 1 (P_1)	P_1	P_1	Sumter (S)
Parent 2 (P_2)	P_2	P_2	Mincu (M)
F_1			
$P_1 \times P_2$	P_1	F_1	S x M
$P_2 \times P_1$	P_2	F_1	M x S
Backcross to P_n			
$P_n \times F_1$	P_n	BC to P_n	S x F_1
$F_1 \times P_n$	F_1	BC to P_n	F_1 x S
F_2	F_1	F_2	F_2-population
F_3	F_2	F_3	F_3-families[y]
F_4	F_3	F_4	F_4-lines[x]

[z] Example of the abbreviated notation used.
[y] Seeds from a single F_2 fruit are an F_3-family.
[x] Seeds from a single selected F_3 plant are an F_4-line.

Table 12. Planting dates and minimum / maximum greenhouse temperature settings[z] for inheritance experiments.

Experiment	Planting date	Temperature
'Sumter' x 'Mincu'		
1986	13 June 1986	21 / 30
1987	2 May 1987	25 / 35
'Sumter' x 'SMR-18'		
1986	10 July 1986	25 / 35
1987	4 April 1987	21 / 30

[z] The actual temperatures maintained were occasionally higher than the maximum setting.

growing media was a bark-loam mix with pH adjusted to 5.9 with lime. Vigorous and healthy plants were obtained by supplying mineral nutrition bi-weekly in the irrigation water and controlling disease and insect pests with registered chemicals when required. Plants were trained to a single stem and one fruit was obtained per plant by appropriate hand pollination. Several plants, randomly arranged, were dedicated to each population. The actual number of plants in each population depended on the population, cross and year of production. The specific number eventually used in the experiments are shown in the results.

Fruits were harvested 43 to 47 days after pollination. Harvested fruits were left on the greenhouse benches for 48 hours before the seeds were extracted, this procedure facilitated easier separation of seeds from the pulp. Seeds from each fruit were kept separate. Seeds were cleaned (without fermentation) and dried as described in the 1985 experiments. However, because the lots were smaller, less drying time was required. Seeds were first dried at 30°C for 20 hours and then held for 4 days at 15°C and 37% RH, resulting in an equilibrium moisture content of about 6.5%. All seeds were then put in coin envelopes and placed in sealed plastic containers and placed in the freezer (-20°C). Seed storage in the freezer was shown in the 1985 study to be an effective means of maintaining the dormant condition.

Seed Storage

Seeds were moved from the freezer and placed at room temperature for after-ripening. The room used for after-ripening in the 1986 experiments was a tissue culture growth room maintained at about 25°C. In 1987 the seeds were stored in a laboratory where the temperature was maintained at about 22°C although it was allowed to fluctuate from about 16°C to 25°C on weekends.

The 1986 seed production for the 'Sumter' x 'Mincu' experiment was divided into two groups which were after-ripened and germination tested separately. The dates on which the germination tests were initiated are shown in Table 13. Within the 1986 'Sumter' x 'Mincu'

Table 13. Dates on which germination tests were initiated for several experiments.

Experiment	Date of germination test (month/day/year)						
	Duration of after-ripening (DORTS[z])						
	25	50	75	100	125	150	250
'Sumter' x 'Mincu'							
1986 GR. 1	8/16/86	9/11/86		10/30/86	11/25/86	1/19/87	4/28/87
1986 GR. 2		3/30/87		3/30/87		3/30/87	7/ 8/87
1987		10/16/87	11/24/87	12/ 5/87		1/25/88	5/ 6/88
'Sumter' x 'SMR-18'							
1986	3/18/87	3/18/87	3/18/87	3/18/87		3/18/87	8/15/88
1987		8/30/87	9/25/87	10/19/87	12/ 8/87	3/19/88	3/19/88

[z] Duration of after-ripening in days of room temperature storage.

group 2 and the 1986 'Sumter' x 'SMR-18' experiments, the 25, 50, 75, 100 and 150 days of after-ripening (DORTS) treatments were tested for germination on the same date. This was possible by sequentially sampling seeds of each genotype from freeze-stored lots. In the other experiments, all the seeds were removed from freeze-storage at one time and the germination tests were conducted sequentially.

Germination Tests

Fifty to 75 seeds were placed in 3 rows on a 25 x 38 cm germination towel (Anchor, St.Paul, MN.) wetted with a 0.2% KNO_3 solution. The towels were rolled loosely and no more than seven were placed in a 5 x 18 x 26cm covered plastic box. The boxes were then placed in a dark incubator set at 15°C ± 1°C. Germination tests were removed from the incubators for less than one minute for counting on days 4, 6, 8, 12, and 18. Seeds were considered germinated if the radicles were >3mm long. On day 18 the germination tests were moved to an elevated temperature (25°C or 30°C). Following 4 days incubation at the higher temperature non-germinated seeds were considered non-viable. Extremely few non-viable seeds were observed (less than 0.1%). Percentage of germination (PG) was corrected for non-viable seeds using the following formula: PG = # of seeds germinated by day 18 / # viable.

The seeds from each fruit (plant) were maintained and tested for germination separately. In 1986 two samples of 15 to 25 seeds each were tested per fruit at each duration of after-ripening. In 1987 three samples of 10 to 25 seeds each were tested per fruit at each duration of after-ripening. Most fruits had over 300 seeds so the majority of the germination tests were based on samples of 25 seeds each.

Statistical Analyses

Percentage of germination (PG) after 18 days of incubation at 15°C was obtained for each experimental unit. PG was arcsin-square root (angular) transformed prior to all analyses (Steel and Torrie,

1980. pg 236). Within each population there were two levels of sampling, fruit within populations ($fruit_{(pop)}$) and germination test samples within fruit ($samples_{(fruit)}$). At each duration of after-ripening the same fruits were tested in each population.

Genetic segregation was not expected to occur for PG between $fruit_{(pop)}$ in the parental, F_1, F_2 and backcross populations. In these populations, therefore, both levels of sub-sampling will contribute only non-genetic variance to the experiment. This non-genetic variance, or experimental error, was composed of between fruit within population variance ($var_{(f)}$) and within fruit or sample variance ($var_{(s)}$). An analysis of variance was conducted on the data from these populations, at each duration of after-ripening. Populations were considered fixed treatment effects and $fruit_{(pop)}$ and $samples_{(fruit)}$ as random sampling and sub-sampling effects. The computational procedures are essentially the same as those shown in Snedecor and Cochran (1967, pg. 291) A sample analysis with the expectations of the mean squares is shown in Table 14.

The broad objectives of this study required making nearly all possible comparisons among population means. Therefore, if the population effect was significant, population means were separated by all possible pair wise T-tests (5% level) for the difference of two means (taking into account unequal replication). This amounts to a LSD test at the 5% level with unequal replication. The error variance used was the mean square for $fruit_{(pop)}$ pooled over all after-ripening times in each experiment. In the actual experiments, the variation within several of the treatments (population x after-ripening time combinations) was zero. Specifically this occurred when all the seeds of a population either germinated (100 PG) or failed to germinate (0 PG) at a duration of after-ripening. Treatments with zero error variance, of course, have the effect of reducing the pooled estimate of experimental error. Therefore to provide a conservative measure of experimental error for treatments with means not 0 PG or 100 PG, the degrees of freedom contributed by populations with zero variance were deducted from the calculation of the error variance. This minor correction actually had no effect on

Table 14. Degrees of freedom (df) and expectations of mean squares (MS) for combined analysis of variance performed on parental, F_1, F_2, and backcross populations.

Source	df	MS	F-test	Expectation of mean square
Population	p-1	MS_p	MS_p/MS_f	$Var_{(s)} + rVar_{(f)} + rC\sum\alpha_i^2/(p-1)$
$Fruit_{(pop)}$	F-p	MS_f	MS_f/MS_s	$Var_{(s)} + rVar_{(f)}$
$Samples_{(fruit)}$	rF-F	MS_s		$Var_{(s)}$

Where:

$Var_{(s)}$ = Variance between samples within fruits.

$Var_{(f)}$ = Variance between fruits within populations.

α_i = effect of population i.

p = number of populations.

F = total number of fruit (all populations).

r = number of samples per fruit.

$$C = \frac{1}{p-1}\left(rF - \frac{(rf_i)^2}{rF}\right)$$

f_i = number of fruit in population i.

the interpretation of the results.

The F_3 and F_4 populations were not included in the analyses outlined above because the variance between fruit means within these populations is expected to have a genetic component of variance as well as a non-genetic (error) component. Only one fruit was obtained per F_3-family, thus there was no way to directly estimate the non-genetic component of the between-fruit variance from the F_3-family data alone. Therefore, for the purpose of comparing the F_3 population mean with the other population means and to detect significant genetic differences among F_3-families, the error variance estimated from the populations where the fruit were not segregating was used.

Chi-square goodness of fit analysis was performed on selected data to test the hypothesis of simple Mendelian inheritance for seed germination at specific storage times. The procedures followed are given in Gomez and Gomez (1984, chapter 11). First, the segregation ratios obtained from replicate fruits within a population were tested for homogeneity. If the fruit were determined to be from a homogeneous population, the data from individual fruit were pooled, and tested for goodness of fit by Chi-square.

Selection Procedure

To obtain additional information on the inheritance of duration of LTGD as well as to provide an estimate of realized heritability, two related selection procedures were devised; a between and within family procedure and a within family only procedure. In the between and within family selection procedure, F_3-families from the 1986 experiments were first ranked according to PG at 100 DORTS (data from other DORTS was used if families were equivalent at 100 DORTS). The highest ranking 10% of the families and the lowest ranking 10% of the families were selected. Within each of these "high" and "low" selected families, 2 to 4 germinated or non-germinated seeds were selected in the respective directions. A special effort was not made to select the most rapidly germinating seeds in the high direction, although selections were made in the first counting when

possible (4 days). In the low direction non-germinated seeds were selected randomly. The F_4 progeny lines from these selections were evaluated in the 1987 experiments.

In the second selection procedure, at least one germinated and one non-germinated seed was selected from each F_3-family in the 1986 experiments. The resulting F_4 progeny lines were also evaluated in 1987. Selection within each family provides an approximate progeny test, for example if the lines resulting from selection in opposite directions differ, the family they were selected from was probably heterozygous ("probably", because non-genetic variability may cause differences also).

Duration of LTGD (after-ripening requirement) was not measured directly, therefore expressing the response to selection for duration of LTGD must be indirectly determined. The PG of the selected lines were compared to the parental, F_1 and F_2 genotypes, which were grown and tested concurrently with them. By such comparisons it was possible to determine the effectiveness of selection in obtaining "parental LTGD types". A formalized estimate of realized heritability (RH) for between and within F_3-family selection for PG at various durations of after-ripening was calculated by the following formula:

$$RH = \frac{(\text{mean of high } F_4\text{-lines}) - (\text{mean of low } F_4\text{-lines})}{100\ PG}$$

This is a modification of a more conventional formula (Fehr, 1987 pg. 102):

$$RH = \frac{(\text{mean of high } F_4\text{-lines}) - (\text{mean of low } F_4\text{-lines})}{(\text{mean of high } F_3\text{-lines}) - (\text{mean of low } F_3\text{-lines})}$$

Where the high and low F_4-lines are progenies of the selected F_3-lines.

