EMBRYO GROWTH IN MATURE CELERY SEEDS

EMBRYO GROEI IN RIJP SELDERIJ ZAAD

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Embryo growth in mature celery seeds

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
DR. H. C. VAN DER PLAS,
IN HET OPENBAAR TE VERDEDIGEN
OP DINSDAG 12 SEPTEMBER 1989
DES NAMIDDAGS TE VIER UUR IN DE AULA
VAN DE LANDBOUWUNIVERSITEIT TE WAGENINGEN.

The investigations were performed at the Seed Technology Department, Nunhems Seeds, The Netherlands.

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List of abbreviations

av	air volume
°C	degree centigrade
cv(s)	cultivar(s)
d.a.p.	days after pollination
dw	dry weight
ea	embryo surface area on longitudinal cross sections
ed	endosperm density
es	endosperm sugar content
ev	endosperm volume
ew	endosperm weight
g (μ,m)	gram (micro, milli)
GA	gibberellin
h	hour
l (μ,m)	liter (micro, milli)
L	light
m (μ,m,c)	meter (micro, milli, centi)
M	molair
min	minutes
nmol	nanomol
p	probability
Pa (K, M)	pascal (kilo, mega)
PEG (6000)	polyetylene glycol (molecular weight 6000)
pd	pericarp density
pv	pericarp volume
pw	pericarp weight
ψ	water potential
ψ_π	osmotic potential
ψ_p	pressure potential
R	regression correlation coefficient
R.H.	relative humidity
ss	seed surface
S (μ)	siemens (micro)
sv	seed volume
sw	seed fresh weight
Σ	sum
t_{50}	time to 50% germination of viable seeds
v	spread in t_{50}

Abstract

Toorn, P. van der (1989). Embryo growth in mature celery seeds. *Doctoral Thesis*, Wageningen, The Netherlands.

Germination of celery seeds is slow, due to the need for embryo growth before radicle protrusion can occur. Germination rate was correlated with embryo growth rate. Celery seeds with different embryo growth rates were obtained with fluid density separation of a seed lot. Low density seeds germinated faster, due to a larger embryo cell size and a higher embryo cell division rate. It was concluded that the embryo cell size was correlated with the osmotic potential of the true seed. A less negative osmotic potential was caused by the higher air volume and lower pericarp volume in the low density seed. It was argued that the higher embryo cell division rate in low density seeds was partly caused by the higher osmotic potential.

During incubation in PEG solutions embryo growth rate in low density seeds was also higher, due to a higher cell division rate. Low density seeds contained more embryo cells after the treatment than high density seeds. It was concluded that the number of embryo cells formed during PEG incubation was partly correlated with the osmotic potential in the true seed.

Not only the embryo length was increased after a PEG incubation, but also the embryo growth rate was increased when seeds were subsequently germinated in water. The increase in embryo growth rate was caused by an increase in cell size, most probably due to a positive effect of PEG incubation on embryo cell wall extensibility.

Seed density was negatively correlated with seed maturity, because during seed ripening on the mother plant the volume of air in the seeds increased. The seed density was not correlated to the umbel position as such, but harvesting of seeds from all umbels at the same time resulted in seeds with different density, due to the different length of the ripening period of the different umbels. Endo-ß-mannanase activity, that is part of the hydrolytic activity in the endosperm, was positively correlated with seed maturity. During germination the endo-ß-mannanase activity increased, but in low density seeds the activity was higher than in high density seeds. The higher hydrolytic activity in low density seeds correlated with the higher embryo cell division rate, both during germination in water and incubation in PEG.

The analysis of structural and physiological parameters of different celery cultivars showed that differences in germination rate and the effect of a priming treatment on the germination rate, could be explained by the same mechanisms as in genetically identical but physiologically different seeds. The general effect of seed priming on celery cultivars is a reduction in mean germination time of about 66%.

General Introduction

Apium graveolens L.

Ancestry and varieties

The cultivated celery belongs to the family *Apiaceae* (syn. *Umbelliferae*) and originates from the wild varieties found in streambeds and bogs in south Eurasia (Orton 1986), the wild celery thrives in a saline habitat (Pressman, Negbi, Sachs and Jacobsen 1977). Breeding and selection have resulted in the green and self-blanching celery (*A. graveolens* L. var. Dulce (Miller) Pers.) characterized by enlarged, succulent petioles borne on a single rosette, and root celery or celeriac (*A. graveolens* L. var. rapaceum (Miller) D.C.), characterized by swollen taproots. In general, seeds of the two domesticates have similar germination and dormancy characteristics (Thomas 1983).

Flowering and seed ripening

Usually, celery is a biennial plant, although cultivars obtained in Asia can be annual (Pressman, Shaked and Negbi 1988). The morphology of flowering parts of celery is complicated due to the many different umbels that are formed. According to the chronology of flowering the umbels can be divided into primary, secundary, tertiary, and quarternary umbels (Thomas, Biddington and O'Toole 1979). In certain cultivars at certain growth conditions there may be even six different umbel categories (personal observation). There is a considerable time lag between flowering of the first and last umbels, resulting in somatic heterochrony (Silvertown 1984) of the seeds from one plant. Normally, in horticultural practice all seeds are harvested at one moment in time. Therefore, a seed lot will consist of seeds of a wide variety of sizes, weights and maturities (Thomas et al. 1979).

Seed structure

A celery "seed" is a schizocarpic fruit; the true seed has a thin compressed seed coat , and contains a small axillary linear embryo, surrounded by living, thick-walled endosperm cells that occupy the bulk of the seed. (Jacobsen and Pressman 1979) (Fig.1). The seed is embedded in a pericarp that consists of lignified maternal tissue. In this thesis "seed" refers at all instances to the schizocarpic fruit and "true seed" refers to the seed of which the pericarp is removed.

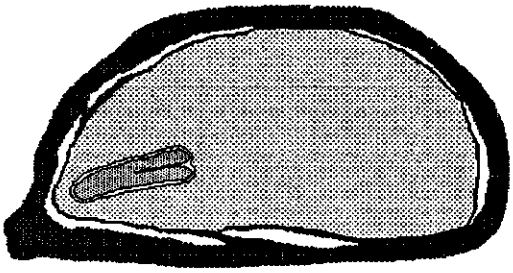


Figure 1. Schematic drawing of a longitudinal cross section of a celery seed.
■ Pericarp; ■ Testa; □ Air; ▨ Endosperm; ▩ Embryo.

Embryo growth and germination

Embryo growth

Like other Umbelliferae (Stokes 1952, Baskin and Baskin 1984) the small, immature embryo of a celery seed grows prior to visible germination from about 30% to 70% of the length of the true seed (Jacobsen and Pressman 1979). The growing embryo needs water, oxygen, light and nutrients. The first three factors have to be available in the environment of the seed. The nutrients are supplied by the endosperm. Jacobsen, Pressman and Pyliotis (1976) showed that in de-embryonated celery seeds endosperm breakdown is specifically induced by applied gibberellin (GA_3 or $GA_{4/7}$). In intact seeds light was a prerequisite for endosperm breakdown (Jacobsen and Pressman 1979). These results indicate that the stimulus for endosperm breakdown emanates from the embryo in respons to light and may be a gibberellin.

Influence of the pericarp on embryo growth

There are no reports on a restriction of water uptake by the pericarp or seed coat of celery seeds, as is the case with other species (Ballard 1973, Rolston 1978). However, oxygen uptake by the embryo may be severly limited by the pericarp. Celery seeds contain coumarins and coumarin derivatives (Ceska, Chaudhary, Warrington and Ashwood-Smith 1987; Jain, Sharma, Gupta and Boyd 1986), which are presumably located in the pericarp. In beet, barley and oat seeds phenolic acids in the tissues that surround the embryo consume the oxygen during imbibition and thereby inhibit germination (Coumans, Côme and Gaspar 1976, Corbineau, Lenoir, Lecat and Côme 1984). Phenolic substances may also inhibit germination directly as has been shown with other species (Nutile 1945, Mayer 1953).

Osmotic regulation of nutrient supply

During germination a large part of the endosperm is digested by the growing embryo. The endosperm breakdown is most likely induced by the embryo through a gibberellin. It is suggested that gibberellin causes the endosperm to produce hydrolases (Jacobsen et al. 1976, Jacobsen and Pressman 1979). Therefore, the embryo growth rate may depend on the rate of endosperm breakdown. In barley seeds the osmotic potential which builds up in the seeds as a result of the endosperm breakdown, regulates the production of the hydrolases by the aleurone layer. (Jones and Armstrong 1971). It is possible that also in celery seeds the osmotic potential in the seeds regulates the endosperm breakdown , and thereby the embryo growth.

Germination

Celery seed germination is slow due to the embryo growth that preceeds radicle protrusion. Germination also occurs irregular, which may be caused by differences in embryo growth rate of seeds within a lot. Optimal germination conditions are alternating temperatures and light (Thompson 1974). Cultivars differ in light requirement and in the ability to germinate at constant temperatures (Pressman et al. 1988).

Celery seed priming

The slow and irregular seed germination makes celery not very suitable for horticultural practice. Due to the high degree of mechanization in modern plant cultivation systems fast and uniform germination in combination with high germination percentages are highly desirable for a cultivated crop.

Seed quality can be improved in several ways. Breeding and selection are the most fundamental approach, but these methods are expensive and time consuming. Moreover certain genetic linkages between desirable and non-desirable characteristics may hinder the improvement of seed quality. In celery, for instance, bolting resistance and seed dormancy showed a positive correlation (Thomas 1978). Treatments during seed production are another possibility to improve seed quality. Spraying of the mother plants with growth regulators ($GA_{4/7}$ in combination with BA, ethephon, daminozide or benomyl) reduced seed dormancy and improved rate and percentage germination of the harvested seeds under laboratory conditions (Thomas and O'Toole 1980, 1981).

However, seed priming (Heydecker 1974) seems the most promising method to improve the quality of celery seeds. Heydecker, Higgins and Gulliver (1973) introduced osmotic priming of seeds as a method to improve the germination rate and uniformity of horicultural crops. The treatment consists of an incubation of the seeds during a certain time (for celery usually 14 days) at a certain temperature (for celery usually 15 °C) in an osmoticum of -1.0 to -1.5 MPa, made up of salt or polyethylene glycol with a molecular weight of 6000 (PEG 6000) dissolved in water. Due to the

osmotic potential of the incubation medium the water uptake of seeds is limited during the treatment. It has been suggested that several metabolic processes that preceed visible germination like mobilization of storage materials occur during the treatment (Khan, Tao, Knypl and Borkowska 1978, Khan, Peck and Samimy 1980/81). However, the seed water content is too low for visible germination to occur.

Considerable efforts have been made to apply the treatment to seeds of celery. Published results of these studies (Salter and Darby 1976, Darby, Salter and Whitlock 1979, Haarman 1980, Brocklehurst, Rankin and Thomas 1982, Brocklehurst and Dearman 1983) indicate that the treatment more than doubles the germination rate, increases the uniformity of germination, and raises the upper-temperature limit for germination.

However, succesful priming in celery is hindered in practice by the different reaction of varieties and even seed lots of one variety to a standard priming treatment (Heydecker and Coolbear 1977, Brocklehurst and Dearman 1983, Cantliffe, Elbella, Guedes, Odell, Perkins-Veazie, Schultheis, Seale, Shuler, Tanne and Watkins 1987, Globerson and Feder 1987). The mechanisms involved in seed priming of celery has as yet not been studied.

The present study

Improvements in rate and uniformity of celery seed germination may be realized by manipulating embryo growth preceeding radicle protrusion. To perform seed manipulations it is essential to know the factors that are involved in the regulation of embryo growth. In earlier work (unpublished results) we found that fast and slow germinating seeds can be separated by fluid density separation (Taylor, McCarthy and Chirco 1982). In the present study we will apply the method to analyze the factors involved in embryo growth of genetic uniform but physiologically different seed batches. The results of the analyses will be used to interpret the effects of priming treatments of those same seed batches. In addition, observations in genetically uniform seeds will be compared to results obtained with genetically different seed batches. Such comparisons may lead to general rules about the control of germination rate and to the explanation of the beneficial effect of priming treatments.

Materials & Methods

Seed lots (chapters 3 to 8)

In the experiments described in chapter 3 seeds of the root celery Monarch lot 9092 (Hild, Germany), the green celery Tall Utah 52-70R lot 10421 (Ferry Morse, USA), and the self blanching celery Latham Selfira lot 30044 (Bejo, Netherlands) were used. Those seeds were all harvested and received in 1985.

For the seed production experiments (chapter 4) seeds of root celery Mars (seedlot number unknown, Hild, Germany), Monarch 9092, Tall Utah 10421, Selfira 30044, and Tall Utah 701607 (Nunhems, Netherlands, produced in 1987) were used.

In the experiments described in chapters 5, 6 and 7 seeds of Monarch lot 9092 were used.

The priming studies (chapter 8) were performed with ten seed lots including cultivars of root celery, green celery and self-blanching celery (Tab. 1). Seeds were kept in plastic containers at 3 °C until use. Moisture content of the seeds of the different cultivars was 8.5 ± 0.6%.

Table 1. Origin of celery cultivars used in the priming experiments described in chapter 8.

type	cultivar	harvest	origin
root celery	Monarch 9092	1985	Nunhems/Hild
	Monarch 9081	1985	Nunhems/Hild
root celery	Alba	1985	Nunhems/Hild
root celery	Arvi	1984	Nickerson
root celery	Iram	1984	Clause
green celery	Tall Utah 10421	1985	Ferry Morse
	Tall Utah 601501	1986	Nunhems
self blanching celery	Elio	1986	Nunhems
self blanching celery	Celebrity	1986	Asmer
self blanching celery	Selfira	1985	Bejo

Seed production (chapter 4)

Seeds from different umbels of the cultivar Mars (a selection from cv. Monarch) were obtained from a production field in Marbach/Neckar, southern Germany, during

the season 1986. From time of flowering (beginning of August) to time of harvest (beginning of October) temperature was recorded by a thermohygrograph. Primary, secundary, tertiary, and quarternary umbels were collected separately from 8 plants on the same day, dried for two weeks in a glasshouse (temperature and R.H. not registred) and subsequently for one week in a commercial seed drier (20°C, 30% R.H.). In the same production field 5 plants were protected by wind shields in order to change the microclimat around the umbels. These umbels were harvested on the same day as the other cultivations and further handled likewise.

