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STUDIES ON GERMINATION AND ARTIFICIAL AGEING
OF SEEDS OF LYCOPERSICON LYCOPERSICUM

MSc(Botany)

UP

1990

**Studies on germination and artificial ageing
of seeds of Lycopersicon lycopersicum**

by

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Submitted as partial fulfilment of the requirements for the
degree

MAGISTER SCIENTIAE (BOTANY)

in the Faculty of Natural Science
Department of Botany
University of Pretoria

March 1990

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ACKNOWLEDGEMENTS

I owe a debt of gratitude to many people for help in preparing this dissertation. Above all I am indebted to my wife, Ronè, and my parents, Philip and Hattie Loubser, for their unfailing physical help, moral assistance and motivation.

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INTRODUCTION

Lycopersicon lycopersicum (L.) Karst. ex Farw. (= L. esculentum Mill.) (the tomato) belongs to the family Solanaceae. According to Strydom et al. (1967) the tomato originated in the Andes mountains of Peru from where it spread extensively in South and Central America. In about 1535 the Spanish Conquistadors brought it from Mexico to Europe. It is believed that tomatoes were not brought to South Africa from Europe, but from the East during the time of Simon van der Stel.

The first reference to tomatoes being grown for culinary use in Europe was made in a book published in 1781. Before this date tomatoes were still listed in European seed catalogues as an ornamental plant and not as a vegetable. It was believed, at that time, that tomatoes were poisonous (Walls, 1975). Although it was first used as a vegetable comparatively recently, its consumption has increased so rapidly that in most countries of the world it can be described as the most important vegetable (Mahmoud and George, 1984).

In South Africa the total value of fresh tomatoes sold on the most important markets has increased from R1 564 000 in 1947/48 to R95 500 000 in 1985/86. The quantity of tomatoes processed by factories varies from year to year, in accordance with the requirements of the fish canning factories, but it may be estimated at an average of 100 000 tons a year, with a cash value of R10 000 000. If the tomatoes sold on the smaller markets are also taken into consideration, the gross value of tomato production in South Africa is estimated at approximately R120 350 000 annually (Department of Agricultural Economics and Marketing, 1986).

It is therefore obvious that tomato production is an integral component of the South African agricultural economy. The international demand for tomatoes increases every year and South African producers should strive for optimal production since the local climate is not generally ideal for tomato growing (Strydom et al., 1967). To enable the tomato producer to establish economically viable yields, the seed should be of the best possible quality.

Like other cultivated crops, the tomato has not escaped the onslaught of pathogens, some of which are seed-borne. Seed treatments to control the spread of seed-borne diseases have therefore become customary. In South Africa, the most common seed treatments are the hot-water treatment against bacterial canker, fungicide treatments to inhibit storage fungi and trisodium phosphate against certain viral diseases (Oosthuizen, 1975; Boelema, 1982).

During the developmental phase of such seed treatment practices, they are primarily evaluated for their effectiveness against the pathogen, parasite or saprophyte for which they are being developed. Often only the short term effects of a treatment on the seed are investigated before it is marketed. The possibility therefore exists that a seed treatment could be generally accepted, or even registered as an effective control measure against seed disease, although it might decrease the germination or vigour of the seed after storage.

The original aim of this study was to study effects of seed treatments on aged tomato seed, but the lack of satisfactory standardised germination and artificial ageing procedures prompted initial investigation of these aspects.

The development of a standardised germination method was necessary to monitor the possible effects of ageing and hot-water treatment on germination of the seeds under optimal conditions. Data on germination capacity were supplemented with measurements of germination rate and seedling organ lengths. These variables were used for investigating the possible inhibitory effect of seed treatments on seedling growth, and are regarded by certain authors as indicative of seedling vigour. The standard germination test is an important tool for assessing the possible effects of ageing and seed treatments. The International Rules for Seed Testing (ISTA Rules, 1985) prescribe two methods for the optimum germination of tomato seed in a seed testing laboratory. It was deemed necessary to standardise a method which would be optimal for the purposes of this study. It was also necessary to standardise the application of water to the germination substrata since the ISTA Rules lack prescriptions in this respect. The application of standard water quantities for germination experiments is essential in repetitive scientific work. These investigations are discussed in Chapter 1.

There is currently no acknowledged method for the artificial ageing of tomato seed. Tomato seed has a relatively long life span during storage under optimal storage conditions (± 20 years). Storability under sub-optimal conditions could, however, be significantly lower. Artificial ageing could therefore be a useful tool in the research on tomato seed owing to the much shorter experimental period concerned. Artificial ageing could, for instance, be used for investigations on the role of storage fungi during ageing, deterioration of seed vigour during ageing, the effects of storage temperature and seed moisture content during storage, and, of particular importance to this study, the evaluation of interaction between seed treatments and ageing.

For reliable interpretation of the effects of tomato seed ageing, especially when the emphasis is on interaction between other factors and ageing, the validity of artificial ageing as a simulation of the natural process should be established. It was therefore decided to compare the effects of the "Controlled Deterioration Test" (Perry, 1981), a possible method for the artificial ageing of tomato seed, with those of seed storage. The results of these investigations are discussed in Chapter 2.

The interaction of the hot-water treatment with ageing was investigated after the above-mentioned investigations had been completed. Two serious diseases of tomato are seed-borne (Wager, 1976; Nedumaran and Vidyasekaran, 1982): Bacterial Canker [Corynebacterium michiganense (E.F. Sm.) H.L. Jens.] and Bacterial Spot [Xanthomonas vesicatoria (Doidge) Dows]. The hot-water treatment is commonly used for disinfecting tomato seed before planting. The effects of this treatment on germinability have not hitherto been well documented. These effects were investigated before and after ageing of the seed and are discussed in Chapter 3. The standardised procedures established in Chapters 1 and 2 were used for the investigation of interactions with seed ageing. Germination data obtained by these procedures were supported by ultrastructural studies of the embryo (Chapters 2 and 3). Since the primary root is regarded as being very sensitive to external influences (Vigil et al., 1984), it was decided to use only the radicle tip for ultrastructural investigations.

CHAPTER 1

STANDARDISATION OF GERMINATION PROCEDURE

1.1 INTRODUCTION

Chalam *et al.* (1967) define germination as the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, indicate its ability to produce a normal plant under favourable conditions.

Seed requires certain conditions for optimal germination and production of normal seedlings. The most important requirements are moisture, temperature and light. The validity of seed germination tests is dependant on the provision of optimal conditions. Temperature and light requirements have been standardised by the International Seed Testing Association (ISTA) for tomato seed germination (ISTA Rules, 1985). The prescription for temperature requirement is an alternating cycle of 16 hours at 20°C and eight hours at 30°C. Light is not an essential factor for germination, although the use thereof may facilitate seedling evaluation. Necrotic areas on the cotyledons and primary leaves are more easily assessed in seedling organs where the chloroplast has become functional in the presence of light.

Different options of substrata are prescribed by the ISTA Rules for tomato seed germination tests, but the amount of water to be applied has not been standardised. Bewley and Black (1982) specify that optimum germination on or between paper substrata requires an optimum amount of water - too little creates external and internal impedances and an excess may restrict oxygen diffusion to the seed. The ISTA Rules provide general prescriptions for species and allow for the investigation of deviations from the Rules to obtain optimal conditions for local cultivars.

Experience has shown that laboratory substrata, especially those not specifically developed for germination testing, may induce phytotoxic effects in seedlings. Such effects may obscure the influence of seed treatments in a germination test and result in serious misinterpretations. Special care should be taken to eliminate those factors which might influence optimal germination. Experimentation was therefore deemed necessary to determine and standardise the optimal moisture and substrata requirements for seed germination of the specific cultivars used in this study.

1.2 STANDARDISATION OF GERMINATION SUBSTRATUM

The different options prescribed by the ISTA Rules (1985) for the germination test on tomato seed are "BP" (between paper) and "TP" (top paper). The purpose of this investigation was to compare three ISTA-approved germination substrata that could be used in these options.

1.2.1 MATERIAL AND METHODS

The two cultivars chosen for this research project represent the two major uses of the tomato fruit in South Africa; tomatoes marketed either as fresh market produce or as a canned product. Floradade is currently the most important cultivar on the fresh market and has the largest production area in the country. It is resistant against two of the races of Fusarium oxysporum Schl. (Fusarium wilt) and to Verticillium dahliae Kleb (Dept. of Agriculture R.S.A., 1983). Other characteristics of Floradade are listed in Table 1.1.

Roma VF is the most important cultivar for the tomato canning industry. The fruit has a firm skin so that even in the ripe stage, it may be transported over long distances without significant damage. Strydom et al. (1967) regards this characteristic to be of primary importance in tomatoes for factory processing since the factories insist that the fruit must ripen on the plants. The dry matter content is higher than in fresh market tomatoes which is also a great asset in the manufacture of paste and puree. The yield of Roma VF is high and it is resistant to Fusarium wilt, but it appears to be more susceptible to tomato canker than other cultivars (Strydom et al., 1967; Boelema, 1982). Other characteristics of Roma VF are listed in Table 1.1.

Table 1.1: Distinctive characteristics of the two tomato cultivars used in this project.

Floradade	Roma VF
determinate growth	determinate growth
fruit is flat-round	fruit is oval to pear-shaped
greenback intensity is present	greenback intensity is absent
abscission layer is present	abscission layer is present
fresh market tomato	canning tomato

Another consideration which determined the choice of cultivars for this project was that Floradade and Roma VF represent two genetic extremes among tomato cultivars.

Seed of Floradade and Roma VF, from a different origin as those used in Chapters 2 and 3, were used for the investigation presented in this chapter. The Floradade sample was obtained from "Dana Seeds" and produced in the East London area. The Roma VF seed sample was certified seed obtained from "Patoma Seeds" and produced in the Kaap Muiden area. All germination determinations and purity analyses were done according to the ISTA Rules (1985).

Only physically pure seed was used for this study. According to the International Rules for Seed Testing (ISTA Rules, 1985), pure seed is that fraction of a seed sample which consists of only true seed of the specific cultivar and includes only seed pieces larger than half the original seed size.

Purity analyses were performed on two replicates of all seed samples according to the ISTA Rules (1985). The percentage by mass of each of the component parts (pure seed, inert matter) was calculated to one decimal place. Percentages were based on the sum of the mass of the components and not on the original mass of the working sample. The sum of the masses of the components was, however, compared with the original mass as a check against loss of material or other error. All replicates examined were within the tolerance prescribed by the ISTA Rules (1985).

The Floradade seed sample consisted of 100% pure seed with a moisture content of 7,0% on a fresh mass basis. The Roma VF sample displayed a purity value of 99,6% owing to 0,4% inert matter. The initial moisture content of the seed was 7,8% on a fresh mass basis.

Three different types of germination substrata were compared:

(a) RP ("roll paper") dipped in water (Fig. 1.1).

Four layers of "roll paper" (254 x 380 mm) ("Anchor" germination paper) were dipped in water, wrung out by hand and placed on each other with the bottom sheet protruding approximately 30 mm at the 380 mm edge. The top sheet was removed and 100 seeds were placed evenly spaced on the third layer. The top sheet was replaced and the protruding part of the bottom sheet folded over the top three layers.

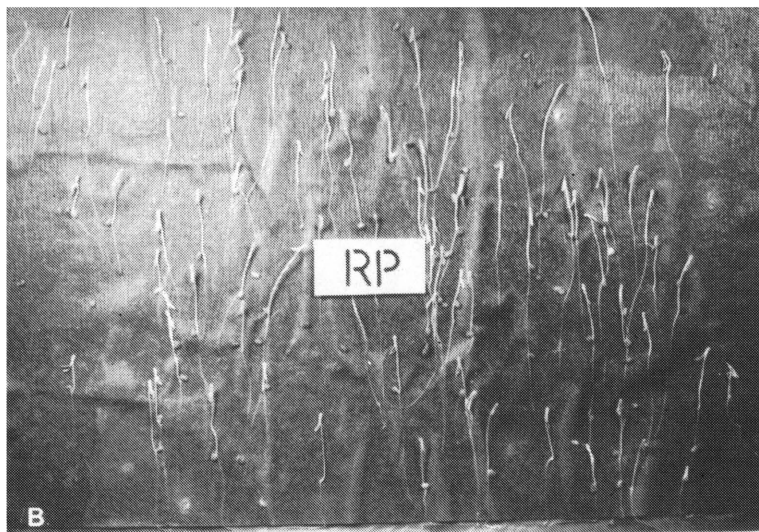


Figure 1.1: The "roll paper" (RP) germination substratum classified as a "between paper" substratum and prescribed by the ISTA Rules (1985) for the germination test on tomato seed. A: substratum, enclosing seeds, is rolled for the purpose of seed germination; B: substratum is opened for the purpose of seedling evaluation.

The four sheets containing the 100 seeds were then rolled from the 254 mm side. The overlapping bottom sheet prevented seeds from falling out when the roll was placed in a vertical position. The rolls were secured with rubber bands at positions approximately one third from the ends. The rolls were placed in polyethylene bags and into plastic drums in a growth chamber. The polyethylene bags were left open at one end for aeration. The plastic drums were sealed with lids thus preventing unnecessary water loss to the atmosphere in the growth chamber.

(b) TP ("top paper") with 14 cm³ water (Fig. 1.2).

One layer of "top paper" (TP) (Schleicher and Schüll No. 598 filter paper discs with a diameter of 150 mm) was placed on one layer cellulose wadding which was cut according to the size and shape of the "top paper" disc. The two layers were placed in a 150 mm diameter glass Petri dish and 14 cm³ water added. One hundred seeds were evenly spaced on top of the paper substratum. The Petri dish was closed and placed in a growth chamber.

(c) PFP ("pleated filter paper") and 50 cm³ water (Fig. 1.3).

"Pleated filter paper" (2000 x 100 mm) (Schleicher and Schüll No. 598 pleated germination paper) consisting of 50 double pleats was placed in a tupperware container [130 mm (length) x 120 mm (width) x 35 mm (height)] and 50 cm³ water added. Two seeds were placed in each fold at positions approximately one third from the edges of the paper. The tupperware containers were closed and placed in a growth chamber.

Germination substrata (a) and (c) are categorised as "between paper".

Four replicates of each germination test were incubated in the dark at 20/30°C (16h/8h) alternating temperatures. The growth chamber temperature was checked twice daily and the temperature and relative humidity (which was kept at 15%) were monitored by means of a thermohygrograph.

Germination counts and seedling evaluations were performed after 5; 7; 9; 11; 13 and 14 days.

Evaluation was performed with reference to the following seed and seedling characteristics:

- (i) The extent of secondary infection of the seedlings.
- (ii) The percentage of normal seedlings, abnormal seedlings, ungerminated fresh seeds and dead seeds.

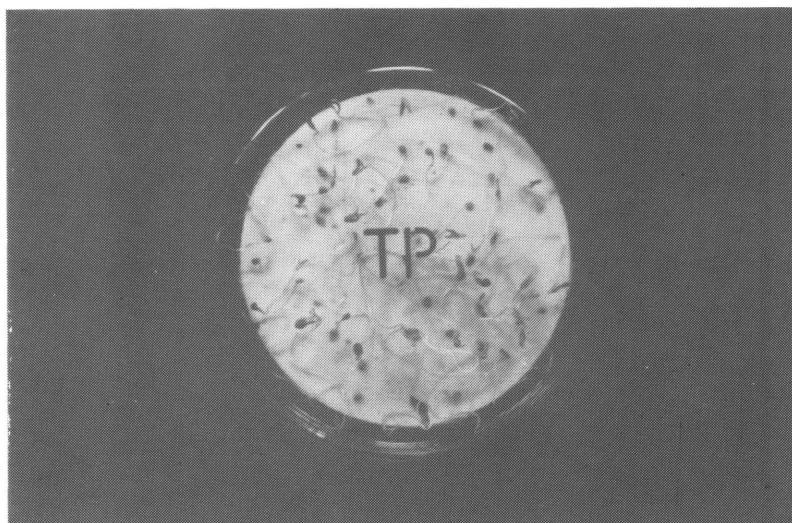


Figure 1.2: The "top paper" (TP) germination substratum in a 150 mm diameter glass Petri dish as prescribed by the ISTA Rules (1985) for the germination test on tomato seed.

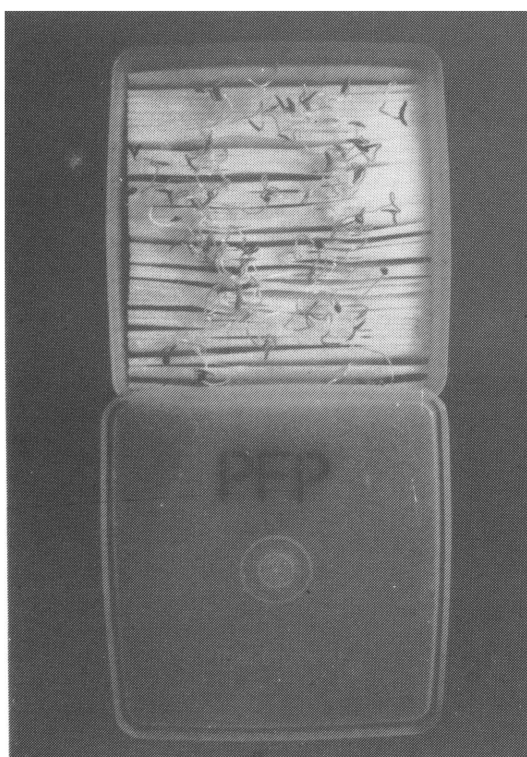


Figure 1.3: The "pleated filter paper" (PFP) germination substratum in a tupperware container classified as a "between paper" substratum and prescribed by the ISTA Rules (1985) for the germination test on tomato seed.

(iii) Looping of the hypocotyl. The ISTA Rules (1985) regard looping of the hypocotyl as an abnormal characteristic in tomato seedlings.

The percentage of normal seedlings after a five day incubation period is referred to as "germination energy" (Department of Agriculture, Canada, 1913).

1.2.2 RESULTS AND DISCUSSION

Secondary infection of Roma VF seedlings occurred only on the top paper substratum and the evaluation of the seedlings was difficult. Floradade displayed a reasonable number of stunted primary roots on top of paper, while this symptom was absent in Roma VF seedlings. This phenomenon could possibly be attributed to the conditions during harvesting of the Floradade seed lot. The acidity of the juice and fine pulp during the fermentation process, is not always effectively controlled and may have been unfavourable for the seed. This could have been the reason for a limited occurrence of secondary infection, although possible damage could also have been caused to the embryo.

It was established by a chi square test that, in the case of Floradade, the different substrata had no significant effect on any of the germination variables determined. (Table 1.2) (Appendix 1.1; pp 118).

Table 1.2: Germination capacity of tomato seed of Floradade and Roma VF after a 14-day incubation period. (RP = roll paper; TP = top paper; PFP = pleated filter paper).

CULTIVAR	FLORADADE			ROMA VF		
	RP	TP	PFP	RP	TP	PFP
Normal seedlings %	77	83	80	72	69	74
Ungerminated fresh seeds %	3	2	2	0	0	0
Abnormal seedlings %	11	8	12	8	16	8
Dead seeds %	9	7	6	20	15	18

The germination variables of Roma VF, however, differed with regard to the ratio between normal seedlings, ungerminated fresh seeds, abnormal seedlings and dead seeds produced in/on the different substrata (Table 1.2) (Appendix 1.1; pp 118). This can be attributed to the greater number of abnormal seedlings found on the top paper substratum compared to the other substrata. There was also a significant difference between the two cultivars with regard to the ratios between germination variables in/on the substrata investigated (Table 1.2) (Appendix 1.1; pp 119 - 120).

The percentage normal seedlings of both cultivars germinated in/on the different substrata was within the tolerance limits allowed by the ISTA Rules (1985). There was, however, a difference concerning the ease with which the seedlings from the different germination methods could be evaluated. According to ISTA specifications, normal seedlings should be removed from the substratum after each germination interval. Seedlings which had germinated in the "pleated filter paper" (BP/PFP) were markedly easier to remove than those which had germinated in/on the other two substrata. A disadvantage of this method was, however, the occurrence of seedlings with loops of the hypocotyl owing to the limited height of the containers generally used for this germination method (Table 1.3). Loops, induced by a suboptimal germination method may be misinterpreted as being the result of an abnormality in the seed.

Table 1.3: The occurrence of tomato seedlings with (+) and without (-) loops of the hypocotyl after different incubation periods.

SUBSTRATUM	CULTIVAR			
	FLORADADE		ROMA VF	
	Incubation period (days)		Incubation period (days)	
	5	7	5	7
RP	-	-	-	-
TP	+	+	+	+
PFP	-	+	-	+

The "roll paper method" (RP), allowed the effective evaluation of seedlings. The cotyledons are visible at a reasonably early stage during the incubation period because of the removal of the seed coat due to the resistance offered by the substratum. The early evaluation of the essential organs of a seedling is an important aspect of the germination test. The conditions for germination as prescribed by the ISTA Rules (1985) are supposedly optimal for radicle emergence and the early development of a seedling. It is, however, important to realize that these conditions are not necessarily favourable for later seedling development. A disadvantage of the roll paper method is the possibility of mistakenly removing fresh, ungerminated seed, attached to the root systems of other seedlings, with the impression that they are empty seed coats. The numerous empty seed coats also hamper the final evaluation of ungerminated seed.

Various difficulties, concerning the handling potential and evaluation of seedlings, were encountered when tomato seed was germinated on "top paper" (TP). Since insufficient resistance was contributed by the substratum, the seedlings did not readily shed their seed coats. The occurrence of secondary loops was very conspicuous owing to the limited height of the Petri dish containers (150 mm diameter; 15 mm height) (Table 1.3). The root systems were completely interlaced which hampered the handling and evaluation of seedlings.

Twenty eight percent more Floradade seedlings and twelve percent more Roma VF seedlings, were able to shed their seed coats after a seven day incubation period in the roll paper (RP) germination substratum in comparison with the performance in/on the other two ISTA-approved germination substrata (Table 1.4).

Table 1.4: Percentage seed coats shed from cotyledons of Floradade and Roma VF after 5 and 7 days in germination tests.

SUBSTRATUM	CULTIVAR			
	FLORADADE		ROMA VF	
	Incubation period (days)		Incubation period (days)	
	5	7	5	7
RP	10%	79%	8%	62%
TP	0%	17%	0%	19%
PFP	7%	51%	5%	50%

As seedlings with intact seed coats could not be evaluated as normal at this stage, the result was a marked difference in the percentage normal seedlings after a five day incubation period with an advantage of 6% and 2% (RP over TP and PFP respectively) for Floradade and 12% and 3% for Roma VF (Fig. 1.4). According to Mann-Whitney-U statistics, the difference in the percentage normal seedlings after a five day incubation period between the two cultivars was significant in the roll paper substrata, although the difference in the pleated filter paper substrata was not statistically significant (Appendix 1.2; pp 122). It was also statistically established that the percentage normal seedlings after a five day incubation period of the two cultivars in the roll paper substrata did not differ significantly from the equivalent variable in the pleated filter paper (Appendix 1.2; pp 121). The percentage normal seedlings after a five day incubation period on the top paper substrata, however, differed significantly from that in the two between paper methods. This difference was of such a magnitude that a statistical analysis was deemed unnecessary.

The "roll paper" (RP) germination substratum provides excellent resistance for the germinating seedling and simulates the natural characteristics of the soil. These characteristics of the substratum also inhibit looping of the hypocotyl and the occurrence of secondary fungus development.

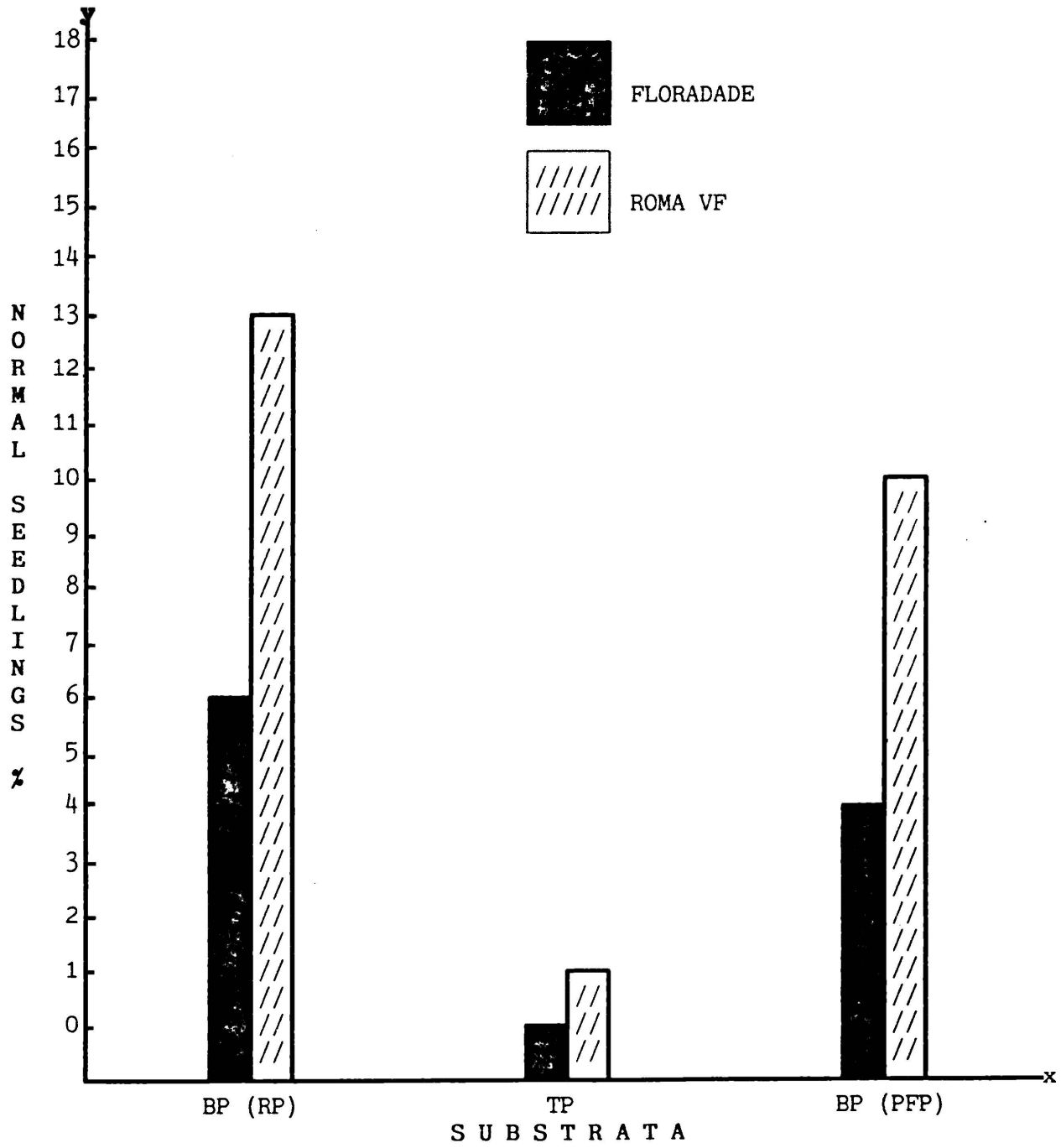


Figure 1.4: The percentage normal seedlings of two tomato cultivars after an incubation period of five days in/on different, ISTA-approved germination substrata.

Another phenomenon was the conspicuous difference in germination energy (percentage normal seedlings after a five day incubation period) between the seed germinated on the top paper substrata on the one hand, and the seed germinated in the pleated filter paper and roll paper substrata on the other. This could possibly be related to differences in mass flow and diffusion of water from the different substrata to the seed, differences in matric potential or differences in phytotoxicity of the different substrata.

The mass flow of water from the different substrata to the seed could be a factor contributing to the significantly lower germination percentage after a five day incubation period of the seed germinated on top paper, since the seeds do not have a similar access to free water as in the between paper substrata. The influences of matric potential and phytotoxicity were not investigated during this study, although the importance of such an investigation should not be underestimated. The surface topography of the seed coat (e.g. hairs) could also influence the water relations of the seed significantly. It is assumed that the factors contributing to differences in the percentage normal seedlings after a germination period of five days are related to differences in the physical properties of the substrata.

It is concluded that, of all the approved substrata prescribed in the ISTA Rules (1985), the "roll paper" method [BP(RP)] represents the best substratum for the germination of Floradade and Roma VF tomato seed. This substratum is superior to the other approved germination substrata mainly due to the resistance offered to the developing seedlings and the superior handling potential of seedlings.

1.3 STANDARDISATION OF WATER APPLICATION TO THE GERMINATION MEDIUM

For seeds of certain crops where "between paper" (BP) is prescribed by ISTA (1985), and applied as "roll paper" (RP), four sheets of the paper are dipped in water and excess water wrung out by hand.

Equal quantities of water are, therefore, not necessarily applied to different replicates and tests, resulting in non-identical conditions. It is essential that scientific investigations be reproducible. The aim of this investigation was to recommend a standardised quantity of water for the "roll paper" (RP) germination procedure for tomato seed.

One hundred and twenty eight sheets of paper were grouped in 32 replicates consisting of four 383 x 254 mm sheets each, and the replicates weighed separately. Each replicate was dipped in water, wrung out by hand and weighed. The difference between dry and wet mass was calculated as the mass of water retained by the germination substratum (Appendix 1.3; pp 123) and was found to be 47,2 cm³. As this is an awkward volume to measure out in routine tests conducted in a seed testing laboratory, it was decided to standardise on a volume of 50 cm³.

The common method used by many seed laboratories in the world (substrata dipped in water and excess water wrung out by hand), is an unreliable watering method. This conclusion could be reached from the statistical analysis of the replicates in Appendix 1.3 (pp 123). The coefficient of variation is approximately seven times higher between replicates after water is added by this method. Such inconsistencies between replicates and even different germination tests, could have serious implications for test results, especially as far as water-sensitive seeds are concerned.

For understandable reasons the ISTA Rules (1985) lack rigid prescriptions regarding detailed methods to be applied by seed laboratories. The International Seed Testing Association, however, encourages continuing investigations by member laboratories to satisfy local requirements. This study is an example of such an investigation and recommendations can now be made to local seed testing laboratories for a detailed, standardised germination procedure for South African-cultivated tomato seed. This procedure was used for subsequent investigations in this study.

CHAPTER 2

ARTIFICIAL AGEING OF TOMATO SEED

2.1 INTRODUCTION

Tomato seed has a relatively long life span during storage. It was decided to apply artificial ageing treatments to eliminate the long time involved for natural ageing to the point of deterioration. To accelerate the ageing process in seeds, the moisture content must be raised, preferably to a known value for reliable scientific interpretation (Justice and Bass, 1979). The International Seed Testing Association (ISTA) has not yet established a suitable method of equilibrating tomato seed to higher moisture contents and it was decided to compare two methods that are commonly used in seed research. One of these methods, imbibition in liquid water, is recommended by ISTA (Perry, 1981) for seeds of various other species. It was decided to compare this method with another acknowledged method in seed research i.e. moisture equilibration at different relative humidities, for their applicability to the moisture equilibration of tomato seed (2.3.2). A second aim of this investigation was to make recommendations to ISTA concerning a reliable method for the artificial ageing of tomato seed.

The acceptability of artificial ageing as an exact simulation of the natural process is currently questioned by many seed scientists (Hailstones and Smith, 1988; Priestly and Leopold, 1983; Ching, 1982 and Harman and Mattick, 1976). To investigate this reservation, tomato seeds were also aged under natural, though sub-optimal, conditions. Standard germination tests, germination rates, seedling organ lengths, ultrastructural investigations of the radicle tip, as well as the assessment of micro-organisms in aged seed samples were utilized to compare the physiological and ultrastructural differences resulting from the two ageing methods.

2.2 MATERIALS AND METHODS

2.2.1 CHOICE AND ORIGIN OF SEED

South African government-certified seed was used in this study. Selected cultivars were obtained from "Mayford Seeds (Pty) Ltd" seed merchants. Production area, harvesting method, seed age and genetic background were taken into consideration in seed selection and uniformity was maintained throughout. Seed samples used for this project were obtained from seed lots produced on seed farms in the Malelane district.

After harvesting the fruits, the seeds were removed in the conventional way by means of fermentation. Tomato fruits were ground up and the seed and fine pulp were separated from the skins and coarse material by sieving. The juice, fine pulp and seeds were allowed to ferment at 20 - 25°C for 25 to 48 hours, or until the jelly-like pulp could be readily washed away. After washing, the seed was dried in thin layers and stored (personal communication: Mr R. Zingle, Mayford Seeds, P.O. Box 160, Lanseria, 1748).

All seed used for this study was produced during August/September 1982 and stored under mild conditions by the producer. The samples were received in this laboratory on 25 July 1984 and subsequently stored in sealed glass bottles at 5°C. According to Work (1952) hardly any deterioration of tomato seeds can be expected to occur during low temperature storage for up to at least 10 to 12 years. The experiments in this study were conducted during 1985 and 1986.

Two cultivars were used for this project namely Floradade and Roma VF. The certification details of the seed lots used appear in Table 2.1.

Table 2.1: Certification details of tomato seed lots used in this project.

Cultivar	Date of sampling	No. of containers	Mass of lot (kg)	Code No.	Certifi- cation No.
Floradade	27 August 1982	5	84	GZW5586	55461
Roma VF	2 October 1982	13	245	GEX5628	55475

2.2.2 CONTROL OF SEED MOISTURE CONTENT

Two methods were compared for increasing the moisture content of tomato seeds to predetermined levels prior to controlled deterioration.

2.2.2.1 Imbibition from liquid water

Imbibition, as prescribed by Perry (1981) for moisture adjustment in certain other species, displayed a disadvantage when conducted on tomato seed. The mass of the seed doubled rapidly after contact with the moistened filter paper, even before the first weighing could be performed. This was assumed to be due to rapid wetting of the seed surface because of the hairy nature of the seed coat. The seed was therefore imbibed for 12 hours and then dried back to the required moisture content in a forced draught oven at 40°C. The latter procedure is not prescribed by Perry (1981).

2.2.2.2 Moisture equilibration in atmospheres of specific humidity

Tomato seeds were equilibrated to elevated moisture contents by means of the static method (Justice and Bass, 1979). Aqueous solutions of sulphuric acid (H_2SO_4) were used to create specific relative humidities for moisture equilibration. The graded solutions were prepared according to the prescriptions of Solomon (1951). The solutions for providing specific relative humidities were placed in a desiccator (300 mm diameter; 300 mm height). Seventy seed samples of 100 seeds each were placed in open containers (65 mm diameter) in the space above the sulphuric acid solution (Fig. 2.1). The seeds were allowed to remain in the desiccator until moisture equilibrium was reached after approximately 14 days. The laboratory temperature was maintained at 20°C by means of an air-conditioning unit.

Seed samples of pure seed (ISTA Rules, 1985) were equilibrated to predetermined moisture contents (see Table 2.2, p 38) in four replicates of 100 seeds each. The control seed samples were kept in sealed containers at 5°C until the equilibration of the other samples was complete.

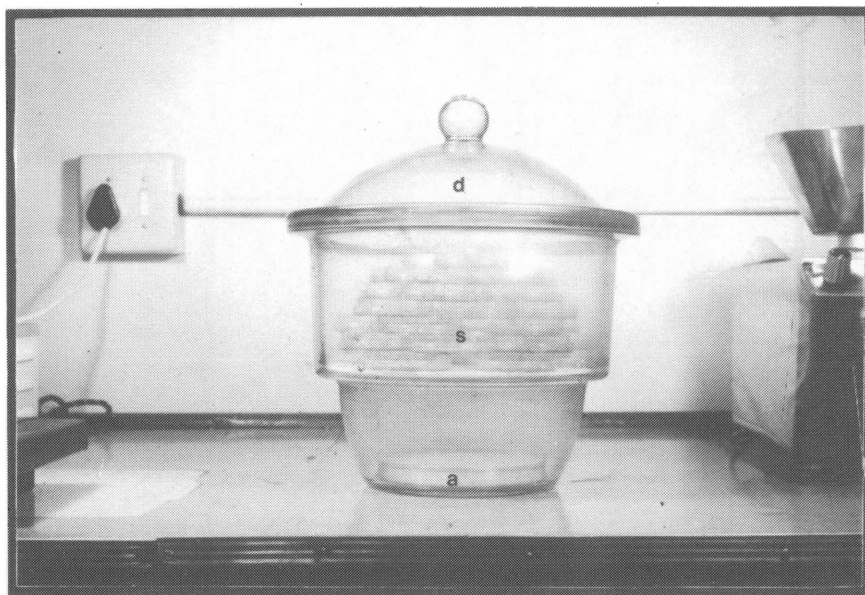


Figure 2.1: Moisture content of tomato seed samples was increased by moisture equilibration over aqueous sulphuric acid solutions in a sealed desiccator (Solomon, 1951). d = desiccator; s = seed samples; a = H_2SO_4 solution.

Moisture determinations on control and moisture-equilibrated seed samples (ISTA Rules, 1985) were conducted on two replicates of 100 seeds each. The high constant temperature oven method as prescribed by the ISTA Rules (1985), was used for all moisture determinations. Duplicate samples containing 100 seeds each were evenly distributed over the surface of aluminium containers (90 mm diameter; 23 mm height). The containers and their covers were weighed before and after filling. The containers were placed on top of their covers, in a forced draught oven maintained at a temperature of 130 - 133°C and dried for one hour. The drying period began at the time the oven temperature returned to the prescribed value. At the end of the prescribed period, the containers were covered and placed in a desiccator to cool for 30 - 45 minutes. After cooling, the containers were weighed with their covers and contents. The relative humidity of the ambient air in the laboratory was monitored and was below 70% during the time the determinations were conducted. The moisture content as a percentage of wet mass was calculated to one decimal place. Sample moisture contents were calculated from the arithmetic mean of the duplicate determinations taking the provision into account that the two duplicate determinations should not differ by more than 0,2%.

After adjustment of seed moisture contents by the two methods described above, seeds were subjected to controlled deterioration (as described below) and their subsequent germination performance compared. On the basis of the results obtained in this study, it was decided to adjust seed moisture content in further experiments on ageing techniques by means of the water vapour equilibration method.

2.2.3 SEED AGEING TECHNIQUES

Different methods to age tomato seed under laboratory conditions were compared:

1. Controlled deterioration [for periods up to 10 days at a temperature of 45°C and seed moisture content of $\pm 20\%$ wet mass basis].
2. Storage under relatively favourable conditions [up to 12 months at a temperature of 20/30°C (16h/8h) and a seed moisture content of $\pm 7\%$ wet mass basis].
3. Storage under suboptimal, though realistic conditions [up to 12 months at a temperature of 20/30°C (16h/8h) and a seed moisture content of $\pm 30\%$ wet mass basis].

2.2.3.1 Controlled deterioration

Controlled deterioration was applied to deteriorate tomato seed without the limitations of the time factor in natural ageing. Controlled deterioration periods did not exceed 10 days in any case.

Matthews and Powell (1981) published the "Controlled Deterioration Test" in the International Seed Testing Association Handbook of Vigour Test Methods. The basis of the test is an ageing technique, similar in principle to accelerated ageing. Controlled deterioration incorporates effective control of seed moisture content and temperature during the period of ageing since the initial seed moisture content is raised to the same level for all samples prior to the period of deterioration at high temperatures.