Realized heritability, as calculated above, does not have a

specific genetic interpretation, however it serves the purpose of illustrating, the proportional response to selection which was made, given a specific selection differential. As selection was also practiced within the selected families, it seemed appropriate to use 100 PG in the denominator (selection differential) in place of the F_3-family difference. This also has the desired effect of indicating the efficiency of selecting for parental type PG, since the parental lines had essentially 100 and 0 PG at 100 DORTS, when the selection took place.

Linkage Analysis

Results from the 1986 F_3-family data suggested that fruit spine color may be linked to the relative duration of LTGD. Recall that 'Sumter' and 'Mincu' had white spines and 'SMR-18' had black spines. In the 'Sumter' x 'SMR-18' cross, spine color was inherited as a single dominate gene for black spine. This is the normal inheritance pattern for most genotypes, although some genotypes may differ at duplicate spine color loci (Gene List, Cucurbit Genetics Cooperative Report, 1985. 8:86.).

Further information on the possible linkage relationship was obtained from the F_2 and backcross to 'Sumter' populations in the 1987 experiment. At 150 DORTS, germinated and non-germinated seeds from each fruit from replications 1 and 3 were transplanted to flats with 2.54cm x 2.54cm cells. To promote early female flowering they were sprayed at the one leaf stage with 250 ppm ethephon (2-chlorethylphosphonic acid). Spine color was determined by examining at least 3 flowers per plant, approximately 3 to 5 days after the petals opened. Several plants died prior to spine color determination. To limit the impact of possible non-random mortality, only data from replications with less than 10% (2 of 25 plants) mortality were used.

A 2 x 2 chi-square contingency test was first conducted to determine if spine color was independent of seed germination at 150 DORTS. If non-independence was indicated, the genetic linkage, expressed in percentage of recombination was calculated by the

following formulas:

$$\text{Backcross:} \quad \text{Recomb. } \% = \frac{\text{number of non-parental individuals}}{\text{total number of individuals}}$$

The product-moment technique developed by Immer (1930) was used for the F_2 population. For coupling the following quantity is first obtained:

$$\frac{(\#\text{ of A\_bb})(\#\text{ of aaB\_})}{(\#\text{ of A\_B\_})(\#\text{ of aabb})}$$

It is then converted to percentage of recombination by tables formulated for this purpose.

The tables given by Immer (1930) also facilitate the estimation of a probable error. However, since LTGD dependent germination was susceptible to classification error, it would be incorrect to assume the estimated linkage values were actually estimated to the precision given by the probable error, which only is a function of sampling probability.

RESULTS

'Sumter' x 'Mincu' Cross

Three experiments were conducted to determine if there was a heritable difference for duration of low temperature germination dormancy (LTGD) between the cultivars Sumter and Mincu. Percentage of germination (PG) was determined at several durations of after-ripening for the parental, reciprocal F_1, F_2 and backcross (BC) populations in each experiment. In all experiments there was a significant population effect for PG at all durations of after-ripening, except at 25 DORTS in the 1986 group 1 experiment (Table 15).

The back transformed population means for PG are shown in Tables 16, 17 and 18. The PG obtained in some of the populations, at a few of the durations of after-ripening, differed among experiments. Although in all three experiments the populations always had the same relationships to one another. Figure 8 has been prepared to aid in the visualization of the LTGD phenomenon. It also provides a convenient means of comparison between the experiments. The major difference among the experiments was that the F_1, F_2 and BC to 'Sumter' populations had a lower PG at 100 DORTS in the 1987 experiment than they had in either of the 1986 experiments. The PG obtained at 150 DORTS in 1987 was comparable to 100 DORTS in the 1986 experiments. This slightly longer after-ripening requirement was probably due to the generally lower seed storage temperature in 1987.

In these experiments the inability of a viable seed to germinate could be completely attributed to the LTGD effect. This was based on the fact that all 'Sumter' lots, in the three experiments, had 100 PG after either 350 or 250 DORTS of after-ripening (Tables 16, 17 and 18). This indicates that they were fully capable of germination under the germination test conditions after the LTGD effect was eliminated by long durations of after-ripening. Similarly, failure to germinate in all populations evaluated in these experiments may, for all practical purposes, be

Table 15. Mean squares for percentage of germination[z] among and within various populations in three 'Sumter' x 'Mincu' experiments.

Source of variation	df	Mean squares: Duration of after-ripening (DORTS)[y] 25	50	75	100	125	150	250
1986 Gr. 1. Experiment								
Populations[x]	9	21ns	1331**		4842***	4806***	3603***	162***
Fruit(populations)	21	15*	285***		21ns	8ns	56***	25ns
Samples(fruit)	31	7	13		14	13	14	58
1986 Gr. 2. Experiment								
Populations[x]	9		3650***		4274***		2855***	
Fruit(populations)	22		189***		46**		104***	
Samples(fruit)	32		47		19		22	
F_3 population								
F_3-families	22		346***		14287***		303***	
Samples(F_3-families)	23		47		72		35	
1987 Experiment								
Populations[x]	7		3317***	13174***	15735***	16802***		
Fruit(populations)	38		298***	258***	152***	119***		
Samples(fruit)	92		20	80	41	31		

[z] Angular transformed.
[y] Days of room temperature storage.
[x] Parental, F_1, F_2, and backcross populations.
***, **, *, ns, significant at the .001, .01 and .05 level, respectively.

Table 16. Mean percentage of germination for several populations in the 1986 group 1 'Sumter' x 'Mincu' experiment.

Population	Number of fruit	Percentage of germination: Duration of after-ripening (DORTS)[z] 50	100	125	150	250
Sumter (S)[y]	4	0 b[x]	0 d	0 d	6 d	89 a
Mincu (M)	4	49 a	100 a	100 a	100 a	100 a
S x F_1 (BC to S)	2	0 b	50 c	55 c	72 c	95 a
F_1 x S	4	0 b	52 c	56 c	79 c	96 a
S x M (F_1)	3	0 b	99 a	100 a	100 ab	100 a
M x S	3	0 b	100 a	99 a	100 ab	100 a
F_2 Population	6	2 b	75 b	76 b	90 bc	98 a
S x M (F_2)	2	2 b	74 bc	85 b	97 ab	97 a
M x S	4	2 b	75 bc	74 bc	86 bc	98 a
M x F_1 (BC to M)	2	8 b	98 a	100 a	100 ab	100 a
F_1 x M	3	0 b	99 a	100 a	100 ab	100 a

[z] Days of room temperature after-ripening.
[y] 'Sumter' had 100 PG at 350 DORTS.
[x] Transformed means separated within columns LSD (0.05).

Table 17. Mean percentage of germination for several populations in the 1986 group 2 'Sumter' x 'Mincu' experiment.

Population	Number of fruit	Percentage of germination: Duration of after-ripening (DORTS)[z] 50	100	150
Sumter (S)[y]	4	0 f[x]	4 e	15 c
Mincu (M)	4	95 a	100 a	100 a
S x F_1 (BC to S)	3	2 f	50 c	76 b
F_1 x S	3	2 f	53 c	76 b
S x M (F_1)	3	9 d	100 a	100 a
M x S	3	22 cd	100 a	100 a
F_2 Population	7	9 def	78 b	95 a
S x M (F_2)	4	4 ef	75 bc	92 ab
M x S	3	18 de	82 b	98 a
F_3-Population	23	13 d	79 b	94 a
M x F_1 (BC to M)	3	55 b	100 a	99 a
F_1 x M	2	18 cd	100 a	100 a

[z] Days of room temperature after-ripening.
[y] 'Sumter' had 100 PG at 250 DORTS.
[x] Transformed means separated within columns LSD (0.05).

Table 18. Mean percentage of germination for several populations in the 1987 'Sumter' x 'Mincu' experiment.

Population	Number of fruit	Percentage of germination: Duration of after-ripening (DORTS)[z] 50	75	100	150
Sumter (S)[y]	9	0 b[x]	1 e	3 e	4 d
Mincu (M)	8	38 a	95 a	100 a	100 a
S x F_1 (BC to S)	4	0 b	5 de	28 d	47 c
F_1 x S	7	0 b	4 e	27 d	46 c
S x M (F_1)	3	3 b	8 cde	75 bc	98 a
M x S	7	1 b	24 bc	90 b	100 a
F_2 Population	8	2 b	23 bc	58 c	79 b
S x M (F_2)	4	2 b	31 b	64 c	83 b
M x S	4	0 b	16 bcd	51 c	75 b

[z] Days of room temperature after-ripening.
[y] 'Sumter' had 84 PG at 200 DORTS and 100 PG at 250 DORTS.
[x] Transformed means separated within columns LSD (0.05).