Seeds from different umbels of cvs. Monarch, Selfira and Tall Utah were harvested from plants grown in ethylene tunnels in Nunhem in the south of the Netherlands during the season 1987. Temperature was recorded from beginning of flowering in June till end of harvest in September. Primary, secundary, tertiary, and quarternary umbels were collected separately from 8 plants and dried for two weeks in a drying cabinet (Van den Berg, Montfoort, The Netherlands) at 25°C and 30% R.H. in the light.

Seeds from different umbels of cv. Tall Utah were harvested from plants grown in a greenhouse in Grientsveen in the south of the Netherlands during the season 1988. Of each umbel type 12 umbels were harvested every week starting 5 weeks after flowering of the umbels. Umbels were dried for two weeks at 25 °C, 30% R.H. in the light.

Seeds were harvested by hand from dried umbels and cleaned using sieves and an intend cylinder. Twin seeds were rubbed between hands. Care was taken to keep the loss of small seeds to a minimum (< 5%).

Density grading (chapters 3 to 8)

The seed separation technique was based on Taylor et al (1982). 50 gram of a seed lot was added to 200 ml of a mixture of hexane and chloroform with a certain calculated density . After 15 s whirling and 40 s rest, the floating seed fraction was removed by decanting the solvents into a strainer. Both floating and sunken seed fractions were dried for 15 min on filter paper. The procedure was repeated with the sunken seed fraction in a solvent mixture with a higher density, until the sunken seed fraction contained less than 2.5 g seed. The density grading was repeated three times for each cultivar.The density of the solvent mixtures was calculated according to Taylor et al (1982) and ranged between 0.90 $mg.mm^{-3}$ and 1.48 $mg.mm^{-3}$.

Mean density of seed fractions harvested from separate umbels or obtained with the seed separation technique (if the difference in density of two succeeding solvent mixtures was larger than 0.02 $mg.mm^{-3}$) was calculated from the weight of 3 replicates of 2 g seeds in air and cloroform, according to the method described by Tupper, Clark and Kunze (1970).

Seed structure parameters (chapters 3,4,5,8)

The mean seed fresh weight (sw) of each density fraction was calculated from the weight of 5 replicates of 100 seeds each (precision 0.1 mg). The mean seed volume (sv)

was calculated from the mean density and the mean seed fresh weight of that fraction.

From each density fraction of the 3 replicate density separation experiments the endosperm length (l) and endosperm width (w) were measured by means of a binocular with 40 times magnification in longitudinal cross sections of 20 seeds (precision 25 µm). From these data, the endosperm volume (ev) was calculated according to the formula:

$$ev = 1.33 \cdot \pi \cdot 0.5 \cdot l \cdot (0.5 \cdot w)^2. \quad (1)$$

The embryo is included in the calculated volume of the endosperm. Formula 1 describes the volume of an elipsoid. To check its validity for whole seeds the formula was used to calculate the seed volume. These data were compared to the volume data calculated from the seed density and seed weight. As an example, the sv of seeds of cv. Monarch lot 9092 with density 1.11 $mg.mm^{-3}$ calculated with formula 1 was 0.590 mm^3, while the sv derived from sw and sd was 0.607 mm^3. The deviation between the two calculated sv values was always less than 5%.

To measure the endosperm density (ed) and the pericarp density (pd), the pericarp was removed by gently abrading the seeds between two sheets of emery-paper (3M-210, P120). Each true seed was immersed individually in a petri dish (Ø 4 cm) containing 10 ml of a hexane-chloroform mixture with density 1.24 $mg.mm^{-3}$. When the true seed floated, it was removed from the mixture, surface dried and immersed in a hexane-chloroform mixture with a density of 1.22 $mg.mm^{-3}$. The procedure was repeated with mixtures of still lower density until the true seed sank. For each measurement 20 seeds were used. The embryo and the testa were included in the measured endosperm density. The collected pericarp material of the 20 seeds was immersed in hexane-chloroform mixtures with increasing density up to pure chloroform (density 1.48 $mg.mm^{-3}$) until it stayed in suspension.

From sw, sv, ev, ed and pd, the endosperm weight (ew), pericarp weight (pw), pericarp volume (pv) and air volume (av) were calculated with the following formulae:

$$ew = ev \cdot ed \quad (2)$$

$$pw = sw - ew \quad (3)$$

$$pv = pw/pd \quad (4)$$

$$av = sv - pv - ev \quad (5)$$

The surface to volume ratio used in chapter 5 was calculated from length (l) and width (w) data of 40 seeds of each fraction. The seed surface (ss) was calculated with the formula:

$$ss = 1.33 \cdot \pi \cdot (0.5 \cdot w^2 + 0.5 \cdot l \cdot w) \quad (6)$$

and the seed volume was calculated with formula 1.

Germination tests (chapters 3 to 8)

All germination tests were carried out in plastic boxes of 7 x 8 x 3 cm, with one layer of blotter paper (730 $g.m^{-2}$) and one layer of filter paper (132 $g.m^{-2}$) (Schut, Renkum, the Netherlands), moistened with 14 ml of distilled water. For each seed

fraction 5 replicates of 50 seeds were used. Germinated seeds were counted daily and removed from the boxes. Germination was defined as protrusion of the radicle through the pericarp.

The time to 50% germination of viable seeds or mean germination time (t_{50}), and spread in time to 50% germination of viable seeds (v) were calculated according to Orchard (1977):

$$t_{50} = \Sigma f \cdot x / \Sigma f \quad (7)$$

$$v = [\Sigma f \cdot (x - t_{50})^2 / (\Sigma f - 1)]^{0.5} \quad (8)$$

where f is the number of seeds germinated on day x. In these formulas days are arimethic days, that is the mean of two counting days.

In Fig. 1 hypothetical examples are depicted of germination time courses of three different seed lots. Seed lot A germinates quite rapidly with a t_{50} of 5.0 days. The v is

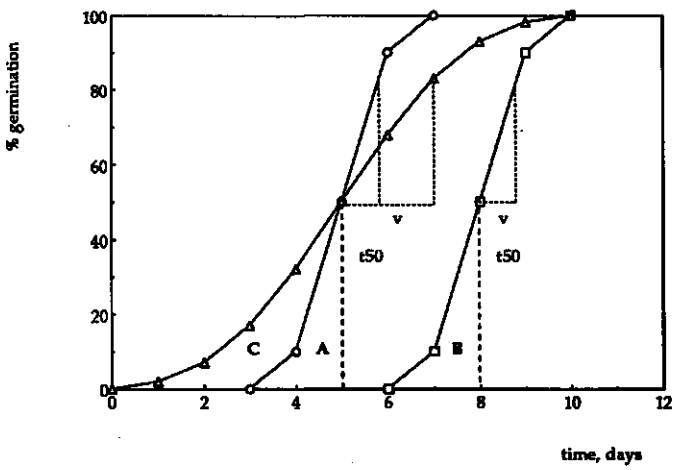


Figure 1. Cumulative germination in time of three imaginary celery seed lots, indicating the time to 50 % germination (t_{50}) and spread in the mean germination time (v), calculated according to the method of Orchard (1977).

0.8 days. Seed lot B germinates much later with a t_{50} of 8.0 days, but v is equal to lot A. Lot C has a t_{50} equal to seed lot A, but v is much larger, 2.0 days. Thus, both germination parameters are required to characterize the germination time course.

Seeds were incubated either at optimal germination conditions (8 h 15°C dark/16 h 20 °C light) (Thompson 1974) or at suboptimal conditions (constant 15°C, light or 8 h 15°C dark/16 h 20°C darkness) in an incubator (Zephyr, Zoetermeer, the Netherlands) with less than 1 °C deviation . In chapter 3 a range of constant temperatures was reali-

zed on a thermogradient table (Zephyr, Zoetermeer, the Netherlands). When germination occurred in the dark, the plastic germination boxes were placed in wooden boxes wrapped in black PVC foil and the final germination percentage was recorded after 21 days.

Germination at high O_2 pressure was tested in 500 ml beakers, each placed inversely in a 9 cm Petri dish and water-locked. Three 5 cm Petri dishes supplied with 1 layer of filter paper (Schleicher & Schüll no. 595) and 1.5 ml distilled water, were piled up in each beaker. A small hole covered with a rubber septum enabled addition of pure O_2 to the beaker with a hypodermic needle. Germination at low O_2 pressure was performed by increasing the amount of water in the germination boxes to 26 ml, a level at which the seeds were flooded.

Seed leaching (chapter 5)

To test the involvement of pericarp bound soluble factors in germination seeds were either pre-incubated during 5 h at 15 °C in a large volume of distilled water (500 seeds per 250 ml) or germination occurred at a hand-made Copenhagen table designed according to ISTA rules (International Seed Testing Association 1985).

Quantitative information on leached components was obtained for seeds of all density fractions (chapter 3). 3 replicates of 200 seeds per density fractions were leached in running tap water for 22 hours at 15 °C. Seed dry weight before and after leaching was measured. The difference between dry weights was the weight of the leached compounds. The amounts of leached electrolytes and, in particular, K^+ ions were measured with seeds of two large density fractions (density 0.97 and 1.15 mg.mm-3). Three replicates of 400 seeds packed in small gauze baskets (New Brunswick Scientific, USA, No. 331X) were incubated at 15 °C in 400 ml of distilled water. The water was stirred constantly. The conductivity of leachates was measured with a conductivity meter P 335 (Portland Electronics Ltd, Oldham, UK) with a dip-type cell PW 9514/60 (Philips, the Netherlands). The amount of leached potassium was measured with a flame photometer (Jenway, England, PFP 7) on 2 replicates of 100 seeds in 10 ml distilled water.

Location of phenols in the seed (chapter 5)

Seeds were incubated for 3 days in water at 15 °C. Seeds were washed and after removal of external water they were embedded in histoplast (Shandon, Zeist, The Netherlands) (melting point 56 °C). Serial sections (10 µm) were cut with a retracting microtome (LKB, Sweden, Rotary-one). The sections were removed from the histoplast, collected in 1 ml 0.5 M Tris buffer (pH 9.0) and stained with 1 ml Gibb's reagent (0.5 mg 2.6-dichloroquinon-4-chlorimide (Sigma, USA) dissolved in 1 ml ehanol). The serial sections were photographed on Kodak Ektachrome 200 film with 50 times magnification by means of a Nikon (Japan) Optiphot microscope. The amount of phenol in the seeds was measured by the method of Stafford (1960) who also applied Gibb's reagent. Fifty seeds were grounded in 3 ml 0.5 M Tris buffer (pH 9.0). After centrifugation

(2000 g during 30 min), 500 µl of the supernatant was used for the measurements. Color intensity was measured with a spectrofotometer using guaiacol (Sigma, USA) as a reference.

Water content (chapters 5,6,8)

Seed moisture content was measured with the oven method according to ISTA rules (International Seed Testing Association 1985) with 3 replicates of about 1000 seeds. Fresh weight was measured after removal of external water with filter paper. Dry weight was measured after drying for 90 min at 130 °C.

Moisture content of true seeds was measured with the oven method on 3 or 5 replicates of 20 seeds. Seeds were incubated at 15 °C in light. Thereafter the pericarp was removed and the fresh weight of the true seeds was measured. To minimize the error in fresh weight measurement as a result of evaporation during preparation (that took about 15 minutes for 20 true seeds) the true seeds were collected in a vial containing 0.5 ml water. The difference in weight of the vial before and after preparation of the true seeds was the true seed fresh weight. To correct for the loss of weight due to evaporation from the 0.5 ml water, a control vial containing the same amount of water was weighted at the same times. Dry weight was measured after drying for 2 hours at 130 °C instead of 90 min because of the excessive amount of water in the vials containing the true seeds.

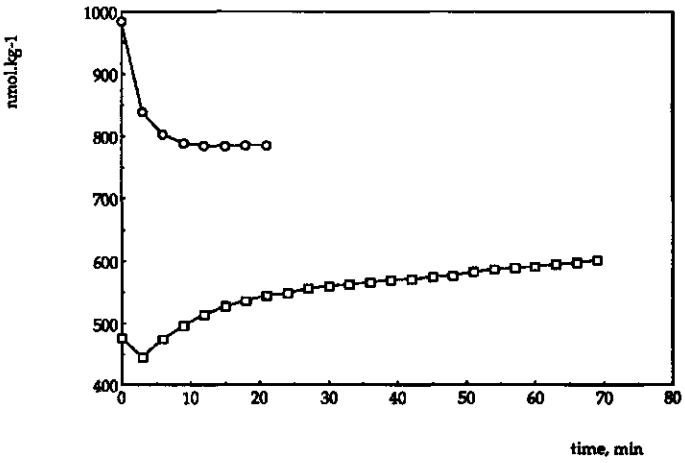


Figure 2. Time course of psychrometer readings after closure of the measuring chamber. Typical examples of ψ (□) and ψ_π (○) readings are shown. In routine measurements readings were taken after 30 min for ψ and after 15 min for ψ_π.

Incubation conditions in studying embryo growth (chapter 6)

To study embryo growth special germination conditions were used: 500 seeds were incubated at 15 °C, 12 h light,12 h dark, in plastic boxes of 18 x 14 x 6 cm, with one layer of blotter paper (730 $g.m^{-2}$), four layers of filter paper (132 $g.m^{-2}$) (Schut, Renkum, the Netherlands), and 100 ml distilled water or a solution of polyethylene glycol, molecular weight 6000 (PEG 6000). The boxes were packed each in plastic bags to reduce evaporation. Osmotic potential of PEG solutions were calculated according to Michel (1983).