Pure seed samples consisting of four replicates of 100 seeds each were equilibrated in desiccators at a relative humidity of 95% to a seed moisture content of $\pm 20\%$ (Solomon, 1951). The latter was chosen as the moisture content to which seed samples were to be equilibrated since Roberts and Ellis (1982) believe that it is at approximately 20% moisture content that changes occur which could have an adverse effect on viability.

After moisture equilibration, the samples were heat-sealed in laminated aluminium foil polyethylene pouches (140 x 100 mm) (Fig. 2.2) (Rumsey, 1962) and submerged in a water bath at 45°C for periods of one to 10 days.

2.2.3.2 Seed storage

Tomato seed is known for its good storage potential. According to Popovska et al. (1981) the germination potential of tomato seed diminishes very slowly when kept under mild storage conditions.

The sub-optimal storage conditions decided upon were representative of conditions which could be experienced in the store room of a seed producer. It was estimated that the temperatures on the shelf of a store in the natural production environment of tomato seed (e.g. the Transvaal Lowveld) would be 20°C during the night and 30°C during the day. To monitor the effects of "natural" deterioration seed was therefore stored in incubators with a 20/30°C (16h/8h) alternating temperature cycle.

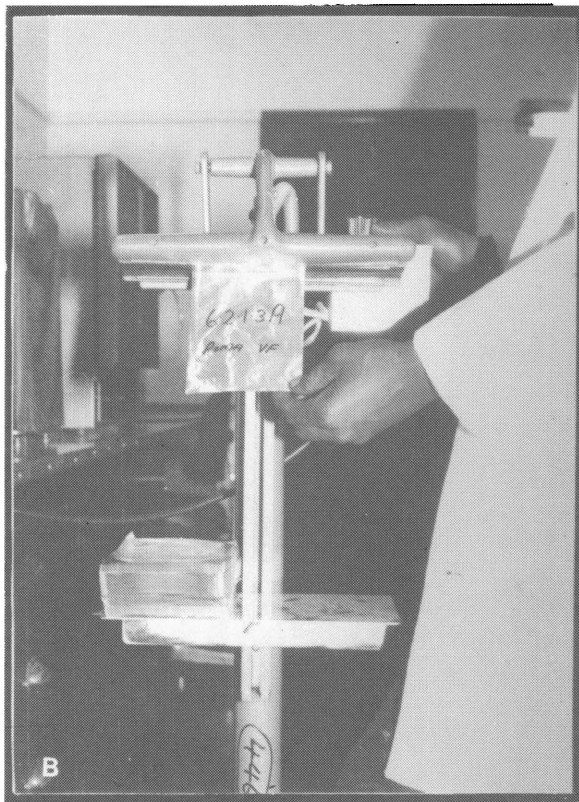


Figure 2.2: Laminated aluminium foil/polyethylene pouches (140 x 100mm)(A) used for the sealing of seed samples (B).

Since tomato seed deteriorates slowly (Popovska *et al.*, 1981), even under sub-optimal temperatures, it was decided to equilibrate them to a relatively high moisture content ($\pm 30\%$) before storage at $20/30^{\circ}\text{C}$ (16h/8h). These storage conditions were necessary to monitor ageing of the seed over a shorter time period than would have been possible under optimum storage conditions. Similar natural storage conditions could, in fact, prevail in certain coastal regions of South Africa. Seed samples were sealed and stored for periods up to 12 months under these conditions. The high moisture content in the seed was obtained after equilibration at 100% relative humidity for 14 days. Four replicates of 100 seeds each were used.

A further storage treatment consisted of seed samples equilibrated to relatively low moisture contents ($\pm 7\%$) which were then stored at $20/30^{\circ}\text{C}$ (16h/8h) for periods up to 12 months. These experiments were also conducted on four replicates of 100 seeds each.

2.2.4 GERMINATION AND GROWTH VARIABLES DETERMINED

2.2.4.1 Germination tests

ISTA Rules (1985) prescribe two types of substrate that can be used for the testing of tomato seed, i.e. "top paper" and "between paper". According to the investigation reported in Chapter 1 paper rolls, which represent "between paper" substrata, with 50 cm^3 water, proved to be the most effective germination medium for tomato seed and was used in all subsequent studies. Distilled, deionized water was used to moisten the germination substrata. Comparative, preliminary germination experiments with ordinary tap water and distilled-deionized water were conducted on tomato seed and it was established that the purified water had no inhibiting effect on the germination capacity or germination rate of at least two tomato cultivars.

Seed samples of pure seed were germinated in a growth chamber at $20/30^{\circ}\text{C}$ (16h/8h) alternating temperatures (ISTA Rules, 1985). The growth chamber temperature was checked twice daily and the temperature and relative humidity were monitored by means of a thermohygrograph. Germination tests were conducted in the dark (Georghiou *et al.*, 1982).

Germination counts were made and seedlings evaluated after five, seven, nine, eleven, thirteen and fourteen days of incubation. Seedlings were classed as normal when all their essential organs were well developed, complete, in proportion and healthy. Seedlings with slight defects or secondary infection (infection by fungi or bacteria from sources other than the parent seed), provided they showed an otherwise satisfactory and proportionate development, were regarded as normal.

Seedlings were classed as abnormal if they did not show the potential to develop into normal plants under favourable conditions. Abnormal seedlings are those with any of the essential organs irreparably damaged, missing, weakly developed, deformed, out of proportion or decayed as a result of primary (i.e. from the parent seed) infection. The classification of normal and abnormal seedlings was conducted in strict accordance with the ISTA Rules (1985).

2.2.4.2 Viability tests

Seeds that had not germinated by the end of the test period (14 days) were subjected to a biochemical test for viability. Tetrazolium testing has been developed to provide rapid estimates of seed viability.

The prepared tetrazolium solutions and salts were stored in a refrigerator at 5°C. The solutions were stored in amber bottles, while the salts were kept in black plastic containers. A pH of 6,5 - 7,0 was maintained in tetrazolium solutions.

The seeds were placed in the dark during staining although Grabe (1970) postulated that light has little effect on the tetrazolium test and may be disregarded as a factor affecting the accuracy of the results.

Since the seeds were already imbibed, the conditioning process for tetrazolium testing was omitted.

The seeds were pierced through the central endosperm around which the embryo is coiled so that the tetrazolium solution could come into contact with the embryo (Moore, 1985) (Fig. 2.3). The seeds were treated in small plastic containers (650 mm diameter, 10 mm height). Sufficient solution was used to cover the seeds. The treatment time in the tetrazolium chloride solution was 12 hours at a temperature of $\pm 20^{\circ}\text{C}$.

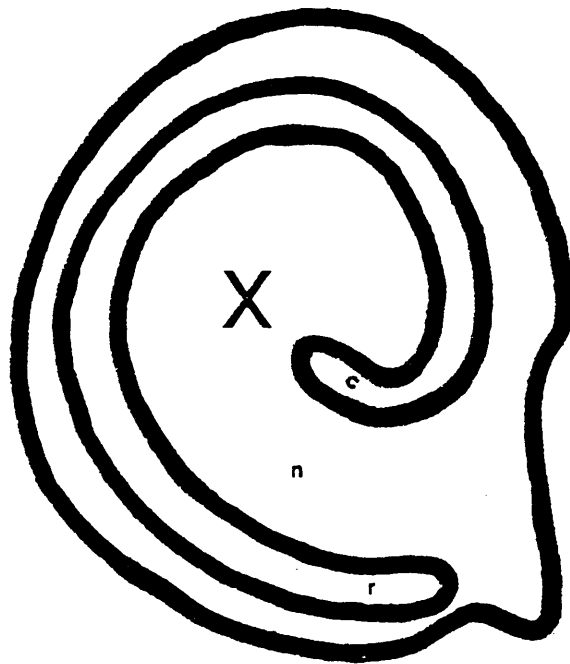


Figure 2.3: An illustration of a bisected tomato seed illustrating the position for piercing during the preparation of the seed for tetrazolium testing. c = cotyledon; r = radicle; n = nutritive tissue; x = position for piercing.

The seeds were rinsed well to remove excess tetrazolium solution and placed in water to prevent the seeds from drying out during evaluation. The seeds were bisected longitudinally and examined with the aid of a dissection microscope. Each embryo was examined and classified according to the ISTA Rules (1985). Seeds which had not germinated, but showed a positive tetrazolium reaction were classed as ungerminated, viable. Seeds which showed a negative reaction were classed as non-viable.

2.2.4.3 Germination rate and seedling growth

To supplement the germination test, germination rates and growth of seedling organs (primary roots and hypocotyls/shoots) were determined (Smith et al., 1973; Perry, 1981).

Germination rate is regarded to be indicative of seed vigour by some authors (Maguire, 1962). Seed lots with similar germination capacity often vary in rate of seedling emergence and growth. According to Verhey (1960) rapid germination is a good indication of high "vitality".

Certain authors even regard first germination count prescribed by the ISTA Rules (1985), as a reliable indicator of seed vigour. This specific estimate of vigour is called germination energy (Department of Agriculture, Canada, 1913). Energy counts of normal tomato seedlings were applied in certain experiments after a five day incubation period i.e. the time of the first count as specified in the ISTA Rules (1985).

Germination rate was calculated by dividing the number of normal seedlings per 100 seeds obtained at each count in the standard germination test by the number of days seeds had been in the germinator. The values obtained at each count were then summed at the end of the germination test to obtain the germination rate (Maguire, 1962):

$$\frac{\text{number of normal seedlings}}{\text{days to first count}} + \text{-----} + \frac{\text{number of normal seedlings}}{\text{days to final count}}$$

As previously mentioned, germination counts were made at two day intervals from the first count after five days to the final count after 14 days. This was a compromise compared to the more conventional methods which prescribe counts every 24 hours. Frequent observations during the initial stages of germination would have amounted to damaging the seedlings because of the germination method (paper rolls) employed.

The lengths of seedlings, primary roots and shoots (hypocotyls) were measured and the ratio of root length to shoot length calculated after seven and 14-day incubation periods (Fig. 2.4). Any seedling judged as "abnormal" according to the ISTA Rules (1985) was not measured (Smith et al., 1973).

Germination capacity, germination rate and seedling growth determinations were conducted on seed samples in four replicates of 100 seeds each.

2.2.5 STATISTICAL CALCULATIONS

The number of replicates in seed testing are prescribed in the International Rules for Seed Testing (ISTA Rules, 1985). The prescriptions in the Rules were followed in this respect. The Rules prescribe four replicates for germination testing, two replicates for tetrazolium testing, and two replicates for moisture determinations.

Statistical analyses were conducted on all relevant data. The following statistics, testing procedures and visual displays were used for the analysis of data (Van Ark, 1981):

1. Levene's test for homogeneous variances
2. Chi-square test
3. Non-parametric U-test of Mann-Whitney
4. Kruskal-Wallis H test
5. Pair-wise t-test
6. Bonferroni significance test
7. Comparative method of Dunn
8. Polynomial regressions
9. Scatter plots.

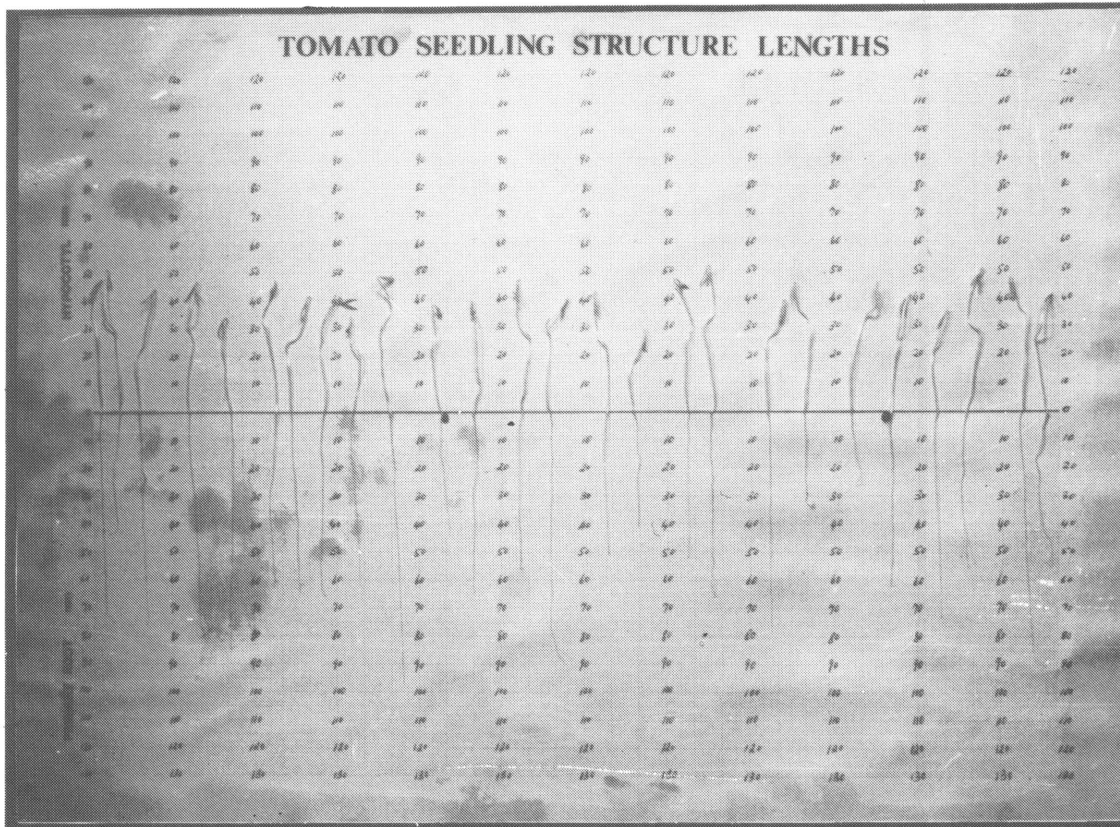


Figure 2.4: Seedlings were placed on graph paper and lengths of hypocotyls and roots determined.

2.2.6 ULTRASTRUCTURAL INVESTIGATIONS

Vigil et al. (1984) suggested that the apical region of the radicle is the most sensitive to adverse conditions. It was therefore decided to prepare only this part of the tomato embryo for ultrastructural investigations.

Ultrastructural investigations were limited to seed samples showing conspicuous anomalies in germination physiology owing to the effects of ageing. Radicle tips of tomato embryos were dissected from dry and imbibed seeds with the aid of a dissection microscope, micro-scalpels and micro-tweezers (Baird et al., 1979). The dissected material was then fixed in two different fixatives.

2.2.6.1 Glutaraldehyde / osmium tetroxide fixation

Glutaraldehyde was used mainly for the stabilisation of proteins and, in particular, of collagen (Bowes and Cater, 1966).

The root tips were fixed by submergence in a 2,5% glutaraldehyde/buffer solution according to the procedure described by O'Brien and Mc Cully (1981), Berjak and Lawton (1973) and Mollenhauer and Totten (1971).

Fresh solutions of fixative were prepared daily and stock solutions of the 25% glutaraldehyde and buffer were stored at 5°C.

The tissue was left in the glutaraldehyde for 12 hours at approximately 20°C after which it was rinsed twice in a 50:50 buffer : distilled water solution for five minutes each.

It is well established that there is a considerable loss of lipids from tissues during glutaraldehyde fixation. Post fixation with osmium tetroxide is therefore recommended by various authors (Paulson and Srivastava, 1968).

Post fixation was performed in a 1% OsO₄ in buffer solution for eight hours at approximately 20°C. Shorter periods of fixation both in glutaraldehyde and osmium tetroxide are recommended by various authors (Paulson and Srivastava, 1968) but were found unsuitable for tomato embryo fixation.

The osmium tetroxide solution was prepared according to the methods of O'Brien and Mc Cully (1981).

The material was once again rinsed twice in a 50:50 buffer : distilled water solution for five minutes each as already described.

The stock solution of osmium tetroxide was kept refrigerated at 5°C.

2.2.6.2 Potassium permanganate fixation

Potassium permanganate was chosen as a second fixative because it is an excellent fixative for the cytoplasm. Organelles, i.e. dictyosomes, mitochondria, plastids and all membrane systems are very well preserved, while other cellular material that might cause visual obstruction is removed by this fixative. Excellent contrast is obtained and no additional contrasting methods are necessary.

A 1% aqueous solution was prepared by dissolving 0,1 g of potassium permanganate crystals in 10 cm³ distilled water. Root tips were fixed in the solution for 12 hours. Fresh solutions were prepared on a daily basis. No post fixation procedures were applied.

Sass (1958) emphasised the necessity of gradual transfer from low to high concentrations of the dehydrant to prevent tissue damage and, for practical reasons, preferred the use of acetone instead of ethanol.

Dehydration of the root tips was implemented by placing the material in 20%; 50%; 70%; 90%; and two 100% acetone solutions for 30 minutes each.

Infiltration of the material with the embedding medium should be gradual for the same reasons specified for dehydration. Infiltration was achieved by adding enough "Spurr" low-viscosity epoxy resin to prepare an approximately 30% embedding medium (Spurr, 1969) (Berjak, 1978).

After eight hours, more resin was added to achieve an approximately 60% embedding medium. This was replaced with clean "Spurr" embedding resin after 12 hours and left for a further eight hours. A final replacement of "Spurr" embedding medium was performed for complete infiltration and removal of all possible acetone residues (Spurr, 1969).

Spurr's resin was used as embedding medium for all the ultrastructural studies, including optical microscopy (Spurr, 1969). Fixed material was embedded in Spurr's resin in five replicates. Of the five replicates, two replicates were prepared for optical microscopy and transmitting electron microscopical investigations.

The Spurr's resin used consisted of 10,0 g vinyl cyclohexene dioxide (ERL - 4206⁴) that was thoroughly mixed with 6,0 g diglycidyl ether of polypropylene glycol (D.E.R.- 736⁵), 26,0 g nonenyl succinic anhydride (NSA⁶) and 0,4 g dimethylaminoethanol (S - 1). Half or quarter quantities, were mixed depending on the circumstances and specific needs. Stock solutions, as well as the NSA⁶ constituent were kept in a desiccator. Root tips were embedded in Spurr's resin in plastic moulds and allowed to polymerize at 70°C for 12 hours.

Embedded root tips in cooled resin blocks were removed from the embedding containers, and trimmed for the re-orientation of the tissue.

Glass knives were used for sectioning purposes. All sections were cut onto distilled water. Special troughs of aluminium tape were prepared and secured to glass knives with melted dental wax.

Ultra thin sections, displaying gold to silver interference colouring ($\pm 0,1$ μm) were used for electron microscope studies. Sections of approximately 1 μm were used for optical microscope investigations (O'Brien and Mc Cully, 1981).

Monitor sections (0,5 μm - 1,0 μm thickness) were prepared from all specimens to aid the electron microscopical studies (Paulson and Srivastava, 1968). Individual sections were removed from the trough in a fine wire loop and transferred with a rolling movement to a drop of distilled water on a glass slide. The sections were dried on a hot tray before staining. A 0,05% toluidine blue dye in benzoate buffer at pH 4,4 was used to stain the thin sections (O'Brien and Mc Cully, 1981) (Berjak and Lawton, 1973) (Briarty *et al.*, 1970). Heating over an alcohol lamp for a few seconds, accelerated the staining process.

The slides were then rinsed for 10 - 60 seconds, until the sections were visually almost free of stain. The slides were once again allowed to dry on a hot tray. Euparal was used as mountant for the permanent preparation of slides for the Reichert Univar optical microscope (Van der Schijff and Robbertse, 1976).

Ultra thin sections for electron microscopy were picked up on 300-mesh copper grids directly from the troughs and allowed to dry on filter paper.

Contrasting of the sections was obtained by submerging the sections in a 5% uranylacetate solution for 10 minutes. After thorough rinsing in distilled water, the sections were submerged in a lead citrate solution, in the absence of carbon dioxide, for three minutes. The grids were then rinsed in a 0,02 N sodium hydroxide solution (Baird *et al.*, 1979). All the grids underwent a final rinse in distilled water and were allowed to dry in air (Venable and Coggeshall, 1965).

The 5% uranylacetate was prepared according to the method prescribed by Watson (1958). The lead citrate solution was prepared according to the methods prescribed by Reynolds (1963). All stock solutions were stored at 5°C. Electron micrographs were obtained with a Philips EM 301 electron microscope, operated at 60 KV.

2.2.7 MICRO-ORGANISM ASSESSMENTS

To ascertain the presence of bacteria and fungi, seed samples representing the different ageing treatments were placed on commercial malt extract agar (22,5 g in 1 dm³ water). A few drops of lactic acid were added to the malt extract agar to inhibit bacterial growth during fungi assessments. The seeds were consequently incubated for 10 days at 25°C in alternating blue and white light/dark cycles (12h/12h).

2.3 RESULTS AND DISCUSSION

2.3.1 MODIFICATION OF SEED WATER CONTENT

The primary aim of developing a suitable procedure to obtain elevated moisture levels in seeds, was for subsequent use in ageing experiments. A second aim was to provide recommendations for ISTA as this organisation has not yet established a suitable method for equilibrating tomato seed at elevated moisture contents for the controlled deterioration test.

The method of Solomon (1951) was found to be reliable for accomplishing elevated moisture levels in tomato seed. A preliminary investigation provided information regarding the moisture content of tomato seed that could be achieved at specific relative humidities in enclosed containers. Relative humidities between 40 - 100% (obtained by the method described in section 2.2.2.2) were investigated. Floradade and Roma VF seed samples in three replicates of 100 seeds each, were enclosed in tupperware containers (130 x 120 x 35 mm) for an equilibration period of two weeks. Moisture determinations as described in 2.2.2 were performed on all the replicates.

The curves shown in Fig. 2.5 conform to the curve described by Justice and Bass (1979). Both curves are exponential and are described by the mathematical equation $y = ax^b e^{cx}$. This curve was used to determine the exact moisture content in seeds of the two different tomato cultivars after equilibration at specific relative humidities. This information was necessary for the comparison of two moisture adjustment methods for controlled deterioration in 2.3.2.

If, for the purpose of controlled deterioration, two methods for obtaining elevated moisture contents in seeds are to be compared, it is necessary to ensure that the moisture contents obtained by the different methods are similar. In section 2.3.2, imbibition, as a method for increasing the moisture content of tomato seeds, is compared to moisture equilibration in different relative humidities. Tomato seeds, in four replicates of 100 seeds each, were subsequently imbibed in liquid water to moisture contents between 5 - 50%. This corresponds to the moisture range that four replicates of 100 seeds each equilibrated to through exposure to relative humidities of between 40 - 100%.

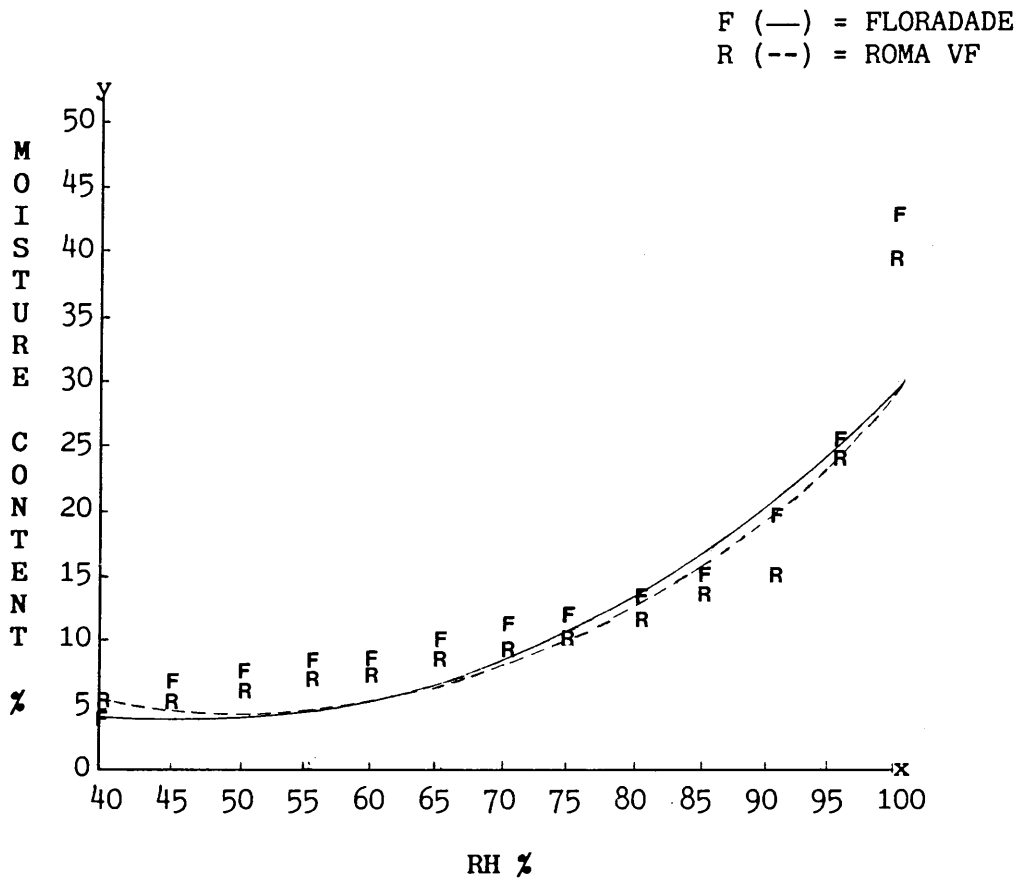


Figure 2.5: The relationship between the relative humidity in an enclosed container and seed moisture content of two tomato cultivars after an equilibration period of 14 days. F: $y = ax^b e^{cx}$; R: $y = ax^b e^{cx}$.

2.3.2 COMPARISON OF TWO MOISTURE ADJUSTMENT METHODS FOR THE CONTROLLED DETERIORATION TEST

Imbibition in liquid water is recommended by ISTA for increasing seed moisture for the controlled deterioration test in various species, but they have not standardised this procedure for tomato seed. Seed moisture content can also be manipulated by equilibrating seeds in an atmosphere of specific relative humidity. This method is slow, taking several weeks to achieve the desired equilibrated moisture content, and, according to Herath et al. (1981), could lead to a loss of seed quality because of the time involved.

The initial moisture content of the Floradade sample was 7,1% and 5,9% for the Roma VF sample on a fresh mass basis.

The moisture content of seeds was adjusted by imbibition on the one hand, and by equilibration at different relative humidities on the other. The samples were then subjected to controlled deterioration according to the method of Matthews and Powell (1981). This method prescribes a time period of 24 hours for effective controlled deterioration.

Figure 2.6 A depicts curves obtained for percentage normal seedlings after 14 days incubation following controlled deterioration subsequent to moisture adjustment to different levels by means of imbibition in liquid water. Similar curves were obtained from germination data obtained after five days of incubation and these data are presented in tabular form (Table 2.2). Similar tendencies for the five and 14-day incubation periods were also found in the case of seeds where moisture adjustment was by means of the equilibration method. The data for the 14-day incubation period are presented in Figure 2.6 B and for the five day period in Table 2.2.

Moisture adjustment by means of imbibition in liquid water resulted in polynomial regressions of the second (Floradade) and third (Roma VF) degrees (Fig. 2.6 A; Appendix 2.1, pp 124 - 125; Appendix 2.2, pp 126 - 127). The mathematical equations for the functions of germination energy are: $y = 130,15 + 7,35x + 0,13x^2$ (Floradade) and $y = 135,36 + 8,43x + 0,30x^2 + 0,003x^3$ (Roma VF).

The mathematical equations for the functions of germination capacity are: $y = 118,71 - 5,38x + 0,10x^2$ (Floradade) and $y = 113,93 - 3,72x + 0,13x^2 - 0,001x^3$ (Roma VF).

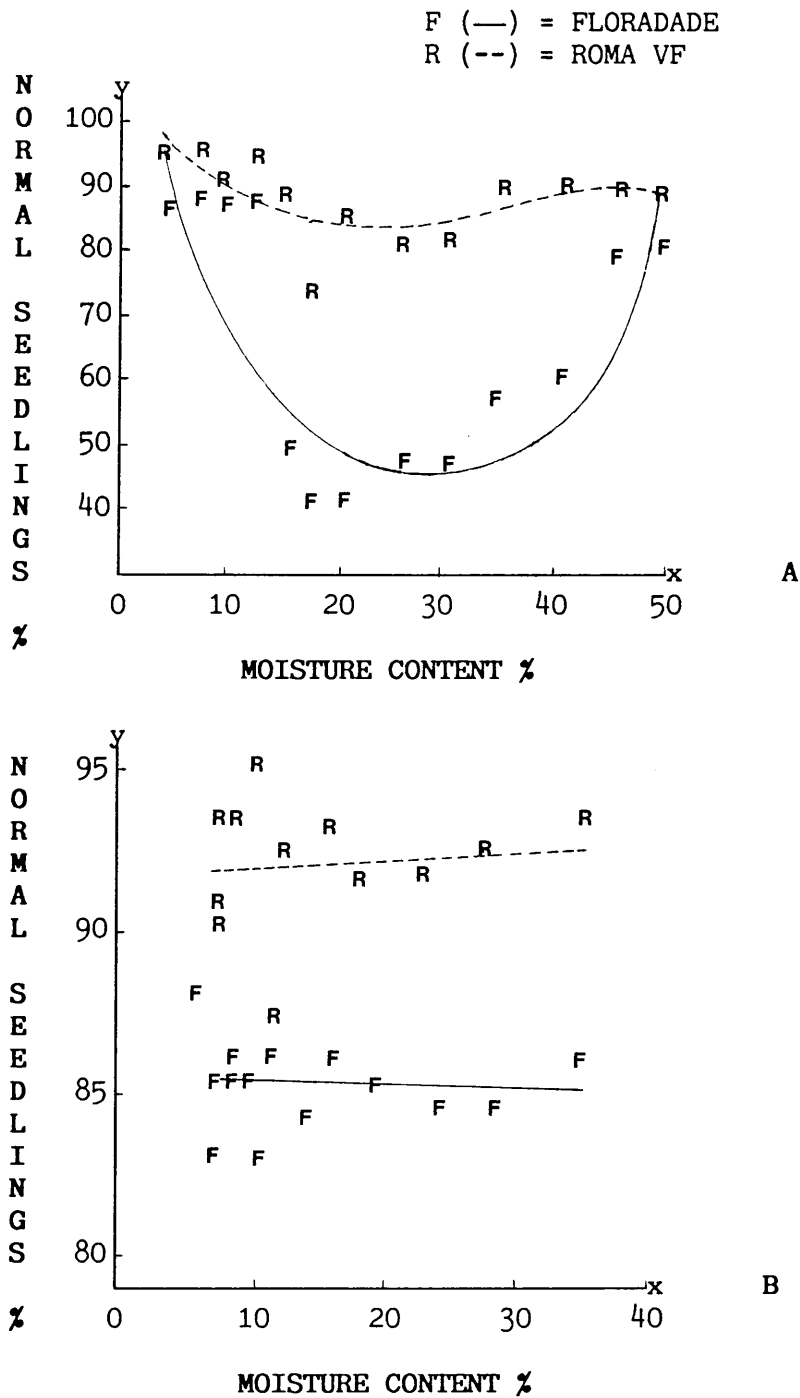


Figure 2.6: The percentage normal seedlings of two tomato cultivars after an incubation period of 14 days following an adjustment of the moisture content of the seed by two different methods prior to controlled deterioration for 24 hours at 45°C. A = Imbibition ($y = 118,71 - 5,38x + 0,10x^2$ for Floradade and $y = 113,93 - 3,72x + 0,13x^2 - 0,001x^3$ for Roma VF); B = Equilibration at different relative humidities.

Table 2.2: The effect of two moisture adjustment methods (imbibition and equilibration at different relative humidities) on the germination of tomato seeds after controlled deterioration for 24 hours at 45°C and an incubation period of five days (germination energy).

Moisture content (%)	Floradade Normal seedlings (%)		Roma VF Normal seedlings (%)	
	Imbibition	Equilibration	Imbibition	Equilibration
Control (Floradade = 7; Roma VF = 6)	86	81	78	83
5	85	87	93	89
6		83		89
7	87	85	93	92
8		84		95
9		84		86
10	86	82	91	
11				91
12	85	83	92	
14				91
15	31	86	71	
17	27		48	89
19		81		
20	24		55	
22				91
23		82		
25	30		60	
28				91
29		82		
30	29		62	
35	36	84	86	92
40	42		89	
45	68		89	
50	77		83	
Fully imbibed	72		93	

For seeds imbibed in liquid water, there was a progressive decrease in germination (in the case of both incubation periods) at the lower moisture contents (14 - 25%) of both cultivars, although the percentage normal seedlings of Floradade was more dramatically affected. A subsequent increase in germination (in the case of both incubation periods) was evident between the moisture content values of 30 - 50%. The decrease in percentage normal seedlings was accompanied by an increase in the percentage abnormal seedlings and vice versa, although many of the abnormal seedlings apparently died at the higher moisture contents of the seed (Table 2.3). Data for seed mortality exhibited a polynomial regression of the second degree in the case of both cultivars (Appendix 2.3; pp 128 - 129).

Moisture content had no significant effect on germination in treatments where adjustment was by equilibration at different relative humidities before controlled deterioration. (Appendices 2.4; 2.5; 2.6; pp 130 - 132). Germination after both five and 14-day periods, did not differ significantly from the mean for each cultivar (Table 2.2) (Fig. 2.6 B).

The results of ageing after imbibition in liquid water confirm the hypothesis of Perry (1981) that the level for effective ageing of vegetable seed is between 19 - 24%. This phenomenon was not evident in the experiments where seed had been equilibrated to similar moisture contents in different relative humidities.

Two explanations are offered for the different responses obtained from the two moisture equilibration methods prior to controlled deterioration:

1. As described in section 2.2.2.1, the conventional method of moisture adjustment by imbibition in liquid water (Perry, 1981) could not be used in the case of tomato seed. Complete imbibition was therefore allowed followed by drying back to the desired moisture content. The imbibition in liquid water treatment consequently entailed both a hydration and dehydration treatment whereas the moisture equilibration treatment entailed only hydration before controlled deterioration. A personal communication of A. Francis and P. Coolbear (Massey University, Palmerston North, New Zealand) revealed that these scientists apparently did not experience the obstacles of imbibition in liquid water as a method to raise the moisture content of tomato seed for artificial ageing.

Table 2.3: The effect of two moisture adjustment methods (imbibition and equilibration in different relative humidities) on seed mortality after controlled deterioration for 24 hours at 45°C and an incubation period of 14 days.

Moisture content (%)	Floradade non-viable seeds (%)		Roma VF non-viable seeds (%)	
	Imbibition	Equilibration	Imbibition	Equilibration
Control (Floradade = 7; Roma VF = 6)	10	7	5	4
5	11	10	5	8
6		14		7
7	9	13	5	5
8		13		3
9		12		11
10	10	14	7	
11				7
12	12	13	7	
14				7
15	45	11	10	
17	57		23	9
19		11		
20	55		13	
22				8
23		12		
25	50		13	
28				7
29		13		
30	47		13	
35	38	12	7	7
40	36		8	
45	19		10	
50	21		9	
Fully imbibed	15		6	

It appears, however, that the above-mentioned scientists increased the moisture content of the seeds by imbibition without aiming for predetermined moisture contents and would therefore not have encountered the problems incurred during this investigation.

2. The moisture contents were determined as percentage wet mass of the entire seed, including endosperm and testa. The fact that exactly similar moisture contents were obtained by the two respective methods does not necessarily imply that the moisture contents in the embryo axes were similar. As mentioned, the adsorption of liquid water to hairs occurred at such a rapid rate that the seeds had to be hydrated fully and then dehydrated to the specific moisture contents required. The tomato embryo is surrounded by endosperm and seed coat (Fig. 2.3). It can therefore be speculated that most of the water loss during drying was from the outer tissue layers of the seed (seed coat and endosperm) with little loss from the embryo axis. The absorption of water vapour on the contrary, is a relatively slow process and the specific moisture contents could be obtained without dehydration. Because of the slow diffusion rate and the lack of capillary movement between seed cells, it is possible that the moisture content of the peripheral seed tissues was higher than that of the centrally located embryo.

Imbibition of tomato seeds from liquid water is not a satisfactory simulation of the actual elevation of moisture content during seed storage. Stored seeds equilibrate to the relative humidity in the atmosphere and direct contact with liquid water is unlikely during ageing. It may also be assumed that cytological changes induced in the seed during imbibition followed by dehydration differ from those induced during exposure of the seeds to a relatively high humidity in the atmosphere. Embryos which have been imbibed, dried, then returned to water, show a rapid initial leakage of electrolytes (Herath *et al.*, 1981). This observation suggests that the differentially permeable membranes of the tonoplast and plasmalemma, which normally retain solutes within cells, lose their integrity during drying and do not act as retentive barriers when seeds are replaced in water. Inadequate hydration of the membrane components in the dried seed probably results in disruption of the ordered membrane structure, which then takes time to reorientate on further hydration for germination. According to Herath *et al.* (1981) changes also occur in the membrane structures of cell organelles during drying and rehydration. Here, there appears to be more permanent structural damage owing to drying which takes even longer to repair; this might require the development of an effective biochemical repair mechanism.

According to the findings in this study, imbibition in liquid water should not be recommended as a method for the moisture adjustment of tomato seeds prior to artificial ageing, since the results obtained after controlled deterioration do not agree with those obtained with moisture equilibration in different relative humidities. The former treatment is also incompatible with the realistic conditions during natural ageing. The disadvantages of equilibration in different relative humidities, mentioned by Heydecker and Coolbear (1977) are the lengthy period of time required for equilibration, condensation at unsteady temperatures, proliferation of micro-organisms and artificial ageing of the seed samples. These disadvantages were not experienced during this investigation. This was borne out by the fact that germination was not significantly reduced after controlled deterioration (Fig. 2.6 B) (Appendices 2.4; 2.5; 2.6; pp 130 - 132).

It was decided to equilibrate tomato seed at 95% relative humidity for the purposes of controlled deterioration in further experiments. Exposure to this relative humidity for 14 days equilibrates tomato seed to a moisture content of 21 - 22% (wet mass basis) (Fig. 2.5) which is within the moisture limits specified by Perry (1981) (19 - 24%) for efficient controlled deterioration. To maintain a relative humidity of 95% in the desiccator for two weeks, 11,02 g of a 98% sulphuric acid solution (H_2SO_4) was added to 88,98 g distilled water (Solomon, 1951).

The 24h controlled deterioration period used in this study did not effectively deteriorate seeds after moisture equilibration by this method and longer periods were investigated as reported in 2.3.3.

2.3.3 EFFECT OF AGEING ON GERMINATION PERFORMANCE

The developers of seed treatment practices often use artificial ageing for the evaluation of the interaction of seed ageing with newly developed seed treatments because of the much shorter period in which evaluation can be completed. In this study, ageing by means of a controlled deterioration treatment, was compared with ageing under sub-optimal, though realistic conditions, since it was anticipated that the mechanisms involved during controlled deterioration and seed storage under sub-optimal conditions might differ. The important factors contributing to deterioration during the controlled deterioration test are high temperature and unfavourable moisture content. The temperature of 45°C and the relatively short ageing period could, however, exclude proliferation of the majority of storage micro-organisms and their role in the ageing process.