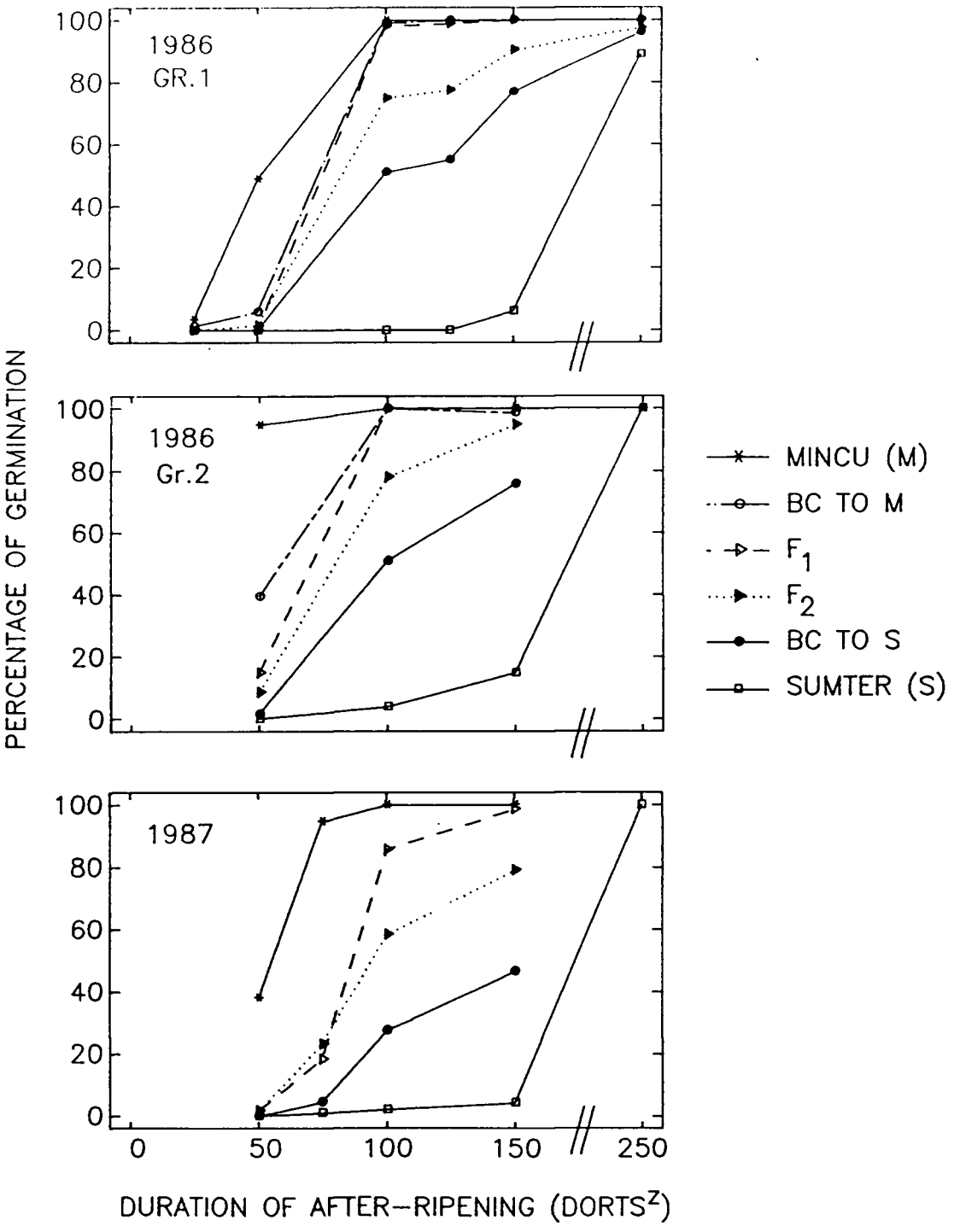

Figure 8. Back transformed percentage of germination for various genetic populations derived from the cross 'Sumter' x 'Mincu' at several durations of after-ripening, in three experiments.

[z] Days of room temperature storage.

attributed solely to LTGD. However, for the F_2 and BC to 'Sumter' populations in the 1987 experiment this must be an assumption, since germination data was not collected on them after 150 DORTS (because the seed supply was depleted).

Because all non-germinated seeds can be assumed to be dormant, observing a significant difference for PG between populations with different genotypes at any intermediate duration of after-ripening, can be interpreted as a significant difference for duration of LTGD. Therefore, the significant population effects shown in Table 15, confirm the existence of genetic variability for duration of LTGD among the populations tested.

There was a large difference in the duration of after-ripening required by the parental cultivars. In all experiments 'Mincu' had 100 PG by 100 DORTS and had significantly greater than zero PG at 50 DORTS (Tables 16 to 18, and Figure 8). 'Sumter' had nearly zero PG for durations of after-ripening of 150 DORTS or shorter. Therefore, 'Mincu' had a LTGD after-ripening requirement which lasted approximately 50 and 100 DORTS, while 'Sumter' required between 150 and 250 DORTS of after-ripening. The durations of LTGD are indicated as approximate ranges due to non-genetic variability for this trait. However, the duration of after-ripening required by seeds of the parental cultivars did not overlap in these experiments.

A difference for PG between reciprocally produced populations only occurred between the BC to 'Mincu' populations at 50 DORTS in the 1986 group 2 experiment (Table 17). This reciprocal cross difference was relatively minor compared to the large effect the embryo genotype had on PG at several durations of after-ripening. Therefore, due to the rarity of reciprocal cross differences, despite ample opportunity to detect them, it is suggested that the maternal genotype had only a slight (if any) effect on the relative duration of LTGD in populations derived from this cross.

A general dominance for germination was observed when the PG of the F_1 and F_2 populations are compared at 100 to 150 DORTS. The fact, that the F_1 population had a higher PG than the F_2 population,

was direct evidence for expression of dominant gene action for the ability to germinate at these intermediate durations of after-ripening, since the gene frequencies in these two populations are identical. Likewise, the expression of relatively short LTGD must be to some extent dominant to long LTGD, given the fact that germinated seeds are expressing shorter LTGD than non-germinating seeds. Because the 'Mincu' population had a significantly higher PG than the F_1 or the BC to 'Mincu' populations at 50 and 75 DORTS, the expression of short LTGD was not completely dominant to long LTGD. This may be described as incomplete dominance for short LTGD.

At 100 and 125 DORTS in 1986, and 150 DORTS in 1987, germination appeared to be completely dominant to non-germination. Furthermore, the ratio of germinating to non-germinating seeds observed at these durations of after-ripening may be explained by the segregation of a single gene as follows. The genotypes in which all seeds have at least one 'Mincu' allele for the hypothesized gene ('Mincu', BC to 'Mincu' and F_1) had very near 100 PG, while genotypes with no 'Mincu' alleles ('Sumter') had very near 0 PG . The F_2 population which was segregating 3 to 1 for seeds with at least one 'Mincu' allele had about 75 PG, and the BC to 'Sumter' population which was segregating 1 to 1 for seeds with at least one 'Mincu' allele had about 50 PG at the durations of after-ripening in question.

Chi-square goodness of fit analysis was used to determine if the observed ratio of germinating (G) to non-germinating (NG) seeds, at the durations of after-ripening in question, was consistent with a single dominant gene hypothesis. The results of the analysis indicate that none of the observed ratios were improbable at the 5% level under the hypothesis (Table 19). In the present situation an adequate fit to an expected segregation ratio, for PG at a single duration of after-ripening, must be carefully interpreted in terms of genetic differences for LTGD. Given the threshold expression, of LTGD the approximate relationship, 100 to 75 to 50 PG, for the F_1 to the F_2 to the BC to 'Sumter' populations, could also be explained in terms of many genes each with a small quantitative effect on the

Table 19. Chi-square goodness of fit analysis, testing a 3 : 1 and 1 : 1 ratio, for germination (G) : non-germination (NG), in F_2 and backcross to 'Sumter' (BC to S) populations, respectively, from a 'Sumter' x 'Mincu' cross.

Population	Number of fruit	Fruit homogeneity[z]	Observed G : NG	Expected G : NG	Chi-square
1986 Gr. 1. (100 DORTS[y])					
F_2	6	5.9 (.31)[x]	223 : 77	225 : 75	0.07 (.79)[x]
BC to S	6	6.6 (.25)	155 : 145	150 : 150	0.33 (.57)
1986 Gr. 1. (125 DORTS)					
F_2	6	2.5 (.78)	231 : 69	225 : 75	0.64 (.42)
BC to S	6	4.4 (.49)	166 : 134	150 : 150	3.41 (.06)
1986 Gr. 2. (100 DORTS)					
F_2	7	10.4 (.17)	243 : 73	237 : 79	0.61 (.44)
BC to S	6	5.6 (.35)	126 : 122	124 : 124	0.06 (.81)
1987 (150 DORTS)					
F_2	8	15.6 (.05)	419 : 121	405 : 135	1.93 (.17)
BC to S	11	9.2 (.61)	359 : 406	382.5 : 382.5	2.88 (.10)

[z] Chi-square test for homogeneity of ratio among replicate fruit (Gomez and Gomez, 1984. pg. 465).
[y] Duration of after-ripening in days of room temperature storage.
[x] Probability level.

duration of LTGD. All germinating seeds at 100 DORTS can not be assumed to have had similar genetic levels of LTGD, nor can every non-germinating seed be assumed to be equally dormant, on the basis of one germination test alone. Also non-genetic variation may obscure correct classification for relative LTGD levels based on individual seed germination. If, however, the assumption is made, for purposes of discussion, that all non-germinating seeds had similar genetic levels of dormancy and all germinating seeds had one of two dormancy levels (either homozygous or heterozygous) at the durations of after-ripening in question, then the single gene hypothesis for germination may be extended to explain the inheritance for the expression of LTGD at these durations of after-ripening. The results obtained from the F_3 families and the selection portion of this study, show that this assumption is probably valid, and should be useful for practical breeding purposes.

The sources of variation for PG in the F_3 population was partitioned into between and within F_3-family (F_3 seeds derived from a single F_2 fruit) components (Table 15). The F_3 population was analyzed separately from the other populations because the $fruit_{(pop)}$ component of variance has a genetic expectation. Significant differences among F_3-families for PG were indicated at 50, 100 and 150 DORTS. The F-test for between F_3 families was equally significant regardless of whether the $fruit_{(pop)}$ mean square from the combined analysis or the $samples_{(fruit)}$ mean square from the F_3 population analysis was used in the denominator.

The frequency distribution of the F_3-families and the parental and F_2 fruit mean ranges (the range of replicate fruit which make up the parental and F_2 seed generations) are shown in Figure 9. The information which was obtained from the F_3-family data was limited by the small number of families evaluated (which was due to incorrect identification of a seed packet at planting time). The relatively large gap in the PG distribution at 100 DORTS supports the hypothesis of relatively simple inheritance (few genes) and a general dominance for germination at 100 DORTS. At 50 DORTS none of