Embryo measurement (chapter 6)

Embryo length and width were measured by means of a binoculair with 40 times magnification. Each observation was the mean of measurements on 40 or 60 longitudinally cut seeds. From embryo length (l) and width (w) the surface area of longitudinal cross sections (ea) was calculated with the formula:

$$ea = 0.5 \cdot l \cdot 0.5 \cdot b \cdot \pi \qquad (9)$$

For the anatomical studies five or ten seeds were used per measurement. On both longitudinal sides of a seed slices were cut to permit entry of chemicals. Seeds were fixed by immersion in 4% formaldehyde for 24 hours under reduced atmospheric pressure (-60 KPa) in a vacuum desiccator. After fixation the seeds were dehydrated in increasing concentrations of alcohol starting with ethanol 50%. The seeds were embedded in glycolmethacrylate according to the method of Gerrits and Smid (1983), in a vacuum desiccator at -60 KPa. Serial sections of 6 µm were cut with a retracting microtome (LKB Rotary-one) provided with a D-knive with tungsten carbide cutting edge. After drying, staining of the serial sections occurred with 0.15 $mg.ml^{-1}$ 4,6-diamidino-2-phenyl-indole (DAPI) dihydrochloride. Serial sections were mounted in DePeX (Gurr).

Between 80 and 100 serial sections were cut from each seed. The serial section with the longest longitudinal cross section through the embryo was photographed on Kodak Ektachrome 200 film with 50 times magnification, using a Nikon Optiphot microscope with Osram HBO mercury lamp 100W/2 and Nikon UV-2a filtercombination.

Slides were projected and of a fixed area in the hypocotyl region just below the apex meristem the number of cells was counted. With these data the number of cells on the total surface area of the cross section of the embryo (formula 9) and mean cell size were calculated.

Water and osmotic potential measurements (chapter 6)

Potentials were determined with a thermocouple psychrometer (Wescor 5500, Logan, USA). Seeds were incubated for 3 days in water or for 7 days in -1.2 MPa PEG at 15 °C in light. At least 3 replicates of 10 seeds were used per measurement. All seed manipulations were performed in a hand made humidity cabinet (22 - 24 °C, > 90%

R.H.) to prevent evaporation. Seeds were shortly rinsed in distilled water and free water was removed with filter paper. True seeds were prepared by removal of the pericarp. Water potential was determined by placing the seeds or the true seeds in the measuring chamber (2.3 mm deep, 7.2 mm diameter). To reduce moisture loss the measuring chamber was covered with a glass cover slip between the placement of the seeds or true seeds in the chamber. 30 min were required to achieve vapour equilibrium (Fig. 2). To measure the osmotic potential of the same tissue it was removed from the psychrometer and frozen inside aluminium foil packets placed in liquid N_2. Frozen tissue was returned to the chambers to thaw before measurement. Vapour equilibrium was reached in 15 min. (Fig. 2). Therefore all readings were taken after 15 min.

Osmolality readings in $mmol.kg^{-1}$ were transformed into MPa by means of a reference curve made with PEG solutions. The osmotic potential of the solutions was calculated according to Michel (1983).

Sugar determination (chapter 7)

100 seeds were grounded for 2 min in a mortar in 4 ml Tris buffer (pH 7.0). Trichloroacetic acid was added to a final concentration of 7%. After centrifugation for 15 min. at 1500 g samples were frozen at -20 °C until use. Reducing carbohydrates were measured with the 2-cyanoacetamide method of Honda, Nishimura, Takahashi, Chiba and Kakehi (1982).

Endo-ß-mannanase activity (chapter 7)

Endo-ß-mannanase activity was assayed viscosimetrically. Enzyme prepararations were made by grounding 50 or 100 seeds in a mortar in 1.5 ml phosphate/citrate buffer (pH 5.0; 0.2/0.1 M) on ice and spun in an Eppendorf centrifuge at 3°C for 15 min. The supernatant was removed and frozen (-20 °C) until use. Locust-bean galactomannan (Sigma Chemical Co., USA) was purified according to Halmer, Bewley and Thorpe (1975) and dissolved in distilled water. The assay mixture consisted of 10.0 ml galacto-mannan substrate, 5.0 ml phosphate/citrate buffer and 200 µl enzyme preparation. Decrease of viscosity of the assay mixture in a glass Oswald viscosimeter at 36 °C, was monitored by measuring the decrease in time of flow of the viscosimeter-graduated volume (10 ml) during 30 min. Initial flow time of the assay mixture was about 160 seconds, flow time of distilled water was 30 seconds. Control incubation was performed with boiled enzyme preparation.

Priming treatments (chapter 8)

To prime, seeds samples of 5 g were packed in nylon gauze and incubated at 15 °C in continuous light in 20 liter PEG 6000 solutions in perspex columns that were constantly aerated. The osmotic potential of PEG solutions was calculated according to Michel (1983). During priming water was added daily to compensate for evaporation. The length of priming treatments varied between experiments.

The relationship between relative proportions of seed parts and germination of celery (*Apium graveolens* L.).

Summary

Fluid density grading was used to separate seed lots of celery cvs. Monarch, Selfira and Tall Utah into different density fractions. Seed structure parameters and germination parameters of the different fractions were measured or calculated. In seeds of all cultivars increased seed density corresponded with a reduction in the relative volume of air. In seeds of cvs. Monarch and Selfira seed density correlated positively with the relative volume of the pericarp and negatively with the remaining constituents of the dry seed, air and endosperm. For these two cultivars the time to 50% germination in light increased with a higher seed density, in seeds of cv. Selfira also the spread in germination time increased. In darkness the higher density fractions of cv. Monarch showed reduced germination percentages at optimal temperatures. The maximum germination temperature of high density seeds was lower than of low density seeds.

The time to 50% germination was a function of the embryo growth rate in the first days of the germination process. It is hypothesized that embryo growth in high density seeds may be inhibited either by pericarp constituents or by the more negative osmotic potential of the seeds.

Introduction

In horticultural practice celery (*Apium graveolens* L.) seeds are notorious for their slow and irregular germination. One of the possibilities to improve the germination rate and uniformity of celery seed lots could be the application of techniques to separate fast germinating from slow germinating seeds. To develop such techniques it is necessary to study the relationship between physical seed parameters and germination.

Taylor et al. (1982) and Taylor and Kenny (1985) introduced a liquid density separation technique based on mixtures of hexane and chloroform to separate seed lots of cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.) into different density fractions. In the present study the method was applied to seed

lots of three celery cultivars. The seed structure and the germination characteristics of the different fractions were studied . A celery "seed" is a schizocarpic fruit; the true seed has a thin-walled, compressed seed coat, and contains a small axillary linear embryo, surrounded by living thick-walled endosperm cells that occupy the bulk of the seed. (Jacobsen & Pressman 1979) (see chapter 1 Fig. 1). Dry seeds contain air between the seed coat and the pericarp and within the pericarp.

Results

The relation between seed density and seed structure

The density separation resulted in 12 seed fractions for cv. Monarch, and 8 seed fractions each for cvs. Tall Utah and Selfira. The fractions with the lowest and highest density contained relatively few seeds; 1.6%, 1.7% and 2.5% of the total number of seeds in the lowest density fraction, and 1.3%, 5.0% and 4.5% in the highest density fraction, in cv. Monarch, Selfira and Tall Utah, respectively, and had low germination percentages due to embryo abortion or the absence of endosperm development. Therefore these fractions were ommitted from further analysis.

The different structural parameters of the other density fractions were measured and calculated. As indicated in chapter 2 the basic observations for the different calculations were in the first place the determinations of seed weight and endosperm width and length. It is shown beneath that those parameteres clearly differed between density fractions. The calculations also required knowledge about the densities of endosperm and pericarp. Those parameters did not differ between density fractions. The mean density of the endosperm mass (including the embryo) for all tested fractions of the three cultivars was 1.214 $mg.mm^{-3} \pm 0.003$ $mg.mm^{-3}$, the fractions did not differ significantly ($P < 0.01$). For all tested fractions the pericarp tissue abraded from the seeds stayed in suspension or sank very slowly in the solvent with the highest density (1.48 $mg.mm^{-3}$). Therefore, we used this value in our further calculations of the seed structure as the density of the pericarp for all seed fractions.

The density of the seeds of cv. Monarch that were used in this study ranged from 0.93 $mg.mm^{-3}$ to 1.165 $mg.mm^{-3}$ (Tab. 1). With increasing seed density the seed weight increased from 0.631 mg to 0.696 mg, and the seed volume decreased from 0.678 mm^3 to 0.598 mm^3. Since the endosperm weight slightly decreased with increasing seed density the increase of seed weight was mainly due to an increase in pericarp weight from 0.325 to 0.434 mg. Because the densities of endosperm and pericarp were constant in all fractions the changes in weight of those seed parts were directly translated into changes in volume. Thus, at higher densities the endosperm volume slightly decreased and the pericarp volume rose considerably. As a consequence, the smaller seed volume at higher seed densities was mainly due to a massive decrease of the air volume from 0.206 to 0.089 mm^3. Fig. 1a illustrates the relative contributions of the

volumes of endosperm, pericarp and air to total seed volume in the fractions of cv. Monarch.

Table 1. Volumes and weights of seeds and seed parts, expressed per seed, and germination parameters of different density fractions of celery cv. Monarch. Mean seed density of total seed lot is 1.052 mg.mm^{-3}.

density fraction, mg.mm^{-3}	weight, mg			volume, mm^3				germination, %	t_{50}, days	v, days
	endosp.	peric.	seed	endosp.	peric.	air	seed			
0.930	0.306	0.325	0.631	0.252	0.220	0.206	0.678	96.7	5.2	0.8
0.980	0.312	0.326	0.638	0.257	0.220	0.174	0.651	97.9	5.2	0.8
1.010	0.316	0.320	0.636	0.260	0.216	0.154	0.630	98.1	5.2	0.7
1.030	0.317	0.324	0.641	0.261	0.219	0.143	0.623	97.2	5.4	0.7
1.050	0.306	0.336	0.642	0.252	0.227	0.133	0.612	98.5	5.8	0.8
1.070	0.310	0.347	0.657	0.255	0.235	0.124	0.614	96.7	6.0	0.8
1.090	0.302	0.364	0.666	0.249	0.246	0.116	0.611	96.2	6.2	0.8
1.110	0.294	0.380	0.674	0.242	0.257	0.108	0.607	95.7	6.5	0.8
1.135	0.287	0.402	0.689	0.236	0.272	0.099	0.607	95.3	6.8	0.8
1.165	0.262	0.434	0.696	0.216	0.293	0.089	0.598	97.1	7.0	0.8
seed lot								96.4	5.9	1.4

The seeds of cv. Selfira (Tab. 2) were much smaller and lighter than the seeds of cv. Monarch. The density of the Selfira seeds ranged from 1.11 mg.mm^{-3} to 1.21 mg.mm^{-3}

Table 2. Volumes and weights of seeds and seed parts, expressed per seed, and germination parameters of different density fractions of celery cv. Selfira. Mean seed density of total seed lot is 1.170 mg.mm^{-3}.

density fraction, mg.mm^{-3}	weight, mg			volume, mm^3				germination, %	t_{50}, days	v, days
	endosp.	peric.	seed	endosp.	peric.	air	seed			
1.110	0.174	0.119	0.293	0.143	0.081	0.040	0.264	94.5	6.5	1.1
1.130	0.168	0.123	0.291	0.138	0.083	0.037	0.258	97.9	6.5	1.2
1.150	0.144	0.147	0.291	0.119	0.099	0.035	0.253	96.8	6.8	1.4
1.170	0.142	0.145	0.287	0.117	0.098	0.030	0.245	95.7	7.2	1.6
1.190	0.142	0.136	0.278	0.117	0.092	0.025	0.234	94.0	7.9	1.7
1.210	0.121	0.145	0.266	0.100	0.098	0.022	0.220	92.1	8.7	2.0
seed lot								94.1	7.4	2.4

With increasing seed density both seed weight and seed volume decreased, from 0.293 to 0.266 mg, and from 0.264 to 0.220 mm^3, respectively. Because the endosperm weights and volumes decreased with increasing density of the fractions the weight and volume of the pericarp had to be increased. As a consequence, the relative proportion of the volume of the pericarp to total seed volume also increased in cv. Selfira (Fig. 1b). The air volume was not so strongly affected as in cv. Monarch.

The mean seed weight and seed volume of cv. Tall Utah were slightly higher than for cv. Selfira, but the seed density was much lower, it ranged from 0.98 $mg.mm^{-3}$ to 1.09 $mg.mm^{-3}$ (Tab. 3). With increasing seed density the seed weight slightly increased but the seed volume remained constant. Since the increase in seed weight was only partly explained by an increase in endosperm weight the pericarp weight also had to be increased. The constant seed volume in combination with slight increases in endosperm and pericarp volumes pointed to a decrease in air volume (Fig 1c).

Table 3. Volumes and weights of seeds and seed parts, expressed per seed, and germination parameters of different density fractions of celery cv. Tall Utah. Mean seed density of total seed lot is 1.033 $mg.mm^{-3}$.

density fraction, $mg.mm^{-3}$	weight, mg			volume, mm^3				germination, %	t_{50}, days	v, days
	endosp.	peric.	seed	endosp.	peric.	air	seed			
0.980	0.174	0.161	0.335	0.143	0.109	0.089	0.341	94.5	6.5	1.3
1.010	0.197	0.152	0.349	0.162	0.103	0.081	0.346	94.7	6.3	1.1
1.030	0.187	0.173	0.360	0.154	0.117	0.078	0.349	95.2	6.4	1.2
1.050	0.200	0.160	0.360	0.165	0.108	0.070	0.343	93.8	6.4	1.2
1.070	0.195	0.171	0.366	0.161	0.115	0.066	0.342	94.8	6.5	1.2
1.090	0.195	0.174	0.369	0.161	0.117	0.060	0.338	92.0	6.7	1.4
seed lot								93.8	6.5	1.8

Regression analyses of the seed structure parameters involved showed that for all cultivars seed density was significantly correlated with relative air volume (Tab. 4). For cvs. Monarch and Selfira also the relative pericarp volume had a significant influence on the seed density. The relative endosperm volume did not affect the seed density in any cultivar.