Seed samples of Floradade and Roma VF were moisture equilibrated in an atmosphere of 95% relative humidity and seed moisture contents of 20,2% and 18,0% respectively were obtained. The seeds were then placed in polyethylene aluminium foil pouches which were heat-sealed and subjected to a process of controlled deterioration as described in 2.2.3.1 for periods of 1 - 10 days at 45°C.

Seed samples for storage up to 12 months were each divided into two sub-samples. The sub-samples of a particular sample were respectively equilibrated to 30% and 7% moisture contents in the seed and stored at 20/30°C (16h/8h). The relatively high level of approximately 30% (31,7% for Floradade and 29,2% for Roma VF) was achieved by equilibration of the seed samples at 100% relative humidity (no H₂SO₄ was added to the water) and a laboratory temperature of 20°C. This high moisture level was induced to age seeds rapidly. Another reason for testing such an unrealistically high moisture level in the seed was to simulate ineffective drying after harvesting or a hot-water treatment. A lower moisture level was achieved by equilibration in the laboratory atmosphere at a temperature of 20°C, and a moisture content of approximately 7% (7,4% for Floradade and 6,5% for Roma VF) was obtained in the seed. The seed samples were stored in sealed polyethylene aluminium foil pouches.

At intervals of 24h, in the controlled deterioration treatments, and one month, in the storage treatments, seed samples were drawn and germination capacity determined after a 14-day incubation period (ISTA Rules, 1985). Tetrazolium tests for viability determinations as described in 2.2.4.2 were performed on all ungerminated seeds. The assessment of ungerminated seeds was necessary to ascertain whether they were viable, but with insufficient energy for germination, or dead.

Germination energy (Department of Agriculture, Canada, 1913), germination rate (Maguire, 1961) and the measurement of seedling organ lengths were also determined. Organ lengths of normally developed seedlings were measured after a seven-day incubation period and discarded. The Kruskal-Wallis analysis for homogeneous variances was used for the global comparison of all seedling measurements. In the event of a significant difference in variance within a specific series of data determined according to Levene's test for variances, the Pairwise T-test for separate variances was applied between data of different series. Pooled variances were used under circumstances where there were no statistically significant differences in homogeneous variance in a specific data series. Dunn's method for pairwise comparisons between different data series was used when the normal distribution of data, as displayed by statistical histograms, was skew.

Figure 2.7 A, based on the statistical analysis in Appendix 2.7 (pp 133 - 134) illustrates the parabolic characteristic of controlled deterioration in the percentage normal seedlings of both cultivars. The parabole is characterised by the mathematical equations $y = 83,34 + 2,33x - 0,97x^2$ (Floradade) and $y = 87,56 + 2,65x - 0,92x^2$ (Roma VF).

There was a general decrease in the number of normal seedlings with time of controlled deterioration. A general increase in the number of abnormal seedlings with time could be detected although this number dropped in Floradade samples after eight days of controlled deterioration (Table 2.4). An increased number of non-viable seeds and ungerminated, viable seed were also recorded (Table 2.5). Although significantly less seeds of Floradade germinated after 10 days of controlled deterioration than was the case with Roma VF, a greater proportion of the ungerminated seeds of Floradade were found to be viable than was the case with ungerminated seeds of Roma VF (Table 2.5). However, tetrazolium tests revealed that some of these seeds displayed only a light colour reaction with the reagent and the intensity of the reaction was too low for valid pattern evaluation. This phenomenon is attributed to the occurrence of many embryonic cells in the process of dying or already dead. Secondary dormancy could also not be suspected.

Table 2.4: Germination of tomato seed after a 14-day incubation period following controlled deterioration at 45°C and ±20% moisture content for different periods.

Period of controlled deterioration (days)	Floradade		Roma VF	
	Normal seedlings (%)	Abnormal seedlings (%)	Normal seedlings (%)	Abnormal seedlings (%)
0(Control)	85	8	93	3
1	82	3	87	3
2	83	2	85	3
3	83	3	83	6
4	81	2	84	5
5	60	14	81	5
6	72	7	75	5
7	46	26	68	3
8	24	21	55	10
9	13	9	59	17
10	0	9	28	19

Table 2.5: Viability of ungerminated tomato seed after a 14-day incubation period following controlled deterioration at 45°C and ±20% moisture content for different periods.

Period of controlled deterioration (days)	Floradade		Roma VF	
	Ungerminated viable (%)	Non-viable (%)	Ungerminated viable (%)	Non-viable (%)
0(Control)	0	7	0	4
1	0	15	0	11
2	0	16	0	12
3	0	14	0	12
4	0	17	0	11
5	1	25	1	13
6	1	20	0	20
7	1	27	2	27
8	8	47	2	33
9	28	50	4	20
10	31	60	7	46

An acceptable period for controlled deterioration determinations could be standardised from these results as the time interval where the percentage normal seedlings was reduced to approximately 50%. The period was seven days for Floradade and eight days for Roma VF (Fig. 2.7 A).

A slight decrease in germination and an equally slight increase in the percentage non-viable seeds was recorded after storage of tomato seed at relatively low ($\pm 7\%$) moisture contents in the seed up to a storage period of 12 months (Tables 2.6, 2.7 and Fig. 2.7 B) (Appendix 2.8; pp 141 - 142). There was a tendency of Roma VF to deteriorate faster than Floradade. Except for the significant difference in germination between the two cultivars before storage the differences during storage were not significant according to the chi-square distribution in Appendix 2.8 (pp 141 - 142).

A sharp decrease in the germination of tomato seed stored at relatively high ($\pm 30\%$) moisture contents in the seed was evident between one and four months of storage (Tables 2.8, 2.9 and Fig. 2.7 C). The germination was almost zero after four months storage. The tetrazolium test for viability indicated no trace of secondary dormancy. The parabolic functions in Fig. 2.7 C, based on the statistical analysis in Appendix 2.9 (pp 143 - 144), display the detrimental effect of seed storage at relatively high seed moisture contents on the germinability of both cultivars. The respective paraboles are characterised by the following mathematical equations:

$y = 84,79 + 3,94x - 6,3x^2$ for Floradade and $y = 92,92 + 3,13x - 6,59x^2$ for Roma VF.

The chi-square distribution in Appendix 2.9 (pp 145 - 146) reveals a significant difference in germination between the two cultivars before storage, but not after different storage periods. The significantly higher number of ungerminated viable seeds in Roma VF after two months storage under relatively unfavourable conditions, could once again be related to the occurrence of significantly more seed in this cultivar that are apparently viable, though incapable of germinating owing to lack of germination "energy".

Table 2.6: Germination of tomato seed after storage at relatively low moisture contents ($\pm 7\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)		Roma VF (%)	
	Normal seedlings	Abnormal seedlings	Normal seedlings	Abnormal Seedlings
0	85	8	93	3
3	87	6	91	4
6	84	5	88	3
9	84	5	85	3
12	83	4	84	5

Table 2.7: Viability of ungerminated tomato seeds after storage at relatively low moisture contents ($\pm 7\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)		Roma VF (%)	
	Ungerm. viable	Non-viable	Ungerm. viable	Non-viable
0	0	7	0	4
3	0	7	0	5
6	0	12	0	10
9	0	11	0	12
12	0	14	0	11

Table 2.8: Germination of tomato seed after storage at relatively high moisture contents ($\pm 30\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)		Roma VF (%)	
	Normal seedlings	Abnormal seedlings	Normal seedlings	Abnormal Seedlings
0	85	8	93	3
1	82	5	88	4
2	69	6	76	6
3	38	12	41	10
4	0	2	1	5
5	0	0	0	1
6	0	0	1	2
9	0	0	0	0
12	0	0	0	0

Table 2.9: Viability of ungerminated tomato seeds after storage at relatively high moisture contents ($\pm 30\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)		Roma VF (%)	
	Ungerm. viable	Non-viable	Ungerm. viable	Non-viable
0	0	8	0	4
1	0	13	0	9
2	1	24	4	16
3	1	50	0	51
4	0	98	0	95
5	0	100	0	100
6	4	96	1	97
9	0	100	0	100
12	0	100	0	100

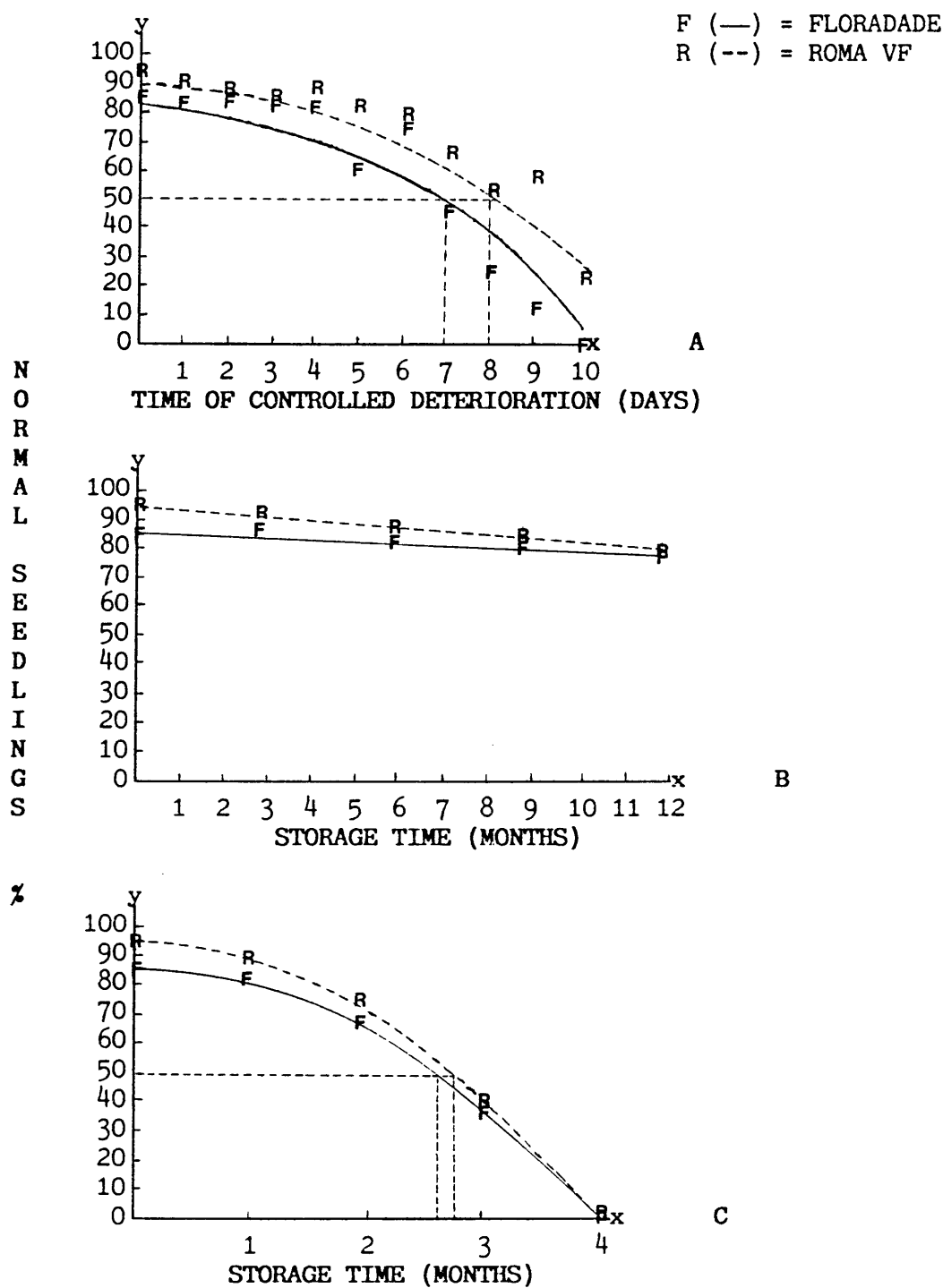


Figure 2.7: The percentage germination (normal seedlings) of two tomato cultivars after different artificial ageing methods and an incubation period of 14 days.

A: Controlled deterioration for different periods at 45°C and ±20% moisture content.
 F: $y = 83,34 + 2,33x - 0,97x^2$; R: $y = 87,56 + 2,65x - 0,92x^2$.

B: Storage for different periods at relatively low moisture contents in the seed (±7%).

C: Storage for different periods at relatively high moisture contents in the seed (±30%).
 F: $y = 84,79 + 3,94x - 6,3x^2$; R: $y = 92,92 + 3,13x - 6,59x^2$.

Tomato seed germination during different periods of controlled deterioration, and during different periods of seed storage at relatively high seed moisture contents, both display parabolic functions (Fig. 2.7 A, C). It is, however, contradictory that Roma VF did not deteriorate faster than Floradade during controlled deterioration, while this occurred during seed storage at both relatively low and high seed moisture contents (Fig. 2.7). Thus, in spite of the initial higher germination capacity of Roma VF, it has a lower storage potential than Floradade. This phenomenon was not demonstrated by controlled deterioration treatments. The germination capacity of Roma VF was, in fact, significantly higher than that of Floradade from day five of controlled deterioration (Appendix 2.7 pp 135 - 140).

The controlled deterioration method published by ISTA (Perry, 1981) has successfully been used to predict performance of Brussels sprouts and onions after commercial storage (Matthews, 1985). The results presented in this chapter indicate that the controlled deterioration method did not accurately predict storability of the two tomato cultivars investigated. The fact that 50% mortality during controlled deterioration is obtained after a longer period in Roma VF (8 days) than in Floradade (7 days), suggests that the Roma VF seedlot has a higher storage potential than Floradade. This is contradicted by the deterioration pattern followed by the seed of the two cultivars during storage (Fig. 2.7). The slight difference in the moisture content obtained in the seed of the two cultivars after equilibration at the same relative humidity, could not be the reason for the difference in performance during the two ageing treatments as Roma VF consistently equilibrated to a slightly lower moisture content in all the experiments.

An increase in the number of abnormal Roma VF seedlings, was observed while this number decreased on days nine and 10 of controlled deterioration in Floradade (Table 2.4). This could be attributed to certain potentially abnormal Floradade seedlings that died during this stage of controlled deterioration, while certain potentially normal Roma VF seedlings became abnormal after controlled deterioration. The number of viable ungerminated seeds during controlled deterioration was also significantly higher in Floradade after 10 days of controlled deterioration than in Roma VF and few ungerminated Roma VF seeds remained viable after controlled deterioration (Table 2.5). The occurrence of ungerminated viable seeds after different storage periods at relatively high seed moisture contents, was conspicuously less than after different periods of controlled deterioration (compare values in Tables 2.5 and 2.9).

The germination after an incubation period of five days (germination energy) is illustrated in Fig. 2.8. Germination energy after different periods of controlled deterioration is characterised by a straight line function (Fig. 2.8 A). This function is described by the mathematical equation $y = 85,0 - 9,4x$ for Floradade and $y = 87,5 - 10,01x$ for Roma VF (Appendix 2.10; pp 147 - 148).

Germination energy after seed storage under relatively favourable conditions displayed no statistically significant difference over time (Fig. 2.8 B) (Appendix 2.11; pp 155 - 157). Germination energy after seed storage for different periods under sub-optimal conditions is characterised by a polynomial regression of the second degree (Fig. 2.8 C) (Appendix 2.12; pp 158 - 159). The following equations describe the regressions: $y = 84,18 - 3,83x - 4,66x^2$ (Floradade) and $y = 86,16 + 3,72x - 6,64x^2$ (Roma VF).

The function of germination energy after controlled deterioration for one to 10 days in Fig. 2.8 A differs from the corresponding function of germination capacity in Fig. 2.7 A and there were, in contrast, no statistically significant differences between the cultivars (Appendices 2.10, pp 149 - 154; 2.11, pp 155 - 157; 2.12, pp 160 - 161). The functions of germination energy after controlled deterioration for one to 10 days (Fig. 2.8 A) also differed from those after seed storage for one to four months under unfavourable conditions (Fig. 2.8 C). This was not the case for germination capacity (compare Fig. 2.7 A and C).

Fig. 2.9 illustrates the effect of controlled deterioration and different storage periods with two different moisture levels in the seed, on the rate of germination. As was the case with germination energy, germination rate also displays a linear function with time of controlled deterioration (Fig. 2.9 A). This function is described by $y = 17,83 - 1,50x$ for Floradade and $y = 19,64 - 1,57x$ for Roma VF (Appendix 2.13; pp 162 - 163). Germination rate declined with time with no statistically significant differences between cultivars (Appendix 2.13; pp 164 - 169).

The graphs of the germination rate of tomato seed after storage for different periods at relatively low and high moisture contents in the seed display similar functions, respectively, as germination capacity after 14 days (compare Figs. 2.7 B, C to Figs. 2.9 B, C) (Appendices 2.8; 2.9; pp 141 - 146 ; 2.14; 2.15; pp 170 - 176) and the conclusions drawn are similar. The mathematical equations for the functions in Figure 2.9 C are: $16,82 + 0,27x - 1,15x^2$ (Floradade); $18,22 + 0,54x - 1,31x^2$ (Roma VF).

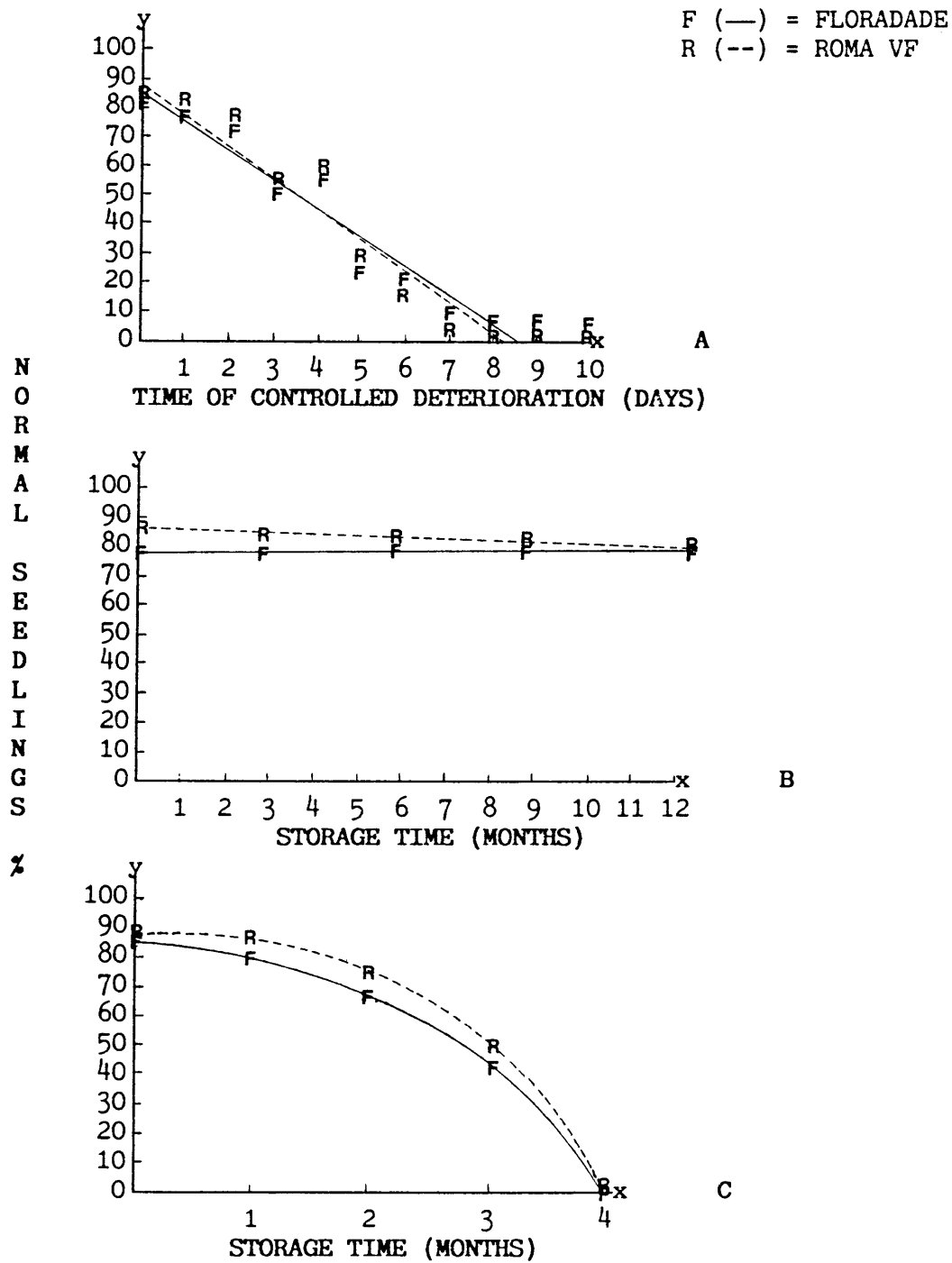


Figure 2.8: The percentage germination energy (normal seedlings after five days) of two tomato cultivars after different artificial ageing methods.
 A: Germination after five days of incubation following controlled deterioration for different periods at 45°C and ±20% moisture content.
 F: $y = 85,0 - 9,4x$; R: $y = 87,5 - 10,0x$.
 B: Storage for different periods at relatively low moisture contents in the seed (±7%).
 C: Germination after five days of incubation following seed storage for different periods at relatively high moisture contents in the seed (±30%).
 F: $y = 84,18 - 3,83x - 4,66x^2$; R: $y = 86,16 + 3,72x - 6,64x^2$.

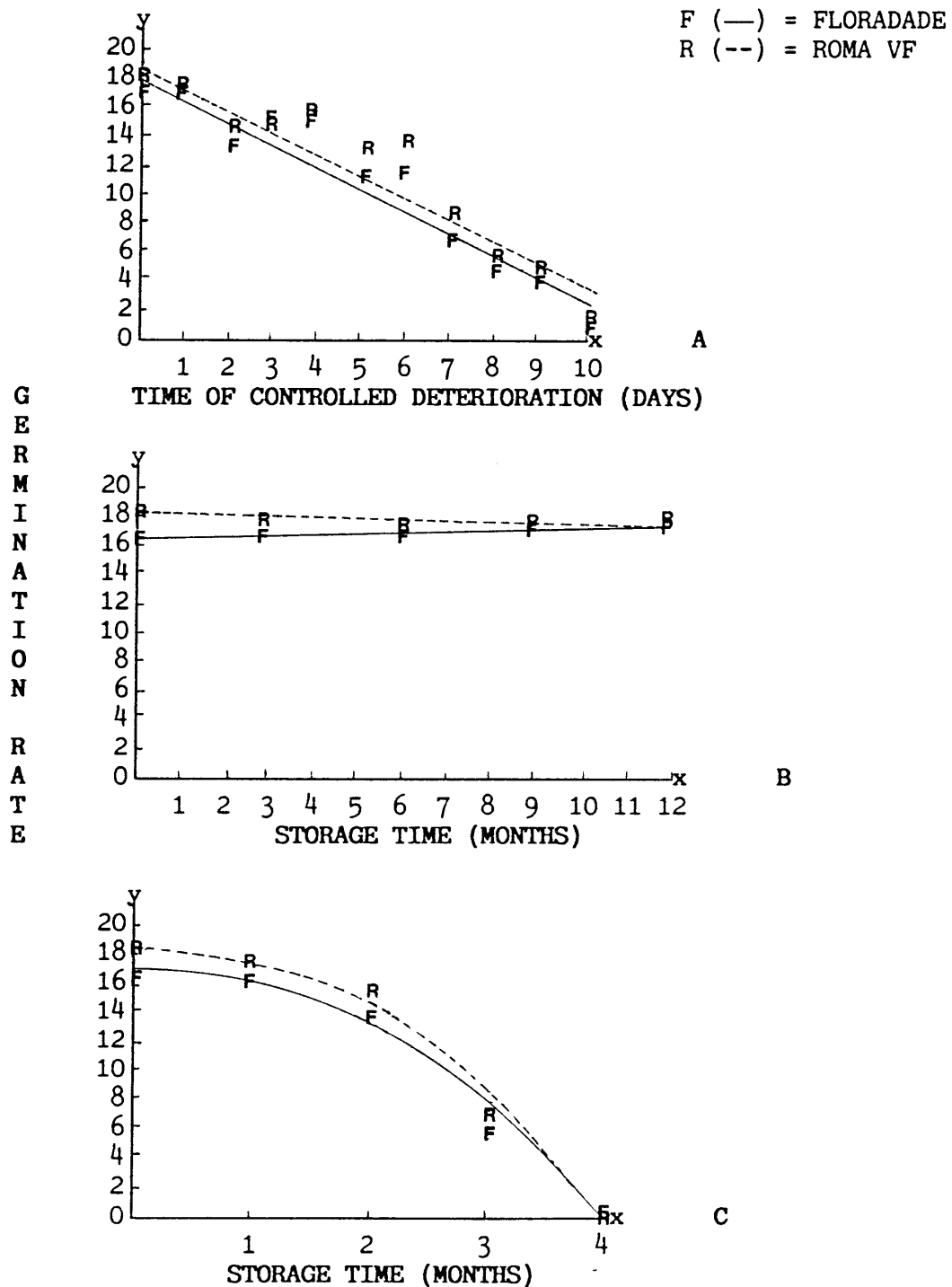


Figure 2.9: The germination rate of two tomato cultivars after different artificial ageing methods.

A: Germination rate during a 14-day incubation period following controlled deterioration for different periods at 45°C and ±20% moisture content.

F: $y = 17,83 - 1,50x$; R: $y = 19,64 - 1,57x$.

B: Germination rate during a 14-day incubation period following storage for different periods at relatively low moisture contents in the seed (±7%).

C: Germination rate during a 14-day incubation period following storage for different periods at relatively high moisture contents in the seed (±30%).

F: $y = 16,82 + 0,27x - 1,15x^2$; R: $y = 18,22 + 0,54x - 1,31x^2$.

There is a difference in the pattern of germination rates of tomato seeds aged by different methods. The germination rates of Floradade and Roma VF seeds after controlled deterioration display a linear function (Fig. 2.9 A), while the rates after different storage periods at relatively high moisture contents in the seed display a parabolic function (Fig. 2.9 C). This can be attributed to a significant decrease in germination rate during the shorter periods of controlled deterioration, while the decrease was relatively less during the shorter periods of seed storage under unfavourable conditions. The germination rate of tomato seed stored for different storage periods at relatively low seed moisture contents displayed no significant differences over time (Fig. 2.9 B). An interesting observation is that the functions of "germination energy" (Fig. 2.8 A and C) respectively correspond with the functions of "germination rate" (Fig. 2.9 A and C). This contradicts the opinion of Verhey (1960) which rejects "germination energy" as an index of speed of germination. The results of this study indicate that the use of germination energy could have value if the determination of germination rate is considered impractical or time consuming.

The statistical tests for homogeneous variances of seedling organ lengths measured after seven days after controlled deterioration for different periods, indicated that root length was homogeneous for Floradade and heterogeneous for Roma VF (Appendix 2.16; pp 177 - 179). Pooled variance was therefore used for the pairwise T-test for Floradade, while separate variance was used for Roma VF. Hypocotyl length was heterogeneous for both Floradade and Roma VF (Appendix 2.19; pp 185 - 187). Separate variances were therefore used for the pairwise T-test.

Seedling organ lengths generally showed a decrease after an incubation period of seven days after the seed samples had been subjected to different periods of controlled deterioration. The decrease in lengths is characterised by polynomial regressions of the second degree (Figs. 2.10 A; 2.11 A) (Appendices 2.16; pp 178 and 180; 2.19; pp 186 and 188). The polynomial regressions of primary root length after different periods of controlled deterioration are described by the following mathematical equations:
 $y = 69,96 + 0,41x - 0,68x^2$ (Floradade); $y = 66,79 + 0,10x - 0,23x^2$ (Roma VF).

The polynomial regressions of hypocotyl length after different periods of controlled deterioration are described by the following mathematical formulas:
 $y = 41,63 - 2,49x - 0,23x^2$ (Floradade); $y = 43,07 - 1,77x - 0,06x^2$ (Roma VF).

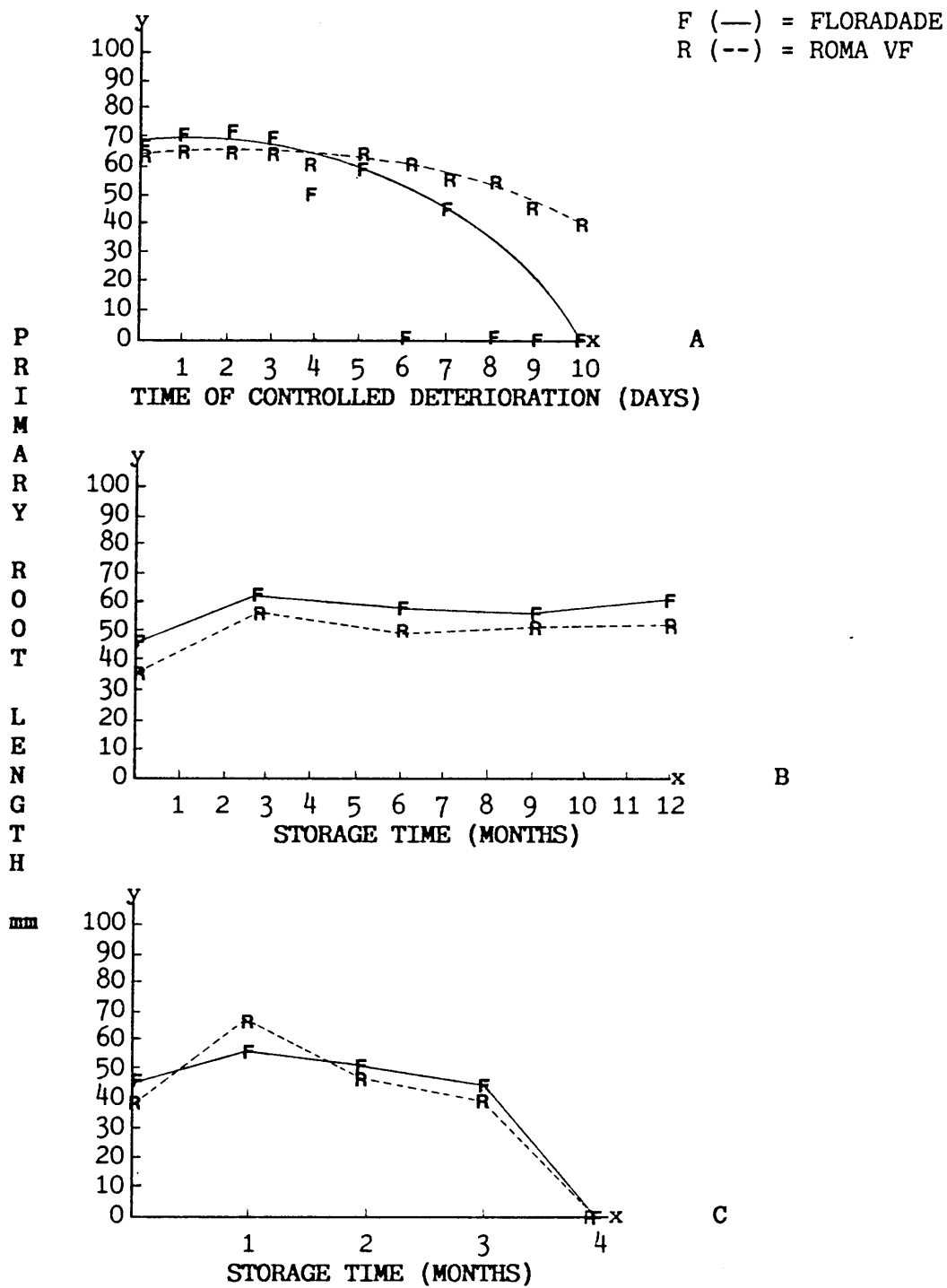


Figure 2.10: Primary root lengths after an incubation period of seven days after the seed samples had been subjected to different methods of artificial ageing.

A: Controlled deterioration for different periods at 45°C and ±20% moisture content.

F: $y = 69,96 + 0,41x - 0,68x^2$; R: $y = 66,79 + 0,10x - 0,23x^2$.

B: Seed storage for different periods at relatively low moisture contents in the seed (±7%).

C: Seed storage for different periods at relatively high moisture contents in the seed (±30%).

The statistical tests for homogeneous variances indicated that all the variances within the different data series of seedling organ lengths, after seed storage under favourable and unfavourable conditions and incubation periods of seven days, were heterogeneous and therefore only the Pairwise T-test for separate variances was used for the comparison of different data series (Appendices 2.17; 2.18; 2.20; 2.21 pp 181 - 184 and 189 - 192).

The variance in the data series for root lengths after different storage periods was significant for Floradade and less significant for Roma VF at relatively low seed moisture contents (Appendix 2.17; pp 181 - 182). At the relatively higher seed moisture contents, the variance within individual data series was highly significant (Appendix 2.18; pp 183 - 184).

It appeared that the length of primary roots increased with time of storage under both conditions (Fig. 2.10 B, C). This increase was highly significant between the unstored control, and all the other storage periods at relatively low moisture contents in the seed (Appendix 2.17; pp 181 - 182). The difference between individual storage periods was, however, less significant (Appendix 2.17; pp 181 - 182).

The Pairwise T-test for separate variances revealed a highly significant increase in root length between the unstored control, and one month storage at relatively high seed moisture contents of the Floradade samples, although the lengths did not differ significantly between the unstored control and the other storage periods (Appendix 2.18; pp 183). This was due to a decrease in the lengths after two and three months storage which was, however, highly significant when compared to the initial increase after one month storage (Appendix 2.18; pp 183).

A stimulatory influence on the growth rate of the hypocotyl was observed after one month storage and was maintained in the case of storage at relatively low moisture contents in the seed (Fig. 2.11 B). This increase in hypocotyl length was highly significant between the unstored controls and the other storage periods in both cultivars (Appendix 2.20; pp 189 - 190).

After a highly significant increase after one month storage, the hypocotyls rapidly decreased in length in the case of storage at a relatively high moisture content of the seed (Fig. 2.11 C). No growth could be determined after an incubation period of seven days, after the seed had been stored for four months at 20/30°C (16h/8h) alternating temperatures.

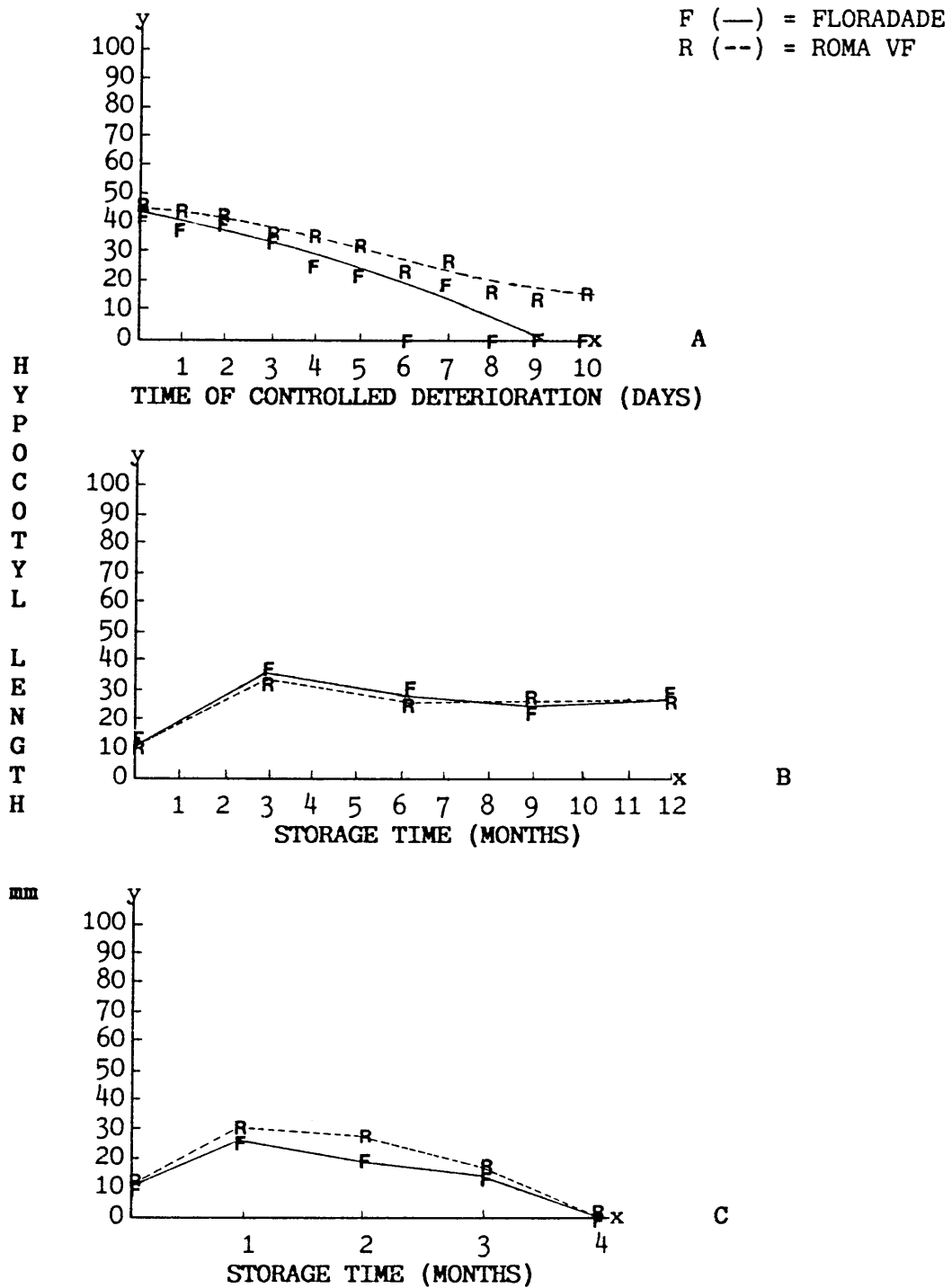


Figure 2.11: Hypocotyl lengths after an incubation period of seven days after the seed samples had been subjected to different methods of artificial ageing. A: Controlled deterioration for different periods at 45°C and ±20% moisture content.

F: $y = 41,63 - 2,49x - 0,23x^2$; R: $y = 43,07 - 1,77x - 0,06x^2$.

B: Seed storage for different periods at relatively low moisture contents in the seed (±7%).

C: Seed storage for different periods at relatively high moisture contents in the seed (±30%).

The Pairwise T-test for separate variances revealed that differences in hypocotyl length of unstored controls were highly significantly less than those of stored samples after one-, two- and three-month storage periods (Appendix 2.21; pp 191 -192). The decrease in hypocotyl length between the respective storage periods from one to three months was also highly significant for both cultivars (Appendix 2.21; pp 191 - 192).

The Kruskal-Wallis one-way analysis of variance between Floradade and Roma VF revealed that the difference in primary root length was highly significant after controlled deterioration for six to ten days. The difference in hypocotyl length was not significant between the cultivars before deterioration but became highly significant from the third day of controlled deterioration.

The Kruskal- Wallis one way analysis of variance between Floradade and Roma VF revealed that primary root length differed significantly between the two cultivars during all storage periods at relatively low moisture contents in the seed, while the differences in hypocotyl lengths were not statistically significant. The primary root lengths show a significant difference between the two cultivars after a storage period of one month, while the difference is not statistically significant after two and three months storage at relatively high moisture contents in the seed.

The hypocotyl lengths showed a highly significant difference between the two cultivars after one and two month storage periods, while the difference in the unstored samples and in those stored for three months at relatively high seed moisture contents, were not statistically significant.