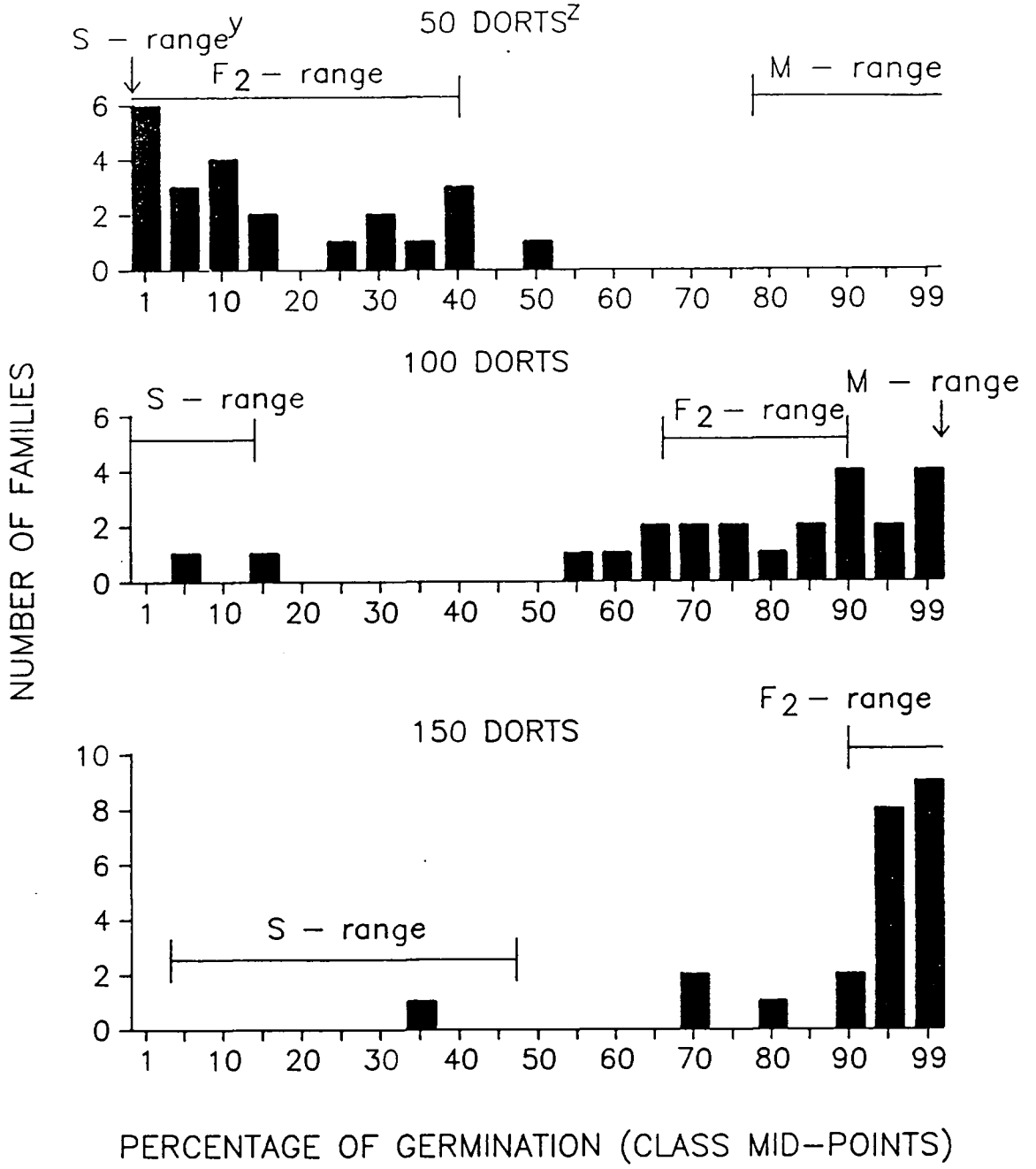

Figure 9. Frequency distribution for percentage of germination for F_3-families from the 1986 'Sumter' x 'Mincu' experiment.

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[z] Duration of after-ripening in days of room temperature storage.
[y] Range of parental (S = 'Sumter'; M = 'Mincu') and F_2 fruit means for percentage of germination.

the F_3-families had a PG within the range of the 'Mincu' fruits. At 150 DORTS only one family was observed within the range of the 'Sumter' fruits. The general lack of parental types suggest that the total heritable difference between the two parents for duration of LTGD was due to the segregation of more than one gene (possibly one major gene and modifiers).

In the 1986 group 2 experiment, 4 germinating (G) seeds were selected from the 3 F_3-families with the highest PG at 100 DORTS; likewise 2 to 4 non-germinating (NG) seeds were selected from the 3 F_3-families with the lowest PG at 100 DORTS (13% bi-directional selection intensity). In addition, one germinating and one non-germinating seed were selected for each of the other families. The resulting F_4 lines were tested in 1987.

The analysis shown in Table 20 breaks out two single degree of freedom comparisons from the between F_4-lines sums of squares for PG in 1987. The first (high vs. low), is a comparison between the progeny lines from the 3 high F_3-families versus the 3 low F_3 families. The second (G vs. NG), is a comparison of lines selected for germination versus non-germination in the middle ranked 17 F_3-families. Both comparisons were highly significant at 75, 100 and 150 DORTS. Significance of the first comparison shows that between and within family selection was effective. A significant mean square for the second comparison reveals that within family selection alone was also effective.

The means (pooled over duplicate progeny lines where necessary) of the F_4 progenies for each F_3-family are shown in Table 21 (note the NG progenies of families 1 to 3 were selected at 50 DORTS). Clearly the progenies selected from the highest and lowest families differed greatly for PG at 100 and 150 DORTS. Considering only the extreme families (1 to 3 and 21 to 23), the G and NG selected lines from the same family did not differ for PG at 100 and 150 DORTS. This indicates that the F_3-families from which they were selected, were true breeding for factors controlling germination at these durations of after-ripening.

Table 20. Mean squares for percentage of germination[z] of selected F_4 lines in the 1987 'Sumter' x 'Mincu' experiment.

		Mean squares			
		Duration of after-ripening (DORTS)[y]			
Source of variation	df	50	75	100	150
F_4-lines	63	225ns	1698***	3556***	3343***
High vs. Low[x]	1	1596*	30208***	78588***	69752***
G vs. NG[w]	1	911ns	24166***	60186***	67287***
Residual	61	190ns	863***	1398***	1206***
Samples(fruit)	128	33	96	26	80
Fruit(populations)[v]	38	298	258	152	119

[z] Angular transformed.
[y] Days of room temperature storage.
[x] Comparison between high and low selected F_3-families; 13% selection intensity between families in each direction, additional selection within families also practiced.
[w] Comparison between F_4 lines from germinating and non-germinating seeds in 17 intermediate families.
[v] Mean squares obtained from populations not segregating for PG (Table 15).
***, **, *, ns, significant at the .001, .01 and .05 level, respectively; fruit(pop) mean square used in denominator.

Table 21. Mean percentages of germination (PG) for progenies resulting from selecting germinating (G) and non-germinating (NG) F_3 seeds in each of 23 F_3 families from the cross 'Sumter' x 'Mincu'.

F_3 families 1986		Number of selected F_4 lines		PG (F_4 progeny lines) 1987				
				100 DORTS			150 DORTS	
100 DORTS[z] Rank	PG	G	NG	G	NG		G	NG
1	100	3	2[y]	100	100	ns	100	100 ns
2	100	4	2[y]	100	100	ns	100	100 ns
3	100	4	2[y]	100	98	ns	100	100 ns
4	98	1	1	62	0	***	75	0 ***
5	93	1	1	87	0	***	91	8 ***
6	93	1	1	78	4	***	81	6 ***
7	90	1	1	100	0	***	100	13 ***
8	90	1	1	50	0	***	60	0 ***
9	90	1	1	0	2	ns	0	5 ns
10	88	1	1	72	0	***	69	2 ***
11	85	1	1	0	0	ns	0	3 ns
12	83	1	1	100	0	***	100	10 ***
13	80	1	1	55	0	***	83	0 ***
14	75	1	1	27	0	***	35	2 ***
15	72	1	1	82	0	***	86	14 ***
16	70	1	1	36	0	***	89	0 ***
17	68	1	1	100	0	***	100	0 ***
18	66	1	1	100	0	***	100	0 ***
19	66	1	1	38	0	***	57	0 ***
20	58	1	1	2	0	ns	33	0 ***
21	56	2	2	0	0	ns	4	5 ns
22	15	2	2	0	2	ns	2	19 ***
23	5	1	4	0	0	ns	0	0ns
Mean	79	33	31	57	1[x]		66	4[x]

[z] Days of room temperature storage (after-ripening).
[y] Selected at 50 DORTS.
[x] Families ranked 1 to 3 not included.
***, **, *, ns; Transformed progeny means due to selection within family are significantly different at the .001, .01, and .05 level, respectivly (paired t-test).

The realized heritability (RH) for PG of F_4 lines at 100 and 150 DORTS in 1987, following bi-directional selection of F_3 families at 100 DORTS in 1986 was 99.3% and 92%, respectively (Table 22). Clearly, the selection procedure was highly effective for this trait, which was further evidence for its simple inheritance. The RH at 75 DORTS was 68.7%. The 'Mincu' population, in 1987, had a mean PG of 95% at 75 DORTS, with individual fruit means of 63 to 100 PG. Thus, 75 DORTS may be taken as near the average threshold duration of after-ripening required by 'Mincu' genotypes. Therefore, the RH at 75 DORTS, when compared to 95 PG for 'Mincu', may be thought of as, the degree to which the selection procedure was effective in selecting for "'Mincu' type" short LTGD on a individual seed basis.

Within F_3-family selection of germinating or non-germinating seeds at 100 DORTS resulted in significant differences for PG of F_4 lines at 75, 100 and 150 DORTS in 1987 (Table 20). Selection of germinating seeds was also effective in obtaining 'Mincu'-like short LTGD lines. First, it is necessary to define a criteria to judge whether a line has "'Mincu'-like" short dormancy. Given that at 50 and 75 DORTS the 'Mincu' population had less than 100 PG, it seems reasonable to assume that PG obtained at these durations of after-ripening was an approximate quantitative measurement of the average degree of dormancy. Obtaining a non-zero or non-100 PG from a non-segregating population (such as 'Mincu') would be due to non-genetic variation. For a character with a threshold expression, such as duration of LTGD, non-genetic variation would only be expressed near the threshold requirement. Thus, if the PG of a F_4-line is within the range of the 'Mincu' fruit means, at all durations of after-ripening, that line could be judged for all practical purposes to have the same degree of LTGD as 'Mincu'. Nine selected F_4-lines from 7 different F_3-families met this criteria (data not shown). Obtaining a 'Mincu' parental type F_4-line by within family selection in 30% of all randomly evaluated families, especially, when in most families only one seed was selected, must be judged as a very high realized heritability parental-type-short LTGD.

Table 22. Realized heritability (RH) for F_4-line percentage of germination, at several durations of after-ripening, following bi-directional selection between and within F_3 families at 100 DORTS from the cross 'Sumter' x 'Mincu'.

$$RH = \frac{\text{(mean of high } F_4\text{-lines)} - \text{(mean of low } F_4\text{-lines)}}{100\ PG}$$

$$RH\ (75\ DORTS^z) = \frac{68.7 - 0.0}{100} = .687$$

$$RH\ (100\ DORTS) = \frac{100.0 - 0.7}{100} = .993$$

$$RH\ (150\ DORTS) = \frac{100.0 - 8.0}{100} = .92$$

[z] Days of room temperature storage (after-ripening).

The PG of all F_4-lines resulting from selection of non-germinating seeds at 100 DORTS (families ranked 4 to 23) had near zero PG at 100 and 150 DORTS. None of these NG lines were outside the range of the 'Sumter' fruit means at 100 and 150 DORTS (Table 21; the range of the 'Sumter' fruit means was 0 to 20 PG and 0 to 44 PG at 100 and 150 DORTS, respectively). This result strongly suggests that all F_3 NG seeds in the 1986 (group 2) 100 DORTS germination test (at least those which were progeny tested; which was essentially a stratified random sample of all NG seeds) were homozygous recessive, or true breeding for the gene(s) responsible for "'Sumter'- like" germination at 100 and 150 DORTS in 1987.