The relation between seed density and germination parameters

The seeds of all density fractions were germinated at 15/20 °C in light. The germination percentage, time to 50% germination (t_{50}), and spread in time to 50% germinati-

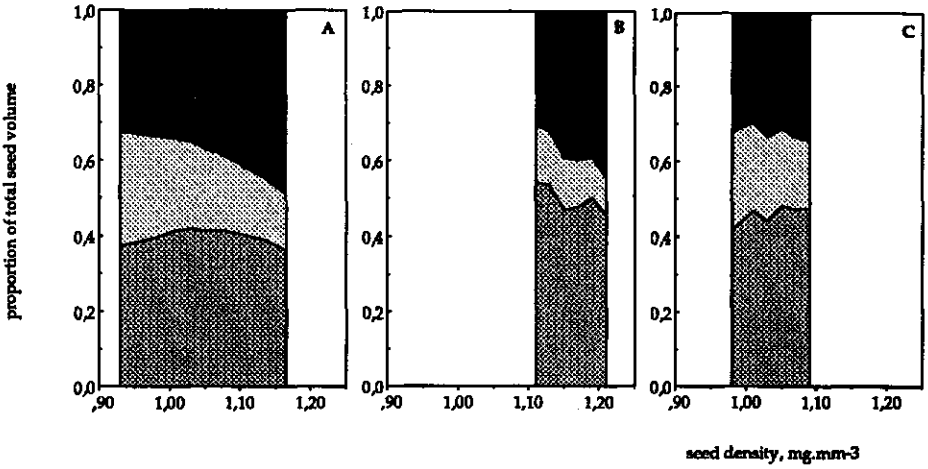


Figure 1. Relative proportion of endosperm, air, and pericarp of cvs. Monarch (A), Selfira (B), and Tall Utah (C) in relation to seed density.
▨ Endosperm; ▩ Air; ■ Pericarp.

Table 4. Regression coefficients between seed density and seed structure parameters of celery cvs. Monarch, Selfira and Tall Utah. Asterix indicates significance ($p < 0.025$)

	regression coefficients		
	relative volumes of		
	endosperm	pericarp	air
Monarch	0.15	0.96*	1.00*
Selfira	0.80	0.94*	0.99*
Tall Utah	0.77	0.65	1.00*

on (v) are presented in Tab. 1, 2 and 3. Final germination percentages of all seed fractions of all three cultivars was hardly affected by seed density.

With increasing seed density, the t_{50} of cv. Monarch rose from 5.2 day to 7.0 day, whereas v varied from 0.7 day to 0.8 day. The t_{50} of cv. Selfira increased from 6.5 to 8.7 day and v from 1.1 day to 2.0 day. The t_{50} of cv. Tall Utah was about 6.4 day and did not vary with seed density. The v of this cultivar was slightly lower than of cv. Selfira, it remained more or less constant.

Calculations of regression coefficients between seed density and either t_{50} or v (Tab. 5 first column) showed that t_{50} correlated with seed density in cvs. Monarch and Selfira whereas v showed a correlation with seed density in Selfira only.

Relations between seed structure parameters and germination parameters

Because in cvs. Monarch and Selfira seed density was a function of the relative volume of air and pericarp (Tab. 4), and was correlated positively with t_{50} (Tab. 5), significant correlations between these factors were expected. Indeed, the relationship between the relative volume of pericarp and the t_{50}, and between relative volume of air and the t_{50}, was significant for both cultivars (Tab. 5). In cv. Tall Utah the t_{50} was not correlated with seed density (Tab. 5), and the latter was only correlated with relative air volume and not with relative pericarp volume (Tab. 4).

For all cultivars no correlation was found between density and relative endosperm volume (Tab. 4), and between the t_{50} and relative endosperm volume (Tab. 5)

These results indicate that the t_{50} is most probably a function of the relative pericarp volume.

In cv. Selfira seed density and v were significantly correlated (Tab. 5). Because the correlation between relative volumes of all three seed parts with v were also significant (Tab. 5) it is not possible to conclude which factor is involved in the control of v.

Table 5. Regression coefficients between seed density, seed structure parameters and germination parameters of celery cvs. Monarch, Selfira and Tall Utah. Asterix indicates significance ($p < 0.025$)

		regression coefficients			
		density	relative volumes of endosperm	pericarp	air
t_{50}	Monarch	0.95*	0.38	0.98*	0.94*
	Selfira	0.95*	0.68	0.85*	0.96*
	Tall Utah	0.62	0.18	0.77	0.58
v	Monarch	0.46	0.49	0.57	0.43
	Selfira	0.99*	0.84*	0.95*	0.97*
	Tall Utah	0.57	0.13	0.74	0.53

Relation between seed density and germination at suboptimal conditions

Apart from germination at optimal conditions (15/20 °C, light) the density fractions 0.98 , 1.03, 1.07, 1.11 and 1.165 mg.mm^{-3} of cv. Monarch were also germinated at

less optimal conditions. A range of constant temperatures from 11 ° to 31 °C was tested in light. Because the temperature-germination curves only differed in the maximum-temperature cut-off-point, the maximum temperature for 90% germination was taken as a parameter for the influence of high temperature on germination (Tab. 6). This test revealed more pronounced differences in germination between the density fractions than at 15/20 °C. The maximum temperature for 90% germination dropped from 25.5 °C for seeds with a density of 0.980 mg.mm^{-3} to 17.9 °C for seeds with density 1.165 mg.mm^{-3}.

The effect of the density of the seeds was also more pronounced when the seeds were germinated at 15/20 °C in darkness instead of light (compare Tab. 6 with Tab. 1).

Table 6. Germination parameters for different density fractions of celery cv. Monarch.

density fraction, mg.mm^{-3}	maximum temperature for 90 % germination,°C	germination at 15/20 °C, dark, %
0.980	25.2	64.3
1.030	24.6	53.5
1.070	23.4	37.2
1.110	20.7	21.5
1.165	17.9	14.9

Relation between t_{50} and embryo growth rate

Because in celery the embryo grows to about twice its size prior to visible germination (Jacobsen and Pressman 1979) differences in t_{50} may be caused by differences in embryo growth rate. Therefore the embryo growth rate between 0 and 3 days after the start of incubation of different density fractions ranging from 0.930 to 1.165 mg.mm^{-3} of cv. Monarch was measured and plotted against t_{50}. The relation was significant ($p < 0.005$) (Fig. 2), indicating that indeed t_{50} depends on the embryo growth rate.

Discussion

The present results showed that the time to 50% germination was a function of the embryo growth rate during the first days of germination (Fig. 2). Therefore influences of seed structure parameters on t_{50} could be interpreted as influences on embryo growth.

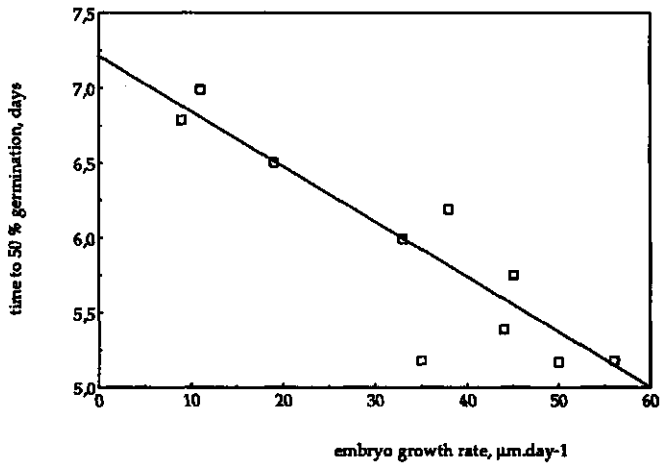


Figure 2. The relation between embryo growth rate and time to 50 % germination in celery seeds. Each point refers to one density fraction of celery cv. Monarch lot 9092. (R=0.90).

The correlation coefficients of the relations between density, seed structure parameters and t_{50} indicate that the relative proportion of the pericarp may be the most important factor involved (Tabs. 4,5).

There are several mechanisms through which the pericarp can influence the embryo growth and subsequent germination. Celery seeds contain coumarins and coumarin-derivatives (Ceska et al. 1987; Jain et al. 1986). If we assume that these compounds are localized in the pericarp, seeds with high density have to contain more of these compounds. It has been shown that phenolic compounds can inhibit seed germination in lettuce (Nutile 1945, Mayer 1953). It has been suggested that a higher temperature increases the inhibitory activity of phenolic compounds (Corbineau, Lecat and Côme 1986). For celery seeds such an interaction should result in a stronger negative influence of supra-optimal temperatures on germination of seeds with a larger proportion of pericarp. The present data are not in contrast to this hypothesis. The phenolic compounds may directly inhibit metabolic processes necessary for embryo growth, or may interact with oxygen uptake (Lenoir, Corbineau and Côme 1983).

It has to be realized, however, that the positive relationship between t_{50} and the relative volume of the pericarp means a similar but negative relationship with the sum of endosperm and air volume. Most likely the air will disappear from the seeds upon imbibition. Therefore, the smaller the relative proportion of the endosperm the slower the germination. In chapter 6 a hypothesis will be tested that relates the relative volume of the endosperm to the osmotic potential of the seed. In a smaller volume the

osmotic potential inside the pericarp may drop to lower negative potentials that are inhibitory to endosperm hydrolysis (Jones and Armstrong 1971) and to embryo growth.

The present data confirmed the usefulness of the fluid density separation technique (Taylor and Kenny 1985, Taylor et al. 1982) in seed research. The method separates genetically uniform but physiologically different seeds.

For cv. Tall Utah the separation did not result in differences in t_{50} of the density fractions. This may be due to seed production or seed processing methods applied by the supplier of the seed lot. It is also possible that this cultivar is more uniform than other cultivars.

Only for cv. Selfira v is correlated with seed density. From the results (Tab. 5) it is not clear which factors may be involved in the regulation of v.

The origin of variation in seed density in celery (*Apium graveolens* L.).

Summary

To obtain information about the origin of variation in seed density in celery, seeds were harvested separately from the different umbels that are formed subsequently on celery plants. The study involved seeds from primary to quarternary umbels, from the cultivars Mars, Monarch, Selfira and Tall Utah. Increasing umbel position resulted in seeds with a lower germination capacity, an extended mean time to 50 % germination and a larger spread in germination time. Seed density also increased with increasing umbel position. Seed density was not correlated to umbel position as such , but to the length of the seed ripening period that differed per umbel type, and to the temperature during ripening. The decrease in seed density during seed development correlated with an increase in air volume in the seeds. It was concluded that the variation in seed density in celery seed lots originated from differences in seed maturity.

Introduction

Celery plants have a complicated flowering structure. The umbels can be divided into four categories that flower after each other (Thomas et al. 1979). Normally, seeds of all umbels are harvested simultaneously. It always occurs at a moment that the seeds of subsequent umbels are still in different stages of development. In comparison to seeds of primary umbels seeds of the quartenary umbels have a lower seed weight, a higher germination capacity, an extended time to 50 % germination (t_{50}), a lower maximum-temperature cut-off-point for germination, and a larger requirement for externally apllied gibberellins when germination occurred in the dark at temperatures otherwise inhibitory to germination (Thomas et al. 1979, Thomas, Gray and Biddington 1978, Thomas and O'Toole 1980, 1981).

It was shown in chapter 3 that the t_{50} is positively correlated with seed density, which in turn is positively correlated to the relative volume of the pericarp and negatively related to the volume of the remaining constituents of the dry seed, the endosperm and the air volume.

In this study it will be analyzed therefore, whether increasing umbel order is positively correlated to a higher seed density. Because differences between umbels can either be due to the umbel position itself or to differences in the length of seed develop-

ment and maturation time, the effect of the latter variable will also be analyzed.

Results

Germination experiments with seeds harvested in 1986 from different umbels of plants of cv. Mars showed that with increasing umbel order the germination percentage decreased from 93.5 % to 85.0 % whereas t_{50} and spread in mean germination time (v) increased, from 6.1 to 10.3 and from 0.9 to 2.0 days, respectively. (Tab.1).

Table 1. Seed parameters of seeds from different umbels of celery cv Mars. Germination parameters were measured at 15/20 °C in light.

	Primary & secondary	Tertiary	Quarternary
number of seeds, % of total	16	44	40
Germination, %	93.5	92.7	85.0
t_{50}, days	6.1	8.5	10.3
v, days	0.9	1.6	2.0
density, $mg.mm^{-3}$	1.08	1.15	1.22
seed weight, mg	0.520	0.530	0.547
seed volume, mm^3	0.481	0.462	0.448
endosperm volume, mm^3	0.201	0.183	0.210
air volume, mm^3	0.094	0.071	0.041
pericarp volume, mm^3	0.186	0.208	0.197

With increasing umbel position the mean seed density rose from 1.08 to 1.22 $mg.mm^{-3}$, and the seed weight from 0.520 to 0.547 mg, whereas the seed volume decreased from 0.481 to 0.448 mm^3. t_{50} and v were significantly correlated with seed density (Tab 2).

For the calculation of the volumes of the different seed parts it was assumed that for all seeds of cv. Mars the densities of endosperm and pericarp were similar to the values determined for the cvs. Monarch, Selfira and Tall Utah (chapter 3), being 1.214 $mg.mm^{-3}$ and 1.48 $mg.mm^{-3}$, respectively. Endosperm and pericarp volume varied inconsistently between seeds harvested from the different umbels (Tab. 1). The air volume decreased from 0.094 to 0.041 mm^3 with increasing umbel position. The relative proportion of air was the only structural parameter that was significantly correlated with seed density ($p < 0.05$) (Tab. 2).