The Pairwise T-test for separate variances in total seedling length after controlled deterioration for different periods or different storage periods and an incubation period of seven days, corresponded to a large extent with the analyses of root and shoot growth. Minor differences could, however, be expected, since the effects of controlled deterioration or storage conditions on root and shoot growth differed.

No specific relationship was found with regard to the ratio of hypocotyl and primary root length after different periods of controlled deterioration or seed storage.

The variance in data on seedling organ lengths after a 14-day incubation period was too heterogeneous for reliable interpretation. (This variable was intended as provision for slower germinating seedlings that were not assessable after seven days).

A comparison of the effects of controlled deterioration and seed storage on seedling growth, reveals that these two artificial ageing methods also differ as far as their effects on primary root and hypocotyl lengths are concerned. After different periods of controlled deterioration, both cultivars displayed polynomial regressions in primary root and hypocotyl lengths (Figs. 2.10 A; 2.11 A). Similar regressions were not observed after different storage periods (Figs. 2.10 B, C; 2.11 B, C). The conspicuous and consistent increase in growth after an initial period of storage was also absent after an initial period of controlled deterioration.

A comparison between the two cultivars with regard to primary root and hypocotyl length after the seed samples had been subjected to controlled deterioration, indicates that growth of Roma VF decreased at a slower rate (Fig. 2.10 A). A similar result was, however, not obtained after seed storage. The more rapid decline in root and hypocotyl lengths of Roma VF after one month storage at a high moisture level (Figs. 2.10 C and 2.11 C) are in accordance with the results obtained with regard to germination capacity (14 days), germination energy and germination rate.

Since the ageing phenomena as portrayed by germination capacity (14 days), germination energy, germination rate and seedling growth differ significantly between the two ageing methods, and since the tetrazolium tests indicate that a secondary dormancy could not be suspected, the reasons for these differences in germination performance between the two ageing methods warrant further investigation. For this reason, ultrastructural investigations were conducted.

Villiers and Edgcumbe (1975) were of the opinion that it is uncertain whether controlled deterioration of seed reflects the adverse effects of unfavourable conditions during storage. They stated that, from a practical point of view, the differences between ordinary chronological ageing and controlled deterioration are accepted as being of degree rather than of kind. The results obtained during this investigation indicate the contrary.

2.3.4 EFFECT OF AGEING ON ULTRASTRUCTURE

Ultrastructural investigations of the radicle tip were conducted in an attempt to interpret germination and vigour differences consequent to controlled deterioration and storage over time.

As described in section 2.2.6, attempts were made to fix both dry and imbibed tomato radicle tips. The fixation of the dry tissue was, however, unsuccessful owing to the lack of penetration by the fixative. This problem was attributed to the overwhelming amount of lipids in cells of the radicle tip. Even in imbibed material, satisfactory fixation was limited although different fixatives were tested. Glutaraldehyde/osmium tetroxide was used for fixing protein and lipid bodies, while potassium permanganate was used to distinguish membrane systems.

Various difficulties were encountered during fixing and the fixing times prescribed in the literature had to be extended by a factor of twelve. Even then, penetration by osmium tetroxide at a room temperature of $\pm 20^{\circ}\text{C}$ was limited to only the outer cell layers. Fixation in potassium permanganate presented numerous obstacles, and successful fixation was only obtained by chance. In many cases, the digestion and oxidation of the overwhelming number of protein and lipid bodies was incomplete and the other organelles in cells were obscured. Partial removal of the protein and lipid bodies occurred at such a late stage during fixation, that other organelles were also damaged to such an extent that they were no longer distinguishable.

Since histology and ultrastructure of tomato have not been comprehensively described in the literature, it was decided to describe the different cell zones in the radicles (imbibed for 24 hours) of control samples before discussing the influence of ageing.

By studying the arrangement of cells in longitudinal sections of the radicle tip, it was possible to attribute the derivation of certain files of cells to groups of initial cells in the apical meristem.

As in many other dicotyledonous root meristems, three groups of initials give rise to the procambium (provascular), ground meristem, protoderm and calyptra, respectively (Spitzer and Lott, 1980). The tomato radicle corresponds with the "open type" as described by Cutter (1972). According to this author three layers of initial cells occur in the promeristem (Fig. 2.12). Since these cells could only be studied in absolute median sections, their ultrastructural investigation was extremely difficult. It was therefore decided to concentrate on the transitional zones surrounding the promeristem. The ultrastructure of the promeristem was, however, also studied when it could be encountered in median sections.

According to Berjak and Villiers (1972^b) the degenerative changes associated with normal senescence, appear to progress from the outermost cells towards the chronologically younger cells. This phenomenon was also encountered in the non-deteriorated controls during this study (Fig. 2.12). The parenchymatous cells become progressively more differentiated as distance from the radicle tip increases. The three principle tissue systems of the embryonic root, the protoderm, ground meristem and procambium, become visually delimited close behind the promeristem (Fig. 2.12). The procambium occurs in a central region and consists of densely staining, elongated cells.

An interesting characteristic of the radicle cell nucleus in imbibed tomato seeds is the unusual distribution of chromatin. The nuclei of both cultivars studied, displayed a peripheral distribution of chromatin clumps in association with the nuclear envelope (Fig. 2.15 A, C). These chromatin clumps display a dark contrast in comparison with the surrounding nuclear matrix when fixed in the glutaraldehyde/osmium tetroxide complex. This was also encountered by Baird *et al.* (1979) in the root tips of *Glycine max* L. (Merr. "Wayne"), though not nearly as well defined as in tomato tissue. Fixation in potassium permanganate, however, resulted in a light contrast of the chromatin clumps in comparison with the surrounding matrix. This phenomenon could have been caused by the unsuccessful fixing of the chromatin material with the latter fixative.

Berjak *et al.* (1984) refer to chromatin clumping in stored seeds of *Zea mays* L. as an age-correlated phenomenon. In tomato radicle cells it was, however, found to occur in all the cells, even in those of the unaged control samples. Whether chromatin clumping occurs in the unimbibed state of the cell nucleus of tomatoes is not known. Two nucleoli (Whaley *et al.*, 1960) often occurred within one nucleus which could probably be interpreted as a common phenomenon in meristematic cells.

Protein bodies and lipids were the only organelles that could be reliably interpreted in the different sections. It was, therefore, decided to concentrate on these organelles for evaluating and interpreting the influence controlled deterioration and seed storage could have on the ultrastructure of the tomato radicle tip. Protein and lipid bodies are spherical with the latter occurring in the common circular arrangement around the protein bodies (Fig. 2.14 B). Lipids also occur on the inside of the plasmalemma to a greater or lesser extent. According to Paulson and Srivastava (1968) protein bodies stain dark blue and lipids greenish-yellow when fixed with glutaraldehyde/osmium tetroxide. Additional staining with toluidine blue intensifies the blue staining of protein bodies, but has no additional reaction with lipids (Fig. 2.13). As these reactions have been found to be specific for protein bodies and lipids, further histochemical confirmation was regarded unnecessary.

2.3.4.1 Protein bodies

The ultrastructure of the protein body in imbibed control samples will be discussed before comparisons with aged material. The protein bodies varied in size between tissues, being the smallest in the promeristem. The protein bodies are bound by a lipoprotein unit membrane (Bewley and Black, 1978). They were oval to circular in section (Fig. 2.14 A, B). In the unimbibed state protein bodies could, however, be irregular in shape (Buttrose, 1973). Although the protein bodies were generally distributed throughout the radicle tip tissue, their content was highest in the cells of the ground meristem. This phenomenon also confirms the findings of Spitzer and Lott (1980).

The protein body in tomato radicles exhibits two internal inclusions, namely crystalloids and globoids (Fig. 2.14 A, B). According to Pernollet (1978) the crystalloid is proteinaceous, sometimes identifiable as a single protein type (Spitzer and Lott, 1980). Many authors agree that there is no membrane bounding of the crystalloid, which appears to be an ordered, partly crystalline protein deposit. On the other hand, the boundary of the globoid is still the subject of dispute. They are non-crystalline, globular structures and the sites of deposition of phytin, the potassium, magnesium and calcium salts of phytic acid (Eggars and Geisman, 1976).

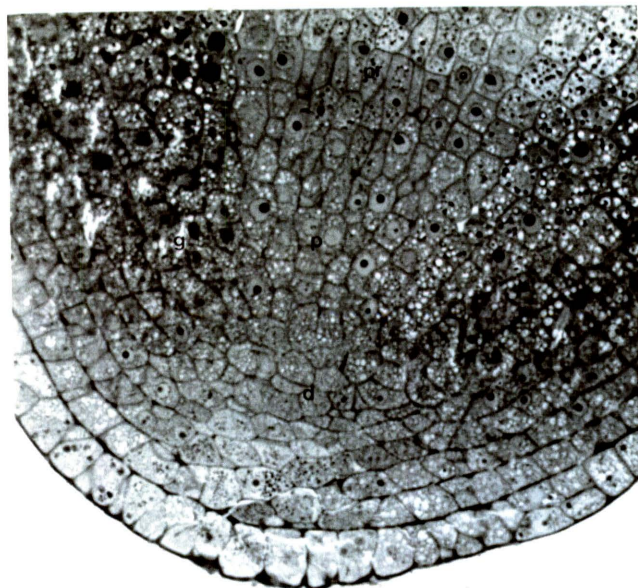


Figure 2.12: A longitudinal section of the radicle tip of a tomato embryo from a control sample to distinguish the position of the promeristem (400 x) (glutaraldehyde/osmium tetroxide fixation).
d = protoderm; p = promeristem; g = ground meristem; pr = procambium.

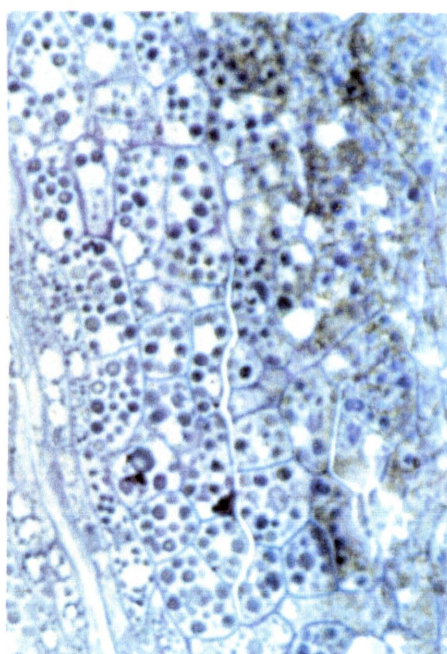


Figure 2.13: A longitudinal section through the radicle tip of the tomato to display the specific colour reactions of osmium tetroxide and toluidine blue with proteins and lipids.
Green = lipid material;
blue = protein bodies.

The protein crystalloid has an irregular or angular shape. A few protein bodies were found which apparently contained more than one protein crystalloid. Spitzer and Lott (1980) regard this as a possible artifact with the two or more regions being part of a single, irregularly shaped protein crystalloid. Lattice systems of the protein crystalloid, as described by the above-mentioned authors, were not observed. Rarely, protein bodies without protein crystalloids were found, but this could also have been due to the angle of sectioning.

Globoid bodies were prevalent although globoid crystals were hardly ever found. According to Paulson and Srivastava (1968) sectioning of the globoid crystals is often difficult. They are hard and resins do not penetrate them well. Parts of, or generally the entire globoid crystal was chipped out during sectioning.

In section, globoids were oval to spherical in shape. An interesting observation during this study was that the protein bodies of Roma VF radicles generally contained a number of smaller globoids, while generally only one relatively large globoid occurred in some of the protein bodies of Floradade radicles (Fig. 2.14). This phenomenon could relate to physiological or genetic differences between the two cultivars. Globoids were never encountered as inclusions of the protein crystalloid. They are transparent regions which do not stain with the dyes used in the present study and correspond to the globoids of classical cytology (Paulson and Srivastava, 1968).

The above-mentioned remarks refer to unaged, imbibed radicle cells. Any anomalies in the ultrastructure of artificially aged radicle cells after a similar period of imbibition are interpreted as signs of deterioration.

2.3.4.1.1 Protein bodies in radicle tissue subjected to controlled deterioration

Protein bodies of tomato radicle tips from seed subjected to controlled deterioration exhibited different patterns of deterioration in embryo tissue during imbibition. In Floradade, certain protein bodies increased conspicuously in size upon seed imbibition and appeared to act in the manner of lysosomes within which protein is broken down, while aggregation of reserve protein occurred in others (Fig. 2.15 B). The protein bodies in Roma VF cells enlarged and coalesced (Fig. 2.16 B) to eventually form larger protein bodies in which the digestion of protein was apparently incomplete. Aggregation of reserve protein was not observed in Roma VF cells.

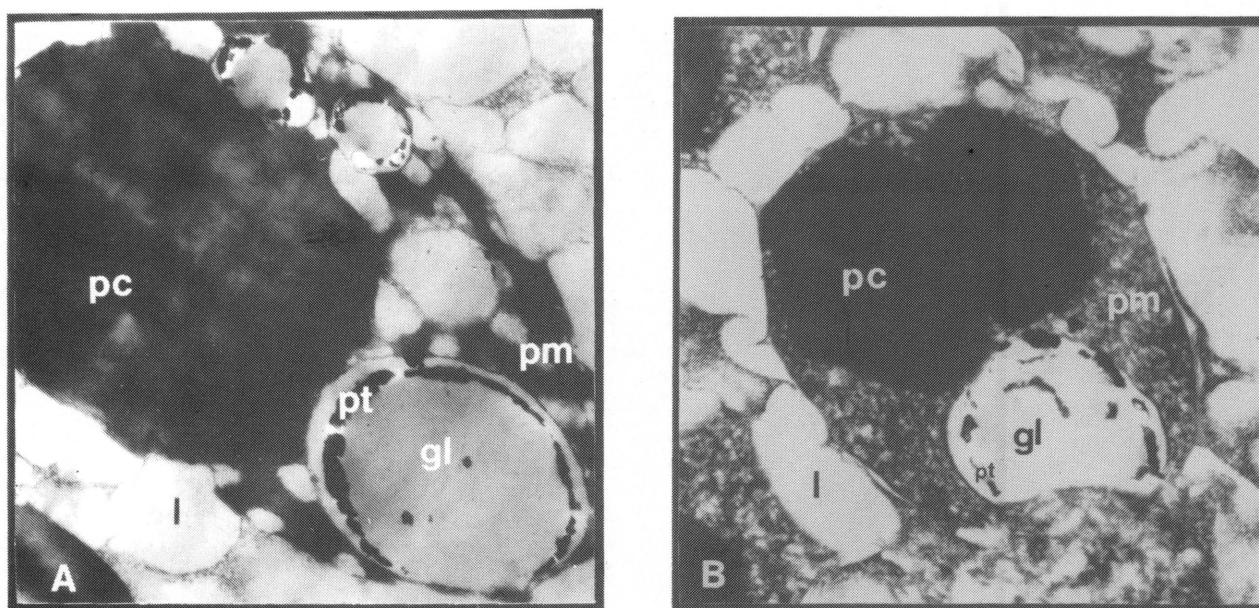


Figure 2.14: Electron micrographs of protein bodies in cells of the ground meristem of tomato radicles illustrating the character of globoids in different cultivars (glutaraldehyde/osmium tetroxide fixation). A = Roma VF (18 000 x); B = Floradade (18 000 x). pc = protein crystalloid; gl = globoid; pm = protein matrix; l = lipid body; pt = phytin.

The disorganisation of the protein bodies into provacuoles (Srivastava and Paulson, 1968) could readily be followed especially in tissue subjected to controlled deterioration. Cells of the radicle root cap, which could otherwise be expected to follow a normal sequence of differentiation from cell division to maturity, became precociously senescent. In certain cells of the calyptra, the provacuoles fused. The degradation of the membranes of the protein bodies were conspicuous within other calyptra cells, with the subsequent formation of an amorfous protein matrix. This is in line with the various reports concerning membrane damage of aged and deteriorated seeds (Berjak and Villiers, 1972^d; Berjak and Villiers, 1972^a) (Berjak *et al.*, 1984).

Myelin-like inclusions often appeared to be incorporated by the provacuoles (Fig. 2.16 B). Berjak and Villiers (1970) reported this to occur in seeds of other species as well. These appeared to be broken down, releasing a fine granular substance. Membrane dissolution and general degradative changes of the cytoplasm, occurred only in certain calyptra cells of deteriorated radicles. This was mostly encountered in the distal cell zones of the calyptra and is rather thought to be the influence of senescence than artificial ageing.

Radicles of both cultivars submitted to controlled deterioration, exhibited lysosomal provacuoles in the ground meristem cells. Vacuolation was, however, more prominent in Roma VF than in Floradade (Figs. 2.15 B; 2.16 B). Protein bodies in ground meristem cells were mostly still intact, although a degradation owing to normal imbibitional processes was more prominent in artificially aged samples than in the control samples (Figs. 2.15 A, B; 2.16 A, B). No protein crystalloids were visible in the Roma VF samples subjected to controlled deterioration for eight days (Fig. 2.16 B). Crystalloids were, however, still visible in the Floradade counterpart although signs of deterioration were also evident (Fig. 2.15 B). Deformation of protein bodies prior to complete hydrolysis of the protein contents, as also observed in other species (Villiers, 1980), could be observed in certain cells of the ground meristem of Floradade radicle tips (Fig. 2.17 B).

Various hydrolases are associated with protein bodies (Berjak and Villiers, 1970). It should be taken into account that some of the enzymes might be contaminants from the cytoplasm (Bewley and Black, 1978) in seed cells subjected to controlled deterioration.

The presence of carbohydrases (Spitzer and Lott, 1980) in protein bodies suggests the possibility that hydrolysis of the dictyosomally-derived polysaccharide creates an osmotic effect resulting in the very marked swelling of these bodies. Subsequently to swelling, or possibly a result of it, the damaged protein body membranes in deteriorated cells, expand disproportionately and form in- and evaginations. The damaged membranes probably fuse on contact and therefore other contents of the cytoplasm could be invaginated and digested by the lytic enzymes in the lysosomal protein body. The damaged membranes also fuse when contact between two lysosomal protein bodies is accomplished. Vacuolation progresses before the proper degradation of protein reserves. It therefore seems that vacuolation is premature and becomes uncontrollable. This could indicate to a certain extent that the accelerated deterioration of these membrane systems, beyond the point of no return (Villiers, 1980), occurs because of the detrimental effects of controlled deterioration. It is also in line with the results of Villiers (1980) who reported general cytoplasmic lysis following dissolution of the protein body membranes in cells of the embryo axis. Observations during this study, however, did not include cells displaying cytoplasmic lysis related to the effects of controlled deterioration. Protein membranes were, therefore, possibly weakened by the treatment, but only to the extent that they expanded disproportionately without breaking.

2.3.4.1.2 Protein bodies in radicle tissue from seed subjected to seed storage under unfavourable conditions

The disintegration of protein in Floradade radicle tissue from seed subjected to storage for three months at relatively high seed moisture contents, resembled that observed after accelerated ageing (Fig. 2.15 C). This is comparable with the so-called imbibitional effects on cells of germinating seeds as described by Berjak and Villiers (1970). It was, however, not observed in control samples (relatively low moisture contents) stored under similar conditions. Control samples were also imbibed prior to fixing and sectioning (Fig. 2.15 A). Cell deterioration was more conspicuous in Roma VF radicle tissue (Fig. 2.16 C) which could possibly be related to the faster decrease in the percentage normal seedlings after a 14-day incubation period, germination rate, and hypocotyl lengths (Figs. 2.7 C; 2.9 C; and 2.11 C).

The ground meristem of radicle tips from seed subjected to storage for three months at relatively high seed moisture contents, showed accelerated disintegration of the protein matrix of protein bodies. This could also be noticed in certain cells undergoing lysis. The retainment of protein crystalloids and globoids was also encountered in this case. Bhandari and Chitralkha (1984) also encountered the persistence of globoids in the differentiated cell of Brassica campestris L. var Sarson.

Ground meristem cells of radicle tips from seed samples subjected to a period of three months storage with relatively low seed moisture contents, showed the ultrastructural changes associated with normal seed imbibition. Lipid bodies were grouped on the inner surface of the plasmalemma and the outer surfaces of the protein body membranes. Fusion of some of the protein bodies occurred, while others, in Floradade tissues especially, adopted extra-ordinary forms in comparison to the usually rounded structure of those of the control samples (Fig. 2.17 C).

After six months storage under unfavourable conditions, the cell content of the calyptra was characterized by the presence of extremely few typical protein bodies. Conspicuous areas of amorfous cytoplasm adjacent to the plasmalemma could also be observed.

Radicle ground meristem cells from seed subjected to six months storage with relatively high seed moisture contents, revealed recognizable signs of dead tissues (Figs. 2.15 D; 2.16 D). Lysis and the general disintegration of the cytoplasm with the exception of certain protein crystalloids, commonly occurred. It was again obvious that deterioration progressed faster in Roma VF tissue than in Floradade (Figs. 2.15 D; 2.16 D). The former displayed lysis of the cytoplasm, while many protein crystalloids were still visible in the Floradade counterpart. This could also explain the faster decrease in germination variables as observed in Figs. 2.7 C; 2.8 C; 2.9 C; 2.10 C; and 2.11 C.

In some way, either through the production of toxins or by one of the mechanisms suggested by Cherry (1983), storage micro-organisms benefit from the consequences of seed storage and grow luxuriantly on seeds.

This is followed by decomposition of proteins to free amino acids. In addition, there is a depletion of some enzymes, the intensification of others and/or production of new multiple molecular forms of enzymes (Cherry, 1983). Many of these enzymes in seeds remain active during the infection period. Thus, the biochemical mechanisms operating in the saprophyte-seed interaction could very efficiently and systematically enhance fungal growth at the expense of the seed reserves.

The assessment of micro-organisms confirmed that Aspergillus, Penicillium, Fusarium spp. and bacteria occurred in all the seed samples stored with relatively high seed moisture contents. The contamination was 23% in Floradade and 55% in Roma VF. This could also present a possible explanation for the accelerated degradation of protein in tissue previously subjected to natural ageing under unfavourable conditions. According to Villiers (1980) this has also been shown to occur in germinating seeds and in seeds of low viability infected with fungi. There was no anatomical evidence of the localization of micro-organisms within radicle tissue, but this could be expected since these organisms are usually found under the seed coat (Anderson and Baker, 1983).

2.3.4.2 Lipid bodies

In embryos of tomato seeds the lipid bodies pack the cells, filling almost all the space not occupied by the other organelles. The distribution of both lipid and protein bodies in the cells of the promeristem was, however, sparse in comparison with the adjacent tissues. Relevant to the controversial views on the origin of the lipid body, is the question as to whether they are bound by membranes (Adams et al., 1983). Observations during this study contributed to evidence in this respect.

2.3.4.2.1 Lipid bodies in radicle tissue from seed subjected to controlled deterioration

After controlled deterioration, radicles seldomly contained cells in which the lipid bodies had become confluent (Figs. 2.15 B; 2.16 B). Lipid bodies in tissue after controlled deterioration therefore appeared relatively unperturbed.

2.3.4.2.2 Lipid bodies in radicle tissue from seed subjected to seed storage under unfavourable conditions

Sections of Roma VF radicle tips from seed stored for three months at relatively high seed moisture contents, contained cells in the process of dying or most probably already dead. Calyptra and ground meristem cells of radicle tips from seed stored for three months at relatively high seed moisture contents displayed confluence of lipids. This resulted in the formation of large masses of homogeneously-staining lipid material (Fig. 2.16 C). Large lipid-derived vesicles were abundant through the apparent autolytic, enzymatic activities within the lipid bodies and the subsequent coalescence of lipid vesicles. This was not observed in the Floradade counterpart after the same storage period and could explain the slower rate of deterioration of these seeds as displayed in Figs. 2.7 C; 2.9 C; and 2.11 C. After a six months storage period, confluence of lipids was also conspicuous in Floradade tissue, while general lysis of the cytoplasm was then already obvious in cells of Roma VF.

The disappearance of lipid droplets occurred concurrently with that of protein bodies (Fig. 2.16 C). It was most noticeable near the cell walls of radicle tip cells where lipid bodies were most abundant. Apparently lipids are selectively lost or migrate from this region prior to germination. It is not clear whether the vesicles were formed as a result of the removal of lipid droplets. Vesicles appear as single, possibly membrane-bound, spheroidal bodies (Srivastava and Paulson, 1968).

As mentioned previously, the origin of lipid bodies is controversial. An early suggestion was that they develop from spherosomes (Bewley and Black, 1978). There is, however, a dispute on whether or not lipid bodies possess a surrounding membrane. As already discussed, many authors deny the presence of a limiting membrane, which therefore distinguishes them from spherosomes. The confluence of lipid bodies could in either cases be attributed to the degradation of membranes owing to ageing, as was observed in the case of protein bodies. If a delimiting membrane is not present, the lipids could become confluent owing to the degradation of other surrounding membrane systems (i.e. endoplasmic reticulum) which previously prevented them from doing so (Bewley and Black, 1978).

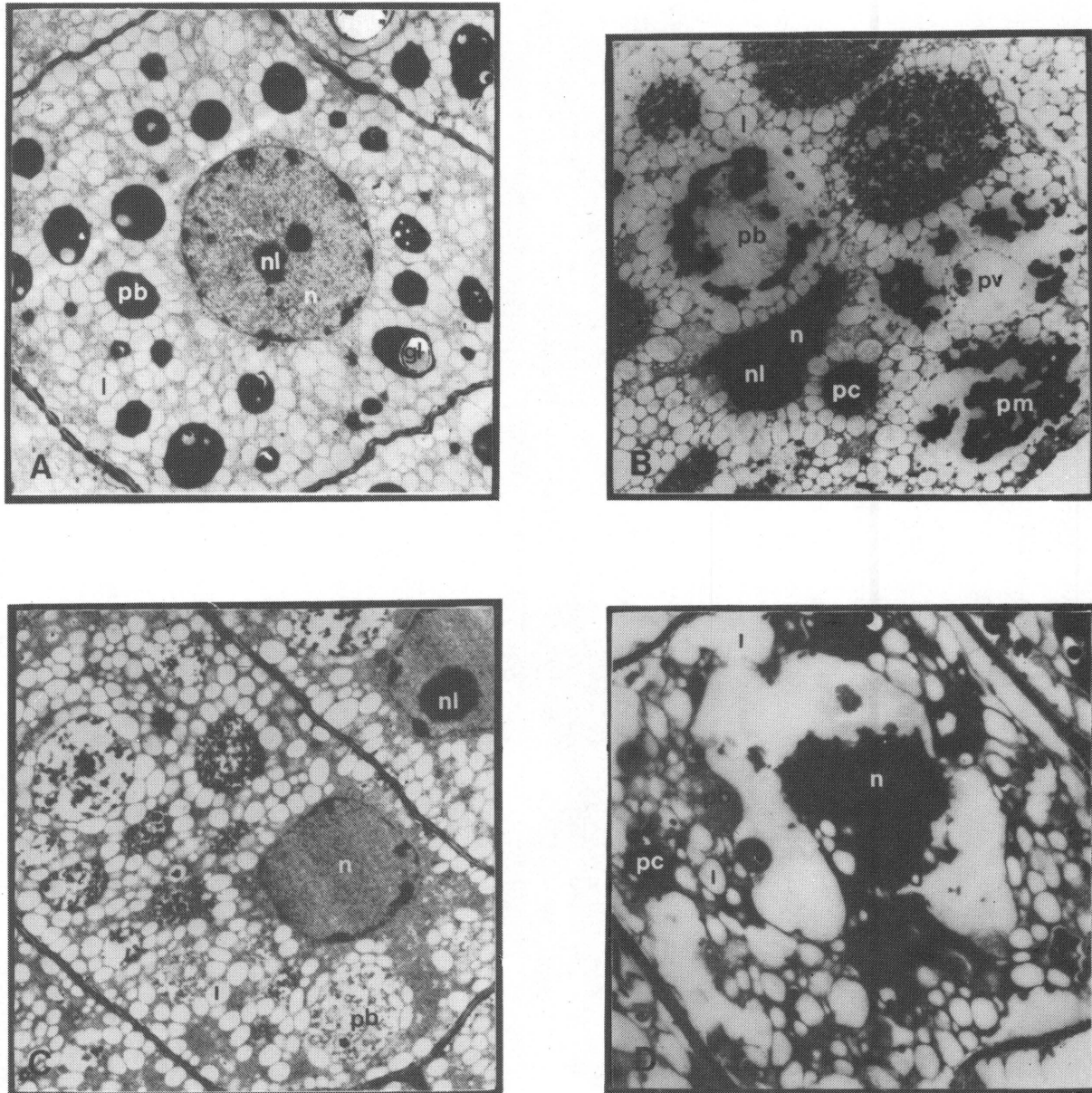


Figure 2.15: Electron micrographs of ground meristem cells in the radicles of Floradade subjected to different methods of artificial ageing (glutaraldehyde/osmium tetroxide fixation).

A: Control (4300 x); B: Controlled deterioration (3600 x); C: Storage for three months at relatively high moisture contents ($\pm 30\%$) (4300 x); D: Storage for six months at relatively high moisture contents ($\pm 30\%$) (3600 x).

pb = protein body; l = lipid; pm = protein matrix; pc = protein crystalloid; pv = provacuole; gl = globoid; n = nucleus; nl = nucleolus.

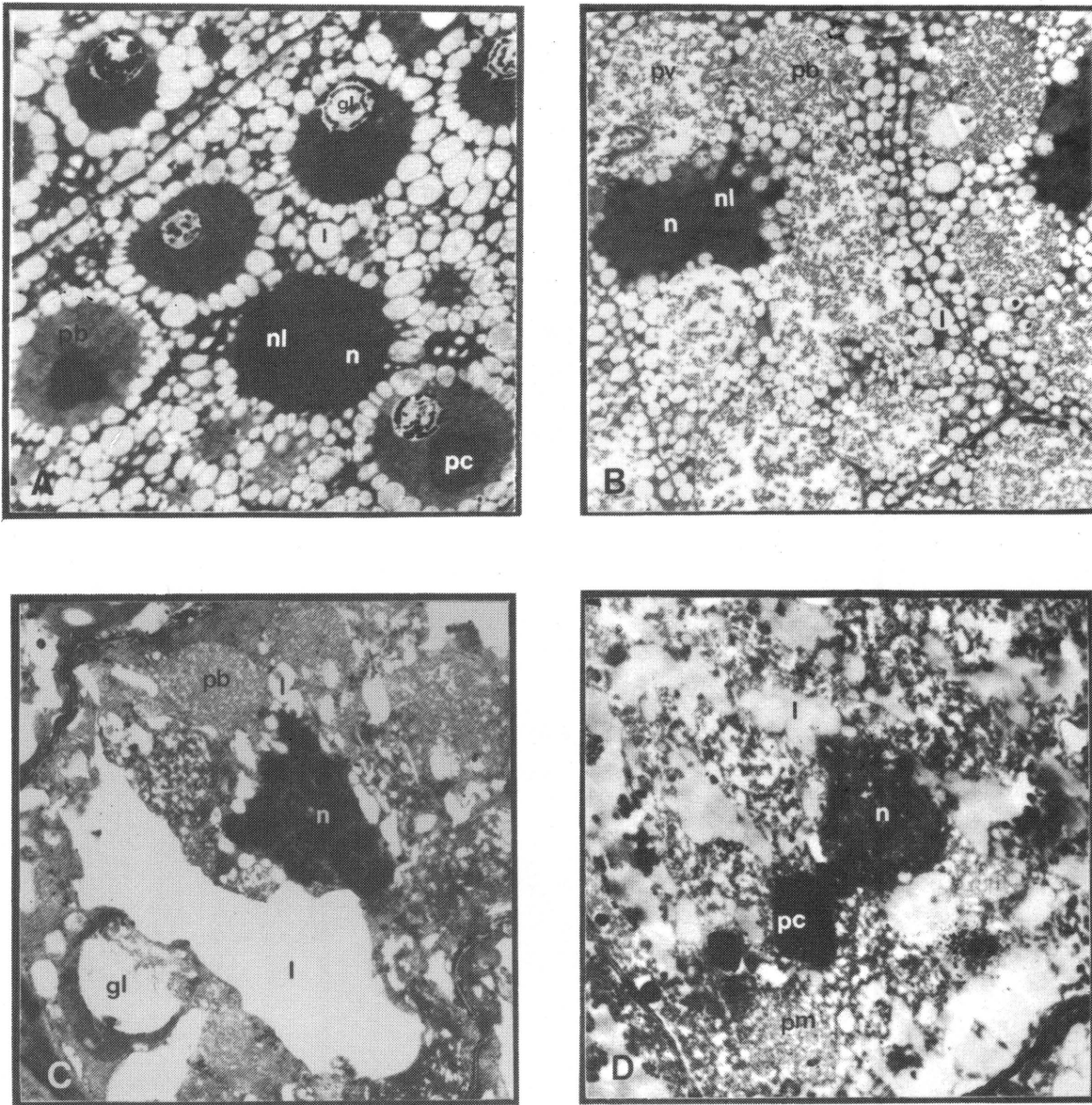


Figure 2.16: Electron micrographs of ground meristem cells in the radicle of Roma VF subjected to different methods of artificial ageing (glutaraldehyde/osmium tetroxide fixation).
A: Control (3600 x); B: Controlled deterioration (3600 x); C: Storage for three months at relatively high moisture contents ($\pm 30\%$) (5900 x); D: Storage for six months at relatively high moisture contents ($\pm 30\%$) (5900 x).
pb = protein body; l = lipid; pm = protein matrix; pc = protein crystalloid; pv = provacuole; gl = globoid; n = nucleus; nl = nucleolus.

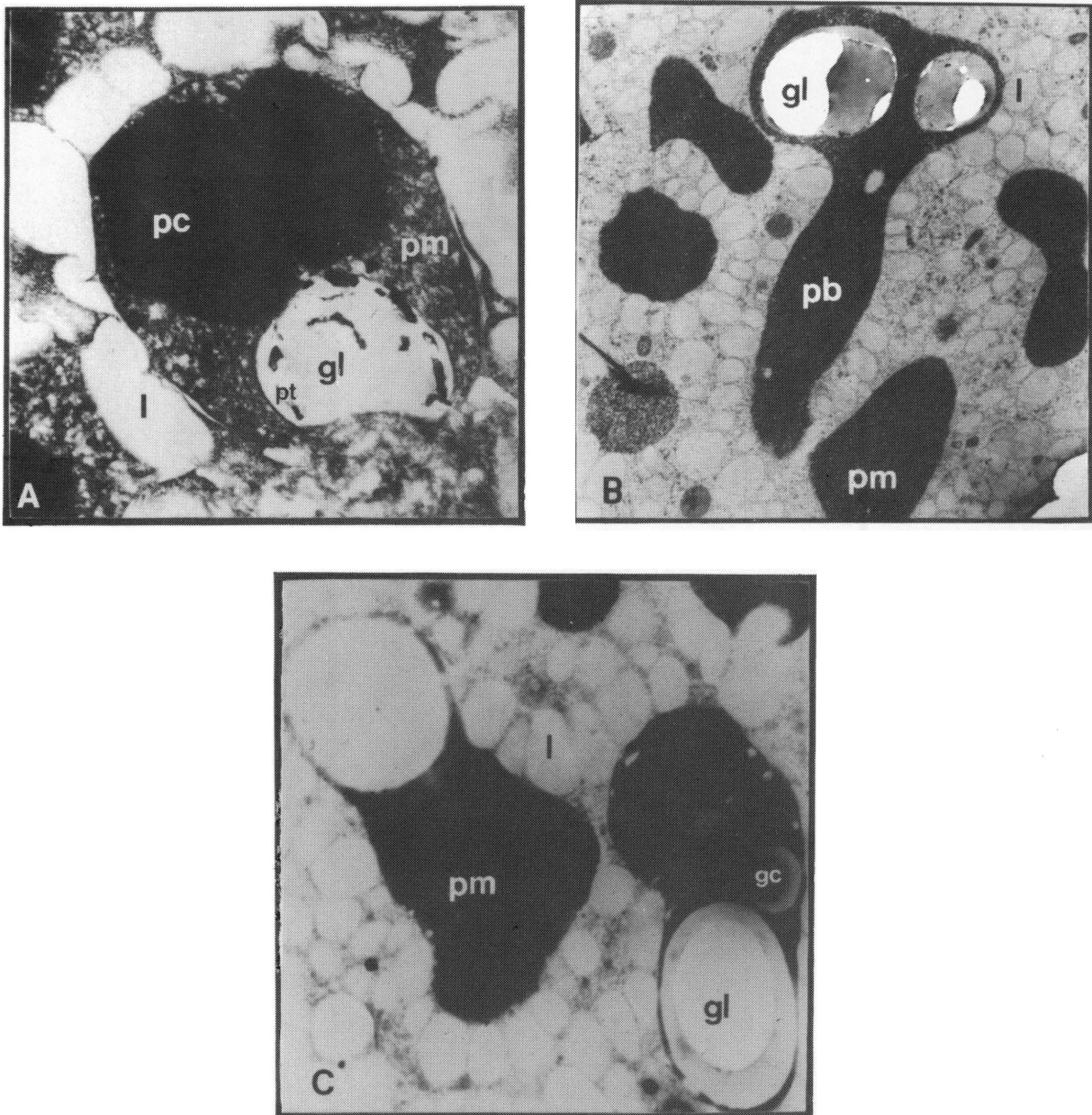


Figure 2.17: Electron micrographs of protein bodies in cells of the ground meristem in tomato radicles (Floradade) illustrating the effect of artificial ageing on the protein body delimiting membranes (glutaraldehyde/osmium tetroxide fixation). A: Control (18000 x); B: Controlled deterioration (4300x); C: Storage for three months at relatively low moisture contents ($\pm 7\%$) (9800 x). p = protein body; l = lipid; pm = protein matrix; pc = protein crystalloid; gl = globoid; gc = globoid crystalloid.

Anderson and Baker (1983) also observed coalescence of spherosomes or lipid bodies, although they speculated that the trigger for such changes could be imbibition and that the occurrence of these phenomena could be enhanced by seed treatments or seed deterioration.

Confluence of lipids in tomato radicle tissue is, however, interpreted as an ageing phenomenon. According to Villiers (1980) the ageing process could damage the half membranes enclosing lipid vesicles.

Cherry (1983) attributes the latter to the presence of fungi and their derived metabolites in the seed. As previously mentioned, fungi, as well as bacteria occurred in all the seed samples stored with relatively high seed moisture contents. It could be speculated that confluence of lipids in aged cells is a secondary influence of ageing attributed to a saprophyte/ageing interaction.

2.3.4.3 General discussion on ultrastructural observations

Observations of the transition between calyptral and promeristem tissues in electron micrographs show that promeristem cells appear less damaged than the adjacent cells of the root cap after controlled deterioration. Although all the cell walls appear distorted, the cells of the promeristem retained their nuclei, while the nuclei in the adjacent calyptral cells were either degraded or altogether disintegrated.