Applying the same type of reasoning to all the F_3-families. It appears that 3 families (ranked 1 to 3) were homozygous for all alleles responsible for a duration of LTGD of 100 DORTS or shorter. This is because in all other families a F_4 line was identified with significantly less than 100 PG at 100 DORTS. If a single dominant gene was responsible for this trait, as was hypothesized earlier, 5.75 of 23 F_3 families would theoretically be expected to be homozygous for all alleles required for expression of 100 PG. The chi-square value for goodness of fit for a 1 to 3 ratio of homozygous dominant to heterozygous and recessive F_2-families for this trait was 1.75 with a probability level of 29%. Thus the single gene hypothesis was not rejected. However, the confidence of assuming the one gene hypotheses is questionable, based on the low number of families tested. The one gene hypotheses does fit the data best, although of the probability level of the chi-square analysis based on two genes was 17%. Following the method outlined in Steel and Torrie (1980 pg. 490), evaluation of 34 families would have been required to chose, with 95% confidence, between the hypotheses of one or two genes, however, 23 families was adequate to differentiate between 1 and 3 genes with greater than 95% confidence.

Obtaining high RH for PG at 100 and 150 DORTS and the relative ease in which "'Mincu'-like" LTGD was selected, also strongly support the hypothesis that the major portion of the genetic

variability for duration of LTGD between 'Sumter' and 'Mincu' expressed in these experiments was simply inherited. A reasonable working hypotheses may be formulated as follows: A single major gene, with embryo expression, accounts for the majority of the genetic variability for duration of LTGD. Expression of this gene, at the level of the underlying dormancy mechanism may be additive or some form of incomplete dominance, however, its expression for PG after about 100 DORTS of after-ripening was completely dominant. Or in other words the expression of the 'Sumter' allele was recessive. However, modifying genes are probably also involved, especially in the expression of "'Mincu'-like" short LTGD.

'Sumter' x 'SMR-18' Cross

Two experiments were conducted, one in 1986 and one in 1987 to determine if there was a heritable difference for duration of LTGD between the cultivars Sumter and SMR-18. The same populations and methods were used as in the 'Sumter' x 'Mincu' cross. There were significant differences for PG between populations at all durations of after-ripening in both years, except 25 DORTS in 1986 (Tables 23 & 24). Back transformed mean PG for each population, separated by LSD's at the 5% level, are shown in Tables 25 and 26 for the 1986 and 1987 experiments, respectively. For those populations and durations of after-ripening in common between the two experiments, the PG was very similar between the two years. The relationships of the populations to one another were identical in the two years.

'Sumter' had less than 5 PG for all durations of after-ripening 150 DORTS or shorter, although by 250 DORTS it had 100 PG (Fig. 10 & Table 26). Since 'Sumter' was capable of 100 PG after long durations of after-ripening, the failure of a seed to germinate in these experiments may be interpreted, as it was in the 'Sumter' x 'Mincu' cross, as completely due to the LTGD effect. However, this interpretation must be assumed for a small percentage of the F_1 x 'Sumter' BC, F_2 and F_3 populations, because they had significantly less than 100 PG at 250 DORTS (the longest duration of after-ripening they were tested at). Therefore the significance of the

Table 23. Mean squares for percentage of germination[z] among and within various populations in the 1986 'Sumter' x 'SMR-18' experiment.

		Mean squares					
		Duration of after-ripening (DORTS[y])					
Source of variation	df	25	50	75	100	150	250
Populations[x]	9	11ns	790***	4649***	6926***	5320***	262**
Fruit(populations)	37	12**	92*	266***	146ns	87ns	103***
Samples(fruit)	47	6	50	52	96	55	19
F_3-population							
F_3 families	26	2ns	8ns	198***	1018***	1069***	757***
Black vs. white[w]	1	-	4ns	377***	2859***	5940***	1485***
Samples(F_3 families)	27	2	8	37	58	36	48

[z]Angular transformed.
[y]Days of room temperature storage.
[x]Parental, F_1, F_2, and backcross populations.
[w]Comparison between families with black and white spines.
***, **, *, ns, significant at the .001, .01 and .05 level, respectively.

Table 24. Mean squares for percentage of germination[z] among and within various populations in the 1987 'Sumter' x 'SMR-18' experiment.

Source of variation	df	Mean squares: Duration of after-ripening (DORTS)[y] 50	75	100	125	150
Populations[x]	6	2653***	21533***	22968***	17866***	16858***
Fruit(populations)	37	405***	99***	89***	72***	102***
Samples(fruit)	90	42	23	27	24	30

[z] Angular transformed.
[y] Days of room temperature storage.
[x] Parental, F_1, F_2, and backcross to 'Sumter' populations.
***, significant at the .001 level.

Table 25. Mean percentage of germination for several populations in the 1986 'Sumter' x 'SMR-18' experiment.

Population	Number of fruit	Percentage of germination: Duration of after-ripening (DORTS)[z] 50	75	100	150	250
Sumter (S)	5	0 b[y]	0 g	2 g	4 d	100 a
SMR-18 (R)	5	29 a	95 a	99 a	100 a	100 a
S x F_1 (BC to S)	3	0 b	0 g	8 fg	49 c	97 abc
F_1 x S	4	0 b	3 fg	19 ef	54 c	88 bc
S x R (F_1)	5	3 b	4 f	19 ef	97 a	100 a
R x S	4	1 b	25 c	94 ab	100 a	100 a
F_2 Population	14	3 b	13 cdef	51 c	82 b	91 bc
S x R (F_2)	7	3 b	10 def	44 cd	82 b	94 abc
R x S	7	2 b	16 cde	58 c	82 b	88 bc
R x F_1 (BC to R)	3	21 a	63 b	100 a	100 a	100 ab
F_1 x R	4	4 b	23 cd	88 b	99 a	100 a
F_3 Population	27	1 b	5 fg	31 e	78 b	91 c
Black spine	22	1	6	37	85	94
White spine	5	0	2	10	41	78

[z] Days of room temperature after-ripening.
[y] Transformed means separated within columns LSD (0.05).

Table 26. Mean percentage of germination for several populations in the 1987 'Sumter' x 'SMR-18' experiment.

Population	Number of fruit	Percentage of germination				
		Duration of after-ripening (DORTS)[z]				
		50	75	100	125	150
Sumter (S)[y]	9	0 b[x]	0 d	0 d	2 e	4 d
SMR-18 (R)	11	20 a	97 a	100 a	100 a	100 a
S x F_1 (BC to S)	6	0 b	2 d	10 c	44 d	45 c
F_1 x S	5	0 b	3 d	12 c	37 d	41 c
S x R (F_1)	3	0 b	3 d	6 cd	88 abc	94 a
R x S	3	1 b	64 b	100 a	100 a	100 a
F_2 Population[w]	7	1 b	17 c	53 b	74 c	75 b

[z] Days of room temperature storage.
[y] 'Sumter' had 80 PG at 200 DORTS and 100 PG at 250 DORTS.
[x] Transformed means separated within columns LSD (0.05).
[w] Reciprocal F_2 populations combined.

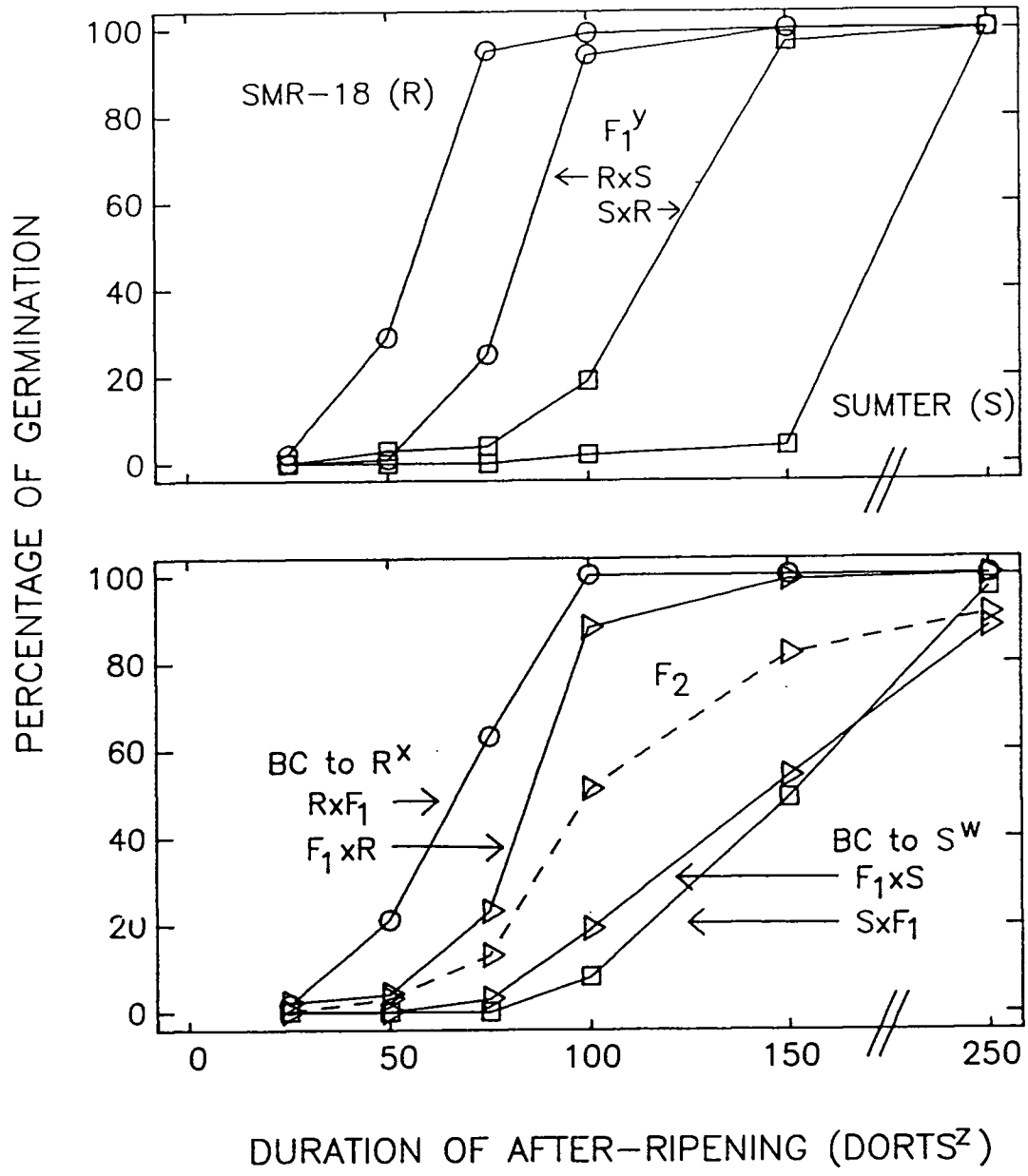

Figure 10. Back transformed percentage of germination for various genetic populations at 6 durations of after-ripening in the 1986 'Sumter' x 'SMR-18' experiment.