Table 2. Regression coefficients of the relations between t_{50} and v with seed density and the relative volumes of seed parts, and between seed density with these volumes, in celery cv. Mars. Detailed data are shown in Tab. 1. Asterix indicates significance ($p < 0.05$).

	regression coefficients			
	Density	relative volumes		
		endosperm	pericarp	air
t_{50}	1.00*	0.62	0.83	0.98*
v	0.99*	0.56	0.87	0.96*
Density	-	0.46	0.78	0.99*

In a separate experiment in the same production field plants were shielded with plastic transparant windshields in order to increase the mean daily temperature. All umbels were harvested and compared with umbels of plants grown without windshields. Mean day-temperatures with and without windshields were 21.8 ° and 19.2 °C respectively. The mean seed density of these plants was 1.12 and 1.16 mg.mm^{-3}.

Seeds from separate umbels were harvested in 1987 from the cvs. Monarch, Selfira and Tall Utah. It is shown in Tab. 3 that with increasing umbel position the seeds had a shorter period of time for development and maturation before the moment of harvest. The higher the umbel order the lower was the mean day temperature during seed maturation. The highest number of seeds was formed on the tertiary umbels. With increasing umbel position the seed weight and the germination percentage decreased, and t_{50} and v increased. The similarity in the observations in the three cultivars suggests that the effect of umbel position on germination was not cultivar dependent.

The variation in density of the seeds of the different umbels of cv. Monarch was analysed. The mean density ranged from 1.10 mg.mm^{-3} for seeds of the primary umbels to 1.16 mg.mm^{-3} for seeds of the quarternary umbels (Fig. 1).

In 1988 seeds of the different umbels of cv. Tall Utah were harvested from 35 to 84 days after flowering (d.a.p.) . Secondary umbels flowered 1 week after the primary umbels, tertiary umbels 2 weeks , and quarternary umbels 4 weeks. All seed analyses were performed after 2 weeks drying of the seeds at 25 °C, 30 % R.H. (chapter 2). The seeds reached their maximum weight 42 d.a.p, it did not change during prolonged seed ripening. Mean weight of the seeds from primary, secondary and teriary umbels was 0.46 ± 0.03 mg; seeds from quarternary umbels had a mean weight of 0.41 ± 0.03 mg. The mean seed density of the seeds decreased during development and maturati-

Table 3. Characteristics of seeds harvested separately from umbels of different order or from all umbels together (whole plants) of celery cvs. Monarch, Selfira and Tall Utah. Seeds were produced in ethylene tunnels in 1987, the mean day-temperature during seed development and maturation was recorded from 14 d.a.p. to the harvest. t_{50} and v of seeds used for the parent plants are given in chapter 3, Tabs. 1,2,3. Germination occurred at 15/20 °C in light.

	Primary umbels	Secondary umbels	Tertiary umbels	Quarternary umbels	Whole plants
cv. MONARCH					
Number of seeds, %	5	32	54	8	100
Germination, %	97.3	87.6	76.6	47.8	70.1
t_{50}, days	5.6	5.7	6.5	7.3	6.4
v, days	0.7	0.6	1.2	1.3	1.1
Seed weigth, mg	0.584	0.552	0.470	0.444	0.574
Harvest, d.a.p.	93	80	73	65	-
Mean day temperature, °C	21.1	20.5	19.8	19.4	-
cv. SELFIRA					
Number of seeds, %	3	23	62	12	100
Germination, %	72.1	43.7	31.7	14.6	54.9
t_{50}, days	6.7	6.8	7.5	8.2	8.4
v, days	1.3	1.7	1.9	-[1]	2.1
Seed weight, mg	0.461	0.434	0.416	0.329	0.378
Harvest, d.a.p.	83	63	63	49	-
Mean day temperature, °C	21.5	20.1	20.1	19.7	-
cv. TALL UTAH					
Number of seeds, %	2	17	61	19	100
Germination, %	61.0	56.3	51.1	38.2	65.8
t_{50}, days	6.4	6.7	6.8	7.7	6.6
v, days	1.4	1.7	1.8	2.3	1.5
Seed weight, mg	0.609	0.602	0.513	0.467	0.513
Harvest, d.a.p.	92	72	65	58	-
Mean day temperature, °C	20.9	19.9	19.6	19.6	-

1) Low germination percentage does not allow calculation of v.

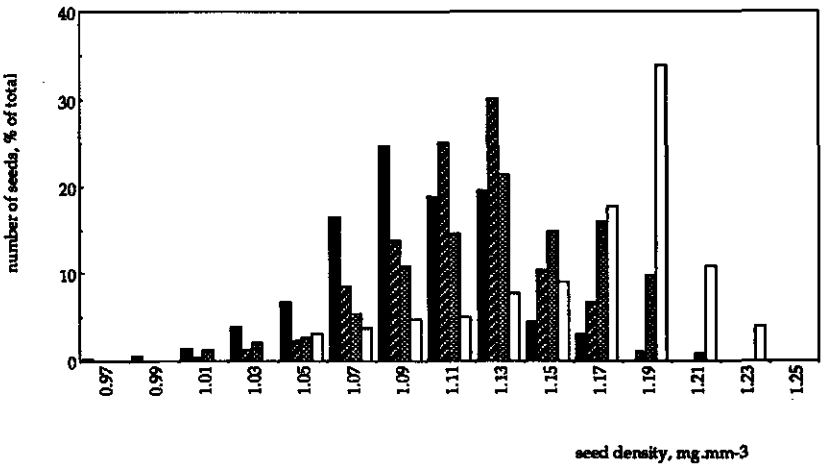


Figure 1. Variation per density interval of the number of seeds, expressed as percentage of the total number of seeds, harvested separately from primary (■), secondary (▩), tertiary (▩) or quartenary (□) umbels of celery cv. Monarch.

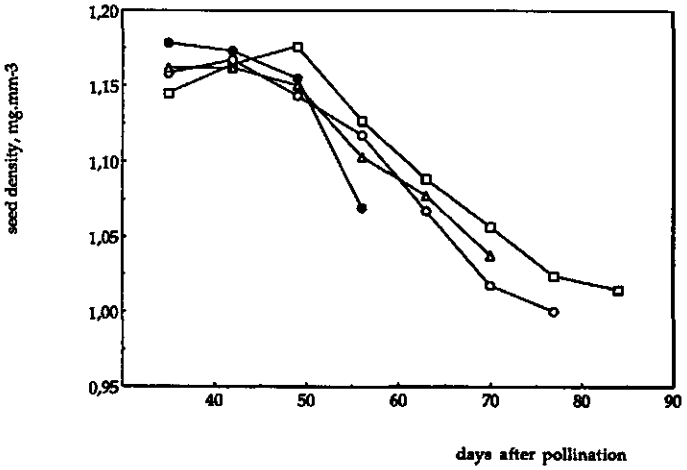


Figure 2. Seed density of seeds harvested from primary (▫), secondary (○), tertiary (△) and quartenary (●) umbels of celery cv. Tall Utah from 35 till 84 days after pollination of the different umbels.

on (Fig. 2). For all umbels, seed density of seeds harvested 35 d.a.p. was 1.16 $mg.mm^{-3}$. Untill 49 d.a.p. it remained at this level, but then decreased to around 1.00 $mg.mm^{-3}$ for seeds harvested 84 d.a.p. It is important to notice that there were no differences in seed density when seeds from different umbel types were harvested after a similar period of development.

For each umbel type the volumes of endosperm and pericarp remained constant between 35 and 84 d.a.p. (Tab. 4). With increasing umbel position the seeds contained less endosperm and more pericarp (Tab. 4). The air volume increased during seed development independently of umbel type. Seed density correlated negatively with relative air volume in the seeds of all different types of umbels (Fig. 3).

Table 4. Mean endosperm and pericarp volume of seeds harvested from different umbels from 35 till 84 d.a.p of celery cv. Tall Utah.

	volume (mm^3)	
umbel	endosperm	pericarp
primary	0.273 ± 0.028	0.077 ± 0.024
secondary	0.260 ± 0.019	0.082 ± 0.032
tertiary	0.233 ± 0.013	0.123 ± 0.016
quarternary	0.206 ± 0.021	0.100 ± 0.030

Discussion

The present results showed for the cvs. Mars, Monarch, Selfira and Tall Utah that increasing umbel position resulted in seeds with a lower germination percentage, an extended t_{50}, and a larger v (Tabs. 1 and 3). These results are in good agreement with earlier studies on celery seed production (Thomas et al. 1978, Thomas, et al. 1979, Thomas and O'Toole 1980, 1981).

The seed density increased with increasing umbel position in cvs. Mars and Monarch (Tab. 1, Fig. 1). The comparison of the density of seeds harvested from plants of cv. Mars grown with and without windshields, and of seeds of cv. Tall Utah harvested from the different umbels at a variable time after flowering (Fig. 2), showed that seed density is not correlated with the umbel position as such, but depends on the progress of seed maturation and on the temperature during that process. In the field umbels of a higher order developed at a lower temperature than the primary ones (Tab. 3). Gray and Steckel (1982, 1983) showed that initial differences in quality of carrot seeds harvested from primary and secondary umbels diminished during pro-

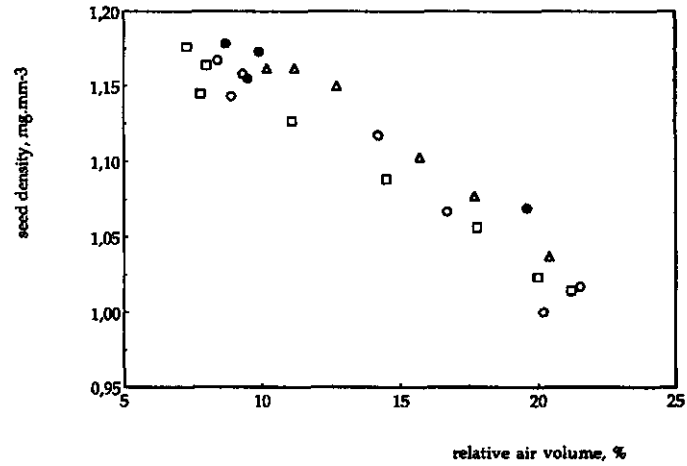


Figure 3. The relation between seed density and relative air volume in seeds of celery cv. Tall Utah harvested from primary (□), secondary (○), tertiary (△) and quartenary (●) umbels from 35 till 84 d.a.p. Regression coefficient of all data is 0.96.

longed seed ripening, and are therefore due to the relatively small difference in time of flowering between the umbels.

In the 1988 production experiment seed weight reached its maximum 6 weeks after flowering and did not change thereafter. The volumes of endosperm and pericarp remained constant after 35 d.a.p. (Tab. 4), but air volume increased. The seed density correlated negatively with the increase in air volume (Fig. 3). In cv. Mars the same correlation was found (Tab. 2). It is concluded that the relationship between seed maturity and seed density is based on the increase in air volume in the seeds during seed development. The increase in air volume may be related to the development of the pericarp.

With increasing umbel position the pericarp volume increased while the endosperm volume decreased (Tab. 4). It is hypothesized that this was related to the temperature during seed development. It was shown by Gray, Steckel, Dearman and Brocklehurst (1988) that during seed development in carrot a higher temperature resulted in a lower pericarp weight.

In general, the studies in celery showed that seed density is negatively correlated to seed quality. It is concluded that the variation in seed density, and therefore in seed quality, of celery seeds originates from differences in seed maturity at harvest. The different timing of pollination , seed development and seed maturation between the different umbels is called somatic heterochrony (Silvertown 1984).

It was shown in chapter 3 that seed density grading separates seeds with low density and high quality from seeds with high density and low quality. The present data show that the separation is based on differences that date back to seed maturation.

When a commercially harvested seed lot was graded with fluid density separation, seed density showed a significant correlation to both relative air volume and relative pericarp volume (chapter 3). In the present data seed density was only correlated with air volume (Tab. 2, Fig. 3). It has to be realized that in a commercially harvested seed the low-density seeds mainly originate from the primary umbels and therefore have according to the present data lower relative pericarp volumes than the high density seeds in the seed lot, that mainly originate from the later formed umbels.

In the present data as well as in the previous chapter the mean germination parameters t_{50} and v differed between the cultivars. In general, cv. Monarch had shortest t_{50} and smallest v, and therefore the best seed quality. Seed quality may be related to the selection pressure in celery breeding programs. The selection in green celery and self blanching celery is aimed at a high number of shoots, whereas in root celery selection is for a low number of shoots and large tubers. These different selection pressures have resulted in green and self blanching cultivars with many shoots , and a large number of umbels and seeds, in contrast to root celery cultivars. Additionally, green and self blanching cultivars are selected for bolting resistance, which may be correllated with seed dormancy (Thomas 1978).

Studies on the relation between the pericarp and germination of celery seeds (*Apium graveolens* L.).

Summary

Several mechanisms for an inhibitory effect of the pericarp on the germination of celery seeds were proposed mainly based on literature data of other species. It has been suggested that phenols originating from the pericarp may directly inhibit embryo growth or indirectly by oxygen fixation in the pericarp, thereby depriving the embryo from oxygen. Also, an inhibition of water uptake by the pericarp has been proposed.

The relation between the pericarp and germination was studied on two celery seed batches originating from the same seed lot but with different pericarp volumes. The amount of leachates correlated with the pericarp volume, but removal of these leachates before or during germination hardly affected the time to 50 % germination. It was further shown that the phenolic substances present in the maternal tissue did not enter the endosperm, therefore a direct inhibition of embryo growth and germination was excluded. The pericarp did neither interfere with oxygen uptake to such an extend that embryo growth was inhibited nor inhibited water uptake by the seeds. It was concluded that that all three proposed mechanisms of germination inhibition by the pericarp could not be accepted for celery seeds.