This also supports the hypothesis of Berjak and Villiers (1972^C) that an acceleration of deterioration by seed ageing affects the distal cells of the radicle more readily than the proximal zones, as the outermost cells have already reached the final stage in the sequence of cellular differentiation during maturation of the seed. The above-mentioned authors also refer to the possibility that meristematic cells are more susceptible to damage during ageing and are replaced from cells normally quiescent and presumably less prone to damage. According to Paulson and Srivastava (1968) lobing of the nucleus is an indication of cell deterioration. Although not as extensive as described by the above-mentioned authors, lobing of the nucleus did occur to a limited extent in the cells of the promeristem. The above-mentioned authors also maintain that limited lobing of nuclei and the concurrent damage incurred, appears to be reversible during early germination. According to Berjak and Villiers (1970) nuclei could appear irregular and lobed in the mature and in the outermost senescing cells. Lobed nuclei were, however, observed in calyptra cells adjacent to the promeristem which could again point to the acceleration of the senescence process to occur

even in relatively immature cells. The cells of the promeristem were practically devoid of lysosome-like vacuoles, while many were observed in the adjacent calyptral cells. According to Villiers (1972) the unusually early development of expanding lysosomes and the presence of other organelles within lysosomes are significant as this could indicate a mechanism for the selective removal of damaged or aberrant organelles.

The phenomenon that cell deterioration is less conspicuous in the promeristem than in the surrounding tissue, possibly indicates the key role of the former in embryo survival under unfavourable conditions. It is also suggested that the slower dividing cells represent the controlling factor of this protective mechanism in the embryo. According to Clowes (1967) these cells may have relatively thick cell walls, thus indicating that they might be less prone to damage than the surrounding meristematic cells.

Radicle tip cells from seed stored for six months at relatively high seed moisture contents, displayed the recognizable signs of death. Cells of the ground meristem from seed stored for six months at relatively low seed moisture contents did not display significant differences from the controls.

A pattern of degradation, which terminated in total autolysis of the cells concerned, was described by Berjak and Lawton (1973) for certain prostelemar cells, in young regions of the root. Deteriorated seeds are reported to leach more of their cellular constituents than non-deteriorated seeds, presumably because of an increase in membrane permeability.

The grainy material that Anderson and Baker (1983) described in the area between the plasmalemma and the cell wall, representing material leached from the protoplast, was only observed in one section of the radicle cells subjected to storage for six months at relatively high moisture content.

Similarities, as well as differences were evident between controlled deterioration and seed storage under relatively unfavourable conditions. Ultrastructural changes of the calyptra cells, encountered during controlled deterioration, were similar to those encountered after storage of the seed samples under unfavourable conditions. Coalescence of lipid vesicles occurred under both conditions, although the phenomenon was limited in tissues from controlled deteriorated seeds. Seed storage under unfavourable conditions [20/30°C (16h/8h) alternating temperature and approximately 30% moisture content] apparently rendered favourable conditions for saprophyte multiplication. As previously mentioned, saprophyte/ageing interactions could cause confluence of lipids. These confluent lipid areas remained on the inside of the plasmalemma and possibly indicate the delay or obstruction of the processes of imbibition. These lipid areas also remained in dying or apparently dead tissue. Although the hydrolysis of protein (Villiers, 1980) seemed to continue in apparently non-viable tissue, the oxidation of lipids requires a significant amount of energy for initiation (Pradet, 1982). Non-viable tissue obviously failed to provide sufficient energy for the initiation of the latter process. The relatively high temperature of controlled deterioration (45°C) at approximately 20% seed moisture content probably created unfavourable conditions for proliferation of micro-organisms. Confluence of lipids therefore seldom occurred.

This explanation was related to the results of assessments on micro-organisms. Aspergillus, Penicillium and Fusarium spp. occurred in all the stored seed samples except in seeds of the Roma VF sample stored with relatively low seed moisture contents ($\pm 7\%$) at 5°C. A high percentage of bacteria occurred in all the stored seed samples. No fungi, and a significantly lower percentage of bacteria (7% in Floradade and 15% in Roma VF), were isolated from the samples that had been subjected to controlled deterioration. According to Neergaard (1977) the favourable temperature and relative humidity for the growth and proliferation of storage fungi are 30 - 33°C and 65 - 90% RH respectively. The author maintains further that storage fungi can still be vigorous and active at 45°C and 95% RH, while most of the field fungi are sensitive to high temperatures and usually disappear under such conditions. The results of this investigation, however, indicated that 45°C as applied in the controlled deterioration test, was too adverse for any fungal growth and proliferation, even with the $\pm 20\%$ moisture content in the seed obtained by equilibration at 95% RH. This relatively high temperature could, however, be responsible for heat injury owing to protein denaturation followed by aggregation (Gopala et al., 1981).

According to Harrow and Mazur (1966) membrane proteins are fibrous, high molecular weight proteins and usually capable of stretching and contracting. Denaturation of these proteins often results in irreversible loss of their biological activity. Denaturation of structural and peripheral proteins of the cellular membranes could, therefore, have caused loss of elasticity and an increase in the permeability of larger molecules.

The loss of elasticity could be responsible for irregularities of the plasmalemma, lobing of the nucleus membranes, and general loss of structure of the protein bodies and provacuoles, causing in- and evagination of the membranes. The partial hydration of radicle cells after equilibration of the seeds to relatively high moisture contents, could be responsible for an advantage in water absorption during imbibition, contributing to the disproportionate size increase of protein bodies/provacuoles. Aggregation of reserve proteins was also visible in certain micrographs.

Sections of the ground meristem prepared from material subjected to controlled deterioration appeared similar, compared to those of more naturally aged radicle tips. An important exception was, however, that the former did not show any signs of lysis. Ultrastructural investigations of the changes resulting from controlled deterioration and seed storage, once again revealed that the physiological mechanisms of deterioration might differ between the two processes. Lyses did not occur in ground meristem cells subjected to controlled deterioration and differences were also observed regarding the lytic activity within the cells.

The lysosomal protein bodies/provacuoles in cells of controlled deteriorated radicle tissue, usually remained intact thus preventing the release of lytic enzymes in the cytoplasm. The retention of the protein body/provacuole membrane could possibly be attributed to the availability of insufficient energy in controlled deteriorated tissue for the initiation of lipid oxidation. The release of free radicals is therefore limited and the degradation of membranes owing to free radicle attack, prevented. Stored tissue could possibly retain enough energy for lipid oxidation to a certain extent. Free radicals could therefore contribute to the degradation of protein body membranes, with the consequent release of lytic enzymes in the cytoplasm causing general lysis.

Suggested models for the different mechanisms involved during the irreparable, degradative ultrastructural changes in radicle cells after controlled deterioration and seed storage under relatively unfavourable conditions, are presented in Figure 2 18 A R

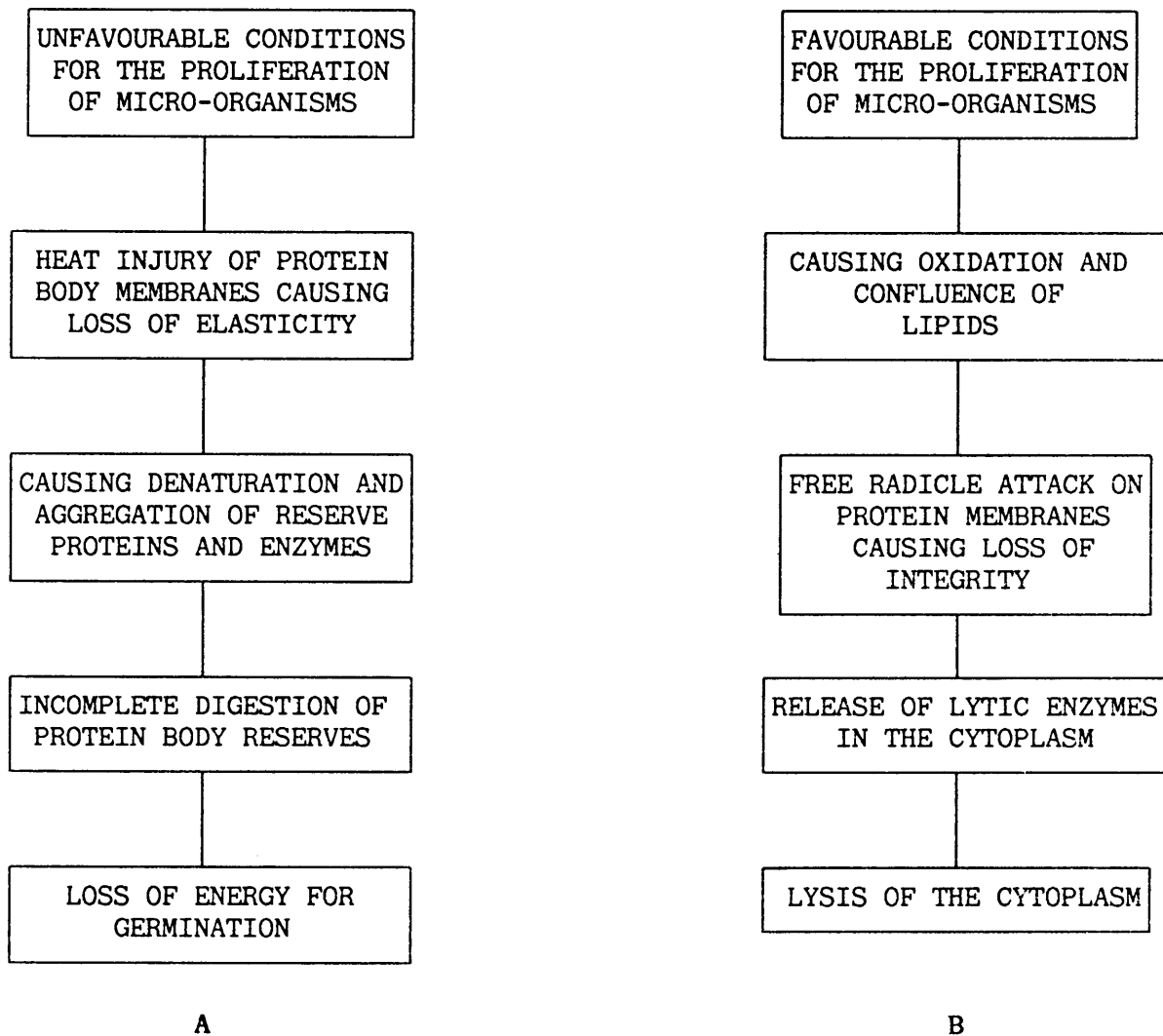


Figure 2.18: Suggested models for the irreparable, degradative ultrastructural changes in radicle cells after different artificial ageing treatments.
A: Controlled deterioration at 45°C and ±20% seed moisture content.
B: Seed storage at 20/30°C (16h/8h) and ±30% seed moisture content.

Many authors assume the process of deterioration which occurs under artificial ageing conditions to be similar, if not identical, to those which occur during natural ageing, the main difference being the speed at which these changes occur (Coolbear et al., 1984; Roberts and Ellis, 1982). Observations made during this study, however, tend to support the views of Hailstones and Smith (1988), Priestly and Leopold (1979 and 1983) Ching (1982) and Harman and Mattick (1976). Seed deterioration owing to long term storage under unfavourable conditions, is primarily the result of lipid changes that subsequently cause an increase in membrane leakiness followed by the irreparable loss of viability. Seed deterioration owing to controlled deterioration seems to be mainly the effect of high temperature damage. Controlled deterioration is therefore of limited usefulness in investigations of the mechanism of seed ageing.

CHAPTER 3

THE EFFECT OF A HOT-WATER TREATMENT ON THE GERMINATION PERFORMANCE AND ULTRASTRUCTURE OF TOMATO SEED

3.1 INTRODUCTION

Tomato seed may be infected with the bacteria, Corynebacterium michiganense (E.F. Sm.) H.L. Jens. which causes bacterial canker. Since the infection is not visible on the seed, but appears only when the seedlings are a few weeks old, infected seed cannot be eliminated before sowing. It is therefore essential that the seed be treated before sowing to prevent seedling infection from this source.

Although the majority of seed-borne diseases of tomato are amenable to control with fungicides, certain pathogens like Bacterial canker can be controlled only by physical means.

Bacterial canker is one of the most difficult tomato diseases to control because of the problem of detecting infected plants owing to the variability of symptom expression, the infectious nature of the disease and the absence of effective chemicals for treatment. The organism has the capacity to survive for long periods in the environment of the crop and may be able to exist at subclinical levels in symptomless plants.

To ensure the health of a tomato crop, a standard method for the control of bacterial canker may be used, namely the hot-water treatment. It is an exacting process and can only be conducted by experienced operators with the appropriate equipment. Seed is soaked in water at a fixed temperature, for a certain period of time, the actual time/temperature combination being that in which the disease is controlled with minimum damage to the seed. The period of exposure is reduced with increasing temperature, but the main practical difficulty of the operation is to maintain a uniform and constant temperature throughout the seed sample during treatment.

Progressively higher temperatures could, however, cause a delay in germination. According to Rumsey (1962) increasing exposure times from 10 minutes to 15 hours could have a significant effect on the rate of germination. It can also be anticipated that seedlings from seed subjected to severe heat treatments may be abnormal in growth and form.

The objectives of the research in this chapter was to investigate the physiological effect of the hot-water treatment on tomato seed germination performance as well as on the ultrastructural disturbances it may cause in the radicle tip.

3.2 MATERIAL AND METHODS

Oosthuizen (1975), Strydom *et al.* (1967), Boelema (1980), Jurchak (1983), Docea and Marinescu (1977) and various other authors have described methods for the bulk hot-water treatment of tomato seeds. These methods can be summarized as follows:

A 25 dm³ container is half filled with clean water and heated over an open fire, stove or gasflame to 50 - 54°C. The container is removed from the fire as soon as this temperature has been reached. The seed, which should be in a cotton bag, is then immersed in the water. The bag is kept immersed and a thermometer is used to stir the water continuously while noting the thermometer reading. The temperature is maintained by the addition of boiling water. The treatment is continued for 25 - 30 minutes, after which the seed is removed and dried in the shade.

The above procedure was simulated in the laboratory. Seed samples consisting of 100 seeds each, were enclosed in small cotton bags (Fig. 3.1) and submerged in hot water at 54°C in a hot-water bath for 25 minutes (Fig. 3.2). After the hot-water treatment, the seeds were dried in the cotton bags in a forced draught oven at 40°C for six hours (Leopold, 1983) (Fig. 3.3). The seed samples were then removed from the cotton bags and equilibrated to two levels of moisture content in the seed. A high level of approximately 30% [31,4% (Floradade) and 29,4% (Roma VF)] was achieved by equilibration of the seed samples at ±100% relative humidity for 14 days.

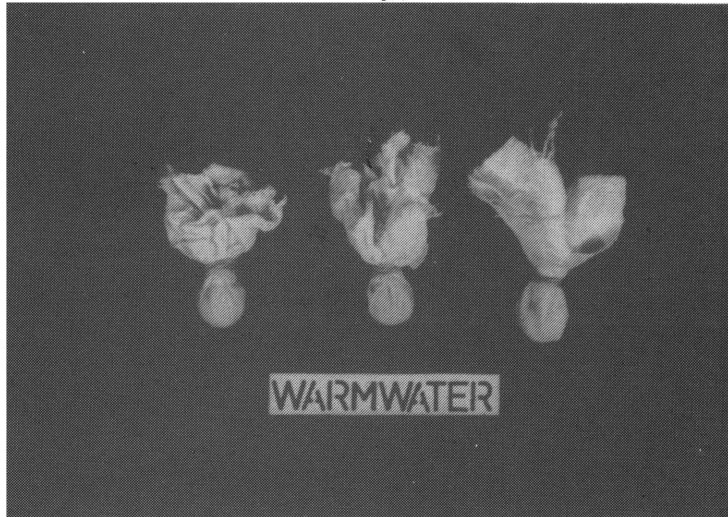


Figure 3.1: For a simulation of the hot-water treatment in the laboratory, each replicate of 100 seeds were placed around a glass marble weight on a piece of cotton cloth. The seeds and weight were subsequently enfolded in the cloth and the edges of the latter tightened with a rubber band.

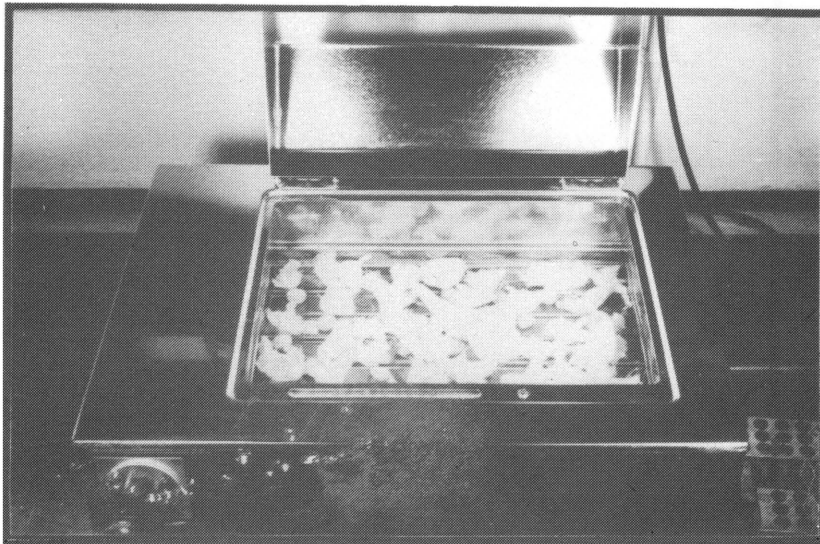


Figure 3.2: Small "cotton bags", each containing 100 tomato seeds and a glass marble weight, were placed in a hot-water bath at 54°C for 25 minutes as a simulation of the hot-water treatment in the laboratory.

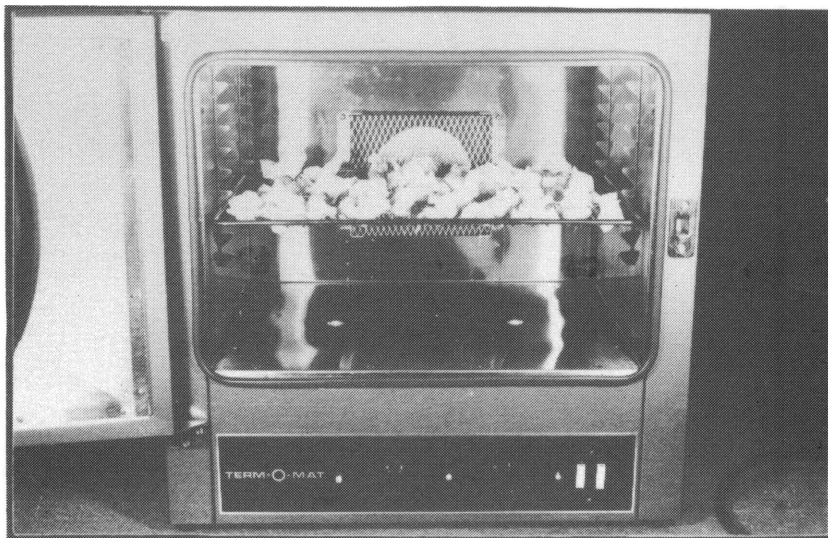


Figure 3.3: The enfolded tomato seeds were dried in a forced draught oven at 40°C for six hours after the hot-water treatment.

The motivation for such an unrealistically high moisture level in the seed was to simulate a possible unfavourable influence of relatively high moisture content on the performance of tomato seed ineffectively dried after the hot-water treatment. Equilibration under controlled laboratory conditions (20°C and 15% RH) resulted in a realistic moisture content of approximately 7% [7,5% (Floradade) and 7,0% (Roma VF)] in the seed.

The samples were heat-sealed in laminated aluminium foil polyethylene pouches as described in Chapter 2 (2.2.3.1) and stored at 20/30°C (16h/8h) alternating temperatures for one to 12 months. Germination capacity, germination rate, seedling organ lengths, as well as ultrastructural investigations were conducted at one month intervals. These procedures were described in Chapter 2 (2.2.4 and 2.2.6). Data on germination performance were analysed mainly by means of polynomial regressions. The statistical procedures as specified in Chapter 2 (2.2.5) were also applied for pairwise tests on the data in this chapter. All experiments were conducted on both Floradade and Roma VF samples. The following sets of four replicates each, were subjected to eight storage periods (1; 2; 3; 4; 5; 6; 9 and 12 months):

- (i) Hot-water treatment + storage at relatively low seed moisture content
- (ii) Control + storage at relatively low seed moisture content
- (iii) Hot-water treatment + storage at relatively high seed moisture content
- (iv) Control + storage at relatively high seed moisture content

After each storage period, germination capacity was determined after a 14-day incubation period at 20/30°C (16h/8h).

3.3 RESULTS AND DISCUSSION

3.3.1 EFFECT OF HOT-WATER TREATMENT ON GERMINATION PERFORMANCE

The germination capacity and germination rate of hot-water treated tomato seed decreased slightly during storage at relatively low seed moisture contents (Figs. 3.4 A, 3,5 A; Tables 3.1 and 3.2). According to the non-parametric U-test of Mann-Whitney, this decrease was not statistically significant for any of the treated samples compared to the performance of untreated controls [Appendices 3.1 (pp 193 - 198); 3.3 (pp 210 - 215)].

Table 3.1: Germination of hot-water treated and untreated tomato seed after storage at relatively low moisture contents ($\pm 7\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)				Roma VF (%)			
	Normal seedlings		Abnormal seedlings		Normal seedlings		Abnormal seedlings	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
0	83	85	11	8	94	93	4	3
3	87	87	6	6	93	91	2	4
6	87	84	7	5	91	88	2	3
9	82	84	4	5	90	85	3	3
12	80	83	6	4	87	84	6	5

Table 3.2: Viability of hot-water treated and untreated ungerminated tomato seeds after storage at relatively low moisture contents ($\pm 7\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)				Roma VF (%)			
	Ungerm. viable		non-viable		Ungerm. viable		non-viable	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
0	0	0	7	7	0	0	2	4
3	0	0	7	7	0	0	5	5
6	0	0	7	12	0	0	7	10
9	0	0	15	11	0	0	8	12
12	0	0	14	14	0	0	7	11

The germination capacity and rate of Roma VF was significantly higher than that of Floradade up to three months storage, but deteriorated faster during the longer periods [Appendices 3.1 (pp 199 - 201); 3.3 (pp 216 - 218)]. As was pointed out in Chapter 2 (pp 49 and 53), this was a common phenomenon in all the storage experiments and is therefore not the consequence of the hot-water treatment, but of seed ageing. These results, therefore, indicate that there was no direct effect of the hot-water treatment on germination performance; nor were any of the cultivars significantly affected under relatively favourable storage conditions.

A highly significant decrease in the percentage normal seedlings that developed from hot-water treated seeds, was observed during the first three months of storage with relatively high moisture contents in the seed (Fig. 3.4 B and Table 3.3) [Appendix 3.2; pp 204 - 207]. Germination rate of hot-water treated seed samples decreased more rapidly than untreated samples during the first two months of seed storage at relatively high moisture contents in the seeds [Fig. 3.5 B and Appendix 3.4 (pp 221 - 224)]. This decrease applied to both cultivars and is described by polynomial regressions of the first degree for Floradade seed samples, and of the second degree for Roma VF. The results in Chapter 2 showed that germination capacity and rate of untreated seed samples of both cultivars subjected to storage at relatively high seed moisture contents, display polynomial regressions of the second degree (compare Figures 2.7 C; 2.9 C and 3.4 B; 3.5 B). The curves for the germination capacity and rate of treated and untreated Roma VF seed, however, differed with regard to their regression coefficients (Figs. 3.4 B, 3.5 B) [compare Appendices 2.9 (pp 143 - 144); 2.15 (pp 173 - 174) and 3.2 (pp 202 - 203); 3.4 (pp 219 - 220)]. The mathematical equations for the relevant curves in Figure 3.4 B are:

$$y = 88,95 - 21,98x \text{ (Floradade, hot-water treated);}$$

$$y = 84,79 + 3,94x - 6,30x^2 \text{ (Floradade, untreated);}$$

$$y = 85,72 - 39,92x + 4,91x^2 \text{ (Roma VF, hot-water treated);}$$

$$y = 92,92 + 3,13x - 6,59x^2 \text{ (Roma VF, untreated).}$$

The mathematical equations for the relevant curves in Figure 3.5 B are:

$$y = 17,06 - 4,47x \text{ (Floradade, hot-water treated);}$$

$$y = 16,82 + 0,27x - 1,15x^2 \text{ (Floradade, untreated);}$$

$$y = 16,41 - 7,96x + 1,00x^2 \text{ (Roma VF, hot-water treated);}$$

$$y = 18,22 + 0,54x - 1,31x^2 \text{ (Roma VF, untreated).}$$

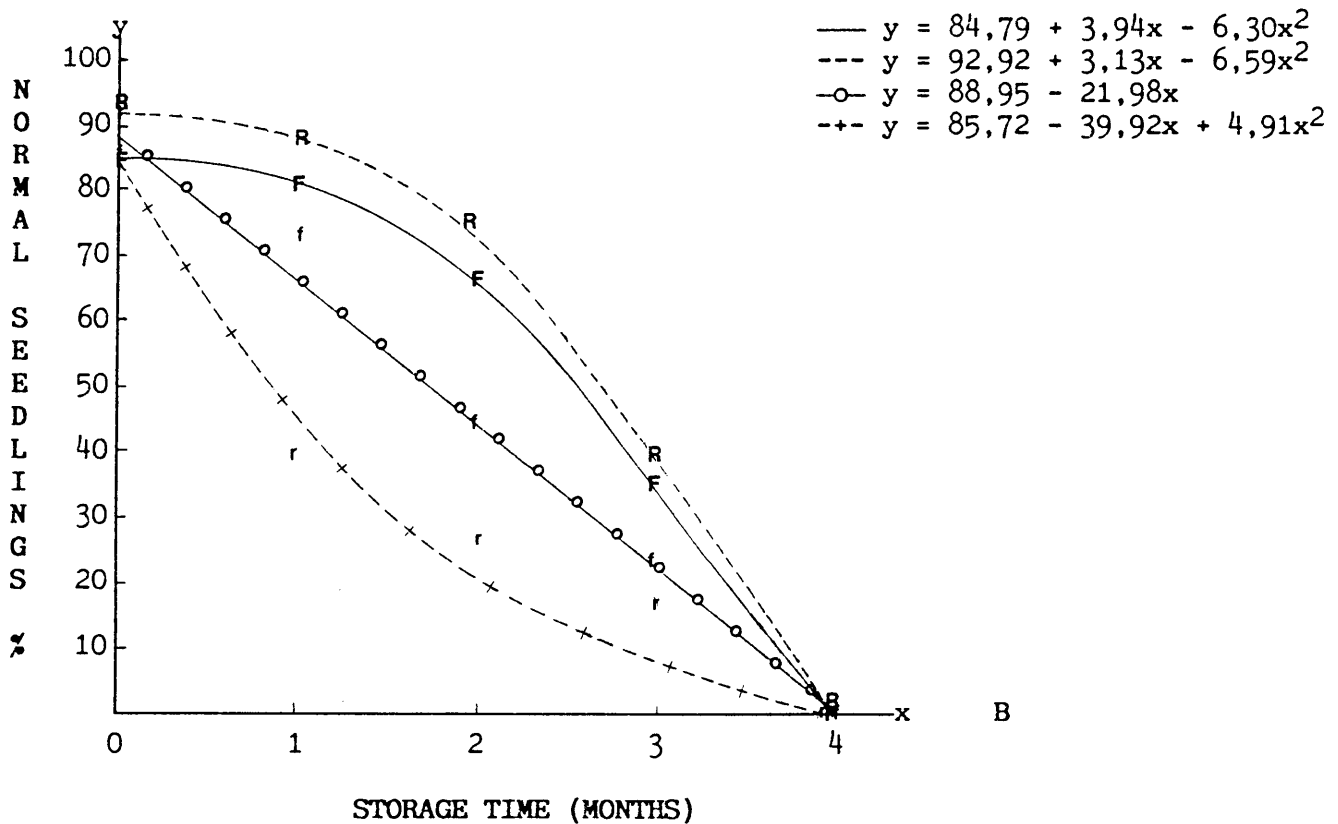
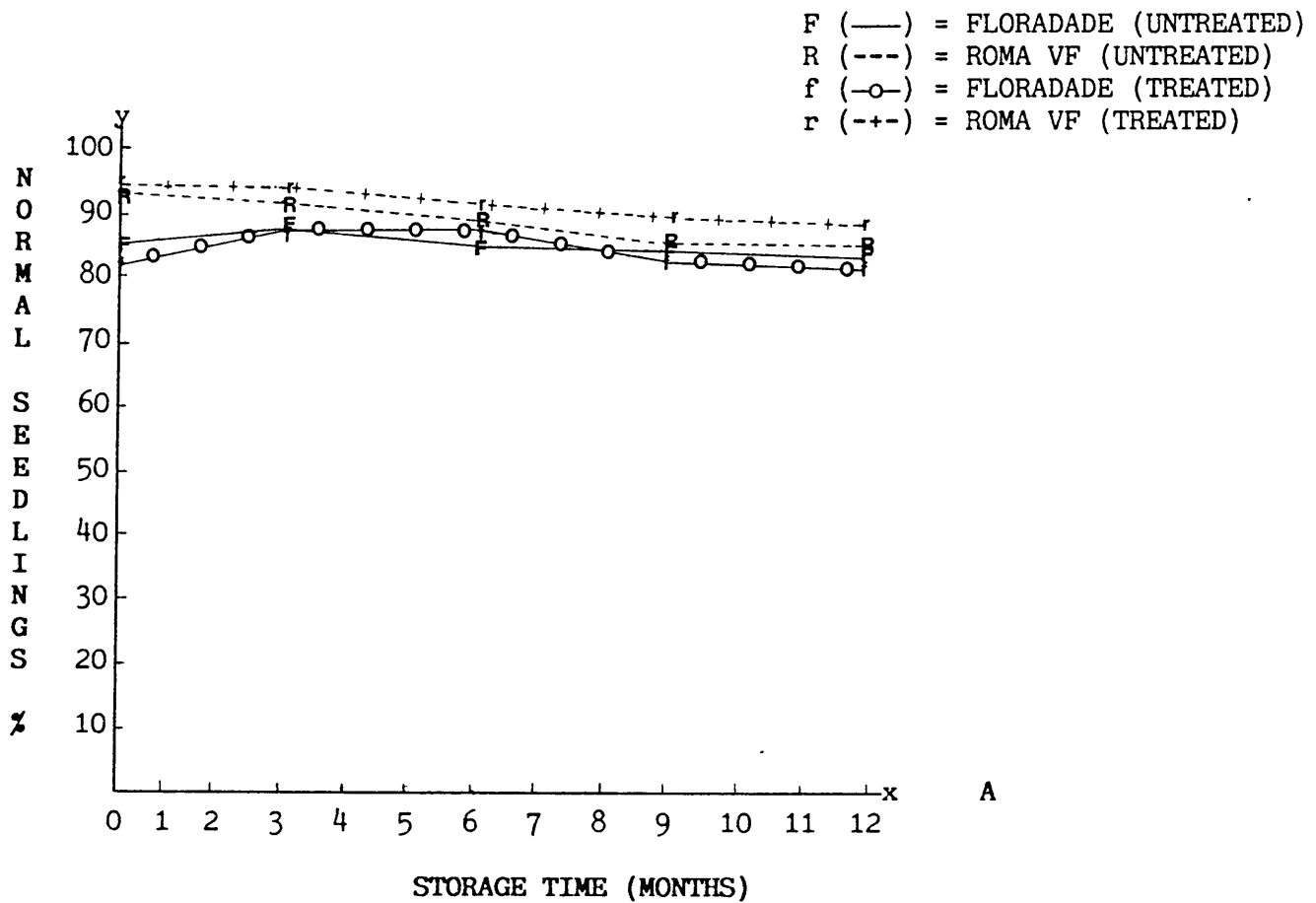


Figure 3.4: The percentage germination (normal seedlings) of hot-water treated and untreated seed samples of two tomato cultivars after seed storage with different seed moisture contents and an incubation period of 14 days.
 A: Storage for different periods at relatively low moisture contents in the seed ($\pm 7\%$).
 B: Storage for different periods at relatively high moisture contents in the seed ($\pm 30\%$).

Table 3.3: Germination of hot-water treated and untreated tomato seed after storage at relatively high moisture contents ($\pm 30\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)				Roma VF (%)			
	Normal seedlings		Abnormal seedlings		Normal seedlings		Abnormal seedlings	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
0	85	85	7	8	91	93	2	3
1	73	82	3	5	39	88	10	4
2	44	69	11	6	28	76	11	6
3	23	38	7	12	19	41	5	10
4	0	0	0	2	0	1	0	5
5	0	0	0	0	0	0	0	1
6	0	0	0	0	0	1	0	2
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0

Table 3.4: Viability of hot-water treated and untreated ungerminated tomato seeds after storage at relatively high moisture contents ($\pm 30\%$) in the seed and an incubation period of 14 days.

Storage time (months)	Floradade (%)				Roma VF (%)			
	Ungerm. viable		non-viable		Ungerm. viable		non-viable	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
0	0	0	8	8	0	0	7	4
1	4	0	21	13	1	0	51	9
2	6	1	39	24	2	4	60	16
3	2	1	69	50	0	0	76	51
4	0	0	100	98	0	0	100	95
5	0	0	100	100	0	0	100	100
6	8	4	92	96	0	1	100	97
9	0	0	100	100	0	0	100	100
12	0	0	100	100	0	0	100	100

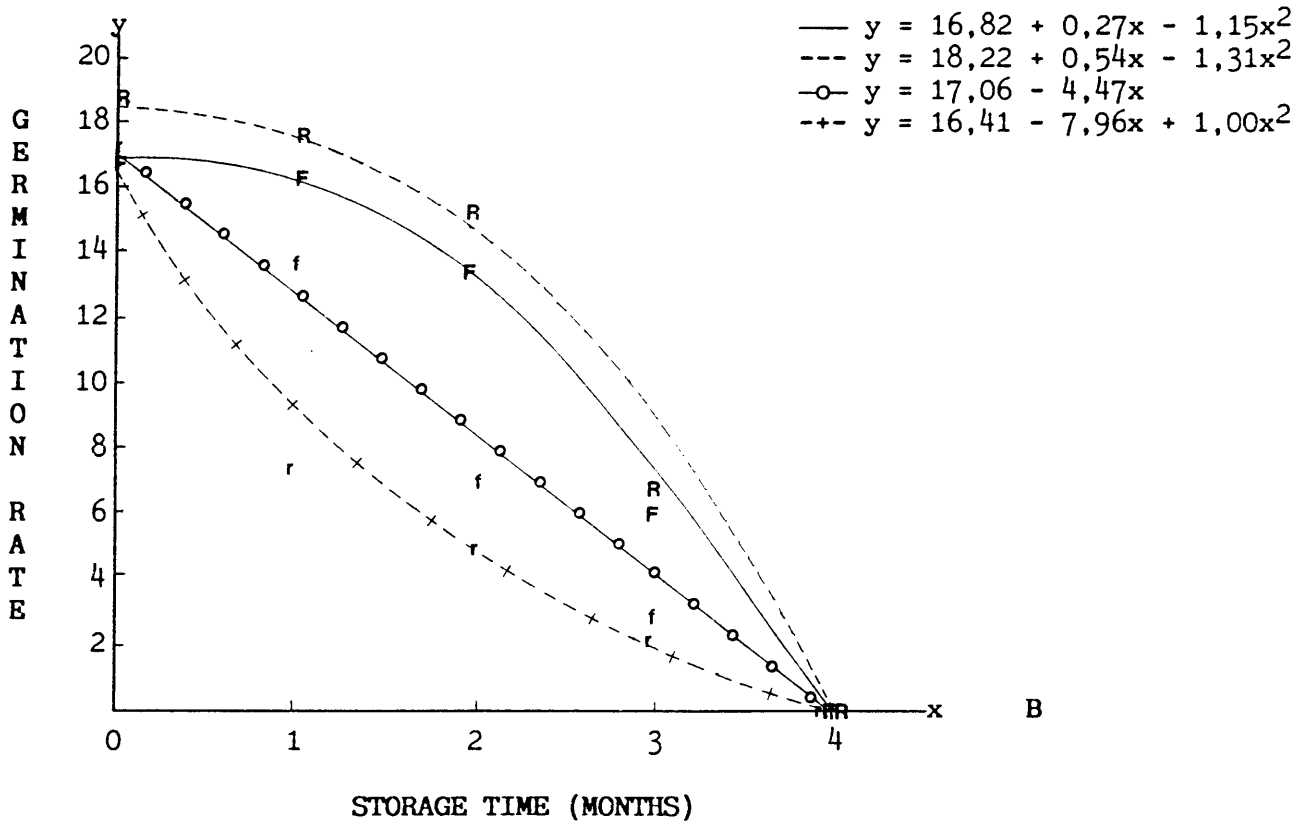
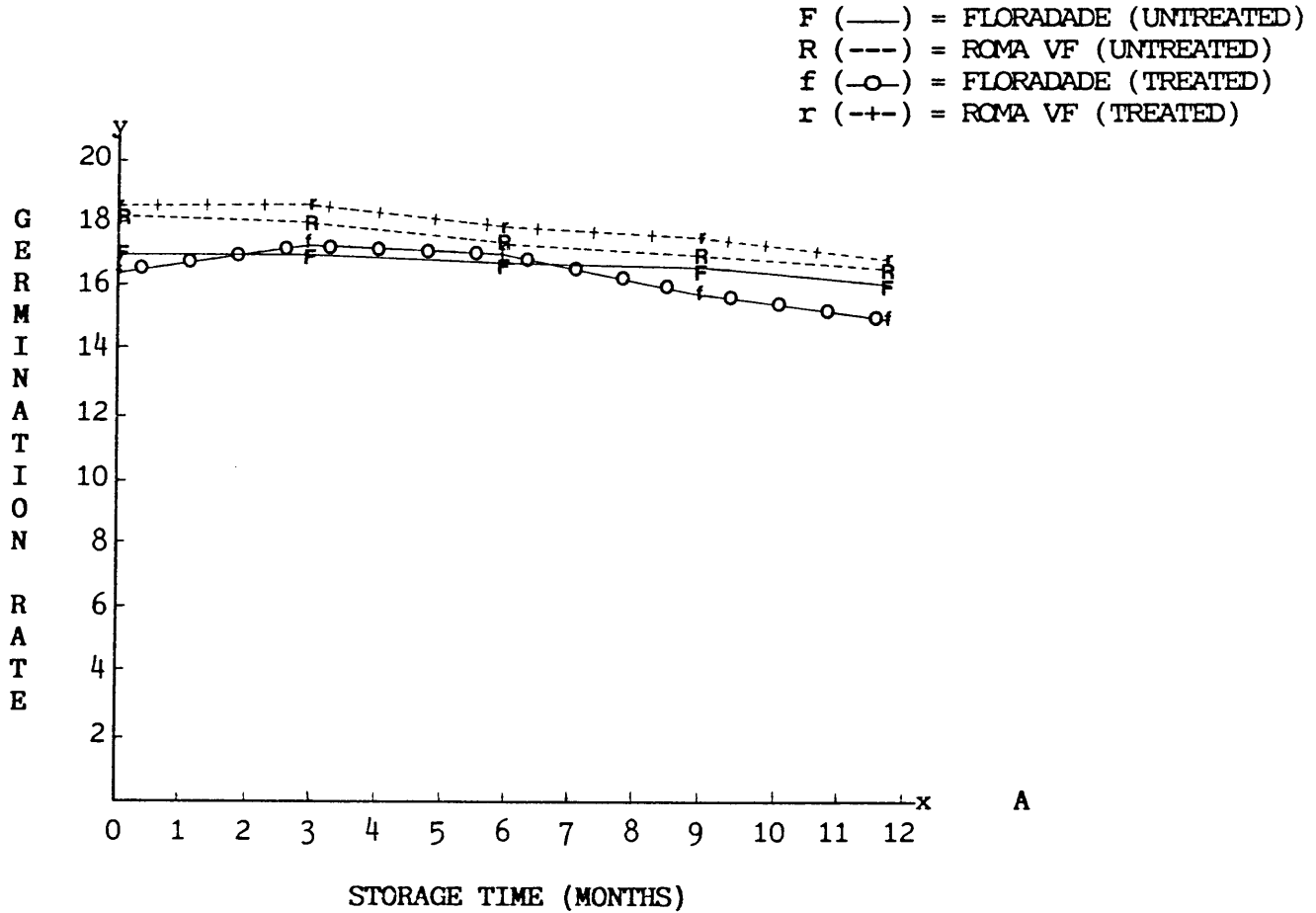


Figure 3.5: The germination rate of hot-water treated and untreated seed samples of two tomato cultivars after seed storage with different seed moisture contents and an incubation period of 14 days.
 A: Storage for different periods at relatively low moisture contents in the seed ($\pm 7\%$).
 B: Storage for different periods at relatively high moisture contents in the seed ($\pm 30\%$).