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[z] Days of room temperature storage.
[y] Reciprocal F_1s = R x S and S x R.
[x] Reciprocal backcrosses to 'SMR-18' = R x F_1 and F_1 x R.
[w] Reciprocal backcrosses to 'Sumter' = F_1 x S and S x F_1.

population effect shown in Tables 23 and 24 was confirmation that genetic variability for duration of LTGD exists among the populations evaluated.

Clearly the parental cultivars differed substantially for the duration of LTGD (Fig. 10). 'SMR-18' had an after-ripening requirement of a about 75 DORTS, with a range of 50 to 100 DORTS. 'SMR-18' and 'Mincu' were not included in the same experiment for any of the experiments reported here, however, they have been directly compared in 2 experiments which are not reported. In these experiments the completely non-dormant state was not detected until 100 DORTS in both cultivars, however, 'Mincu' consistently had a higher PG at 50 DORTS. This suggests the LTGD requirement of 'Mincu' may be slightly shorter than 'SMR-18'. 'Sumter' had a LTGD of 150 to 250 DORTS in the 'Sumter' x 'SMR-18' experiments, similar to what was observed in the 'Sumter' x 'Mincu' experiments.

Reciprocal F_1 and BC to 'SMR-18' populations differed for PG at 75 and 100 DORTS and 50, 75 and 100 DORTS, in 1986 and 1987, respectively (Tables 25 and 26). The BC to 'Sumter' and F_2 reciprocal populations did not differ for PG at any duration of after-ripening. No reciprocal cross differences were observed at durations of after-ripening of 125 DORTS of longer. This evidence strongly suggests, at least part of the heritable difference for duration of LTGD between 'Sumter' and 'SMR-18', was expressed by the maternal genotype.

The embryo genotype also contributed to variation among populations for PG, and thus, duration of LTGD. At 75 DORTS in 1986 'SMR-18' had 95 PG while the 'SMR-18' x F_1 (BC to 'SMR-18') and the 'SMR-18' x 'Sumter' (F_1) populations had 63 and 25 PG respectively (Table 25). All three populations have a common maternal genotype, yet the PG of each were different. Many significant differences for PG were also observed among the populations with 'Sumter' as the seed parent ('Sumter', 'Sumter' x 'SMR-18' (F_1) and 'Sumter' x F_1) and the F_1 as the seed parent (F_2, F_1 x 'SMR-18' and F_1 x 'Sumter'; Tables 25 and 26). These differences among embryo genotypes with the same maternal genotype are also evident in Figure 10 (note the

symbols indicate maternal genotype).

The relative duration of LTGD, of both the reciprocal F_1 populations was significantly longer than the short dormancy parent ('SMR-18'). Likewise the BC to 'SMR-18' populations also had a slightly longer LTGD than 'SMR-18' itself. The BC to 'Sumter' populations were nearly completely dormant until 100 DORTS, at which time they had between 8 and 19 PG, which was significantly greater than 'Sumter', and about equal the F_1 with 'Sumter' as the seed parent. At 150 DORTS (125 DORTS also, in 1987 experiment) the 'Sumter' x 'SMR-18' (F_1) population had much higher PG than the BC to 'Sumter' and F_2 populations.

This data taken together can only be interpreted in approximate genetic terms, which is complicated by both maternal and embryo expressed effects. First, at 125 and 150 DORTS, where the maternal effect was no longer detected, the F_2 populations had a lower PG than the F_1 populations, and a higher PG than the BC to 'Sumter' populations. This indicates a general expression of dominance for germination at 125 and 150 DORTS by the embryo expressed genetic differences. However, the 'SMR-18' x 'Sumter' (F_1) and the 'SMR-18' x F_1 (BC to 'SMR-18') have a lower PG than 'SMR-18' at 50 or 75 DORTS, indicating the embryo expressed alleles for short LTGD are incompletely dominant to long LTGD alleles, or modifying genes are partially responsible for 'SMR-18'-like short LTGD. In contrast, the expression of relatively long LTGD appears to be dominant to some extent to short LTGD for the maternal expressed genetic differences for LTGD. This was suggested by finding significant reciprocal cross differences between the BC to 'SMR-18' populations, but not between the BC to 'Sumter' populations (Fig. 10 and Table 25).

The data obtained at 150 DORTS in both years suggested that germination at this duration of after-ripening may be due to a single embryo expressed dominant gene, similar to that observed in the 'Sumter' x 'Mincu' cross. The observation that PG for the F_2 population remained steady at 75 PG between 125 and 150 DORTS in 1987 was especially suggestive for this hypothesis (Table 26).

Accordingly, the observed ratios of G : NG seeds in the F_2 and BC to 'Sumter' populations were tested for goodness of fit to the hypothesized ratios of 3 : 1 and 1 : 1, respectively (Table 27). The results of the chi-square analyses were variable. The fit was good for the F_2 in 1987 and the BC to 'Sumter' in 1986, but the observed ratios for the F_2 in 1987 and the BC to 'Sumter' in 1986 were rejected as improbable (P-value .008 and .02 respectively). The test for fruit homogeneity was also significant (.01 level) thus suggesting the segregation ratios among fruits in these populations were not the same. This was actually equivalent to the known significant $fruit_{(pop)}$ variance for PG, or non-genetic variability (Tables 23 and 24). Results, from all experiments conducted to date, clearly indicate the existence of non-genetic variation for duration of LTGD. Chi-square analysis assumes that individuals can be classified without error. Thus, the rejection of a segregation ratio by chi-square analysis does not necessarily indicate that the hypothesis is incorrect, it may just be a reflection of incorrect classification of several individuals. In actuality many of the individual fruit segregation ratios did fit the hypothesized ratios in the 1986 F_2 and 1987 BC to 'Sumter' populations. For the 1986 F_2, exclusion of 4 of 14 fruit (3 too high and 1 too low) was sufficient to obtain a probable chi-square value. Likewise, omission of 3 of 11 BC to 'Sumter' fruits in 1987 was sufficient to obtain a probable chi-square value (Table 27).

Allowing for some non-genetic variation, the PG data obtained in both years does suggest at least the possibility that germination at 150 DORTS was determined by the segregation of a single completely dominant gene. This conclusion however can only partly explain the total heritable differences for duration of LTGD. Clearly, 'SMR-18'-like short dormancy also depends in part to incompletely dominant factors and a maternal component. Also, the single gene hypothesis depends on the assumption that all germinated seeds at 150 DORTS, had at most one of two short LTGD duration phenotypes (heterozygous and homozygous), and that all non-germinated seeds had genetically equal long durations of LTGD, if

Table 27. Chi-square goodness of fit analysis, testing a 3 : 1 and 1 : 1 ratio, for germination (G) : non-germination (NG) at 150 DORTS, in F_2 and backcross to 'Sumter' (BC to S) populations, respectively, from two 'Sumter' x 'SMR-18' experiments.

Population	Number of fruit	Fruit homogeneity[z]	Observed G : NG	Expected G : NG	Chi-square
1986 F_2	14	28.2 (.009)[y]	399 : 99	373.5 : 124.5	6.96 (.008)[y]
10 fruits[x]	10	10.1 (.43)	265 : 73	253.5 : 84.5	2.09 (.15)
1986 BC to S	7	12.1 (.09)	129 : 121	125 : 125	0.25 (.62)
1987 F_2	7	12.2 (.09)	330 : 120	337.5 : 112.5	0.67 (.41)
1987 BC to S	11	31.5 (.001)	399 : 466	432.5 : 432.5	5.19 (.02)
8 fruits[x]	8	12.1 (.09)	272 : 298	285 : 285	1.19 (.28)

[z] Chi-square test for homogeneity of ratio among replicate fruit (Gomez and Gomez, 1984. pg. 465).
[y] Probability level.
[x] Excluding the fruits with the highest individual chi-squares.

they could be measured (see 'Sumter' x 'Mincu' results for discussion).

The frequency distribution for PG of the F_3-families supports the hypothesis of a general dominance for germination at 150 DORTS (Figure 11). At 150 DORTS, 3 F_3-families were in the range of the 'Sumter' parental fruits means, 16 families were within the range of the F_2 population fruit means and 6 had 100 PG, which was equal to the 'SMR-18' parent. If the remaining 2 families with 32 and 48 PG are classified as 'Sumter'-like, then the ratio of 5 : 16 : 6 was observed for 'Sumter'-like : F_2-like :'SMR-18'-like PG at 150 DORTS. The theoretical segregation ratio for 27 F_2-families, considering a single completely dominant gene is 6.75 : 13.5 : 6.75. The chi-square statistic for the observed fit was 0.55, which has a probability level of 76%. Thus, assuming the classification to be valid, the F_3-family data supports the BC to 'Sumter' and F_2 population data, suggesting germination at 150 DORTS was simply inherited. None of the F_3-families had PG within the range of the 'SMR-18' fruit means at 75 DORTS, thus, inheritance of 'SMR-18'-like short LTGD appears to be more complex, and certainly involves additional genes (modifiers), some of which have a maternal expression.

A selection experiment very similar to the one described in the 'Sumter' x 'Mincu' cross was also carried out in this cross. The actual number of F_4-lines, selected from each F_3 family in 1986, at 100 DORTS, is shown in Table 28. Note, that for F_3-families with zero PG at 100 DORTS, lines selected for germination were not obtained. Table 29 shows the mean squares for between F_4-line PG, and one degree of freedom comparisons for between and within family selection (high vs. low) and within family selection (G vs. NG). Both types of selection significantly influenced the PG at 75, 100, 125 and 150 DORTS. High vs. low family selection was also effective in influencing PG at 50 DORTS.

The realized heritability for bi-directional between and within F_3-family selection was very high, 0.95, 0.97 and 0.96 when measured as PG at 100, 125 and 150 DORTS, respectively (Table 30).