Introduction

Compared to most other horticultural species, celery seed germination is slow and irregular. Germination is slow because the embryo in a mature celery seed is underdeveloped and has to grow at the expense of the endosperm prior to the start of visible germination (chapter 3). The irregularity in the moment of germination in a seed lot is due to the large differences in seed maturity (chapter 4). The seed density decreases during the ripening of the seeds on the mother plant. Therefore fluid density grading separates a seed lot in immature high-density and mature low-density seeds (chapter 4). Increased ripening is, apart from decreased density, also correlated to quicker germination expressed in the mean time to 50 % germination (t_{50}). Therefore density grading also separates a seed lot in slow and fast germinating seeds (chapter 3). The density of a celery seed depends on the relations between the volumes of endosperm, air

and pericarp and the density of these constituents. It was also shown the t_{50} is a function of the embryo growth rate during the first part of the germination process, and since the t_{50} correlates positively with the relative pericarp volume, the pericarp could be involved in the regulation of embryo growth.

Seed leachates of *Umbellifereae* can be inhibitory to the rate of germination (Chaturvedi and Muralia 1975). In particular celery seeds contain several coumarins and coumarin derivatives (Ceska et al. 1987, Garg, Gupta and Sharma 1978, 1979a, 1979b, 1979c, 1980, Jain et al. 1986). And since "Coumarin is one of the most potent inhibitors known" (Evenari 1949), a direct chemical inhibition of embryo growth by these subtances might be possible. However, for this mechanism to be true for celery seeds, it has to be proven that seeds with thicker pericarps contain more phenolic compounds, that these compounds can enter the endosperm, and finally, that they inhibit embryo growth

Apart from the direct chemical inhibition it also has been suggested that coumarin and other phenolic compounds inhibit germination by fixation of oxygen in the pericarp. Such a mechanism of inhibition has been demonstrated in beet, barley and oat seeds, where phenolic acids that are located in the tissues that surround the embryo consume the passing oxygen during imbibition and thereby inhibit germination (Corbineau et al. 1984, Coumans et al. 1976, Roubaix and Lazar 1957, Lenoir et al.1983). For this mechanism uptake by the true seed is not neccessary because coumarins act *in situ* in the pericarp.

The pericarp can play an important role in the regulation of the transfer of water to the embryo as has been decribed for species of *Leguminosae, Trifolieae* and other families (Ballard 1973, Rolston 1978). Differences in the thickness of the pericarp may be related to differences in hydration rate of the endosperm and embryo, causing differences in embryo growth rate.

In the present study the correlation between pericarp and embryo growth was analyzed in two seed fractions of celery with the same genetic constitution but with different relative pericarp volume. The two seed batches were separated by fluid density grading (chapter 3).

Results

leachates , conductivity and K^+

Density grading of seed lot 9092 of cv. Monarch with 0.02 $mg.mm^{-3}$ density intervals of the separation solutions resulted in 12 density fractions (chapter 3). The decrease in seed dry weight of these fractions after 22 hr leaching in running tab water was positively correlated to the volume of the pericarp (Fig. 1), indicating that the decrease in seed weight was most probably due to leachates from the pericarp.

All other experiments were concentrated on two seed fractions with mean seed densities of 0.98 and 1.15 $mg.mm^{-3}$. As was shown in chapter 3 an increase in seed

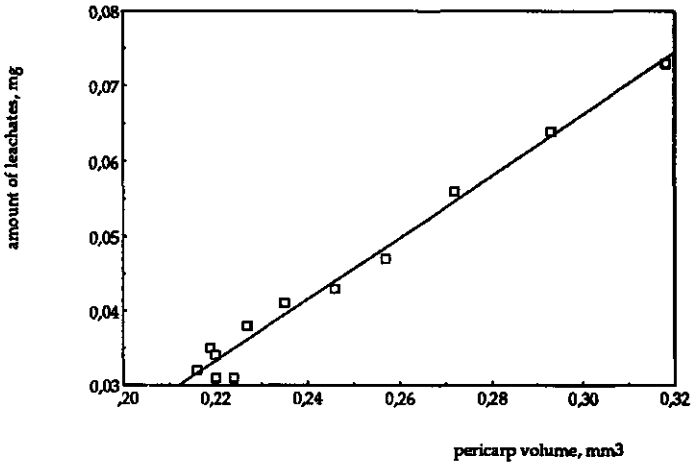


Figure 1. Decrease in seed weight after 22 hours leaching in running tab water at 15 °C in light of 12 density fractions of celery cv. Monarch lot 9092, related to the pericarp volume of the fractions.

density was correlated to a rise in weight and volume of the pericarp and a reduction of air volume (Tab. 1). The volume of the pericarp of the low and high density fractions was 30 % and 41 % of total seed volume, repectively.

Table 1. Seed structure parameters of the two density fractions of celery cv. Monarch lot 9092 used in this study.

density fraction, $mg.mm^{-3}$	mean density, $mg.mm^{-3}$	weight, mg			volume, mm^3				surface/ volume ratio
		endosp.	peric.	seed	endosp.	peric.	air	seed	
0.90 - 1.02	0.98	0.327	0.300	0.627	0.269	0.203	0.170	0.642	5.3
1.10 - 1.18	1.15	0.338	0.366	0.704	0.278	0.247	0.086	0.612	5.4

There was considerable leakage of electrolytes during the first half hour of imbibition in a large volume of distilled water, it was about five times higher in high than in low density seeds (Tab. 2). After half an hour the leakage rapidly dropped. The lea-

Table 2. Increase in conductivity (1 seed.ml^{-1}) and decrease in seed weight of two density fractions of celery cv. Monarch lot 9092.

incubation time, h	density 0.98 mg.mm^{-3}		density 1.15 mg.mm^{-3}	
	conductivity, µS.hr^{-1}	decrease in seed weight, µg.hr^{-1}	conductivity, µS.hr^{-1}	decrease in seed weight, µg.hr^{-1}
0-0.5	10.2	72	49.4	74
0.5-5	1.3	5.3	1.4	13.3
5-25	0.31	-0.4	0.27	-1.0

kage of non-electrolytes as judged from the decrease in seed dry weight was also most pronounced during the first half hour of imbibition, but this leakage continued somewhat longer in time. Leakage of these compounds was twice as large from high than from low density seeds (Tab. 2). After 5 hours the decrease in seed weight stopped. Total leakage resulted in a decrease in dry seed weight of about 9% in seeds with a mean density of 0.977 mg.mm^{-3} and of about 11% in seeds with a mean density of 1.15 mg.mm^{-3}.

After 5 hours of imbibition leachates from low density seeds contained 2.2 µg potassium and from high density seeds 5.2 µg. For both fractions the amount of leaked potassium after 5 h of imbibition was only 4% of the total amount of potassium present in the seeds (Tab 3).

Table 3. Amount of potassium in seed leachates and seeds of two density fractions of celery cv. Monarch lot 9092.

	potassium, µg	
	density 0.98 mg.mm^{-3}	density 1.15 mg.mm^{-3}
K^+ in leachates	2.2	5.2
K^+ in seeds	63	120

Effect of leakage on germination time

Germination experiments at optimal conditions showed a difference in t_{50} of 1.5 days between seeds of the two density fractions (Tab. 4). It was tested if removal of the

Table 4. Effect of seed leaching before or during germination test at 15/20 °C, light on the germination parameters of two density fractions of celery cv. Monarch lot 9092.
Germination occurred either at standard germination conditions on filter paper in plastic boxes, without or with 5 h leaching in distilled water, or on a hand-made Copenhagen table equipement that allowed constant leaching during the germination test. Decrease in seed dry weight was determined after 1 day incubation.

	density 0.98 mg.mm^{-3}				density 1.15 mg.mm^{-3}			
	decrease in seed dw, µg	germination, %	t_{50}, days	v, days	decrease in seed dw, µg	germination, %	t_{50}, days	v, days
control	20	97.0	5.3	0.8	46	97.4	6.8	1.0
leaching before germination test*	31	96.0	5.6	1.0	59	97.1	6.9	1.0
copenhagen table	30	96.3	5.5	0.9	56	96.4	7.0	1.3

* Figures are corrected for leaching time.

seed leachates had any effect upon the t_{50}. Leachates were either removed before the germination test by leaching in distilled water for 5 h (see Tab. 2), or removed during the germination test. The latter occurred by means of a germination box that was based on the "Copenhagen table" design (International Seed Testing Association 1985), where seed leachates are removed from the surrounding of the seed by a constant flow of water along the seeds caused by evaporation of water at the edges of the filter paper that functions as substratum for the seeds and as supply of water near the center of the filter paper. As indicated by the decrease in seed dry weight 1 day after the start of the germination test both leaching methods lead to an increase in seed leakage.

The germination percentages were not affected by seed leaching. t_{50} values were very slightly increased by both treatments and v became somewhat larger by a few treatments. In none of the treatments seed leaching reduced neither t_{50} nor v, as should have been expected when the leachates contained germination inhibiting compounds. The difference in t_{50} between the two seed fractions were also not influenced by seed leaching in spite of the large differences in the amount of leachates (Tab. 2). It is concluded from these results that the substances that leak from the seeds do not inhibit germination. Therefore, it is unlikely that differences in pericarp volume cause the differences in t_{50} through the leakage of these compounds.

Location and quantity of phenolic substances in the seed

A condition for a supposed effect of substances from the pericarp on embryo growth is that they must be able to enter the endosperm. Histological sections of the seeds were stained with Gibbs reagens to locate the phenolic substances. It was shown that Gibbs-positive phenols were present particularly in the testa and also to some extend in the pericarp (Fig. 2). Obviously phenolic substances were not present in the endosperm, even not in the neighbouring cells. Thus, phenols localized in the maternal tissue do not reach the embryo or endosperm.

The amount of Gibbs positive phenols in the two density fractions was spectrofotometrically quantified. Low density seeds contained 0.516 µg guaiacol equivalents whereas high density seeds contained 0.714 µg. After 3 days incubation in water at 15 °C these amounts were 0.456 µg and 0.576 µg.

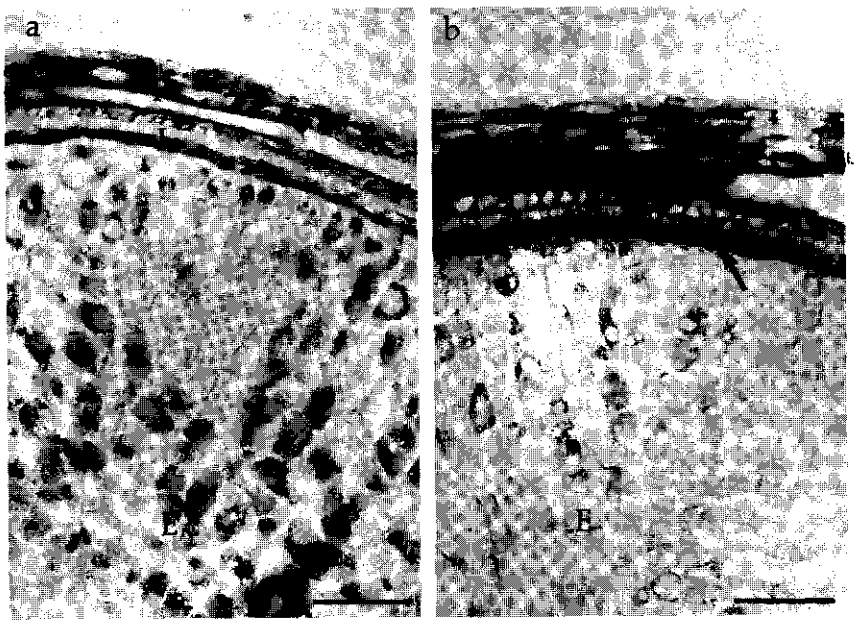


Figure 2 A, B. Localization of Gibbs-positive phenols in seeds of celery cv. Monarch lot 9092. Seeds were incubated during 3 days at 15 °C in light before sectioning and staining. A) control, not stained. B) stained with Gibbs reagens. The arrows indicate the location of the phenols. E = endosperm, I = testa, P = pericarp.

Effect of O_2 pressure on germination

Seeds of the two density fractions were germinated at 15 °C, light, either in pure oxygen (Tab. 5) or totally immersed in water, which strongly reduced the O_2 pressure

Table 5.Influence of high O_2 pressure on germination of seeds with low and high density of celery cv. Monarch lot 9092, at 15 °C, light.

	density 0.98 mg.mm^{-3}			density 1.15 mg.mm^{-3}		
	germination, %	t_{50}, days	v, days	germination, %	t_{50}, days	v, days
air	91.9	6.3	1.1	72.0	8.5	1.1
100 % O_2	91.7	6.9	0.9	90.4	8.1	0.8

Table 6. Influence of low O_2 pressure on germination of seeds with low and high density of celery cv. Monarch lot 9092 at 15 °C, light. Seeds were germinated in the standard germination boxes to which either the standard 14 ml distilled water was added or 28 ml that completely submerged the seeds.

	density 0.98 mg.mm^{-3}			density 1.15 mg.mm^{-3}		
	germination, %	t_{50}, days	v, days	germination, %	t_{50}, days	v, days
control (14 ml)	96.0	6.4	1.0	95.2	8.3	1.3
low O_2 (28 ml)	92.5	6.8	1.1	92.6	8.8	1.5

(Tab. 6). Due to the suboptimal temperature t_{50} increased with 1 day for seeds with low density and 1.5 day for seeds with high density as compared to germination at 15/20 °C (Tab. 4). Due to the conditions of the germination experiment at high O_2 pressure, germination percentages were slightly inhibited for both density fractions (Tab.5). In low density seeds t_{50} was slightly increased and v slighly decreased whereas in high density seeds both t_{50} and v were somewhat lower. However, the differences were small, and the large difference in t_{50} between the two density fractions remained.

Also at low O_2 pressure the germination of both density fractions was somewhat inhibited and t_{50} and v slightly increased (Tab. 6). But also this treatment did not remove the differences between the two fractions . It is concluded that immersion in water leading to a limitation of oxygen availability is hardly inhibitory to germination.

These experiments indicate that the pericarp does not interfere with oxygen up-

take to such an extend that embryo growth is inhibited.

Imbibition rate

The time course of water uptake at 15 °C in the light was determined. Seeds of the two density fractions did not differ in imbibition rate (Fig. 3).