Germination capacity and rate were zero after four months storage for both cultivars. The germination capacity of hot-water treated seed samples decreased at a significantly greater rate than untreated samples for up to two months storage [Appendices 3.2 (pp 204 - 207); 3.4 (pp 221 - 224)]. The non-parametric U-test of Mann-Whitney revealed that the germination performance of Floradade was significantly higher than that of Roma VF for individual replicates stored for one month at relatively high seed moisture contents, while the polynomial regressions indicated that this difference could still be relevant after two and three months storage under similar conditions. The rapid decrease in germination performance was more pronounced in the Roma VF sample, which reflects its inability to maintain its initial higher germinability under unfavourable storage conditions, as was established in the previous chapter. In contrast to the conclusion reached regarding the influence of the hot-water treatment in interaction with seed storage at relatively low seed moisture contents, the hot-water treatment, and not ageing, was the cause of the initial greater decrease in germination performance of the Roma VF seed stored at relatively high seed moisture content. Rumsey (1962) conducted similar experiments on tomato seed of the "Pearson" cultivar, although these experiments involved temperature treatments without hydration of the seeds with hot water. According to his experiments, a sudden decrease in germination occurred after seed with a moisture content of 38,3% had been subjected to 55°C for 30 minutes. This indicates that temperature (with or without hydration of the seeds with hot water) in interaction with a relatively high seed moisture content could be responsible for the initial greater decrease in germination performance. Hydration/dehydration, therefore, seem to be unimportant factors in this respect.

The presence of ungerminated yet viable seed after storage at relatively high moisture contents in the seed (Table 3.4) could have been due to reduced germination energy owing to unfavourable storage conditions.

The relative lengths of primary roots and hypocotyls were determined as described in Chapter 2 (2.2.4.3). The ratio of hypocotyl and primary root lengths was calculated and the total seedling length was also determined.

Normally developed seedlings were measured after a seven day incubation period and discarded. The slower germinating seedlings were measured after a 14-day incubation period. All the distinctly developed normal seedlings were removed from the germination substratum and individually measured. The data were individually compared for each incubation period.

The results indicated that there was no significant effect of the hot-water treatment in interaction with seed storage on seedling organ lengths (Appendices 3.5 and 3.6; pp 227 - 228). Although Heydecker and Coolbear (1977) expect at least an increased ratio after soaking and drying treatments, this variable also showed no significant effect of the hot-water treatment (Appendices 3.5; pp 227 and 3.6; pp 228).

The results of this study show that the hot-water treatment can be applied with safety if the prescribed procedures are followed and if the seeds are redried under controlled conditions to a moisture content of approximately 7% before storage. However, there appears to be a highly significant interaction between the hot-water treatment and seed storage if the treated seed is equilibrated to a relatively high moisture content of approximately 30% prior to storage. A similar situation could occur under practical conditions with improper control over drying, or where drying is followed by exposure of seeds to a high relative humidity.

3.3.2 EFFECT OF HOT-WATER TREATMENT ON ULTRASTRUCTURE

During the hot-water treatment, tomato seeds may enter the initial phase of cell hydration and in addition, are exposed to a relatively high temperature. After this treatment the seeds are dried back to a realistic moisture content for sowing or storage purposes. The aim of this ultrastructural investigation was to find possible reasons why hot-water treated seed deteriorated faster than untreated seed during storage under unfavourable conditions.

Thin sections of the radicle tip were prepared for both optical microscope and transmission electron microscope studies as described in Chapter 2 (2.2.6). The two fixing methods used for this study, resulted in differences in the assessment of ultrastructural information. Potassium permanganate accentuated membrane systems through oxidation of most of the protein bodies and lipids. Organelles that were obscured after fixing of the material in glutaraldehyde and osmium tetroxide, then became readily visible. Since the emphasis in this study was on protein bodies and lipids, only the ultrastructure of sections fixed in glutaraldehyde/osmium tetroxide will be discussed. The different treatments that were investigated appear in Table 3.5.

Table 3.5: An outline of the ultrastructural investigation, with reference to electron micrographs illustrating the effects of different treatments on the ultrastructure of radicle tips of two tomato cultivars.

No storage	Storage [7% seed moisture content 20/30°C (16h/8h)]		Storage [30% seed moisture content 20/30°C (16h/8h)]	
	Time in months	Electron micrograph	Time in months	Electron micrograph
Control (no hot-water treatment) Figures 3.6 A (Floradade) and 3.7 A (Roma VF) 1*	3	Fig. 3.6C (Floradade) Fig. 3.7C (Roma VF) 3*	3	Fig. 3.6E (Floradade) Fig. 3.7E (Roma VF) 5*
	6	Fig. 3.6G (Floradade) Fig. 3.7G (Roma VF) 7*	6	Fig. 3.6I (Floradade) Fig. 3.7I (Roma VF) 9*
Hot-water treatment (54°C for 25 minutes) Figures 3.6 B (Floradade) and 3.7 B (Roma VF) 2*	3	Fig. 3.6D (Floradade) Fig. 3.7D (Roma VF) 4*	3	Fig. 3.6F (Floradade) Fig. 3.7F (Roma VF) 6*
	6	Fig. 3.6H (Floradade) Fig. 3.7H (Roma VF) 8*	6	Fig. 3.6J (Floradade) Fig. 3.7J (Roma VF) 10*

* Treatment numbers

The investigation of radicle tip cells from unstored seed treated with hot-water (treatment 2 in Table 3.5) revealed that they were in a more advanced phase of imbibition than the untreated controls (treatment 1 in Table 3.5) (Figs. 3.6 A, B; 3.7 A, B). It was particularly apparent in Roma VF tissue (Fig. 3.7 B). After drying, the dehydrated membranes in the cells apparently did not return completely to their original condition (before rehydration) and possibly resumed the process of imbibition with an advantage over the untreated controls.

Untreated seed samples subjected to a period of three months storage with relatively low seed moisture contents (treatment 3 in Table 3.5), showed the anticipated ultrastructural changes consequent to seed imbibition and did not differ significantly from the unstored controls (Figs. 3.6 C; 3.7 C). Floradade seed samples treated with hot water prior to three months storage at relatively low seed moisture contents (treatment 4 in Table 3.5), displayed large, prominent provacuoles (Fig. 3.6 D). Roma VF seed samples, after the same treatment, displayed the characteristic disintegration of the protein matrix in protein bodies of normally developing cells after seed imbibition and cell hydration (Fig. 3.7 D).

Untreated seed subjected to storage for three months at relatively high seed moisture contents (treatment 5 in Table 3.5), also revealed degradation of the protein matrix (Figs. 3.6 E; 3.7 E) in protein bodies, although less prominent than in the hot-water treated samples (treatment 6 in Table 3.5) (Figs. 3.6 F; 3.7 F). Confluent lipid areas occurred in Roma VF radicle cells from untreated seed samples stored for three months at relatively high seed moisture contents (Fig. 3.7 E), but were absent in Floradade tissue stored under similar conditions (Fig. 3.6 E). Anderson and Baker (1983) made similar observations of coalescence of spherosomes or lipid bodies, although they speculated that the trigger for such changes could be imbibition and that the occurrence of this phenomenon could be enhanced by seed treatments or seed deterioration.

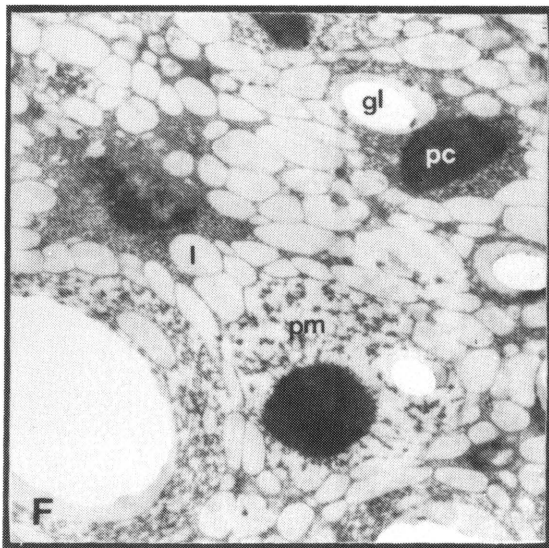
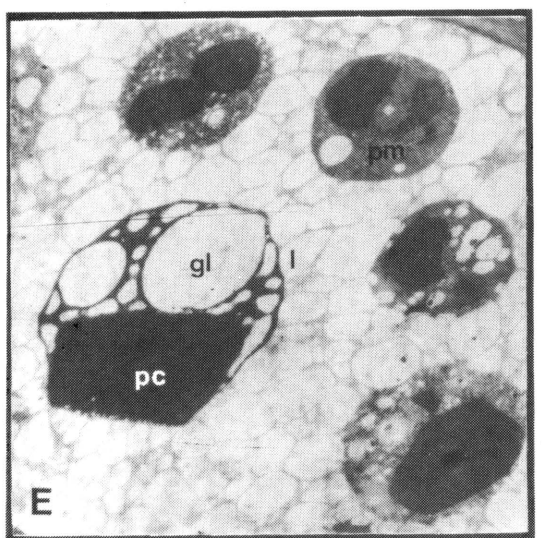
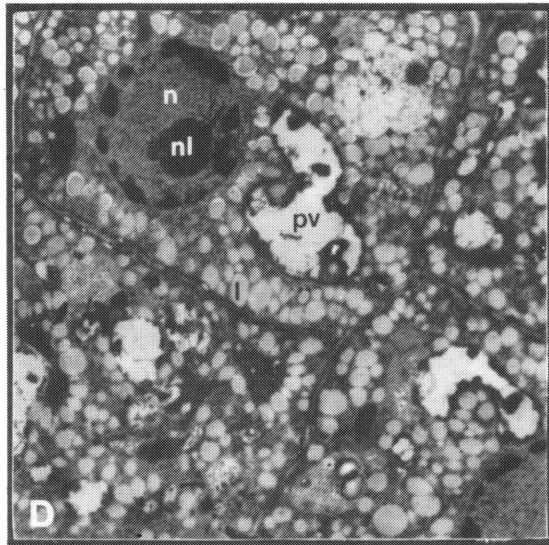
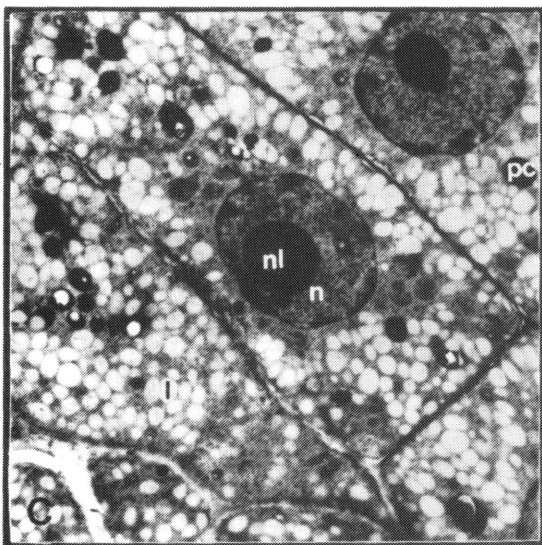
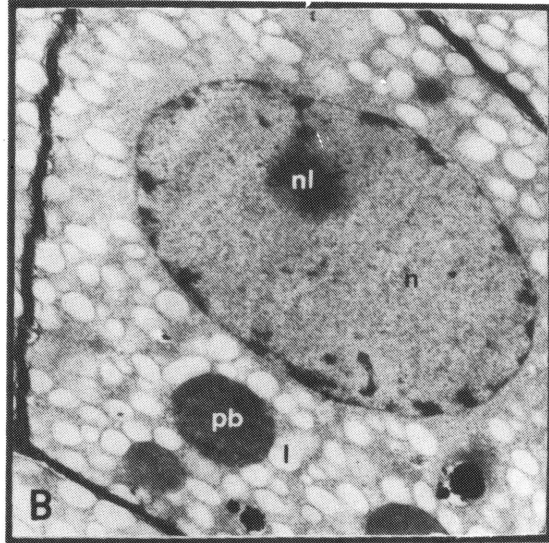
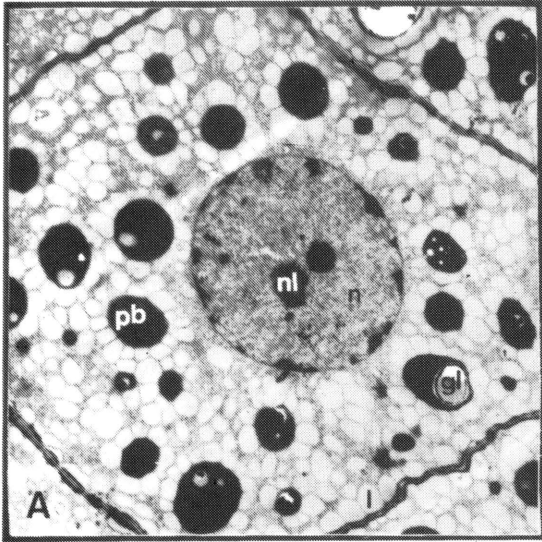
Sections of radicle tips from hot-water treated seed stored for three months at relatively high seed moisture contents displayed the enhanced disintegration of protein in Floradade samples (Fig. 3.6 F) and the occurrence of general cytoplasmic lysis in Roma VF (Fig. 3.7 F). An interesting observation was the apparent retainment of protein crystalloides, while the protein matrix disintegrated.

Untreated seed samples subjected to storage for six months at relatively low seed moisture contents (treatment 7 in Table 3.5), exhibited the ultrastructural characteristics of briefly imbibed embryonic tissue, as was observed in the untreated samples after three months storage (Figs. 3.6 G; 3.7 G). Seed samples treated with hot-water and stored for six months at relatively low moisture contents in the seed (treatment 8 in Table 3.5), displayed cells with an extraordinary deformation of protein bodies, especially in Floradade tissue (Figs. 3.6 H; 3.7 H). This was not observed in the untreated samples (Figs. 3.6 G; 3.7 G). This could be an indication of membrane damage inflicted by the hydration/dehydration treatment at relatively high temperatures and the incapability of the cells to recover during the subsequent storage period.

Sections of the ground meristem of radicle tips from untreated seed subjected to six months storage at relatively high seed moisture contents (treatment 9 in Table 3.5) displayed confluence of lipids and general lysis of the cytoplasm (Figs. 3.6 I; 3.7 I).

Sections of radicle tissue from hot-water treated seed samples stored for six months with relatively high moisture contents in the seed (treatment 10 in Table 3.5), revealed the indisputable signs of dead tissue. Deteriorated seeds are also reported to leach more of their cellular constituents than non-deteriorated seeds, presumably because of an increase in membrane permeability (Anderson and Baker, 1983). The grainy material found in the area between the plasmalemma and the cell wall, probably represented material leached from the protoplast (Figs. 3.6 J; 3.7 J). In contrast with the untreated samples, plasmolysis and the general disintegration of the cytoplasm with the exception of the protein crystalloids commonly occurred, while confluence of lipids and general lysis of the cytoplasm were the more obvious changes in the untreated, aged seed samples.

Anderson *et al.* (1970) attributed the coalescence of lipid bodies (Figs. 3.6 I, 3.7 E and 3.7 I) and the withdrawal of the protoplast from the cell wall, to the presence of storage fungi within the seed tissues. A possible explanation for the coalescence of lipids is that the lipid membrane (if present), was ruptured and the lipid contents of the organelle flowed together to form the amorphous mass. A second possibility was mentioned by Anderson *et al.* in 1970 that some fungal metabolite (lipase or surface-tension breaking compounds) liquifies the lipid and causes its coalescence. A combination of both explanations might have been involved.



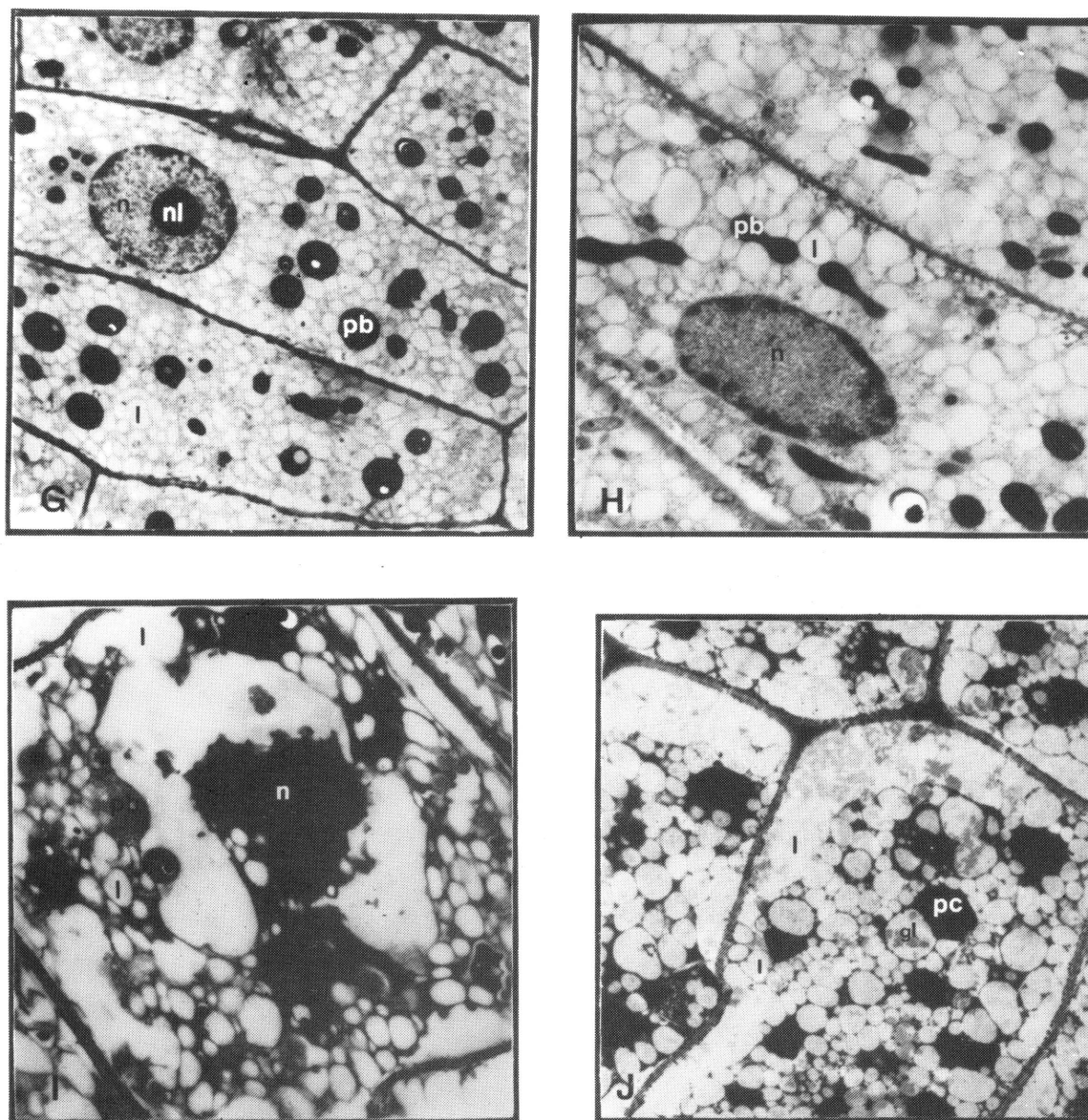
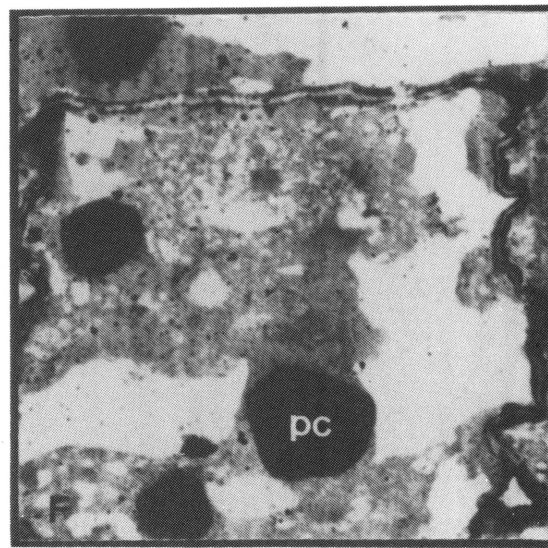
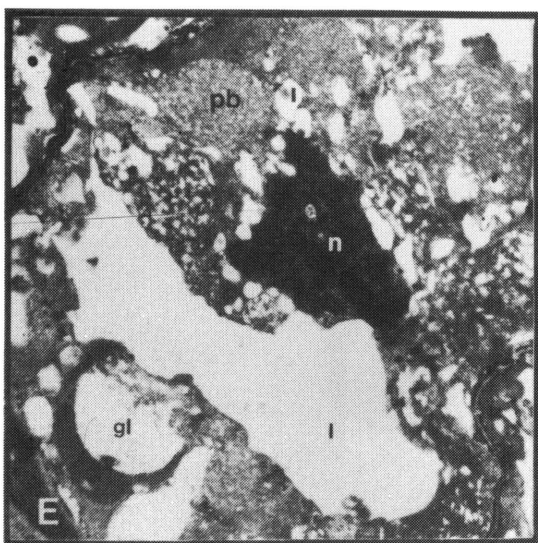
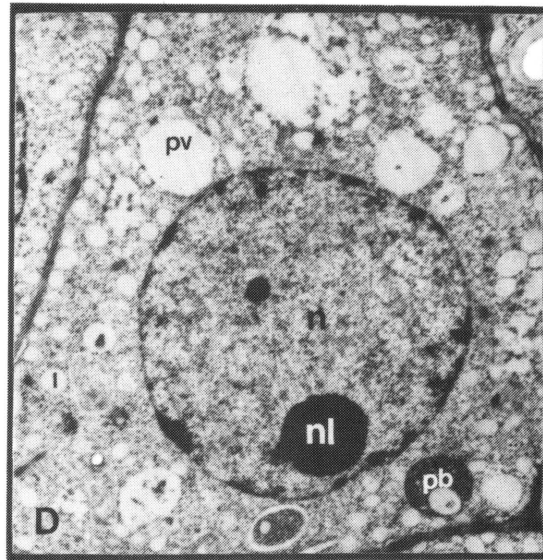
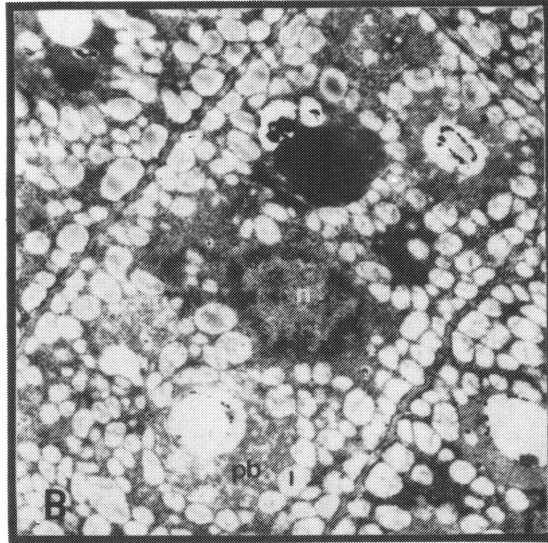
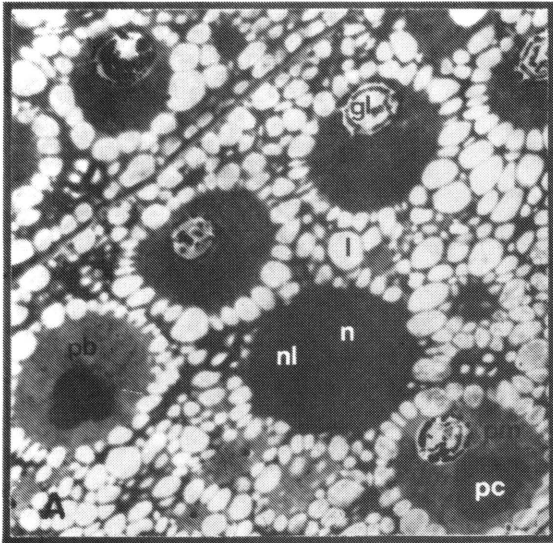


Figure 3.6: Electron micrographs of Floradade radicle cells in the area immediately surrounding the promeristem from hot-water treated and untreated seed subjected to storage with two different moisture contents in the seed. (glutaraldehyde/osmium tetroxide fixation).

A: Control (4300 x); B: Hot-water treatment and unstored (7500 x); C: Storage for three months at relatively low moisture contents ($\pm 7\%$) (3600 x); D: Hot-water treatment and storage for three months at relatively low moisture contents ($\pm 7\%$) (4300 x); E: Storage for three months at relatively high moisture contents ($\pm 30\%$) (5900 x); F: Hot-water treatment and storage for three months at relatively high moisture contents ($\pm 30\%$) (7500 x); G: Storage for six months at relatively low moisture contents ($\pm 7\%$) (3600 x); H: Hot-water treatment and storage for six months at relatively low moisture contents ($\pm 7\%$) (4300 x); I: Storage for six months at relatively high moisture contents ($\pm 30\%$) (5900 x); J: Hot-water treatment and storage for six months at relatively high moisture contents ($\pm 30\%$) (3600 x).

pb = protein body; l = lipid; pm = protein matrix; pc = protein crystalloid; pv = provacuole; gl = globoid; n = nucleus; nl = nucleolus.



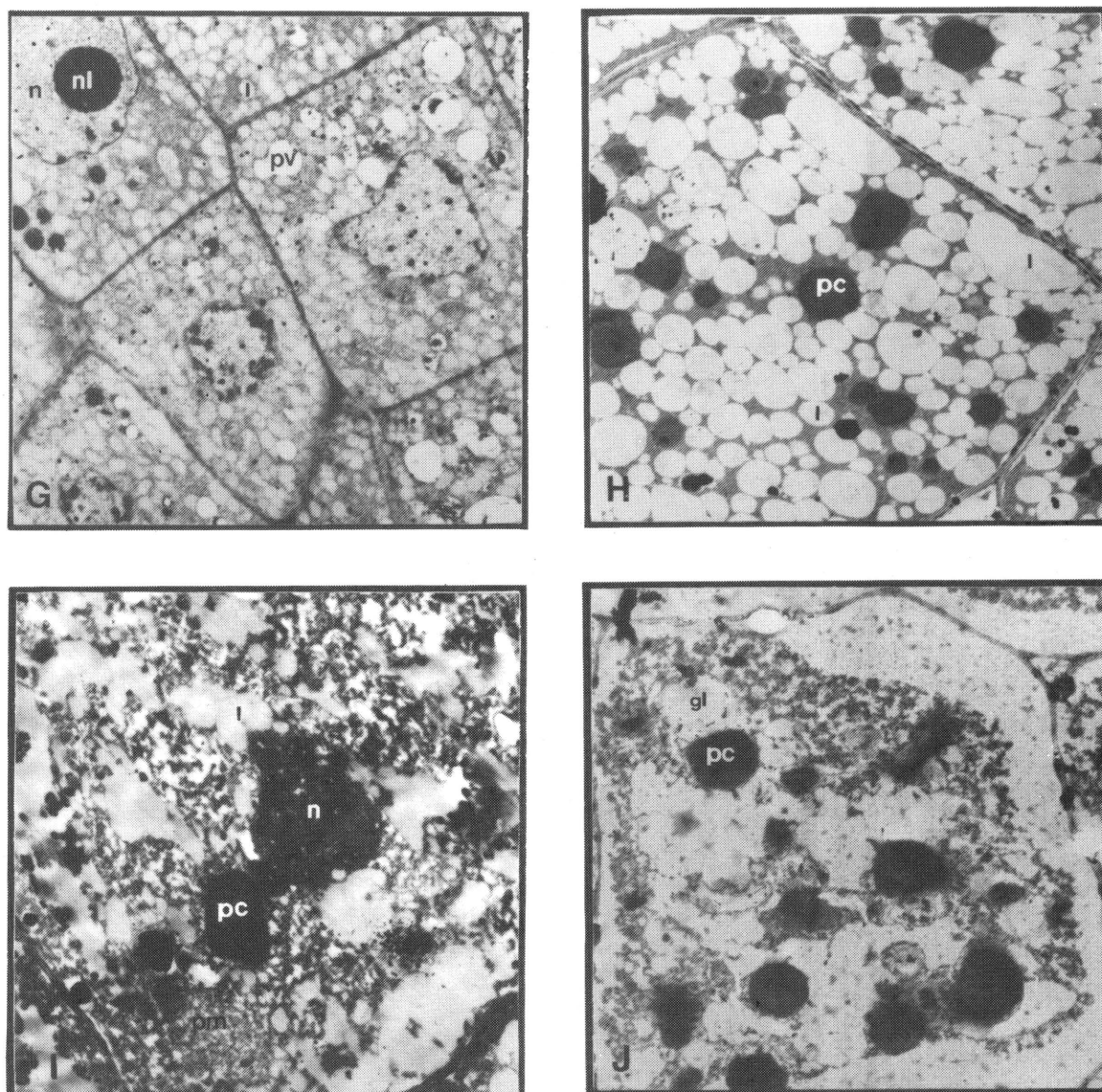


Figure 3.7: Electron micrographs of Roma VF radicle cells in the area immediately surrounding the promeristem from hot-water treated and untreated seed subjected to storage with two different moisture contents in the seed (glutaraldehyde/osmium tetroxide fixation).

A: Control (3600 x); B: Hot-water treatment and unstored (5900 x); C: Storage for three months at relatively low moisture contents ($\pm 7\%$) (5900 x); D: Hot-water treatment and storage for three months at relatively low moisture contents ($\pm 7\%$) (5900 x); E: Storage for three months at relatively high moisture contents ($\pm 30\%$) (5900 x); F: Hot-water treatment and storage for three months at relatively high moisture contents ($\pm 30\%$) (7500 x).; G: Storage for six months at relatively low moisture contents ($\pm 7\%$) (3600 x); H: Hot-water treatment and storage for six months at relatively low moisture contents ($\pm 7\%$) (4300 x); I: Storage for six months at relatively high moisture contents ($\pm 30\%$) (5900 x); J: Hot-water treatment and storage for six months at relatively high moisture contents ($\pm 30\%$) (3600 x).

pb = protein body; l = lipid; pm = protein matrix; pc = protein crystalloid; pv = provacuole; gl = globoid; n = nucleus; nl = nucleolus.

This explanation could be regarded as a possibility, since the assessments of micro-organisms conducted as described in Chapter 2 (2.2.7 and 2.3.4.3), indicated that Aspergillus, Penicillium, Fusarium spp. and a relatively high percentage of bacterial infection, occurred in all the stored seed samples. No fungi, however, were isolated from any of the samples that had been subjected to the hot-water treatment, although the percentage of bacterial infected seed increased significantly after treatment. As mentioned in the previous chapter, the favourable temperature and relative humidity for the growth and proliferation of storage fungi are 30 - 33°C and 65 - 90% respectively (Neergaard, 1977). The results of this investigation indicate that 54°C as applied in the hot-water treatment, was too adverse for any fungal growth and proliferation, even when followed by storage at ±30% moisture content in the seed. It should, however, be stressed that not all bacteria were eradicated by the hot-water treatment. The surviving population were saprophytes and representative of endospore-producing bacteria. The significantly higher percentage of infected seed after the hot-water treatment could have been the result of contamination during the hot-water soak.

The interaction of bacterial activity, membrane damage and repair, the degradation of seed reserves (proteins and lipids) and the production of heat shock proteins, could present possible causative factors for the significantly lower germination performance of treated seed samples during the first two months of storage under relatively unfavourable conditions, as well as for the significant difference in germination capacity and rate between the two cultivars.

Extremely favourable conditions for the proliferation of the surviving bacteria population was created due to the relatively high moisture content maintained during storage. The elimination by the hot-water treatment of all, or most of the other potential micro competitors, contributed further to these favourable conditions.

During the hot-water treatment, seeds are soaked in water and the cells may enter the initial hydration phase of imbibition. This may lead to the initiation of cell structure reorganisation. These processes are terminated by dehydration. The consequent equilibration of the seed to a relatively high moisture content for storage experiments, does not raise the seed moisture content high enough for the repair mechanisms to be activated as would have been the case after hydration in liquid water. The cells are therefore vulnerable and the membranes weakened.

This could have facilitated the transport of enzymes released by bacteria across membranes. The seed reserves could have been depleted by these enzymes for utilisation by the bacteria at the expense of the seed. This could present one explanation for the significantly reduced germination performance after the hot-water treatment in interaction with seed storage under relatively unfavourable conditions. This is, however, speculative and will require an in depth investigation of the interaction between seed-borne bacteria and seed deterioration.

As established in the seed storage experiments in Chapter 2, the Roma VF seed exhibits a significantly higher sensitivity to unfavourable storage conditions than Floradade, although the former displays a significantly superior germinability under favourable germination conditions. Bacteria assessments indicated that the percentage infected seed was significantly higher in all the Roma VF samples, which could have been responsible for the significantly lower germination performance under relatively unfavourable storage conditions. It could also be speculated that the Roma VF seed had a lower adaptability for the production of heat shock proteins under stress conditions. According to Lindquist and Craig (1988), all organisms respond to heat by inducing the synthesis of a group of these proteins. According to these authors, the response is the most highly conserved genetic system known. Several of the proteins induced by heat are induced by a variety of other stresses. The most compelling argument that shock proteins have a protective function is the strong correlation between their induction and the induction of thermotolerance (Nover and Scharf, 1984). The Floradade seed was possibly able to produce more of these heat shock proteins after the hot-water treatment for more effective protection during unfavourable storage conditions. This proposed mechanism is also a possibility for further investigations.

Plasmolysis was also more prominent in deteriorated Roma VF cells than in Floradade. This is the most definite sign of non viable tissue (Berjak and Villiers, 1972^d) and described by Villiers (1980) as the "point of no return" to cellular repair mechanisms.

It can therefore be concluded that, although seed storage at relatively high seed moisture contents was the most detrimental influence on seed longevity, it was significantly enhanced by the interaction with the hot-water treatment.

GENERAL CONCLUSIONS

Seed germination is probably the most important variable in an assessment of the effects of controlled deterioration, seed storage, and a hot-water treatment. The ISTA Rules (1985) prescribe general methods for routine testing, although these methods do not necessarily represent the optimal conditions for individual cultivars of the species. They should therefore be critically analysed for their applicability to local cultivars and for purposes other than routine seed testing. Limitations imposed by the germination method itself have to be excluded when the effects of controlled deterioration, seed storage, or the hot-water treatment is investigated.

During comparisons of different approved substrata for optimal germination (Chapter 1), different methods to raise the moisture content in tomato seed for controlled deterioration studies (Chapter 2), different ageing methods (Chapter 2), and seed storage in interaction with a hot-water treatment (Chapter 3), it was obvious that the two investigated cultivars differed with regard to their reaction to different treatments. Although Roma VF displayed a better germinability in the controls, the decrease in germination capacity was more rapid than in Floradade during storage under unfavourable conditions and after treatment with hot water. This phenomenon, however, did not occur after controlled deterioration, which suggests that the mechanism of deterioration in the latter treatment possibly differs from the other treatments in this study.

Germination energy, germination rate and seedling growth indicated the effects of treatments more clearly than germination capacity. These variables also indicated important differences in the deterioration pattern induced by different treatments. It was possible to explain some of the differences in deterioration pattern by ultrastructural investigations of the radicle tip. The following general conclusions are presented:

1. STANDARDISATION OF A GERMINATION PROCEDURE FOR SOUTH AFRICAN TOMATO CULTIVARS

The results presented in Chapter 1 resulted in the standardisation of a germination procedure for South African tomato cultivars. This led to the introduction of this particular procedure to the South African official Seed Testing Station. This procedure has also been recommended to the seed trade and is currently applied in many seed laboratories in the country. The most important reasons for choosing the roll paper method are the physical properties of this substratum, which are more optimal for imbibition and seedling development.

2. CONTROLLED DETERIORATION IS NOT A RELIABLE SIMULATION OF SEED STORAGE UNDER UNFAVOURABLE STORAGE CONDITIONS

The use of a rapid ageing technique such as controlled deterioration is useful for rapid assessment of the effects of seed treatments during ageing, although special care should be taken in the interpretation of results. The controlled deterioration test, as prescribed by Matthews and Powell (1981), is not effective for tomato seed as reliable moisture adjustment by means of imbibition cannot be achieved. The modified version of this method which was developed for the purposes of this study, indicated that the decrease in germination capacity under relatively unfavourable storage conditions can be simulated. Other variables of germination performance (germination energy, germination rate and seedling organ lengths) can, however, differ between the two ageing methods.

The fact that seed-borne micro-organisms are significantly reduced in seeds after controlled deterioration, in comparison with their occurrence in stored samples, as well as the unrealistically high temperature involved in the controlled deterioration test, could be important factors responsible for the different mechanisms of seed deterioration experienced during the different ageing methods. The adaptation of the controlled deterioration test used in this study can, therefore, not be recommended as a rapid ageing technique in tomato seed research.

3. THE HOT-WATER TREATMENT OF TOMATO SEEDS AGAINST BACTERIAL CANKER IS NOT DETRIMENTAL TO SEED VIABILITY WHEN APPLIED BEFORE SEED STORAGE UNDER FAVOURABLE CONDITIONS

The use of a hot-water treatment (54°C for 25 minutes) can be used with safety prior to storage at relatively low seed moisture contents ($\pm 7\%$) for up to 12 months, under average day/night temperature conditions during South African summers. This could imply that hot-water treated tomato seed may be stored for up to two years without significant decrease in viability. It is unlikely that viability will decrease significantly during the winter months, provided that a relatively low seed moisture content is maintained. The hot-water treatment, however, reduces seed viability when applied prior to seed storage under relatively unfavourable conditions [$\pm 30\%$ seed moisture content and 20/30°C (16h/8h)]. Tomato seed can be expected to lose viability within six months under such conditions.