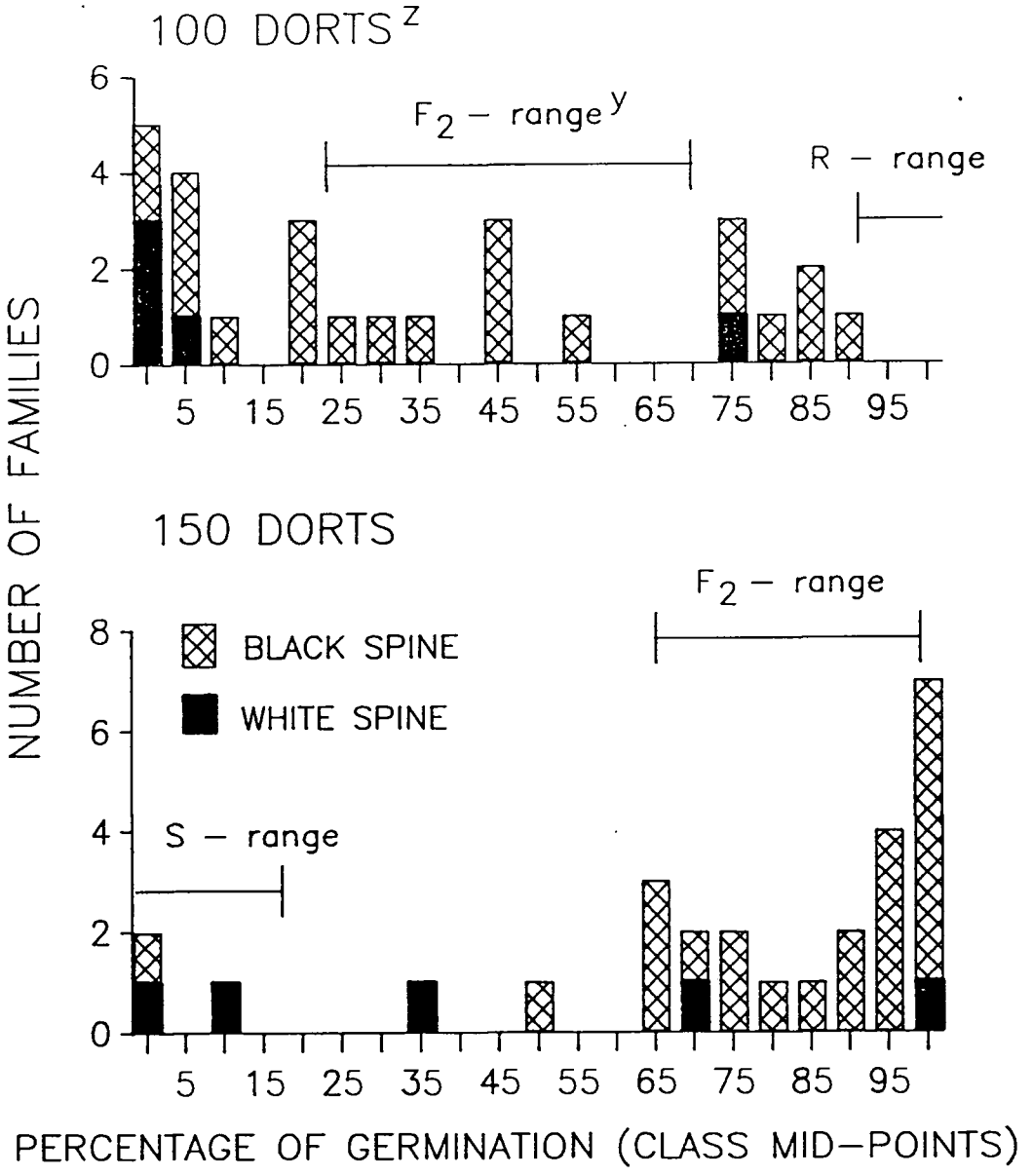

Figure 11. Frequency distribution for percentage of germination for F_3-families from the 1986 'Sumter' x 'SMR-18' experiment.

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z Duration of after-ripening in days of room temperature storage.

y Range of parental (S = 'Sumter'; M = 'Mincu') and F_2 fruit means for percentage of germination.

Table 28. Mean percentages of germination (PG) for progenies resulting from selecting germinating (G) and non-germinating (NG) F_3 seeds in each of 26 F_3 families from the cross 'Sumter' x 'SMR-18'.

F_3 families 1986		Number of selected F_4 lines		PG (F_4 progeny lines) 1987				
100 DORTS[z]				100 DORTS			150 DORTS	
Rank	PG	G	NG	G	NG		G	NG
1	92	4	2	91	17	***[y]	98	38 ***
2	87	4	2	97	88	*	100	100 ns
3	83	4	2	100	83	***	100	100 ns
4	81	1	1	62	3	***	100	81 **
5	78	1	1	100	92	ns	100	100 ns
6	76	3	2	88	0	***	100	9 ***
7	73	1	1	100	2	***	100	25 ***
8	56	1	1	100	96	ns	100	100 ns
9	45	2	1	41	41	ns	75	79 ns
10	44	1	1	100	2	***	100	97 ns
11	44	1	1	50	0	***	81	0 ***
12	34	1	1	40	35	ns	83	73 ns
13	26	1	1	100	28	***	100	64 ***
14	22	1	1	100	6	***	100	40 ***
15	20	1	1	98	33	***	100	72 ***
16	18	1	1	73	43	**	85	91 ns
17	10	1	1	13	0	**	82	57 **
18	7	1	1	3	96	***	98	100 ns
19	6	1	1	2	0	ns	0	2 ns
20	5	1	1	100	43	***	100	82 **
21	5	1	1	94	28	***	100	81 **
22	0	0	4		23			57
23	0	0	3		0			1
24	0	0	4		2			5
25	0	0	3		3			4
26	0	0	4		0			0
Mean	31	33	43	86	31		96	69

[z] Days of room temperature storage (after-ripening)

[y] ***, **, *, ns; Transformed progeny means due to selection within family are significantly different at the .001, .01, and .05 level, respectivly (paired t-test).

Table 29. Mean squares for percentage of germination[z] among selected F_4 lines in the 1987 'Sumter' x 'SMR-18' experiment.

Source of variation	df	Mean squares: Duration of after-ripening (DORTS[y]) 50	75	100	125	150
F_4-lines	75	243ns	1740***	3102***	3211***	2979***
High vs. Low[x]	1	3111**	64813***	99654***	107641***	103252***
G vs. NG[w]	1	109ns	39377***	50477***	36756***	27426***
Residual	73	235ns	360***	1131***	1321***	1271***
Samples(fruit)	152	4	35	52	45	26
Fruits(populations)[v]	90	405	99	89	72	102

[z] Angular transformed.
[y] Days of room temperature storage.
[x] Comparison between high and low selected F_3-families; 12% selection intensity between families in each direction, additional selection within families also practiced.
[w] Comparison between F_4 lines from germinating and non-germinating seeds in 21 families.
[v] Mean squares obtained from populations not segregating for PG (Table 24).
***, **, *, ns, significant at the .001, .01 and .05 level, respectively; fruit(pop) mean square used in denominator.

Table 30. Realized heritability (RH) for F_4-line percentage of germination, at several durations of after-ripening, following bi-directional selection between and within F_3 families at 100 DORTS from the cross 'Sumter' x 'SMR-18'.

$$RH = \frac{(\text{mean of high } F_4\text{-lines}) - (\text{mean of low } F_4\text{-lines})}{100\ PG}$$

$$RH\ (75\ DORTS^z) = \frac{74.0 - 1.1}{100} = .729$$

$$RH\ (100\ DORTS) = \frac{96.4 - 1.8}{100} = .946$$

$$RH\ (125\ DORTS) = \frac{98.1 - 1.8}{100} = .965$$

$$RH\ (150\ DORTS) = \frac{98.7 - 2.9}{100} = .958$$

[z] Days of room temperature storage (after-ripening).

The F_3 families ranked 1-3, clearly were not homozygous for all genes responsible for 'SMR-18'-like short LTGD, because lines resulting from NG seeds had significantly lower PG at 75, 100, 125, and 150 DORTS than the G selection lines (Table 28; data for 75 and 125 DORTS not shown). This result agrees with that observed on the basis of the F_3-families alone.

The results shown in Table 28 indicate that within family selection was relatively effective in obtaining F_4 lines with 100 PG by 100 to 150 DORTS, even when in some cases only one in 20 seeds had germinated at 100 DORTS (families ranked 20 and 21). The success for selection of low PG lines, however, was much poorer than that obtained in the 'Sumter' x 'Mincu' selection experiment (Table 21). This is not much of a practical concern, but can easily be explained, by the fact that selection in the 'Sumter' x 'SMR-18' experiment took place prior to the hypothesized expression of single gene inheritance for PG at a key duration of after-ripening (150 DORTS), while in the 'Sumter' x 'Mincu' experiment, selection coincided with the duration of after-ripening expressing simple inheritance.

The real value of within family selection was, that 10 F_4-lines from 6 different F_3-families were obtained with PG equivalent or above that of 'SMR-18' at all durations of after-ripening. The duration of LTGD for the 'SMR-18' population was estimated to be between 50 and 75 DORTS in 1987 (Table 26). Thus F_4-lines with PG within the range of the 'SMR-18' fruit means at 50 and 75 DORTS and with 100 PG at 100 DORTS, may be considered to have 'SMR-18'-like short LTGD.

The results of these experiments, taken together, suggest the following working hypothesis: A single gene difference with embryo expression for duration of LTGD between 'Sumter' and 'SMR-18' was sufficient to explain the expression of LTGD at 150 DORTS. However, for expression of 'SMR-18'-like short LTGD a few additional genes are likely involved. At least one of these additional genes has a maternal expression. Based on the relatively high proportion of F_3-families (23%) in which mild selection was effective in obtaining

'SMR-18'-like short LTGD F_4-lines, the number of important segregating genes for LTGD appears to be fairly low.

Percentage of germination for the 1986 F_3-families appeared not to be independent of fruit spine color (Fig. 11). The significance of a one degree of freedom comparison, for PG between F_3-families with white and black spine color, support this suggestion (Table 23). Spine color was inherited as a single gene, with black completely dominant to white in this cross. The F_3-family data suggested, if any relationship existed at all, it was probably due to linkage and not pleiotropy, since a few apparent crossover types were observed. From the F_3-family data it was impossible to determine whether spine color was linked to a maternal or embryo expressed factor for duration of LTGD. To gain further information on this possible linkage relationship, germinating (G) and non-germinating (NG) F_2 and BC to 'Sumter' seeds, were transplanted to the greenhouse, and subsequently scored for spine color, following the 1987-150 DORTS germination test.

It was previously hypothesized, and accepted with some reservation, that germination at 150 DORTS in the 1987 experiment was due to the segregation of a single gene with complete dominance for germination (Table 27). The 1987 BC to 'Sumter' population deviated significantly from a 1 : 1 ratio when data from all fruits were included, but the fit was satisfactory after excluding the three most deviant fruits (Table 27). Data from these three fruits were also excluded from the analysis shown in Table 31, although including them had very little effect on the estimated percent recombination (17.6 with them compared to 18.3 without them).

Both traits fit the expected 1 : 1 and 3 : 1 segregation ratios for the BC to 'Sumter' and F_2 populations, respectively, when considered independently (Tables 31 and 32). Very high chi-square values were obtained for the test for independent assortment, indicating that the 2 traits were linked. The calculated linkage distances were 18.3 and 11.98 recombination units, respectively, from the BC to 'Sumter' and F_2 populations. The fact that these two estimates are not exactly equivalent should be of little concern,

Table 31. Linkage analysis for fruit spine color and germination at 150 DORTS in a backcross to 'Sumter' population from the 1987 'Sumter' x 'SMR-18' experiment.