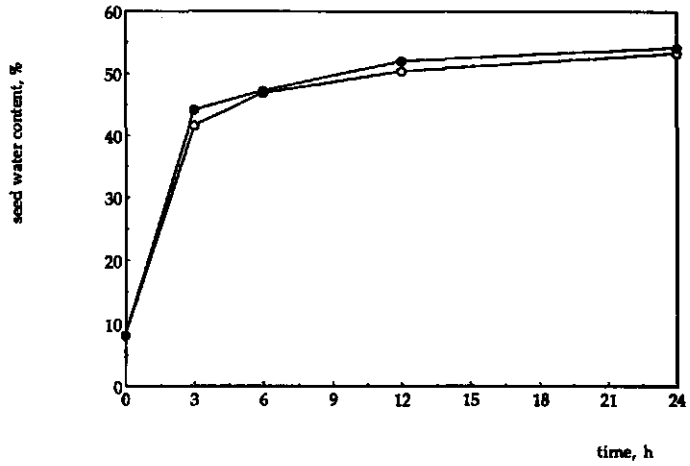


Figure 3. Increase in moisture content in time during incubation at 15 °C of celery cv. Monarch lot 9092 low density (○) and high density (●) seeds.

Discussion

Leakage

The present results showed that during the first hours of imbibition celery seeds lost up to 11% of their dry weight due to leakage . Part of the leakage is often attributed to imbibitional damage of living endosperm and embryo cells. Simon and Mathavan (1986) found that within 1 hour after start of imbibition more than 60% of the total potassium content of celery seeds leaked out. In general, potassium leakage is an indication for leakage from the cytoplasm. The authors suggested that the high imbibitional damage is a result of the high surface to volume ratio of small seeds that causes relatively a high proportion of damaged cells. In contrast our results showed that only 4% of the total amount of potassium is leaked out (Tab. 3). Weges (1987) showed that in lettuce seeds potassium only leakes from the testa-pericarp. The endo-

sperm was found impermeable to potassium transport from both directions. The present data also showed that in spite of a difference in surface to volume ratio of 3% in favour of the high density seeds (Tab.1), the potassium leakage was twice as high (Tab. 3). These results suggest that the volume of the pericarp determines largely the amount of leachates (Fig. 1), and that true imbibitional damage only plays a minor role.

The influence of the pericarp on embryo growth

The results in Tab. 3 show that leachate removal before or during the germination test hardly influenced t_{50} of germinating celery seeds. These results are in good agreement with the work of Thomas (1983), but contrast to Hovadik and Kratochvilova (1979) and Finch-Savage (1984) who reported an increase in germination rate of celery after seed leaching and to Taylor (1949) who concluded for a decreased germination rate.

On first view the present conclusion that leachates do not influence germination in celery seeds seems in contrast to the reports that mention an inhibitory effect of applied leachates (Chaturvedi and Muralia 1975) on germination rate and to the studies mentioned above that report inhibiting effects of applied coumarin and other phenolic compounds. However, to inhibit embryo growth and germination compounds from maternal tissues have to enter the endosperm and embryo. The present results indicate that such is not the case with phenols from the pericarp and testa of celery seeds. It is supposed that in many inhibitor studies the compounds either were applied in non-physiological concentrations, or had their effect at first after the start of visible growth. Chaturvedi and Muralia (1975) reported that seed leachates of Umbelliferae seeds inhibit root and hypocotyl growth in the phase after visible germination and Tarragan (1953) and Goodwin and Taves (1950) showed that coumarin can inhibit root growth.

Our present results show that Gibbs positive phenolic substances are present in the testa and the pericarp. Garg et al (1978, 1979a,b,c, 1980) and Ahluwalia, Boyd, Jain, Khanduri and Sharma (1988) have shown that several of the coumarins, furanocoumarins and furanocoumarin-glucosides present in celery seeds are Gibbs positive (celeroin, rutaretin, apiumetin, apiumoside). The present determinations showed that high density seeds with a thicker pericarp contained more of these phenols than low density seeds. After 3 days incubation in water the amounts are reduced by about 10 to 15%, probably due to leakage.

Battle and Whittington (1969) suggested that the different phenolic acids in seeds are intermediates of lignin biosynthesis. Therefore, immature seeds might contain more of these acids because the pathway of lignin biosynthesis is highly active. It was shown in chapter 4 that during maturation on the mother plant the seed density of the seeds decreased. Taking into consideration the positive correlation between seed density and relative pericarp volume it is assumed that the amount of phenols decreased during maturation of the seeds.

Although the phenolic substances do not enter the endosperm, they may still be inhibitory to embryo growth by interfering with the oxygen uptake. As we mentioned before oxygen fixation by phenolics in the pericarp is reported for several species. The present results show, however, that oxygen is not the limiting factor for embryo growth in celery. If oxygen is partly consumed in the maternal tissue it does not occur to such an extend that it becomes critical for embryo growth.

Our present results did also show that the pericarp is not limiting to the water uptake of the seeds.

In general, it is concluded that the pericarp did neither directly nor indirectly inhibit the germination rate of celery seeds. Such in spite of the positive correlation between the relative volume of the pericarp and t_{50} values. It has to be realized, however, that t_{50} is negatively related to the volume of the endosperm and the air-filled space in the dry seed. Therefore, it will be studied in the next chapter whether the rates of embryo growth and germination are related to water relations of the seeds.

Analysis of embryo growth in mature celery seeds (*Apium graveolens* L.).

Summary

Embryo growth in celery seeds of cv. Monarch with low and high densities was studied during incubation in water and PEG. Embryo growth in water was characterized by both cell division and an increase in cell size, while embryo growth in PEG solutions solely occurred through cell division. Low density seeds differed from high density seeds in a higher cell division rate both during incubation in water and PEG, whereas in water also the embryo cell size was larger. As a consequence, the low density seeds reached in water the embryo length of 1.5 mm, that was critical for radicle protrusion, after 6.1 days, 1.7 days before the high density seeds. After 13 days of incubation in -1.2 MPa PEG the embryos in both fractions reached their maximum length of 0.9 and 0.7 mm in low and high density seeds, respectively.

During incubation in water low density seeds had a higher true-seed water content, and a less negative ψ_π than high density seeds. It was concluded that these differences were caused by a lower $\psi_{p,pericarp}$. When the pericarp was removed the differences in water content and ψ_π dissapeared, the cell size in both fractions became similar, and the difference in t_{50} was reduced. A similar difference in ψ_π was also present when the seeds were incubated in PEG. It is concluded that also in PEG ψ_π correlated with $\psi_{p,pericarp}$. It is concluded that the difference in embryo growth rate between the two fractions, both during incubation in water and in PEG, was partly caused by the difference in ψ_π. It is argued that ψ_π of the true seeds affected the embryo cell size and the embryo cell division rate, and the embryo cell number after PEG incubation.

When incubation in PEG was followed after 17 days by incubation in water, the embryo growth was faster as without a pre-incubation in PEG in both fractions; also the cell size increased. The differences between the fractions persisted, however. It is concluded that embryo cell wall extensibility increased during PEG-incubation.

Introduction

A celery "seed", being morphologically a schizocarpic fruit, consists largely of endosperm surrounded by a thin seed coat ("true seed") and a relativly thick pericarp.

The small axile-linear embryo is located at one end of the endosperm (Jacobsen & Pressman 1979). The embryo is about one third of the length of the endosperm. It grows to about twice its size before visible germination occurs. The need for embryo growth in the mature seed, shown by Stokes (1952) and Baskin & Baskin (1984) in other Umbellifer species, may be the main reason for the slow germination rate of celery seeds. The study of different seed fractions separated by fluid density grading revealed a negative relationship between the embryo growth rate and the relative proportion of pericarp tissue (chapter 3). It was proven that the relation did not involve chemical inhibition, oxygen fixation or restriction of water uptake by the pericarp (chapter 5).

It was indicated, however, that the negative relationship between embryo growth rate and relative proportion of pericarp tissue also means a similar but positive relationship between embryo growth rate and the relative proportion of the remaining seed parts, air and endosperm (chapter 3). It was hypothesized that upon imbibition the osmotic potential in seeds with a smaller true seed volume may drop to lower negative potentials. An inhibitory effect of low osmotic potentials on endosperm hydrolysis has been shown in barley aleurone layers (Jones and Armstrong 1971). Osmotic conditions may also inhibit embryo growth.

It has been shown by a number of authors (Salter and Darby 1976, Khan et al. 1978, Darby et al. 1979, Haarman 1980, Khan et al. 1980/81, Nakamura, Teranishi and Aoki 1982, Brocklehurst and Dearman 1983, Brocklehurst et al. 1982, Cantliffe et al. 1987, Globerson and Feder 1987) that incubation of celery seeds in polyethylene glycol (PEG) solutions reduces the t_{50} of subsequent germination. Because the embryo has to grow inside the seed before germination can occur it seems likely that embryo growth occurs during PEG incubation.

It is hypothesized that embryo growth in mature celery seeds during germination is caused by both cell division and cell elongation. Most probably the embryo growth during PEG incubation occurs exclusively due to cell division, because cell expansion is limited under these conditions (Haber and Luippold 1960).

In this study embryo development and water relations were studied during incubation in water with or without a pre-incubation in PEG, and during PEG incubation, in two seed fractions of celery with the same genetic constitution but with different density. The fractions were separated by fluid density grading (chapter 3).

Results

Embryo development during incubation in water

Embryo length, and the number and size of embryo cells were measured during incubation of celery seeds cv. Monarch in water at 15 °C in light (Fig. 1). The embryo growth rate in seeds with lower density was somewhat faster than in high density seeds (Fig. 1a). Embryo growth in seeds of both fractions was clearly inhibited after the fourth day. At that time the embryo length in seeds with low and high density was

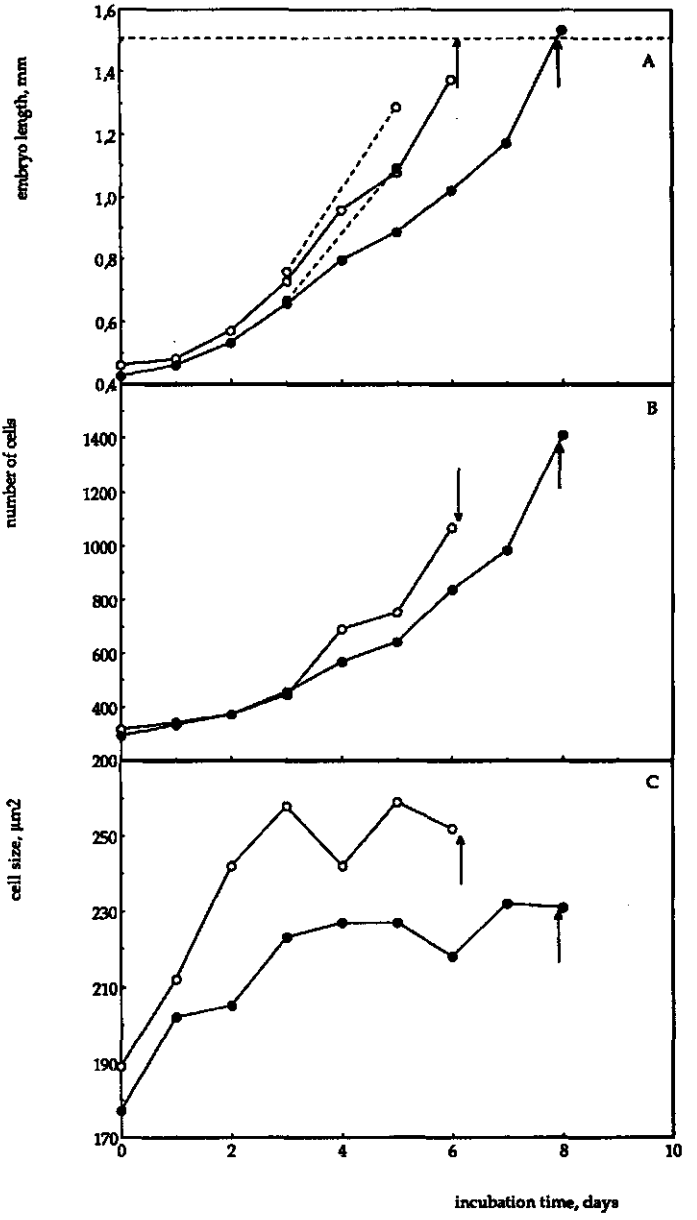


Figure 1. Intraseminal embryo growth in seeds of celery cv. Monarch lot 9092 with density 0.98 $mg.mm^{-3}$ (○) or 1.15 $mg.mm^{-3}$ (●) during incubation in water at 15 °C in light. A. Increase in embryo length; dotted lines indicate embryo growth in true seeds (the pericarp was removed at the start of the incubation). B. Increase in cell number in a longitudinal cross section of the embryo. C. Increase in cell size as measured on the longitudinal cross section.
Arrows indicate mean time to radicle protrusion (t_{50})

0.95 and 0.75 mm, respectively. When the pericarp was removed at the start of the incubation, this inhibition did not occur (Fig. 1a). Embryo growth in the seed resulted in a t_{50} of 6.1 day for seeds with low density and 7.8 day for seeds with high density. The embryo length at the moment of radicle protrusion was 1.5 mm for both fractions.

During germination the number of cells was counted in a longitudinal cross section of the embryo, in a standard surface area of the hypocotyl just below the apical meristem that contained cortical and vascular cells. From these data the mean cell size in this area was calculated.

Comparison of different areas on the longitudinal cross sections showed that the ratio of cortical to vascular tissue was 1 to 1, and similar in the whole embryo, exept for the four meristems, that occupied small areas on the sections. Therefore, the mean cell size of the subapical region was regarded as representative for the mean cell size on the whole longitudinal embryo cross section. The cell number on the embryo cross sections was calculated from the number of cells in the standard surface area, and the total surface area of the cross section.