Another important observation made during this study, is that a hot-water treatment prior to seed storage may replace the need for fungicides during storage, because of the effective eradication of seed-borne fungi. It should, however, be emphasized that, although bacterial canker should also be effectively eradicated by the treatment, a hot-water soak at 54°C for 25 minutes is not effective in the elimination of endospore forming bacteria. These bacteria remain viable and seed contamination may even be expected to increase as a result of treatment. These bacteria had no detrimental effect on seed viability during storage under relatively favourable conditions. Storage conditions that are unfavourable for seed, however, may be extremely favourable for the proliferation of micro-organisms at the expense of the seed reserves. The increased percentage of contaminated seed after the hot-water treatment could, therefore, have contributed significantly to the rapid deterioration of seed viability under unfavourable storage conditions.

SUMMARY

Studies on germination and artificial ageing of seeds of Lycopersicon lycopersicum.

Promotor: Prof. H.A. van de Venter

Co-promotor: Prof. P.J. Robbertse

Department: Botany

Degree: M.Sc. (Botany)

Commencement: January 1985

Seed of two cultivars namely Floradade (fresh produce cultivar) and Roma VF (canning cultivar) was used in this study. Experiments were undertaken to standardise the optimal water and substratum requirements for the germination of the seed. Two choices, representing three different substrata, are currently prescribed by the International Seed Testing Association (ISTA Rules, 1985) for the germination test on tomatoes. Best results were obtained with the roll paper method, described by ISTA as a "between paper" substratum. The water application for this germination substratum was standardised at 50 cm³ for four 383 x 254 mm sheets of "Anchor" germination paper containing 100 seeds.

In investigations on methods for adjusting the moisture content of tomato seed prior to controlled deterioration treatments, seed imbibition in liquid water was found to be unsuitable. The seed coat is hairy and water is rapidly adsorbed. The consequent increase in mass of the seed is not necessarily the result of water absorbed through the seed coat. Full hydration and subsequent dehydration to the desired water content is therefore necessary. Controlled deterioration tests comparing moisture adjustment by means of imbibition from liquid water with moisture adjustment by means of equilibration in a relatively humid atmosphere, yielded dissimilar results. The latter method was used in subsequent studies on artificial ageing, since it is a more reliable simulation of seed moisture adjustment during storage.

A modified version of the controlled deterioration test was applied as an accelerated ageing technique over a maximum short term period of eight days, while seed storage was applied over a longer period of 12 months. The purpose of this investigation was to compare the physiological and ultrastructural changes in the seed caused by the different ageing methods.

The conclusions drawn from these investigations support the school of thought that the physiological and ultrastructural consequences of artificial ageing differ from those resulting from natural ageing over time. Rapid ageing techniques are therefore not recommended for the assessment of effects of seed treatments during seed storage. Investigations on the effects of a hot-water treatment of tomato seed, in interaction with ageing treatments, led to the conclusion that a hot-water treatment comprising 54°C for 25 minutes does not affect the viability of tomato seed during a storage period of at least one year, provided that the seed moisture content is maintained at a relatively low level ($\pm 7\%$).

OPSOMMING

Studies met betrekking tot kieming en kunsmatige veroudering van saad van Lycopersicon lycopersicum.

Promotor: Prof. H.A. van de Venter
Mede-promotor: Prof. P.J. Robbertse
Departement: Plantkunde
Graad: M.Sc. (Plantkunde)
Aanvang: Januarie 1985.

Saad van twee kultivars naamlik Floradade (varsmarkkultivar) en Roma VF (inmaakkultivar) is gebruik vir hierdie studie. Eksperimente is uitgevoer om die optimale water- en substratumvereistes vir ontkieming te standaardiseer. Twee keuses, wat drie verskillende substratums verteenwoordig, word tans deur die Internasionale Saadtoetsassosiasie (ISTA Rules, 1985) vir die ontkiemingstoets op tamaties voorgeskryf. Die beste resultate is met die rolpapiermetode verkry. Hierdie metode word deur ISTA as 'n "between paper" substratum beskryf. Die watertoediening vir 100 sade in hierdie ontkiemingssubstratum, is op 50 cm³ gestandaardiseer vir vier 383 x 254 mm velle "Anchor" ontkiemingspapier.

Tydens die ondersoek van metodes om die voginhoud van tamatiesaad te verander, was saadimbibisie in vrye water ondoeltreffend. Die saadhuid is harig en water adsorbeer maklik. Die gevolglike toename in saadmassa is dus nie noodwendig die gevolg van wateropname deur die saadhuid nie. Volledige hidrasie, gevolg deur dehidrasie na die verlangde voginhoud, is dus nodig. Beheerde agteruitgangstoetse, waartydens vogveranderings wat deur middel van imbibisie verkry, vergelyk is met die vogveranderings na ekwilibrasie in 'n relatiewe vogtige atmosfeer, het verskillende resultate gelewer. Laasgenoemde metode is gebruik in verdere eksperimente met kunsmatige veroudering, aangesien dit 'n meer betroubare nabootsing van moontlike saadvogveranderings gedurende opberging verteenwoordig.

'n Gemodifiseerde weergawe van die beheerde agteruitgangstoets is gebruik as 'n versnelde verouderingstegniek oor 'n maksimum korttermyn periode van agt dae. Saadopberging is oor 'n langer tydperk van 12 maande ondersoek. Die doel van hierdie ondersoeke was om die fisiologiese en ultrastrukturele veranderings wat in die saad deur verskillende verouderingsmetodes veroorsaak word te vergelyk.

Die gevolgtrekkings wat uit hierdie ondersoek gemaak is, ondersteun sekere denkrigtings dat daar 'n verskil is tussen die fisiologiese en ultrastrukturele gevolge van kunsmatige veroudering en natuurlike veroudering oor tyd. Versnelde verouderingstegnieke word gevolglik nie aanbeveel vir die evaluering van die invloed van saadbehandelings tydens saadopberging nie. Ondersoeke met betrekking tot die invloed van 'n warmwaterbehandeling van tamatiesaad in wisselwerking met verouderingsbehandelings, het gelei tot die gevolgtrekking dat 'n warmwater behandeling van 54°C vir 25 minute nie die kiemkragtigheid van die saad tydens opberging vir ten minste een jaar beïnvloed nie. Dit is egter slegs geldig indien die voginhoud van die saad relatief laag gehou word ($\pm 7\%$).

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APPENDIX 1.1: GERMINATION AFTER 14 DAYS - COMPARISON OF SUBSTRATA AND CULTIVARS

COMPARISON OF SUBSTRATA - FLORADADE

Explanation of error codes: Error code 0 = normal analysis
 Error code 1 = expected value smaller than 1,0
 Error code 3 = degrees of freedom equal to 0,0
 Error code 4 = expected value zero

CODES	RP	TP	PFP
Normal seedlings	309,00	333,00	320,00
Ungerminated fresh seeds	12,00	8,00	8,00
Abnormal seedlings	43,00	33,00	49,00
Dead seeds	37,00	26,00	26,00

Chi square for main table test value = 12,596
 Chi square: 0,78908E+01 D.F.: 6 5 % level: NON SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,01666667

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
RP	TP	3	4,9324	1,00000000	0
RP	PFP	3	3,2994	1,00000000	0
TP	PFP	3	3,3696	1,00000000	0

COMPARISON OF SUBSTRATA - ROMA VF

CODES	RP	TP	PFP
Normal seedlings	287,00	278,00	295,00
Ungerminated fresh seeds	2,00	1,00	1,00
Abnormal seedlings	31,00	63,00	32,00
Dead seeds	80,00	60,00	73,00

Chi square for main table test value = 12,596
 Chi square: 0,19643E+02 D.F.: 6 5 % level: SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,01666667

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
RP	TP	3	14,2226	0,00262549	0
RP	PFP	3	0,7782	1,00000000	0
TP	PFP	3	11,8896	0,00780202	1

COMPARISON OF CULTIVARS - ROLL PAPER

Explanation of error codes: Error code 0 = normal analysis
 Error code 1 = expected value smaller than 1,0
 Error code 3 = degrees of freedom equal to 0,0
 Error code 4 = expected value zero

CODES	FLORADADE	ROMA VF
Normal seedlings	309,00	287,00
Ungerminated fresh seeds	12,00	2,00
Abnormal seedlings	43,00	31,00
Dead seeds	37,00	80,00

Chi square for main table test value = 7,817
 Chi square: 0,25703E+02 D.F.: 3 5 % level: SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	3	25,7031	0,00001102	0

COMPARISON OF CULTIVARS - TOP PAPER

CODES	FLORADADE	ROMA VF
Normal seedlings	320,00	295,00
Ungerminated fresh seeds	8,00	1,00
Abnormal seedlings	49,00	32,00
Dead seeds	26,00	73,00

Chi square for main table test value = 7,817
 Chi square: 0,32337E+02 D.F.: 3 5 % level: SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	3	32,3370	0,00000044	0

COMPARISON OF CULTIVARS - PLEATED FILTER PAPER

CODES	FLORADADE	ROMA VF
Normal seedlings	333,00	278,00
Ungerminated fresh seeds	8,00	1,00
Abnormal seedlings	33,00	63,00
Dead seeds	26,00	60,00

Chi square for main table test value = 7,817

Chi square: 0,33207E+02 D.F.: 3 5 % level: SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	3	33,2074	0,00000029	0

APPENDIX 1.2: GERMINATION AFTER FIVE DAYS - COMPARISON OF SUBSTRATA AND CULTIVARS

COMPARISON OF ROLL AND FILTER PAPER - FLORADADE

The non-parametric U-test of Mann-Whitney

First sample		Second sample	
Observation	Rank	Observation	Rank
9,0000	7,5	1,0000	1,5
3,0000	3,5	1,0000	1,5
8,0000	6,0	3,0000	3,5
5,0000	5,0	9,0000	7,5
<hr/>		<hr/>	
Rank totals	22,0		14,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF ROLL AND FILTER PAPER - ROMA VF

The non-parametric U-test of Mann-Whitney

First sample		Second sample	
Observation	Rank	Observation	Rank
10,0000	5,0	5,0000	1,0
17,0000	8,0	16,0000	7,0
14,0000	6,0	8,0000	2,0
9,0000	3,5	9,0000	3,5
<hr/>		<hr/>	
Rank totals	22,5		13,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 3,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - ROLL PAPER

The non-parametric U-test of Mann-Whitney

First sample		Second sample	
Observation	Rank	Observation	Rank
9,0000	4,5	10,0000	6,0
3,0000	1,0	17,0000	8,0
8,0000	3,0	14,0000	7,0
5,0000	2,0	9,0000	4,5
<hr/>		<hr/>	
Rank totals	10,5		25,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - PLEATED FILTER PAPER

The non-parametric U-test of Mann-Whitney

First sample		Second sample	
Observation	Rank	Observation	Rank
1,0000	1,5	5,0000	4,0
1,0000	1,5	16,0000	8,0
3,0000	3,0	8,0000	5,0
9,0000	6,5	9,0000	6,5
<hr/>		<hr/>	
Rank totals	12,5		23,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 1.3: STANDARDISATION OF WATER APPLICATION TO THE GERMINATION MEDIUM

REPLICATE	DRY MASS (g)	WET MASS (g)	WATERMASS (g)
1	25,20	66,37	41,17
2	24,77	65,61	40,84
3	24,93	64,44	39,51
4	25,12	65,23	40,11
5	25,06	70,03	44,97
6	25,07	70,51	45,44
7	24,65	70,34	45,69
8	24,73	71,46	46,73
9	25,75	70,94	45,19
10	24,91	70,73	45,82
11	24,80	66,93	42,13
12	24,83	66,68	41,85
13	25,22	69,72	44,50
14	24,87	73,24	48,37
15	24,95	73,88	48,93
16	24,70	74,87	50,17
17	24,68	70,80	46,12
18	25,18	76,08	50,90
19	25,02	74,81	49,79
20	25,14	75,55	50,41
21	25,54	74,30	48,76
22	25,39	71,35	45,96
23	25,19	73,87	48,68
24	25,10	71,60	46,50
25	25,42	79,73	54,31
26	25,75	77,36	51,61
27	25,79	78,20	52,41
28	25,36	74,45	49,09
29	25,00	76,06	51,06
30	24,90	70,25	45,35
31	24,62	79,22	54,60
32	24,87	78,24	53,37

VARIABLE NAME	MEAN	STANDARD DEVIATION	ST. ERR OF MEAN	COEFF. OF VARIATION	S M A L L E S T VALUE	Z-SCORE	L A R G E S T VALUE	Z-SCORE	RANGE
Dry mass	25,078	0,323	0,0570	0,01286	24,620	-1,42	25,790	2,21	1,170
Wet mass	72,277	4,208	0,7439	0,05822	64,440	-1,86	79,730	1,77	15,290
Water mass	47,198	4,127	0,7295	0,08743	39,510	-1,86	54,600	1,79	15,090

APPENDIX 2.1: STATISTICAL ANALYSIS OF THE EFFECT OF IMBIBING SEED TO DIFFERENT MOISTURE LEVELS PRIOR TO CONTROLLED DETERIORATION * GERMINATION ENERGY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	130,14957	11,579	11,24**
1	7,34923	1,099	-6,69**
2	0,12879	0,020	6,34**

* significant at p = 0,05

** significant at p = 0,01

RESIDUAL MEAN SQUARE = 168,34583 (D.F. = 11)

MULTIPLE R-SQUARE = 0,80534

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	8414,10872	5	1682,82174	12,25	0,0014*
1	7516,59672	4	1879,14918	13,68	0,0012*
2	752,69858	3	250,89953	1,83	0,2204
3	694,49578	2	347,24789	2,53	0,1410
4	410,94269	1	410,94269	2,99	0,1220
Residual	1099,10557	8	137,38820		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 3 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	135,35795	17,877	7,57**
1	8,43132	2,817	-2,99*
2	0,29685	0,120	2,48*
3	0,00295	0,002	-2,02

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 128,71908 (D.F. = 10)

MULTIPLE R-SQUARE = 0,59322

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	2569,40246	5	513,88049	6,48	0,0107*
1	2569,30883	4	642,32721	8,10	0,0065*
2	1176,65547	3	392,21849	4,95	0,0314*
3	652,87896	2	326,43948	4,12	0,0590
4	236,83205	1	236,83205	2,99	0,1220
Residual	634,31182	8	79,28898		

* significant

APPENDIX 2.2: STATISTICAL ANALYSIS OF THE EFFECT OF IMBIBING SEED TO DIFFERENT MOISTURE LEVELS PRIOR TO CONTROLLED DETERIORATION * GERMINATION CAPACITY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	118,71163	9,166	12,95**
1	-5,38153	0,870	-6,19**
2	0,09515	0,016	5,92**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 105,48504 (D.F. = 11)

MULTIPLE R-SQUARE = 0,77829

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	4723,89495	5	944,77899	14,83	0,0007*
1	4342,95277	4	1085,73819	17,04	0,0006*
2	650,73037	3	216,91012	3,41	0,0736
3	534,47093	2	267,23546	4,20	0,0568
4	231,12194	1	231,12194	3,63	0,0933
Residual	509,60505	8	63,70063		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 3 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	113,93117	7,729	14,74**
1	-3,72126	1,218	-3,06*
2	0,13329	0,052	2,58*
3	-0,00138	0,001	-2,17

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 24,06123 (D.F. = 10)

MULTIPLE R-SQUARE = 0,57516

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	407,32690	5	81,56538	4,12	0,0379*
1	397,48690	4	99,37173	5,01	0,0255*
2	195,70941	3	65,23647	3,29	0,0790
3	82,08260	2	41,04130	2,07	0,1884
4	34,34070	1	34,34070	1,73	0,2245
Residual	158,53024	8	19,81628		

* significant

APPENDIX 2.3: STATISTICAL ANALYSIS OF THE EFFECT OF IMBIBING SEED TO DIFFERENT MOISTURE LEVELS PRIOR TO CONTROLLED DETERIORATION * MORTALITY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	17,79839	9,398	-1,89
1	4,82281	0,892	5,41**
2	0,08512	0,016	-5,16**

* significant at p = 0,05

** significant at p = 0,01

RESIDUAL MEAN SQUARE = 110,89333 (D.F. = 11)

MULTIPLE R-SQUARE = 0,72869

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	3990,79818	5	798,15964	12,64	0,0012*
1	3669,32292	4	917,33073	14,53	0,0010*
2	714,62484	3	238,20828	3,77	0,0591
3	517,10991	2	258,55495	4,09	0,0596
4	265,52966	1	265,52966	4,20	0,0744
Residual	505,20182	8	63,15023		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	1,18833	3,786	0,31
1	0,85733	0,359	2,39*
2	-0,01519	0,007	-2,29*

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 17,99995 (D.F. = 11)

MULTIPLE R-SQUARE = 0,34266

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	200,90938	5	40,18188	3,20	0,0699*
1	191,78666	4	47,94667	3,82	0,0504*
2	97,69457	3	32,56486	2,60	0,1247
3	40,41372	2	20,20686	1,61	0,2582
4	36,74449	1	36,74449	2,93	0,1253
Residual	100,30491	8	12,53811		

* significant

APPENDIX 2.4: STATISTICAL ANALYSIS OF THE EFFECT OF EQUILIBRATING SEED TO DIFFERENT MOISTURE LEVELS AT DIFFERENT RELATIVE HUMIDITIES PRIOR TO CONTROLLED DETERIORATION * GERMINATION ENERGY

GOODNESS OF FIT TEST - FLORADADE

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	15,83526	5	3,16705	0,82	0,5700
1	14,19753	4	3,54938	0,92	0,4997
2	11,05628	3	3,68543	0,95	0,4610
3	10,39220	2	5,19610	1,34	0,3148
4	7,56223	1	7,56223	1,95	0,2001
Residual	31,02189	8	3,87774		

* significant

GOODNESS OF FIT TEST - ROMA VF

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	10,45361	5	2,09072	0,18	0,9643
1	0,60986	4	0,15247	0,01	0,9996
2	0,58097	3	0,19366	0,02	0,9969
3	0,18091	2	0,09045	0,01	0,9924
4	0,00274	1	0,00274	0,00	0,9883
Residual	95,26068	8	11,90758		

* significant

APPENDIX 2.5: STATISTICAL ANALYSIS OF THE EFFECT OF EQUILIBRATING SEED TO DIFFERENT MOISTURE LEVELS AT DIFFERENT RELATIVE HUMIDITIES PRIOR TO CONTROLLED DETERIORATION * GERMINATION CAPACITY

GOODNESS OF FIT TEST - FLORADADE

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	8,07455	5	1,61491	0,81	0,5731
1	7,88045	4	1,97011	0,99	0,4655
2	5,30095	3	1,76698	0,89	0,4877
3	5,29981	2	2,64991	1,33	0,3169
4	1,25586	1	1,25586	0,63	0,4500
Residual	15,92545	8	1,99068		

* significant

GOODNESS OF FIT TEST - ROMA VF

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	2,12660	5	0,42532	0,08	0,9941
1	1,61231	4	0,40308	0,07	0,9885
2	0,52197	3	0,17399	0,03	0,9919
3	0,17331	2	0,08666	0,02	0,9845
4	0,04525	1	0,04525	0,01	0,9301
Residual	44,23055	8	5,52882		

* significant

APPENDIX 2.6: STATISTICAL ANALYSIS OF THE EFFECT OF EQUILIBRATING SEED TO DIFFERENT MOISTURE LEVELS AT DIFFERENT RELATIVE HUMIDITIES PRIOR TO CONTROLLED DETERIORATION * MORTALITY

GOODNESS OF FIT TEST - FLORADADE

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	4,11286	5	0,82257	0,15	0,9754
1	4,06617	4	1,01654	0,18	0,9416
2	4,06267	3	1,35422	0,24	0,8649
3	3,88038	2	1,94019	0,35	0,7174
4	0,00038	1	0,00038	0,00	0,9936
Residual	44,81571	8	5,60196		

* significant

GOODNESS OF FIT TEST - ROMA VF

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	9,95948	5	1,99190	0,29	0,9039
1	9,73091	4	2,43273	0,36	0,8320
2	8,57817	3	2,85939	0,42	0,7434
3	8,30641	2	4,15321	0,61	0,5664
4	3,18080	1	3,18080	0,47	0,5133
Residual	54,39766	8	6,79971		

* significant

APPENDIX 2.7: STATISTICAL ANALYSIS OF THE EFFECT OF CONTROLLED DETERIORATION ON GERMINATION CAPACITY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	83,33566	5,112	16,30**
1	2,33077	2,379	0,98
2	-0,96853	0,229	-4,23**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 45,029023 (D.F. = 8)

MULTIPLE R-SQUARE = 0,94937

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	6754,67692	5	3377,33846	75,00	0,0000
1	804,84965	4	804,84965	17,87	0,0029*
Residual	360,23217	8	45,02902		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	87,55944	2,911	30,08**
1	2,65431	1,354	1,96
2	-0,91725	0,130	-7,03**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 14,596273 (D.F. = 8)

MULTIPLE R-SQUARE = 0,97882

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	5395,41166	5	2697,70583	184,82	0,0000
1	721,87529	4	721,87529	49,46	0,0001*
Residual	116,77016	8	14,59627		

* significant

COMPARISON OF CULTIVARS - SEED SAMPLES NOT SUBJECTED TO CONTROLLED DETERIORATION

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
90,0000	4,0	96,0000	8,0
81,0000	1,0	91,0000	5,0
87,0000	3,0	94,0000	7,0
83,0000	2,0	92,0000	6,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR ONE DAY

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
84,0000	4,0	83,0000	3,0
82,0000	2,0	88,0000	6,5
75,0000	1,0	91,0000	8,0
88,0000	6,5	85,0000	5,0
<hr/>		<hr/>	
Rank totals	13,5		22,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 3,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TWO DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
86,0000	5,5	81,0000	4,0
78,0000	1,0	87,0000	7,0
86,0000	5,5	91,0000	8,0
80,0000	2,5	80,0000	2,5
Rank totals		13,5	22,5

Mann-Whitney U-statistic = 3,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR THREE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
82,0000	4,5	80,0000	1,0
81,0000	2,5	81,0000	2,5
84,0000	6,5	87,0000	8,0
84,0000	6,5	82,0000	4,5
Rank totals		18,0	8,0

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FOUR DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
80,0000	2,0	88,0000	8,0
77,0000	1,0	84,0000	6,0
82,0000	3,5	82,0000	3,5
85,0000	7,0	83,0000	5,0
Rank totals		13,5	22,5

Mann-Whitney U-statistic = 3,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FIVE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
62,0000	2,0	85,0000	8,0
73,0000	4,5	73,0000	4,5
63,0000	3,0	84,0000	7,0
41,0000	1,0	82,0000	6,0
Rank totals		10,5	25,5

Mann-Whitney U-statistic = 0,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SIX DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
72,0000	2,5	75,0000	7,0
71,0000	1,0	74,0000	6,0
72,0000	2,5	79,0000	8,0
72,0000	2,5	73,0000	4,5
Rank totals		10,5	25,5

Mann-Whitney U-statistic = 0,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SEVEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
62,0000	4,0	67,0000	6,0
56,0000	3,0	69,0000	7,0
43,0000	2,0	74,0000	8,0
21,0000	1,0	63,0000	5,0
Rank totals		10,0	26,0

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR EIGHT DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
48,0000	4,0	65,0000	7,5
49,0000	5,0	60,0000	6,0
0,0000	1,5	65,0000	7,5
0,0000	1,5	30,0000	3,0
<hr/>		<hr/>	
Rank totals	12,0		24,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR NINE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
34,0000	4,0	47,0000	5,0
8,0000	2,0	61,0000	7,0
0,0000	1,0	59,0000	6,0
9,0000	3,0	70,0000	8,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
0,0000	2,5	25,0000	6,0
0,0000	2,5	40,0000	8,0
0,0000	2,5	32,0000	7,0
0,0000	2,5	14,0000	5,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.8: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE FOR DIFFERENT PERIODS AT RELATIVELY LOW SEED MOISTURE CONTENTS ON GERMINATION CAPACITY

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES

Explanation of error codes: Error code 0 = normal analysis
 Error code 1 = expected value smaller than 1,0
 Error code 3 = degrees of freedom equal to 0,0
 Error code 4 = expected value zero

CODES	FLORADADE	ROMA VF
Normal seedlings	339,00	373,00
Ungerminated seeds	29,00	16,00
Abnormal seedlings	32,00	10,00

Chi square for main table test value = 5,995
 Chi square: 0,16902E+02 D.F.: 2 5 % level: SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	16,9017	0,00021371	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	348,00	365,00
Ungerminated seeds	27,00	21,00
Abnormal seedlings	25,00	14,00

Chi square for main table test value = 5,995
 Chi square: 0,42579E+01 D.F.: 2 5 % level: NON SIG Error code: 0
 Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	4,2579	1,00000000	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR SIX MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	334,00	351,00
Ungerminated seeds	48,00	38,00
Abnormal seedlings	18,00	11,00

Chi square for main table test value = 5,995

Chi square: 0,32743E+01 D.F.: 2 5 % level: NON SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	3,2743	1,00000000	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR NINE MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	335,00	339,00
Ungerminated seeds	44,00	49,00
Abnormal seedlings	21,00	12,00

Chi square for main table test value = 5,995

Chi square: 0,27471E+01 D.F.: 2 5 % level: NON SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	2,7471	1,00000000	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR 12 MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	330,00	337,00
Ungerminated seeds	54,00	43,00
Abnormal seedlings	16,00	20,00

Chi square for main table test value = 5,995

Chi square: 0,17653E+01 D.F.: 2 5 % level: NON SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	1,7653	1,00000000	0

APPENDIX 2.9: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE FOR DIFFERENT PERIODS AT RELATIVELY HIGH SEED MOISTURE CONTENTS ON GERMINATION CAPACITY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	84,79286	1,565	54,19**
1	3,93929	1,854	2,13
2	-6,30357	0,444	-14,19**

* significant at p = 0,05

** significant at p = 0,01

MULTIPLE R-SQUARE = 0,9978

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-Ratio	TAIL PROBABILITY
2	5082,55000	2	2541,27000	919,32	0,0011*
Error	5,52857	2	2,76429		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	92,92143	2,973	31,26**
1	3,13214	3,522	0,89
2	-6,58929	0,844	-7,81**

* significant at $p = 0,05$

** significant at $p = 0,01$

MULTIPLE R-SQUARE = 0,9934

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-Ratio	TAIL PROBABILITY
2	6001,87000	2	3000,93000	300,74	0,0033*
Error	19,95710	2	9,97857		

* significant

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES

Explanation of error codes: Error code 0 = normal analysis
Error code 1 = expected value smaller than 1,0
Error code 3 = degrees of freedom equal to 0,0
Error code 4 = expected value zero

CODES	FLORADADE	ROMA VF
Normal seedlings	339,00	373,00
Ungerminated seeds	31,00	16,00
Abnormal seedlings	32,00	10,00

Chi square for main table test value = 5,995
Chi square: 0,17924E+02 D.F.: 2 5 % level: SIG Error code: 0
Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	17,9237	0,00012821	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR ONE MONTH

CODES	FLORADADE	ROMA VF
Normal seedlings	328,00	351,00
Ungerminated seeds	53,00	34,00
Abnormal seedlings	19,00	15,00

Chi square for main table test value = 5,995
Chi square: 0,53991E+01 D.F.: 2 5 % level: NON SIG Error code: 0
Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	2	5,3991	1,00000000	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR TWO MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	276,00	304,00
Ungerminated viable seeds	4,00	15,00
Abnormal seedlings	25,00	24,00
Dead seeds	95,00	65,00

Chi square for main table test value = 7,817

Chi square: 0,13288E+02 D.F.: 3 5 % level: SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	3	13,2876	0,00406800	0

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

CODES	FLORADADE	ROMA VF
Normal seedlings	153,00	162,00
Ungerminated viable seeds	2,00	1,00
Abnormal seedlings	46,00	38,00
Dead seeds	199,00	202,00

Chi square for main table test value = 7,817

Chi square: 0,13636E+01 D.F.: 3 5 % level: NON SIG Error code: 0

Chi square for sub tables. Comparison of columns. Test value = 0,05000000

CODES with regard to	CODES	D.F.	Chi square	5 % level	Error code
Floradade	Roma VF	3	1,3636	1,00000000	0

APPENDIX 2.10: STATISTICAL ANALYSIS OF THE EFFECT OF CONTROLLED DETERIORATION ON GERMINATION ENERGY

RESULTS FOR POLYNOMIAL OF DEGREE 1 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	85,00000	6,047	14,06**
1	-9,40000	1,022	-9,20**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 114,93333 (D.F. = 9)

MULTIPLE R-SQUARE = 0,90381

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	9719,60000	1	9719,60000	84,57	0,0000*
Residual	1034,40000	9	114,93333		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 1 - ROMA V F

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	87,50000	6,176	14,17**
1	-10,00909	1,044	-9,59**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 119,85758 (D.F. = 9)

MULTIPLE R-SQUARE = 0,91084

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	11020,00909	1	11020,00909	91,94	0,0000*
Residual	1078,71818	9	119,85758		

* significant

COMPARISON OF CULTIVARS - SEED SAMPLES NOT SUBJECTED TO CONTROLLED DETERIORATION

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
86,0000	6,0	77,0000	3,0
74,0000	1,0	76,0000	2,0
83,0000	5,0	90,0000	7,5
79,0000	4,0	90,0000	7,5
<hr/>		<hr/>	
Rank totals	16,0		20,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR ONE DAY

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
80,0000	4,0	79,0000	2,0
80,0000	4,0	80,0000	4,0
72,0000	1,0	81,0000	6,0
87,0000	8,0	83,0000	7,0
<hr/>		<hr/>	
Rank totals	17,0		19,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TWO DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
85,0000	8,0	76,0000	4,0
53,0000	1,0	70,0000	2,0
78,0000	5,0	82,0000	7,0
79,0000	6,0	75,0000	3,0
<hr/>		<hr/>	
Rank totals	20,0		16,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR THREE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
75,0000	7,0	59,0000	3,0
17,0000	1,0	21,0000	2,0
71,0000	6,0	80,0000	8,0
60,0000	4,5	60,0000	4,5
<hr/>		<hr/>	
Rank totals	18,5		17,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FOUR DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
66,0000	6,0	65,0000	5,0
60,0000	2,5	60,0000	2,5
70,0000	7,0	76,0000	8,0
60,0000	2,5	60,0000	2,5
Rank totals		18,0	

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FIVE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
37,0000	5,0	53,0000	8,0
40,0000	6,0	50,0000	7,0
0,0000	2,5	0,0000	2,5
0,0000	2,5	0,0000	2,5
Rank totals		16,0	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SIX DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
67,0000	8,0	52,0000	7,0
10,0000	5,0	25,0000	6,0
6,0000	4,0	0,0000	2,0
0,0000	2,0	0,0000	-2,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SEVEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
2,0000	4,0	0,0000	2,0
3,0000	5,0	0,0000	2,0
10,0000	6,0	0,0000	2,0
20,0000	8,0	12,0000	7,0
<hr/>		<hr/>	
Rank totals	23,0		13,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR EIGHT DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
9,0000	7,0	0,0000	3,0
20,0000	8,0	6,0000	6,0
0,0000	3,0	0,0000	3,0
0,0000	3,0	0,0000	3,0
Rank totals		21,0	
		15,0	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR NINE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
23,0000	8,0	0,0000	4,0
0,0000	4,0	0,0000	4,0
0,0000	4,0	0,0000	4,0
0,0000	4,0	0,0000	4,0
Rank totals		20,0	
		16,0	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
0,0000	3,5	0,0000	3,5
5,0000	7,0	9,0000	8,0
0,0000	3,5	0,0000	3,5
0,0000	3,5	0,0000	3,5
<hr/>		<hr/>	
Rank totals	17,5		18,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.11: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE FOR DIFFERENT PERIODS AT RELATIVELY LOW SEED MOISTURE CONTENTS ON GERMINATION ENERGY

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
64,0000	1,0	77,0000	2,5
77,0000	2,5	76,0000	2,0
85,0000	6,0	90,0000	7,5
83,0000	5,0	90,0000	7,5
Rank totals		14,5	19,5

Mann-Whitney U-statistic = 6,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR THREE MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
80,0000	3,5	84,0000	7,0
80,0000	3,5	87,0000	8,0
81,0000	5,5	81,0000	5,5
71,0000	1,0	78,0000	2,0
Rank totals		13,5	22,5

Mann-Whitney U-statistic = 3,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR SIX MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
84,0000	4,0	87,0000	7,0
70,0000	1,0	73,0000	2,0
88,0000	8,0	82,0000	3,0
86,0000	6,0	85,0000	5,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR NINE MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
82,0000	4,5	77,0000	1,0
84,0000	8,0	83,0000	6,5
83,0000	6,5	79,0000	2,0
82,0000	4,5	80,0000	3,0
<hr/>		<hr/>	
Rank totals	23,5		12,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR TWELVE MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
78,0000	2,0	86,0000	8,0
84,0000	6,5	73,0000	1,0
81,0000	4,0	84,0000	6,5
79,0000	3,0	82,0000	5,0
Rank totals		15,5	20,5

Mann-Whitney U-statistic = 5,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.12: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE FOR DIFFERENT PERIODS AT RELATIVELY HIGH SEED MOISTURE CONTENTS ON GERMINATION ENERGY

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	84,17857	5,776	14,57**
1	-3,83214	6,842	-0,56
2	-4,66071	1,640	-2,84*

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 150,67521 (D.F. = 17)

MULTIPLE R-SQUARE = 0,89320

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	21421,47143	2	10710,73571	71,08	0,0000*
1	1216,44643	1	1216,44643	8,07	0,0113*
Residual	2561,47857	17	150,67521		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	86,16429	5,530	15,58**
1	3,72143	6,551	0,57
2	-6,64286	1,570	-4,23**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 138,10042 (D.F. = 17)

MULTIPLE R-SQUARE = 0,90866

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	23356,04286	2	11678,02143	84,56	0,0000*
1	2471,14286	1	2471,14286	17,89	0,0006*
Residual	2347,70714	17	138,10042		

* significant

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
86,0000	8,0	75,0000	3,0
74,0000	2,0	85,0000	7,0
83,0000	5,5	83,0000	5,5
79,0000	4,0	70,0000	1,0
Rank totals 19,5		16,5	

Mann-Whitney U-statistic = 6,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR ONE MONTH AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
60,0000	5,0	37,0000	3,0
65,0000	6,0	28,0000	1,0
68,0000	7,0	34,0000	2,0
70,0000	8,0	40,0000	4,0
Rank totals 26,0		10,0	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR TWO MONTHS AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
24,0000	4,0	35,0000	7,0
18,0000	2,5	28,0000	5,5
50,0000	8,0	17,0000	1,0
28,0000	5,5	18,0000	2,5
Rank totals		20,0	16,0

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR THREE MONTHS AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
1,0000	4,5	6,0000	7,0
5,0000	6,0	0,0000	2,0
0,0000	2,0	0,0000	2,0
1,0000	4,5	15,0000	8,0
Rank totals		17,0	19,0

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.13: STATISTICAL ANALYSIS OF THE EFFECT OF CONTROLLED DETERIORATION FOR DIFFERENT PERIODS ON GERMINATION RATE

RESULTS FOR POLYNOMIAL OF DEGREE 1 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	17,83364	1,097	16,25**
1	-1,50436	0,186	-8,11**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 3,78345 (D.F. = 9)

MULTIPLE R-SQUARE = 0,87968

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	258,78546	2	129,39273	42,76	0,0001*
1	9,84337	1	9,84337	3,25	0,1090
Residual	24,20770	8	3,02596		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 1 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	19,64227	0,973	20,19**
1	-1,56918	0,164	-9,54**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 2,97333 (D.F. = 9)

MULTIPLE R-SQUARE = 0,91009

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	286,95589	2	143,47795	107,67	0,0000*
1	16,09942	1	16,09942	12,08	0,0084*
Residual	10,66056	8	1,33257		

* significant

COMPARISON OF CULTIVARS - SEED SAMPLES NOT SUBJECTED TO CONTROLLED DETERIORATION

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
17,6429	5,0	18,0714	6,0
15,5857	1,0	17,2286	4,0
17,0857	3,0	18,5714	8,0
16,3714	2,0	18,2857	7,0
<hr/>		<hr/>	
Rank totals	11,0		25,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 1,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR ONE DAY

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,4429	4,0	16,3714	3,0
16,2429	2,0	17,1000	6,0
14,8286	1,0	17,5857	8,0
17,4714	7,0	16,7714	5,0
<hr/>		<hr/>	
Rank totals	14,0		22,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TWO DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
0,1429	1,0	15,9143	4,0
14,1000	2,0	16,4286	6,0
16,7429	7,0	17,6857	8,0
15,9429	5,0	15,6000	3,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR THREE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,0000	7,0	14,6429	3,0
12,5429	1,0	12,6429	2,0
15,9429	6,0	17,0000	8,0
15,2286	5,0	14,7571	4,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FOUR DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,1571	3,0	16,1571	8,0
14,3000	1,0	15,0143	2,0
15,6286	6,0	15,9000	7,0
15,2857	5,0	15,2000	4,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR FIVE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
10,9714	3,0	13,2429	7,0
14,3429	8,0	12,9143	6,0
8,8714	2,0	11,9571	5,0
5,4857	1,0	11,3714	4,0
<hr/>		<hr/>	
Rank totals	14,0		22,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SIX DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
14,1857	7,0	23,8571	8,0
10,5000	4,0	11,3857	6,0
10,4857	3,0	10,9000	5,0
9,1286	1,0	9,4286	2,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR SEVEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
8,4000	5,0	8,4429	6,0
9,3857	7,0	8,3857	4,0
5,5000	1,0	9,9143	8,0
6,7714	2,0	7,5429	3,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR EIGHT DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
8,9429	8,0	8,3000	6,0
8,7714	7,0	6,2286	4,0
0,0000	1,5	6,4571	5,0
0,0000	1,5	4,1143	3,0
Rank totals 18,0		18,0	

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR NINE DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
10,4286	8,0	5,6000	5,0
6,8571	6,0	8,1857	7,0
1,0143	3,0	4,2000	4,0
0,0000	1,0	0,7000	2,0
Rank totals 18,0		18,0	

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES SUBJECTED TO CONTROLLED DETERIORATION FOR TEN DAYS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
0,0000	2,5	2,3571	6,0
4,6000	7,0	5,5857	8,0
0,0000	2,5	0,0000	2,5
0,0000	2,5	1,0000	5,0
<hr/>		<hr/>	
Rank totals	14,5		21,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.14: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE FOR DIFFERENT PERIODS AT RELATIVELY LOW SEED MOISTURE CONTENTS ON GERMINATION RATE

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,7481	1,0	18,0825	6,0
16,2254	2,0	17,2592	5,0
17,1429	4,0	18,5714	8,0
16,8857	3,0	18,2857	7,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR THREE MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,7302	2,0	17,9905	7,0
17,5079	5,0	18,1143	8,0
17,2794	4,0	17,5333	6,0
15,6762	1,0	17,0396	3,0
<hr/>		<hr/>	
Rank totals	12,0		24,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR SIX MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
17,0540	4,0	17,9714	7,0
14,3968	1,0	15,6794	2,0
17,6000	6,0	16,6540	3,0
17,3111	5,0	18,3333	8,0
<hr/>		<hr/>	
Rank totals	16,0		20,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR NINE MONTHS AT RELATIVELY LOW MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,6540	4,0	16,3683	1,0
16,8000	8,0	16,7429	6,0
16,7111	5,0	16,8476	7,0
16,5429	2,0	16,5714	3,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR 12 MONTHS AT RELATIVELY LOW
MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,0286	2,0	17,2000	7,0
16,8000	6,0	15,4571	1,0
16,4857	4,0	17,3714	8,0
16,2286	3,0	16,6857	5,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO
TAILED)