Test	Fruit homogeneity[z]	Chi-square[y]
Spine color	5.83 (0.56)[x]	0.45 (0.50)[x]
Germination	13.74 (0.06)	0.10 (0.75)
Independence[w]		90.20 (< 0.001)

$$\text{Recombination \%} = \frac{18 + 23}{244} = 18.3 \pm 1.7\%$$

[z] Chi-square test for homogeneity of ratio among 8 fruit (Gomez and Gomez, 1984. pg. 465).
[y] Chi-square goodness of fit to 1 : 1 ratio; 224 individuals.
[x] Probability level.
[w] 2 x 2 contingency test for character independence.

Table 32. Linkage analysis for fruit spine color and germination at 150 DORTS in a F_2 population from the 1987 'Sumter' x 'SMR-18' experiment.

Test	Fruit homogeneity[z]	Chi-square[y]
Spine color	2.46 (0.78)[x]	0.12 (0.73)[x]
Germination	4.59 (0.47)	0.33 (0.57)
Independence[w]		109.4 (< 0.001)

$$\text{Ratio of } \frac{(A\_bb)(aaB\_)}{(A\_B\_)(aabb)} = \frac{(16)(10)}{(158)(43)} = .02355$$

$$\text{Recombination \%}^{v} = 11.98 \pm 1.6\%$$

[z] Chi-square test for homogeneity of ratio among 7 fruit (Gomez and Gomez, 1984. pg. 465).
[y] Chi-square goodness of fit to 3 : 1 ratio; 227 individuals.
[x] Probability level.
[w] 2 x 2 contingency test for character independence.
[v] Calculated by the product-moment method from Tables by Immer (1930).

since they both must be considered more approximate than their probable errors indicate. This is because, LTGD dependent germination was not measured without error, also the relatively large mortality may also have effected the results to some extent. These results, clearly indicate that the spine color locus is linked to a major embryo expressed gene determining the duration of LTGD in this cross.

DISCUSSION

Through plant breeding, the cucumber plant has been greatly improved in many characteristics. Once-over mechanical harvest of processing cucumbers has been made feasible by the introduction of predominantly female hybrid cultivars which have a concentrated fruit set. However, if seedling emergence is non-uniform, there is little hope for a high yielding, concentrated fruit set to occur. Cucumber seeds are especially susceptible to non-uniform emergence under cool soil conditions. Therefore, the incorporation of superior low temperature germination ability into processing cucumber cultivars is an important breeding objective (Nienhuis et al., 1983). The low temperature germination dormancy (LTGD) phenomenon is an important factor contributing to low temperature germination ability, especially when germination is attempted after short seed storage durations. A long range solution to many of the potential problems associated with LTGD, would be to develop cultivars or breeding lines with a minimum after-ripening requirement. An immediate concern exists for improving or understanding the long LTGD requirement of the cultivar 'Sumter', because of its importance and desirability as a pollinator, when blended with predominantly female hybrid seed.

The first and primary objective of this study was to confirm the existence of genetic variability for duration of LTGD among cucumber cultivars. Highly significant population effects for PG were observed at durations of after-ripening between 50 and 150 DORTS in these experiments (Tables 15, 23 and 24), confirming the existence of genetic variability for length of LTGD among the populations evaluated, under the conditions of the experiments. Because LTGD is a complex trait, several potentially important factors had to be controlled in order to separate genetic variability from non-genetic variability in the present study. Therefore, the results must be interpreted with this in mind. The conclusions arrived at in this study may not be applicable if duration of LTGD is measured on seeds produced or evaluated under

different conditions.

The parental lines used differed substantially for duration of LTGD. 'Mincu' and 'SMR-18', which will be referred to as short LTGD parents, required 100 DORTS or less after-ripening to reach 100 PG (completely non-dormant). 'Sumter' on the other hand was completely dormant for at least 150 DORTS, and was not detected to reach the completely non-dormant state until 250 or 350 DORTS (Figs 8 and 10). Thus, the LTGD requirement for seeds of the short dormancy cultivars did not over lap with the requirement of 'Sumter' seeds for duration of LTGD.

The second objective was to determine if the heritable differences for LTGD are an expression of the maternal and/or the embryo genotype. Cucumber seeds are composed of two genetically distinct tissues. The seed coat and nucellar membrane are maternal, while the embryo is filial and reflects the genes of both the maternal and pollen parent. The maternal plant itself might also affect the the duration of LTGD, since the seed is dependent on it for photosynthate and mineral nutrition. The seed may also be influenced by the maternal plant by translocation of plant growth regulating substances. An understanding of the relative importance of the maternal versus the embryo genotype for expression of LTGD is helpful for efficient design of a breeding program for short LTGD.

The heritable differences for duration of LTGD between 'Sumter' and 'Mincu' appeared, for practical purposes, to be only expressed by the embryo genotype. Thus, selection procedures for LTGD in populations derived from a cross between these cultivars should emphasize the embryo genotype. Selection will also be simplified by segregation of only one of the two seed genotypes.

In the 'Sumter' by 'SMR-18' cross heritable differences for duration of LTGD were observed to be both maternal and embryo expressed. This may complicate the selection among individuals and populations from this cross as well as the specific determination of the nature of the heritable differences. However, it appeared that the maternal effects were most important in defining short dormancy of 'SMR-18', and were less important than the embryo effects in

defining the very long LTGD of 'Sumter'. Thus, among the cultivars 'Mincu', 'SMR-18' and 'Sumter' the embryo genotype expressed the most important heritable differences. This suggests, that at least part, if not all, of the underlying LTGD mechanism is expressed by the embryo. The results obtained in the 1985 embryo excision experiment also support this hypothesis (Table 9).

The third objective of this study was to determine appropriate selection procedures for the manipulation of LTGD. This broad objective was only partially addressed, although useful pertinent information was obtained. The choice of selection procedures is a function of the objectives of the selection program, the number of genes involved with expression of the trait, the type of gene expression (additive - dominance - recessive) and the degree to which the phenotype reflects the genotype (heritability).

The objective of a selection program for LTGD would be to obtain lines or cultivars with LTGD as short as possible, providing short LTGD is not negatively associated with other characteristics. A possible negative association is premature sprouting of seeds in the fruit. Some information on this possible association has been obtained. As part of the overall LTGD project at SUNSEEDS, other genetic populations have been studied. One of these was a set of F_3-families derived from the cross 'Mincu' x 'PI 109484'. 'PI 109484' has a shorter LTGD than 'Mincu' or 'SMR-18'. Many of these F_3-families expressed premature sprouting and this characteristic was associated with very short or near absence of LTGD in those seeds which did not sprout prematurely. Thus, it appears selection for extremely short LTGD may not be desirable, unless another source of it can be found. The level of LTGD found in 'Mincu' and 'SMR-18' may be the most desirable, since these cultivars have been used for many years and no adverse seed storage or premature sprouting problems have been encountered. The very long LTGD of 'Sumter' appears to be undesirable. Therefore, a practical breeding goal, may be to screen breeding lines, and important inbreds, for their duration of LTGD relative to 'SMR-18' and 'Sumter'. Lines which carry long LTGD as in 'Sumter' could be crossed a source such as

'Mincu' or 'SMR-18' to achieve at least this level of short LTGD.

Based on the information obtained from this study, the majority of the genetic variability for duration of LTGD between 'Sumter' and 'Mincu' may be due to as few as one major gene, however, based on the general lack of parental type F_3-families, additional minor genes are likely involved. Therefore, the recovery of 'Mincu'-like short LTGD in a selection situation may be slightly more difficult than just the elimination of a single recessive allele from the population.

There also appeared to be relatively few segregating genes involved in the differences in LTGD between 'Sumter' and 'SMR-18'. The data fairly strongly suggested at least two major genes are involved, although additional modifying genes are possible for 'SMR-18'-like short LTGD. At least one major gene has embryo expression and at least one has maternal expression.

A major segregating gene, with embryo expression for duration of LTGD, was linked to the spine color locus. The linkage distance was estimated to be about 12 to 18 recombination units. Currently white spine cucumber cultivars are preferred, therefore this linkage relationship must be taken into account if a black spine line (such as 'SMR-18') is used as a source of short LTGD in a breeding program. The fact that a linkage relationship was identified also strongly suggests the importance of a single chromosomal segment in the determination of a major portion of the genetic variability for the duration of LTGD.

The general type of gene expression observed can be described as incomplete dominance for short LTGD. The term incomplete dominance was chosen, because at the durations of after-ripening at which the parental lines were maximally separated for PG (100 to 150 DORTS; Figs. 8 and 10), germination was essentially completely dominant to non-germination. This dominance for PG at these key durations of after-ripening, suggest that expression of short LTGD was also mostly dominant to long LTGD. The term partial is required, because at shorter durations of after-ripening (50 to 75 DORTS) homozygous short LTGD parental lines had higher PG and thus

shorter LTGD than heterozygous F_1 seeds.

The transient expression of incomplete dominance for short LTGD may have a direct bearing on the development of the most efficient selection procedure for short LTGD. If the durations of after-ripening at which complete and incomplete dominance are expressed can be identified, then it is theoretically possible to differentiate between families and individuals which have both desired short LTGD alleles from those which are heterozygous at the LTGD locus (or loci).

A simple between and within family selection procedure was developed and tested. At a between family selection intensity of just over 10% in each cross, over 95% selection efficiency was realized. Obtaining this high level of selection success supports the hypothesis that only a few major genes condition differences for duration of LTGD in this material. It also means that transfer of 'Mincu' and 'SMR-18' type short LTGD in to germplasm like 'Sumter' which have relatively long LTGD should be simple and rapid. 'Mincu' and 'SMR-18' short dormancy alleles may not respond similarly in all genetic backgrounds, but this has not been determined.

There are two important aspects of the selection procedure developed in this study which were probably key to its success. First, the seed production and handling procedures were very uniform among selection and selected units. Close attention to uniform seed maturity and allowing only one fruit to develop per plant, were probably very important in reducing non-genetic variation. Secondly, the selection took place when the parental lines expressed maximal difference for percentage of germination. Identifying this key duration of after-ripening was possible by testing all populations at several durations of after-ripening. However, in a breeding program it would be possible to screen and select for short LTGD in one germination test as follows: Following seed harvest and drying the material to be selected may be placed in freeze-storage. Grown concurrently with this material would be parental lines or other populations with known relative durations of LTGD. These indicator populations may be tested for germination at several

durations of after-ripening, thus identifying the optimum duration of after-ripening to screen the material to be selected. If time is a consideration the material to be selected, may be taken out of freeze-storage 2 or 3 weeks after the indicator populations, because results of from laboratory germination tests can be obtained in 2 or 3 weeks. A possible improvement in the selection procedure would be to use soil and a growth chamber to run the tests. The duration of after-ripening optimum for making selections for short LTGD, in the crosses tested in this study, would be at the point where the short dormancy parent has high PG but before the F_1 population had high PG, in other words at the point where expression of short LTGD is incompletely dominant.

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