APPENDIX 2.15: STATISTICAL ANALYSIS OF THE EFFECT OF STORAGE FOR DIFFERENT PERIODS AT RELATIVELY HIGH SEED MOISTURE CONTENTS ON GERMINATION RATE

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	16,81871	1,032	16,30**
1	0,27282	1,222	0,22
2	-1,15339	0,293	-3,94**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 4,80939 (D.F. = 17)

MULTIPLE R-SQUARE = 0,91015

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	828,18207	2	414,09103	89,10	0,0000*
1	74,49764	1	74,49764	15,49	0,0011*
Residual	81,75961	17	4,80939		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	18,22164	0,882	20,67**
1	0,54296	1,044	0,52
2	-1,30518	0,250	-5,21**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 3,51046 (D.F. = 17)

MULTIPLE R-SQUARE = 0,94208

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	970,64930	2	485,32465	138,25	0,0000*
1	95,39550	1	95,39550	27,17	0,0001*
Residual	59,67775	17	3,51046		

* significant

COMPARISON OF CULTIVARS - UNSTORED SEED SAMPLES AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,7481	1,0	18,0825	6,0
16,2254	2,0	17,2592	5,0
17,1429	4,0	18,5714	8,0
16,8857	3,0	18,2857	7,0
Rank totals		26,0	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR ONE MONTH AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,7429	1,0	17,8000	8,0
16,6286	4,0	17,4857	6,0
16,6000	3,0	16,9429	5,0
16,4000	2,0	17,7111	7,0
Rank totals		26,0	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR TWO MONTHS AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,2600	7,0	14,5671	6,0
12,5714	2,0	14,1429	5,0
12,1714	1,0	13,9111	4,0
13,7397	3,0	17,5429	8,0
Rank totals		13,0	23,0

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED SAMPLES STORED FOR THREE MONTHS AT RELATIVELY HIGH MOISTURE CONTENTS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
3,6016	3,0	9,2098	7,0
6,9314	4,0	7,4003	5,0
1,3276	1,0	1,5895	2,0
11,8952	8,0	9,0263	6,0
Rank totals		16,0	20,0

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 2.16: STATISTICAL ANALYSIS OF THE EFFECT OF CONTROLLED DETERIORATION ON THE RELATIVE LENGTHS OF PRIMARY ROOTS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 6,1460; F VALUE: 0,89; TAIL PROBABILITY: 0,5013; VARIANCE: HOMOGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	POOLED VARIANCE		DIFF. OF MEANS
	T-VAL	P-VAL	
Control vs.			
1 day	-2,55	0,0110	-0,073
2 days	-1,92	0,0555	-0,057
3 days	-1,40	0,1624	-0,041
4 days	9,90	0,0000***	0,380
5 days	6,83	0,0000***	0,239
7 days	11,10	0,0000***	0,439
1 day vs.			
2 days	0,55	0,5792	0,016
3 days	1,08	0,2800	0,032
4 days	11,81	0,0000***	0,453
5 days	8,92	0,0000***	0,312
7 days	12,95	0,0000***	0,512
2 days vs.			
3 days	0,51	0,6104	0,015
4 days	11,19	0,0000***	0,436
5 days	8,28	0,0000***	0,296
7 days	12,33	0,0000***	0,495
3 days vs.			
4 days	10,81	0,0000***	0,421
5 days	7,86	0,0000***	0,280
7 days	11,97	0,0000***	0,480
4 days vs.			
5 days	-3,25	0,0012*	-0,141
7 days	1,25	0,2101	0,059
5 days vs.			
7 days	4,50	0,0000***	0,199

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	69,96320	2,454	28,51**
1	0,40970	1,871	0,22
2	-0,68131	0,282	-2,41*

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 2333,17000 (D.F. = 25)

MULTIPLE R-SQUARE = 0,63177

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	115399,32790	3	38466,44263	21,47	0,0000*
1	28908,22514	2	14454,11257	8,07	0,0021*
2	15324,93087	1	15324,93087	8,55	0,0074*
Residual	43004,31907	24	1791,84663		

* significant

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS
LEVENE'S TEST FOR VARIANCES

D.F.: 10,1460; F VALUE: 8,60; TAIL PROBABILITY: 0,0000;
VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF.OF MEANS	TIME GROUP	SEPARATE VARIANCE			DIFF.OF MEANS
	T-VAL	D.F.	P-VAL			T-VAL	D.F.	P-VAL	
Control vs.					1 day vs.				
1 day	2,53	533	0,0117	0,144	2 days	-2,24	538	0,0254	-0,123
2 days	0,41	555	0,6806	0,021	3 days	-3,34	492	0,0009*	-0,173
3 days	0,62	518	0,5369	-0,029	4 days	-1,92	495	0,0556	-0,114
4 days	0,54	473	0,5914	0,030	5 days	3,23	517	0,0013	0,189
5 days	6,09	503	0,0000***	0,333	6 days	8,66	165	0,0000***	0,627
6 days	11,13	142	0,0000***	0,771	7 days	-0,37	531	0,7093	-0,022
7 days	2,23	519	0,0262	0,122	8 days	8,83	83	0,0000***	1,033
8 days	10,22	78	0,0000***	1,177	9 days	12,49	198	0,0000***	0,833
9 days	15,43	170	0,0000***	0,977	10 days	2,87	101	0,0050	0,238
10 days	4,75	89	0,0000***	0,382					
2 days vs.					3 days vs.				
3 days	-1,12	590	0,2646	-0,050	4 days	1,17	424	0,2412	0,059
4 days	0,17	471	0,8667	0,009	5 days	7,35	466	0,0000***	0,362
5 days	5,95	511	0,0000***	0,312	6 days	12,29	115	0,0000***	0,800
6 days	11,10	131	0,0000***	0,750	7 days	13,06	486	0,0023	0,151
7 days	1,92	530	0,0549	0,101	8 days	10,70	72	0,0000***	1,206
8 days	10,13	75	0,0000***	1,156	9 days	17,11	133	0,0000***	1,006
9 days	15,55	155	0,0000***	0,956	10 days	5,35	75	0,0000***	0,412
10 days	4,58	84	0,0000***	0,361					
4 days vs.					5 days vs.				
5 days	5,29	463	0,0000***	0,303	6 days	6,22	150	0,0000***	0,438
6 days	10,39	155	0,0000***	0,741	7 days	-3,74	499	0,0002*	-0,211
7 days	1,61	475	0,1086	0,092	8 days	7,28	80	0,0000***	0,844
8 days	9,85	81	0,0000***	1,147	9 days	9,96	179	0,0000***	0,644
9 days	14,44	185	0,0000***	0,947	10 days	0,61	94	0,5443	0,050
10 days	4,29	97	0,0000**	0,352					
6 days vs.					7 days vs.				
7 days	-9,18	152	0,0000***	-0,649	8 days	9,09	80	0,0000***	1,055
8 days	3,29	97	0,0014	0,406	9 days	13,18	182	0,0000***	0,855
9 days	2,66	149	0,0086	0,206	10 days	3,19	94	0,0019	0,260
10 days	-4,23	116	0,0000**	-0,389					
8 days vs.					9 days vs.				
9 days	-1,66	90	0,0997	-0,200	10 days	-6,79	107	0,0000***	-0,595
10 days	-6,11	106	0,0000***	-0,795					

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:
 *** 0,001 significance
 ** 0,01 significance
 * 0,05 significance
 no significance

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	66,79362	2,631	25,38**
1	0,10126	1,410	0,07
2	-0,23220	0,154	-1,51

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 3010,89114 (D.F. = 35)

MULTIPLE R-SQUARE = 0,38605

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	68436,86002	3	22812,28667	7,52	0,0005*
1	9047,83206	2	4523,91603	1,49	0,2396
2	2173,65751	1	2173,65751	0,72	0,4034
Residual	103207,532377	34	3035,51566		

* significant

APPENDIX 2.17: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE AT RELATIVELY LOW SEED MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF PRIMARY ROOTS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 4,1656; F VALUE: 4,91; TAIL PROBABILITY: 0,0006;
VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
3 month	-13,88	646	0,0000***	-18,663
6 months	-10,82	618	0,0000***	-14,946
9 months	-10,78	651	0,0000***	-14,229
12 days	-12,07	655	0,0000***	-15,541
3 months vs.				
6 months	2,47	653	0,0137	3,717
9 months	3,06	665	0,0023*	4,434
12 months	2,20	659	0,0279	3,123
6 months vs.				
9 months	0,48	648	0,6284	0,717
12 months	-0,41	640	0,6825	-0,594
9 months vs.				
12 months	-0,94	659	0,3471	-1,312

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 4,1728; F VALUE: 2,45; TAIL PROBABILITY: 0,0442;
 VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
3 month	-17,74	707	0,0000***	-20,818
6 months	-13,78	670	0,0000***	-17,149
9 months	-15,82	672	0,0000***	-19,021
12 days	-14,38	679	0,0000***	-17,313
3 months vs.				
6 months	2,82	682	0,0049*	3,669
9 months	1,42	677	0,1548	1,797
12 months	2,78	683	0,0055	3,505
6 months vs.				
9 months	-1,41	670	0,1418	-1,872
12 months	-0,12	675	0,9018	-0,164
9 months vs.				
12 months	1,32	666	0,1858	1,708

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

APPENDIX 2.18: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE AT RELATIVELY HIGH SEED MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF PRIMARY ROOTS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 3,1031; F VALUE: 13,05; TAIL PROBABILITY: 0,0000;
VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
1 month	-10,46	621	0,0000***	-14,141
2 months	-1,21	601	0,2287	-1,441
3 months	0,64	171	0,5221	1,168
1 month vs.				
2 months	9,46	574	0,0000***	12,700
3 months	7,97	204	0,0000***	15,309
2 months vs.				
3 months	1,44	168	0,1520	2,609

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 3,1143; F VALUE: 27,91; TAIL PROBABILITY: 0,0000;
 VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
1 month	-21,56	654	0,0000***	-26,844
2 months	-9,09	651	0,0000***	-8,964
3 months	-2,82	249	0,0052*	-4,375
1 month vs.				
2 months	15,73	542	0,0000***	17,880
3 months	13,59	299	0,0000***	22,469
2 months vs.				
3 months	3,13	204	0,0020*	4,589

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

APPENDIX 2.19: STATISTICAL ANALYSIS OF THE EFFECT OF CONTROLLED DETERIORATION ON THE RELATIVE LENGTHS OF HYPOCOTYLS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 6,1460; F VALUE: 5,58; TAIL PROBABILITY: 0,0000;
VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
1 day	2,54	543	0,0114	0,531
2 days	-0,76	545	0,4480	-0,179
3 days	8,14	533	0,0000***	1,717
4 days	17,03	227	0,0000***	4,927
5 days	19,37	328	0,0000***	5,115
7 days	21,93	217	0,0000***	6,203
1 day vs.				
2 days	-3,38	496	0,0008*	-0,709
3 days	6,51	547	0,0000***	1,186
4 days	16,33	180	0,0000***	4,396
5 days	18,96	260	0,0000***	4,584
7 days	21,64	170	0,0000***	5,672
2 days vs.				
3 days	8,95	489	0,0000***	1,896
4 days	17,61	226	0,0000***	5,105
5 days	20,00	322	0,0000***	5,294
7 days	22,52	216	0,0000***	6,381
3 days vs.				
4 days	11,86	182	0,0000***	3,210
5 days	13,96	263	0,0000***	3,398
7 days	17,01	173	0,0000***	4,486
4 days vs.				
5 days	0,60	239	0,5489	0,188
7 days	3,87	214	0,0001**	1,276
5 days vs.				
7 days	3,53	230	0,0005*	1,087

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

*** 0,001 significance
** 0,01 significance
* 0,05 significance
no significance

RESULTS FOR POLYNOMIAL OF DEGREE 2 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	41,63233	1,510	27,57**
1	-2,48466	1,151	-2,16
2	-0,22723	0,227	-1,31

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 883,27127 (D.F. = 25)

MULTIPLE R-SQUARE = 0,80442

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	99558,17313	3	33186,05771	59,68	0,0000*
1	10247,14473	2	5123,57236	9,21	0,0011*
2	8736,17609	1	8736,17609	15,71	0,0006*
Residual	13345,60570	24	556,06690		

* significant

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 10,2143; F VALUE: 3,90; TAIL PROBABILITY: 0,0000;
 VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF HYPOCOTYL LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF.OF MEANS	TIME GROUP	SEPARATE VARIANCE			DIFF.OF MEANS
	T-VAL	D.F.	P-VAL			T-VAL	D.F.	P-VAL	
Control vs.					1 day vs.				
1 day	2,83	535	0,0048	0,266	2 days	-1,65	555	0,0992	-0,138
2 days	1,46	521	0,1461	0,128	3 days	0,26	523	0,7962	0,021
3 days	3,40	484	0,0007*	0,287	4 days	2,93	485	0,0035	0,271
4 days	5,57	487	0,0000***	0,537	5 days	7,52	514	0,0000***	0,680
5 days	10,00	508	0,0000***	0,946	6 days	11,45	130	0,0000***	1,438
6 days	13,24	142	0,0000***	1,704	7 days	8,05	532	0,0000***	0,704
7 days	10,58	515	0,0000***	0,970	8 days	16,72	90	0,0000***	2,562
8 days	18,16	96	0,0000***	2,828	9 days	21,67	175	0,0000***	2,284
9 days	23,39	193	0,0000***	2,550	10 days	6,42	92	0,0000***	0,853
10 days	8,25	99	0,0000***	1,119					
2 days vs.					3 days vs.				
3 days	2,19	596	0,0291	0,159	4 days	3,03	426	0,0026	0,250
4 days	4,73	464	0,0000***	0,409	5 days	8,21	471	0,0000***	0,659
5 days	9,70	506	0,0000***	0,818	6 days	11,95	106	0,0000***	1,418
6 days	12,99	115	0,0000***	1,576	7 days	8,89	513	0,0000***	0,683
7 days	10,39	543	0,0000***	0,842	8 days	17,23	78	0,0000***	2,541
8 days	18,04	83	0,0000***	2,700	9 days	23,36	133	0,0000***	2,263
9 days	24,17	149	0,0000***	2,422	10 days	6,60	76	0,0000***	0,832
10 days	7,70	82	0,0000***	0,991					
4 days vs.					5 days vs.				
5 days	4,40	462	0,0000***	0,409	6 days	6,01	132	0,0000***	0,759
6 days	9,15	136	0,0000***	1,167	7 days	0,27	496	0,7847	0,024
7 days	4,81	465	0,0000***	0,433	8 days	12,25	91	0,0000***	1,882
8 days	14,80	94	0,0000***	2,291	9 days	15,14	176	0,0000***	1,604
9 days	18,70	182	0,0000***	2,013	10 days	1,30	93	0,1970	0,173
10 days	4,33	96	0,0000**	0,582					
6 days vs.					7 days vs.				
7 days	-5,92	124	0,0000***	-0,734	8 days	12,24	87	0,0000***	1,858
8 days	6,36	124	0,0000***	1,123	9 days	15,28	164	0,0000***	1,580
9 days	6,16	142	0,0000***	0,845	10 days	1,14	88	0,2591	0,149
10 days	-3,68	124	0,0003*	-0,586					
8 days vs.					9 days vs.				
9 days	-1,71	105	0,0906	-0,278	10 days	-9,95	108	0,0000***	-1,431
10 days	-9,40	117	0,0000***	-1,709					

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	43,06803	1,842	23,38**
1	-1,76646	0,987	-1,79
2	-0,06406	0,108	-0,60

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 1475,06064 (D.F. = 35)

MULTIPLE R-SQUARE = 0,62796

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	89963,36556	3	29987,78852	20,89	0,0000*
1	3344,87801	2	1672,43901	1,17	0,3240
2	2821,69705	1	2821,69705	1,97	0,1700
Residual	48805,42517	34	1435,45368		

* significant

APPENDIX 2.20: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE AT RELATIVELY LOW SEED MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF HYPOCOTYLS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 4,1656; F VALUE: 22,38; TAIL PROBABILITY: 0,0000;
VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
3 month	-37,82	553	0,0000***	-21,915
6 months	-28,97	482	0,0000***	-19,158
9 months	-27,04	517	0,0000***	-16,925
12 days	-28,88	511	0,0000***	-18,164
3 months vs.				
6 months	3,59	633	0,0004**	2,757
9 months	6,76	658	0,0000***	4,990
12 months	5,06	654	0,0000***	3,751
6 months vs.				
9 months	2,78	649	0,0056	2,233
12 months	-0,41	640	0,6825	-0,594
9 months vs.				
12 months	-1,59	660	0,1115	-1,239

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 4,1728; F VALUE: 26,66; TAIL PROBABILITY: 0,0000;
 VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
3 month	-37,08	570	0,0000***	-20,922
6 months	-31,28	526	0,0000***	-18,919
9 months	-31,07	533	0,0000***	-17,897
12 days	-30,86	528	0,0000***	-18,257
3 months vs.				
6 months	2,78	683	0,0056	2,002
9 months	4,34	678	0,0000***	3,025
12 months	3,75	681	0,0002**	2,665
6 months vs.				
9 months	1,40	670	0,1618	1,023
12 months	0,89	676	0,3726	0,662
9 months vs.				
12 months	-0,50	666	0,6167	-0,360

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

APPENDIX 2.21: STATISTICAL ANALYSIS OF THE EFFECT OF SEED STORAGE AT RELATIVELY HIGH SEED MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF HYPOCOTYLS

TEST FOR HOMOGENEOUS VARIANCES - FLORADADE AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 3,1031; F VALUE: 25,23; TAIL PROBABILITY: 0,0000;

VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
1 month	-28,83	561	0,0000***	-15,606
2 months	-13,59	423	0,0000***	-8,531
3 months	-5,21	145	0,0000***	-4,137
1 month vs.				
2 months	9,96	540	0,0000***	7,075
3 months	13,33	193	0,0000***	11,469
2 months vs.				
3 months	4,79	232	0,0000***	4,394

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

*** 0,001 significance

** 0,01 significance

* 0,05 significance

no significance

TEST FOR HOMOGENEOUS VARIANCES - ROMA VF AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR VARIANCES

D.F.: 3,1143; F VALUE: 37,00; TAIL PROBABILITY: 0,0000;
 VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME GROUP	SEPARATE VARIANCE			DIFF. OF MEANS
	T-VAL	D.F.	P-VAL	
Control vs.				
1 month	-41,86	620	0,0000***	-20,293
2 months	-26,77	456	0,0000***	-16,518
3 months	-7,57	193	0,0000***	-5,832
1 month vs.				
2 months	5,62	550	0,0000***	3,776
3 months	17,74	235	0,0000***	14,462
2 months vs.				
3 months	11,87	312	0,0000***	10,686

NOTATION FOR BONFERRONI SIGNIFICANCE LEVELS:

- *** 0,001 significance
- ** 0,01 significance
- * 0,05 significance
- no significance

APPENDIX 3.1: STATISTICAL ANALYSIS OF THE INTERACTION OF THE HOT-WATER TREATMENT AND SEED STORAGE AT RELATIVELY LOW SEED MOISTURE CONTENTS WITH REGARD TO GERMINATION CAPACITY.

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
82,0000	2,0	85,0000	6,5
83,0000	3,5	83,0000	3,5
81,0000	1,0	86,0000	8,0
84,0000	5,0	85,0000	6,5
<hr/>		<hr/>	
Rank totals	11,5		24,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 1,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
85,0000	2,5	86,0000	4,0
88,0000	5,0	91,0000	8,0
89,0000	6,5	89,0000	6,5
85,0000	2,5	82,0000	1,0
<hr/>		<hr/>	
Rank totals	16,5		19,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - SIX MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
81,0000	2,0	86,0000	3,5
91,0000	8,0	73,0000	1,0
86,0000	3,5	88,0000	6,0
90,0000	7,0	87,0000	5,0
<hr/>		<hr/>	
Rank totals	20,5		15,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - NINE MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
88,0000	8,0	84,0000	5,5
78,0000	2,0	84,0000	5,5
84,0000	5,5	84,0000	5,5
76,0000	1,0	83,0000	3,0
<hr/>		<hr/>	
Rank totals	16,5		19,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWELVE MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
79,0000	2,0	81,0000	3,5
77,0000	1,0	84,0000	8,0
82,0000	5,5	83,0000	7,0
81,0000	3,5	82,0000	5,5
<hr/>		<hr/>	
Rank totals	12,0		24,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
90,0000	1,0	96,0000	7,0
94,0000	4,5	91,0000	2,0
97,0000	8,0	94,0000	4,5
95,0000	6,0	92,0000	3,0
<hr/>		<hr/>	
Rank totals	19,5		16,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
94,0000	7,0	93,0000	6,0
91,0000	3,0	92,0000	5,0
91,0000	3,0	91,0000	3,0
96,0000	8,0	89,0000	1,0
<hr/>		<hr/>	
Rank totals	21,0		15,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - SIX MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
88,0000	4,0	91,0000	6,0
85,0000	3,0	81,0000	1,0
100,0000	8,0	84,0000	2,0
90,0000	5,0	95,0000	7,0
Rank totals		20,0	16,0

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - NINE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
87,0000	4,0	91,0000	7,5
89,0000	5,5	84,0000	2,0
91,0000	7,5	89,0000	5,5
84,0000	2,0	84,0000	2,0
Rank totals		19,0	17,0

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWELVE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
89,0000	8,0	86,0000	4,5
86,0000	4,5	79,0000	1,0
86,0000	4,5	88,0000	7,0
86,0000	4,5	84,0000	2,0
<hr/>		<hr/>	
Rank totals	21,5		14,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - UNSTORED SEED

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
82,0000	2,0	90,0000	5,0
83,0000	3,0	94,0000	6,0
81,0000	1,0	97,0000	8,0
84,0000	4,0	95,0000	7,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
85,0000	1,5	94,0000	7,0
88,0000	3,0	91,0000	5,5
89,0000	4,0	91,0000	5,5
85,0000	1,5	96,0000	8,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR SIX MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
81,0000	1,0	88,0000	4,0
91,0000	6,0	85,0000	2,0
86,0000	3,0	100,0000	8,0
90,0000	5,0	92,0000	7,0
Rank totals 15,0		21,0	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR NINE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
88,0000	6,0	91,0000	8,0
78,0000	2,0	84,0000	4,0
84,0000	4,0	89,0000	7,0
76,0000	1,0	84,0000	4,0
Rank totals 13,0		23,0	

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR TWELVE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
79,0000	2,0	89,0000	8,0
77,0000	1,0	86,0000	6,0
82,0000	4,0	86,0000	6,0
81,0000	3,0	86,0000	6,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO
TAILED)

APPENDIX 3.2: STATISTICAL ANALYSIS OF THE INTERACTION OF THE HOT-WATER TREATMENT AND SEED STORAGE AT RELATIVELY HIGH SEED MOISTURE CONTENTS WITH REGARD TO GERMINATION CAPACITY.

RESULTS FOR POLYNOMIAL OF DEGREE 1 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	88,95000	2,837	31,35**
1	-21,97500	1,158	-18,97**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 53,66528 (D.F. = 18)

MULTIPLE R-SQUARE = 0,95237

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	19366,18571	2	9683,09286	179,74	0,0000*
1	50,16071	1	50,16071	0,93	0,3481
Residual	915,81429	17	53,87143		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	85,72143	6,049	14,17**
1	-39,91786	7,165	-5,57**
2	4,91071	1,718	2,86*

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 165,23992 (D.F. = 17)

MULTIPLE R-SQUARE = 0,86365

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	17793,47143	2	8896,73571	53,84	0,0000*
1	1350,44643	1	1350,44643	8,17	0,0109*
Residual	2809,07857	17	165,23992		

* significant

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
90,0000	8,0	85,0000	4,5
81,0000	1,0	83,0000	2,5
87,0000	7,0	86,0000	6,0
83,0000	2,5	85,0000	4,5
<hr/>		<hr/>	
Rank totals	18,5		17,5
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,5

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - ONE MONTH STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
69,0000	1,0	79,0000	5,0
72,0000	2,0	84,0000	8,0
74,0000	3,0	83,0000	7,0
75,0000	4,0	82,0000	6,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWO MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
38,0000	2,0	80,0000	8,0
35,0000	1,0	64,0000	6,0
54,0000	4,0	62,0000	5,0
49,0000	3,0	70,0000	7,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO
TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
22,0000	3,0	29,0000	5,0
27,0000	4,0	44,0000	7,0
7,0000	1,0	13,0000	2,0
37,0000	6,0	67,0000	8,0
<hr/>		<hr/>	
Rank totals	14,0		22,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO
TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
82,0000	1,0	96,0000	7,5
95,0000	6,0	91,0000	2,0
96,0000	7,5	94,0000	5,0
92,0000	3,5	92,0000	3,5
<hr/>		<hr/>	
Rank totals	18,0		18,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - ONE MONTH STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
40,0000	3,0	89,0000	7,5
31,0000	1,0	88,0000	6,0
36,0000	2,0	85,0000	5,0
48,0000	4,0	89,0000	7,5
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWO MONTHS STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
39,0000	4,0	75,0000	7,0
33,0000	3,0	71,0000	6,0
20,0000	1,0	70,0000	5,0
21,0000	2,0	88,0000	8,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
32,0000	4,0	57,0000	8,0
0,0000	1,0	43,0000	6,0
3,0000	2,0	13,0000	3,0
39,0000	5,0	49,0000	7,0
<hr/>		<hr/>	
Rank totals	12,0		24,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - UNSTORED SEED

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
90,0000	5,0	82,0000	2,0
81,0000	1,0	95,0000	7,0
87,0000	4,0	96,0000	8,0
83,0000	3,0	92,0000	6,0
<hr/>		<hr/>	
Rank totals	13,0		23,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR ONE MONTH

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
69,0000	5,0	40,0000	3,0
72,0000	6,0	31,0000	1,0
74,0000	7,0	36,0000	2,0
75,0000	8,0	48,0000	4,0
<hr/>		<hr/>	
Rank totals	26,0		10,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR TWO MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
38,0000	5,0	39,0000	6,0
35,0000	4,0	33,0000	3,0
54,0000	8,0	20,0000	1,0
49,0000	7,0	21,0000	2,0
<hr/>		<hr/>	
Rank totals	24,0		12,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
22,0000	4,0	32,0000	6,0
27,0000	5,0	0,0000	1,0
7,0000	3,0	3,0000	2,0
37,0000	7,0	39,0000	8,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 3.3: STATISTICAL ANALYSIS OF THE INTERACTION OF THE HOT-WATER TREATMENT AND SEED STORAGE AT RELATIVELY LOW SEED MOISTURE CONTENTS WITH REGARD TO GERMINATION RATE

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
16,1079	2,0	15,7481	1,0
16,3143	5,0	16,2254	4,0
16,1111	3,0	17,1429	8,0
16,7111	6,0	16,8857	7,0
<hr/>		<hr/>	
Rank totals	16,0		20,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
16,5683	2,0	16,7302	3,0
17,6000	7,0	17,5079	6,0
17,8000	8,0	17,2794	5,0
16,8222	4,0	15,6762	1,0
<hr/>		<hr/>	
Rank totals	21,0		15,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - SIX MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
15,4841	2,0	17,0540	3,0
17,7163	8,0	14,3968	1,0
17,1429	4,0	17,6000	6,0
17,6254	7,0	17,3111	5,0
<hr/>		<hr/>	
Rank totals	21,0		15,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - NINE MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
17,2789	8,0	16,6540	5,0
15,1143	2,0	16,8000	7,0
16,6222	4,0	16,7111	6,0
14,7100	1,0	16,5429	3,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWELVE MONTHS STORAGE -
FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
14,9178	1,0	16,0286	4,0
15,0040	2,0	16,8000	8,0
15,9189	3,0	16,4857	7,0
16,0909	5,0	16,2286	6,0
<hr/>		<hr/>	
Rank totals	11,0		25,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 1,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
17,7397	2,0	18,0825	3,0
18,4254	5,0	17,2592	1,0
19,0571	8,0	18,5714	6,0
18,8857	7,0	18,2857	4,0
Rank totals 22,0		14,0	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
18,6857	7,0	17,9905	4,0
18,0909	5,0	18,1143	6,0
17,8571	3,0	17,5333	2,0
18,9397	8,0	17,0396	1,0
Rank totals 23,0		13,0	

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - SIX MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
16,9247	4,0	17,9714	6,0
16,5683	2,0	15,6794	1,0
18,7619	8,0	16,6540	3,0
17,5393	5,0	18,3333	7,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - NINE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
17,7429	6,0	16,3683	1,0
16,9683	5,0	16,7429	3,0
17,9143	8,0	16,8476	4,0
17,8218	7,0	16,5714	2,0
<hr/>		<hr/>	
Rank totals	26,0		10,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO
TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWELVE MONTHS STORAGE -
ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
16,4420	2,0	17,2000	7,0
16,8967	6,0	15,4571	1,0
16,8484	5,0	17,3714	8,0
16,6508	3,0	16,6857	4,0
<hr/>		<hr/>	
Rank totals	16,0		20,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 6,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - UNSTORED SEED

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,1079	1,0	17,7397	5,0
16,3143	3,0	18,4254	6,0
16,1111	2,0	19,0571	8,0
16,7111	4,0	18,8857	7,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
16,5683	1,0	18,6857	7,0
17,6000	3,0	18,0909	6,0
17,8000	4,0	17,8571	5,0
16,8222	2,0	18,9397	8,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR SIX MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
15,4841	1,0	16,9247	3,0
17,7163	7,0	16,5683	2,0
17,1429	4,0	18,7619	8,0
17,6254	6,0	17,5393	5,0
<hr/>		<hr/>	
Rank totals	18,0		18,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 8,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR NINE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
17,2789	5,0	17,7429	6,0
15,1143	2,0	16,9683	4,0
16,6222	3,0	17,9143	8,0
14,7100	1,0	17,8214	7,0
<hr/>		<hr/>	
Rank totals	11,0		25,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 1,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050
(TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR TWELVE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
14,9178	1,0	16,4420	5,0
15,0040	2,0	16,8967	8,0
15,9189	3,0	16,8484	7,0
16,0909	4,0	16,6508	6,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO
TAILED)

APPENDIX 3.4: STATISTICAL ANALYSIS OF THE INTERACTION OF THE HOT-WATER TREATMENT AND SEED STORAGE AT RELATIVELY HIGH SEED MOISTURE CONTENTS WITH REGARD TO GERMINATION RATE

RESULTS FOR POLYNOMIAL OF DEGREE 1 - FLORADADE

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR	T VALUE
0	17,05900	0,607	28,09**
1	-4,46675	0,248	-18,01**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 2,45944 (D.F. = 18)

MULTIPLE R-SQUARE = 0,94744

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	799,43517	2	399,71758	158,36	0,0000*
1	1,36094	1	1,36094	0,54	0,4728
Residual	42,90893	17	2,52405		

* significant

RESULTS FOR POLYNOMIAL OF DEGREE 2 - ROMA VF

POLYNOMIAL COEFFICIENTS	POLYNOMIAL IN X		
	DEGREE	REGRESSION COEFFICIENT	STANDARD ERROR
0	16,41321	0,971	16,91**
1	-7,95693	1,150	-6,92**
2	1,00286	0,276	3,64**

* significant at $p = 0,05$

** significant at $p = 0,01$

RESIDUAL MEAN SQUARE = 4,25614 (D.F. = 17)

MULTIPLE R-SQUARE = 0,90370

GOODNESS OF FIT TEST

For the polynomial of each degree, a test is made for additional information in the orthogonal polynomials of higher degree. The numerator sum of squares for each of these tests is the sum of squares attributed to all orthogonal polynomials of higher degree and the denominator sum of squares is the residual sum of squares from the fit to the highest degree polynomial (fit to all orthogonal polynomials). A significant F-statistic thus indicates that a higher degree polynomial should be considered.

DEGREE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROBABILITY
0	678,99927	2	339,49963	79,77	0,0000*
1	56,32046	1	56,32046	13,23	0,0020*
Residual	72,35439	17	4,25614		

* significant

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
17,6762	8,0	15,7481	2,0
15,6413	1,0	16,2254	3,0
17,1079	6,0	17,1429	7,0
16,3714	4,0	16,8857	5,0
<hr/>		<hr/>	
Rank totals	19,0		17,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - ONE MONTH STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
13,0384	1,0	15,7429	5,0
13,9683	2,0	16,6286	8,0
14,1189	3,0	16,6000	7,0
14,6825	4,0	16,4000	6,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWO MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
6,3116	2,0	15,2600	8,0
4,9822	1,0	12,5714	6,0
10,3761	4,0	12,1714	5,0
7,4877	3,0	13,7397	7,0
Rank totals 10,0		26,0	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE - FLORADADE

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
2,3052	3,0	3,6016	5,0
3,5786	4,0	6,9314	7,0
0,6286	1,0	1,3276	2,0
4,2162	6,0	11,8952	8,0
Rank totals 14,0		22,0	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - UNSTORED - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
15,8730	1,0	18,0825	4,0
18,2698	6,0	17,2592	3,0
18,2667	5,0	18,5714	8,0
16,7619	2,0	18,2857	7,0
Rank totals 14,0		22,0	

Mann-Whitney U-statistic = 4,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - ONE MONTH STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
7,7651	3,0	17,8000	8,0
5,9333	1,0	17,4857	6,0
7,0540	2,0	16,9429	5,0
9,0592	4,0	17,7111	7,0
Rank totals 10,0		26,0	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - TWO MONTHS STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
7,2650	4,0	14,5671	7,0
6,0872	3,0	14,1429	6,0
3,7309	1,0	13,9111	5,0
3,9309	2,0	17,5429	8,0
<hr/>		<hr/>	
Rank totals	10,0		26,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF TREATED AND UNTREATED SEED SAMPLES - THREE MONTHS STORAGE - ROMA VF

The non-parametric U-test of Mann-Whitney

Treated		Untreated	
Observation	Rank	Observation	Rank
4,0355	4,0	9,2098	8,0
0,0000	1,0	7,4003	6,0
0,1818	2,0	1,5895	3,0
6,1111	5,0	9,0263	7,0
<hr/>		<hr/>	
Rank totals	12,0		24,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 2,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - UNSTORED SEED

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
17,6762	6,0	15,8730	2,0
15,6413	1,0	18,2698	8,0
17,1079	5,0	18,2667	7,0
16,3714	3,0	16,7619	4,0
<hr/>		<hr/>	
Rank totals	15,0		21,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 5,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR ONE MONTH

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
13,0384	5,0	7,7651	3,0
13,9683	6,0	5,9333	1,0
14,1189	7,0	7,0540	2,0
14,6825	8,0	9,0592	4,0
<hr/>		<hr/>	
Rank totals	26,0		10,0
<hr/>		<hr/>	

Mann-Whitney U-statistic = 0,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR TWO MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
6,3116	5,0	7,2650	6,0
4,9822	3,0	6,0872	4,0
10,3761	8,0	3,7309	1,0
7,4877	7,0	3,9309	2,0
Rank totals 23,0		13,0	

Mann-Whitney U-statistic = 3,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

COMPARISON OF CULTIVARS - SEED STORAGE FOR THREE MONTHS

The non-parametric U-test of Mann-Whitney

Floradade		Roma VF	
Observation	Rank	Observation	Rank
2,3052	4,0	4,0355	6,0
3,5786	5,0	0,0000	1,0
0,6286	3,0	0,1818	2,0
4,2162	7,0	6,1111	8,0
Rank totals 19,0		17,0	

Mann-Whitney U-statistic = 7,0

N1 = 4; N2 = 4

THE DIFFERENCE BETWEEN THE RANK TOTALS IS NOT SIGNIFICANT AT P = 0,050 (TWO TAILED)

APPENDIX 3.5: STATISTICAL ANALYSIS OF THE EFFECT OF HOT-WATER TREATMENT IN INTERACTION WITH SEED STORAGE AT RELATIVELY LOW MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF SEEDLING ORGANS

PRIMARY ROOT LENGTH AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR HOMOGENEOUS VARIANCES

D.F.: 4,15; F VALUE: 0,51; TAIL PROBABILITY: 0,73055 VARIANCE: HOMOGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	POOLED VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,06	0,09	3	3
3	0,05	0,01	3	3
6	0,17	0,12	3	3
9	0,04	0,002	3	3
12	0,002	0,02	3	3

* = significant

HYPOCOTYL LENGTH AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR HOMOGENEOUS VARIANCES

D.F.: 4,15; F VALUE: 1,00; TAIL PROBABILITY: 0,49805 VARIANCE: HOMOGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	POOLED VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,02	0,23	3	3
3	0,39	0,34	3	3
6	0,02	0,20	3	3
9	0,13	0,12	3	3
12	0,03	0,01	3	3

* = significant

HYPOCOTYL/PRIMARY ROOT RATIO AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR HOMOGENEOUS VARIANCES

D.F.: 4,15; F VALUE: 3,70; TAIL PROBABILITY: 0,0274; VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	SEPARATE VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,003	0,004	3	3
3	0,02	0,008	3	3
6	0,01	0,02	3	2
9	0,01	0,008	0,03	3
12	0,001	0,004	0,03	2

* = significant

APPENDIX 3.6: STATISTICAL ANALYSIS OF THE EFFECT OF HOT-WATER TREATMENT IN INTERACTION WITH SEED STORAGE AT RELATIVELY HIGH MOISTURE CONTENTS ON THE RELATIVE LENGTHS OF SEEDLING ORGANS

PRIMARY ROOT LENGTH AFTER AN INCUBATION PERIOD OF SEVEN DAYS

TEST FOR HOMOGENEOUS VARIANCES

D.F.: 3,11; F VALUE: 46,5; TAIL PROBABILITY: 0,0005; VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	SEPARATE VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,45	0,50	3	3
1	0,23	0,66	3	3
2	0,26	0,35	3	3
3	0,37	0,23	3	3

* = significant

HYPOCOTYL LENGTH AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR HOMOGENEOUS VARIANCES

D.F.: 3,11; F VALUE: 73,2; TAIL PROBABILITY: 0,0000; VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	SEPARATE VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,19	0,35	3	3
1	1,47	2,72	3	3
2	0,09	0,01	3	3
3	2,00	0,33	3	3

* = significant

HYPOCOTYL/PRIMARY ROOT RATIO AFTER AN INCUBATION PERIOD OF SEVEN DAYS

LEVENE'S TEST FOR HOMOGENEOUS VARIANCES

D.F.: 3,11; F VALUE: 22,8; TAIL PROBABILITY: 0,0015; VARIANCE: HETEROGENEOUS

PAIRWISE T-TEST OF PRIMARY ROOT LENGTH GROUPED BY TIME

TIME (months)	SEPARATE VARIANCE			
	T-VAL		D.F.	
	Floradade	Roma VF	Floradade	Roma VF
0	0,002	0,01	3	3
1	0,02	0,05	0,03	3
2	0,01	0,002	3	3
3	0,01	0,01	2	3

* = significant