Measurements of cortical and vascular cells separately showed that cortical cells were about 10% larger than vascular cells throughout the germination process.

According to this method longitudinal cross sections of embryos of both fractions contained at 3 h after the start of the incubation about 300 cells (Fig. 1b). After 3 days of incubation the number of cells was increased to 450 in both fractions. Thereafter, the number of cells per embryo became different between the two fractions. Cell division rate in low density seeds was higher than of high density seeds. At the time of radicle protrusion in low density seeds, the embryos of these seeds contained about 1100 cells in the cross sections, whereas embryos of high density seeds at that time contained about 850 cells. At the time of radicle protrusion in high density seeds the embryos of those seeds contained 1400 cells in the cross sections.

Apart from cell number also cell size changed during embryo growth. The mean surface area of the hypocotyl cells in longitudinal cross sections at the start of imbibition was 189 and 177 μm^2, respectively, for the two fractions. During the first three days of incubation it increased to 250 μm^2 in seeds with low density and to 225 μm^2 in seeds with high density. (Fig. 1c). This difference in cell size was the reason why high density seeds needed more cells to reach the embryo length of 1.5 mm than low density seeds.

It is concluded that high density seeds have an extended t_{50} as compared to low density seeds because the cell division rate is slower and the size of embryo cells is smaller.

Embryo development during PEG incubation

Embryo length, and number and size of embryo cells, were also measured during incubation in -1.2 MPa PEG (Fig. 2). In both fractions embryo growth did occur, but at a much lower rate than in water (compare Fig. 1a to 2a). Again embryo growth rate in seeds with the lower density was higher than in high density seeds (Fig. 2a). After 13

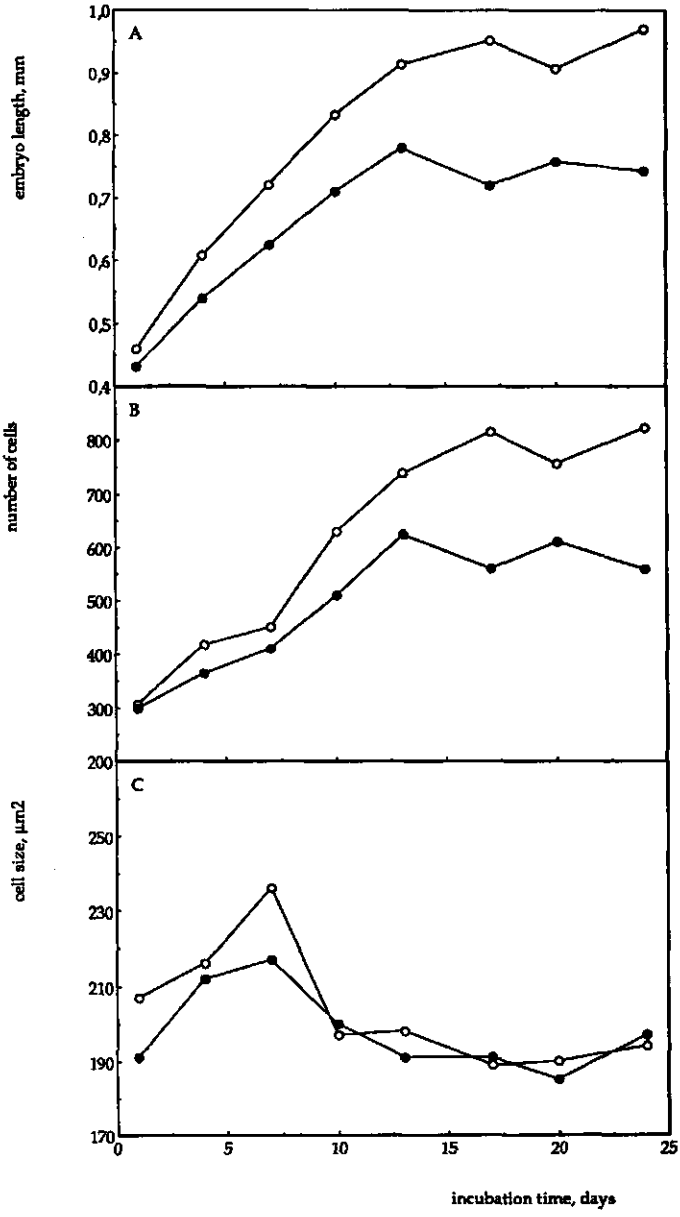


Figure 2. Intraseminal embryo growth in seeds of celery cv. Monarch lot 9092 with density 0.98 $mg.mm^{-3}$ (o) or 1.15 $mg.mm^{-3}$ (•) during incubation in -1.2 MPa PEG at 15 °C in light. A. Increase in embryo length. B. Increase in cell number in a longitudinal cross section of the embryo. C. Change in cell size as measured on the longitudinal cross section. Notice the different diversion of the axes in comparison to Fig. 1.

days incubation embryo growth ceased in seeds of both fractions at a length of 0.75 and 0.95 mm for the high and low density seeds, respectively.

After 1 day of incubation in PEG the number of cells per embryo was in both fractions about 330 (Fig. 2b). During 13 days of incubation the number of cells in seeds with low and high density increased to about 800 and 600, respectively. Mean cell division rate was 34 cells.day^{-1} and 25 cells.day^{-1}, respectively, for low and high density seeds. This was only about 27% of the cell division rate in seeds of both fractions during incubation in water (Fig. 1b).

After 1 day of incubation the cell size was about 200 μm^2. From day 1 to day 7 the cell size increased in low and high density seeds to 240 and 220 μm^2, respectively, and decreased thereafter to 190 μm^2 in seeds of both fractions (Fig. 2c).

Embryo development during incubation in water after PEG incubation

Seeds of the two density fractions were incubated in -1.2 MPa PEG for 17 days at 15 °C in light. Thereafter, the seeds were washed in running tap water, tissue-dried and further incubated in water at 15 °C in light. Embryo length, and the number and size of embryo cells, were measured during this second period of incubation (Fig. 3).

In this particular experiment the embryo length after the PEG incubation was 0.85 mm and 0.65 mm, respectively, for the low and high density seeds (Fig. 3a). These values were sligthly lower than the embryo lengths observed after 17 days of incubation in the first PEG incubation experiment (Fig. 2a). This difference was probably caused by a slight decrease in the potential of the incubation solution. Also the embryo cell numbers in both fractions after the PEG incubation, 625 and 480, respectively, in low and high density seeds (Fig. 3b), were a bit lower than in the first experiment (Fig. 2b). The cell size after incubation was 200 μm^2 in seeds of both fractions (Fig. 3c), which is about the same as in the first experiment (Fig. 2c).

Upon transfer of the seeds from the PEG solution to water, the embryo length increased in 1.7 and 2.8 days, in low and high density seeds, respectively, to the embryo length that corresponded with radicle protrusion, 1.5 mm (Fig. 3a). These incubation times were shorter than the times needed for a similar increase in embryo length during incubation in water without pre-incubation in PEG. In untreated seeds it took about 2.7 days to increase the embryo length from 0.85 to 1.5 mm in low density seeds and 4.8 days to increase the embryo length from 0.65 to 1.5 mm in high density seeds (Fig. 1a and 3a). Thus, the increase in embryo growth rate after PEG incubation resulted in low density seeds in a gain of incubation time of 1.0 days and in high density seeds of 2.0 days.

A further comparison between the growth pattern of PEG treated and untreated embryos showed that from the moment on that both types of embryos contained the same number of cells, cell division rate was not different until the moment of radicle protrusion (Fig. 3b). However, the embryo cell size in treated seeds increased rapidly during incubation in water, from 200 to 330 μm^2 in low density seeds, and from 200 to 310 μm^2 in high density seeds (Fig. 3c). During the growth of untreated embryos the

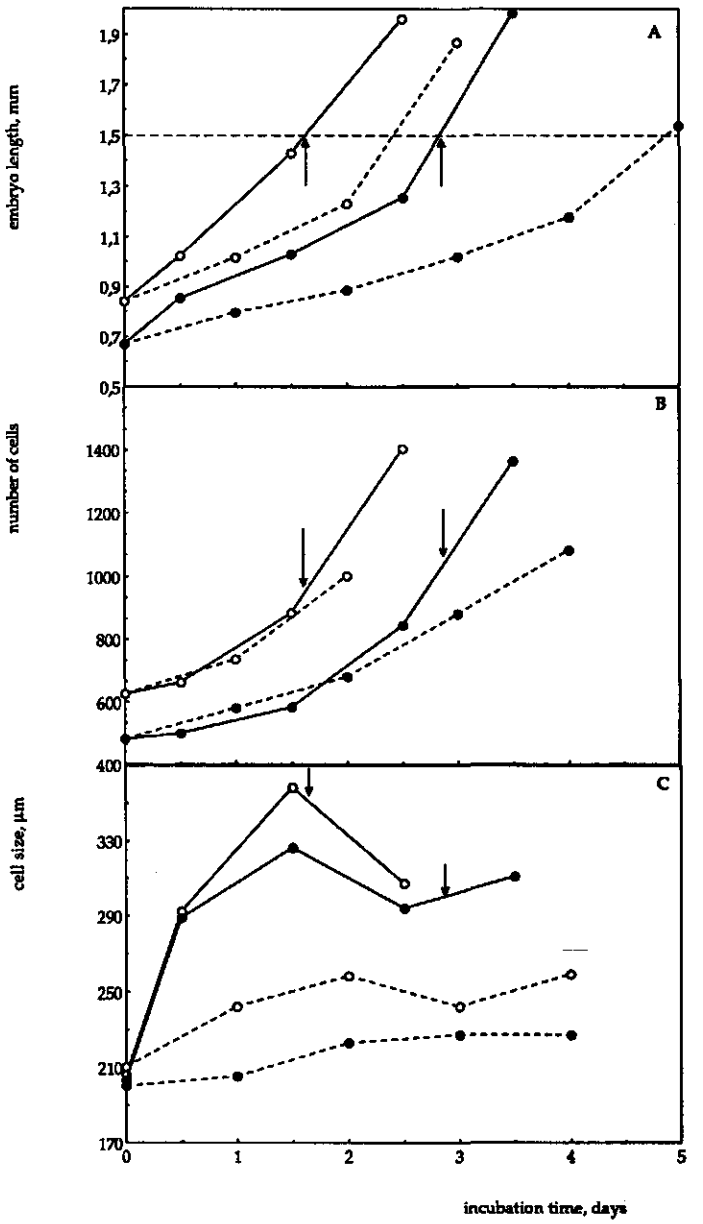


Figure 3. Intraseminal embryo growth in seeds of celery cv. Monarch lot 9092 with density 0.98 $mg.mm^{-3}$ (o) or 1.15 $mg.mm^{-3}$ (•) during incubation in water at 15 °C in light, after a 17 day incubation in -1.2 MPa PEG at 15 °C in light. A. Increase in embryo length. B. Increase in cell number in a longitudinal cross section of the embryo. C. Increase in cell size as measured on the longitudinal cross section. Dotted lines indicate increases in embryo length, number of cells and cell size in untreated embryos; these data were derived from Fig. 1.
Arrows indicate mean time to radicle protrusion (t_{50})

cell size in low density seeds was about 250 μm^2 and in high density seeds about 220 μm^2 (Fig. 1c). The increase was therefore 24% and 41%, respectively, in low and high density seeds. It is concluded that the higher rate of embryo growth in water of PEG-preincubated seeds, when compared to untreated seeds, is due to a larger embryo cell size.

Because the cell size was larger the number of cells necessary to reach the embryo length for visible germination, 1.5 mm, was lower. Only 950 and 1150 cells were needed to reach this length in low and high density seeds, respectively, instead of 1100 and 1400 in untreated seeds. When only the incubation period in water is taken as a measure, the reduction in time to reach the embryo length of 1.5 mm was 4.4 days for low density seeds and 5.0 day for high density seeds, a relative reduction of about 66% in both fractions.

Effect of pericarp removal on embryo development during incubation in water

When the pericarp was removed at the start of the incubation it took the true seeds of low and high density fractions 5.2 day and 6.0 day, respectively, to reach an embryo length of 1.5 mm (Tab. 1). The effect of pericarp removal was twice as strong for high density seeds. The initial difference in t_{50} between the two fractions of 1.7 day is reduced to 0.8 day.

Pericarp removal at the beginning of the incubation slightly decreased the number of cells in longitudinal cross sections of embryos after 3 and 5 days incubation (Tab. 2), but the cell size increased by the same treatment with 21 and 19 µm on day 3 and with 53 and 82 µm on day 5 for low and high density seeds, respectively (Tab 2). As a result cell size in true seeds became equal in both fractions on day 5. Thus, the stimulation of embryo growth after pericarp removal was correlated with an increase in the cell size.

Tabel 1. Incubation time at 15 °C in light at which embryo length is 1.5 mm. This length corresponds with 50 % radicle protrusion in intact seeds (t_{50}). For true seeds, the pericarp was removed at the start of the incubation.

seed density, $mg.mm^{-3}$	Incubation time, days		
	seed	true seed	difference
0.98	6.1	5.2	0.9
1.15	7.8	6.0	1.8
difference	1.7	0.8	

Table 2. Mean embryo cell size and number in longitudinal cross sections of embryos in seeds and true seeds of celery cv. Monarch after 3 and 5 days incubation at 15 °C in light. Pericarp was removed before the start of imbibition.

incubation time, days	density 0.98 mg.m^{-3}				density 1.15 mg.mm^{-3}			
	seed		true seed		seed		true seed	
	mean cell size, µm^2	mean cell number	mean cell size, µm^2	mean cell number	mean cell size, µm^2	mean cell number	mean cell size, µm^2	mean cell number
3	258	457	279	444	223	448	242	413
5	259	710	312	762	227	674	309	657

Effect of the external osmotic potential on the t_{50}

Germination experiments with low density seeds in a range of osmotic potentials showed that when an external osmotic potential of -0.25 MPa was applied, the t_{50} of low density seeds became equal to the t_{50} of high density seeds germinated in water (Fig. 4).

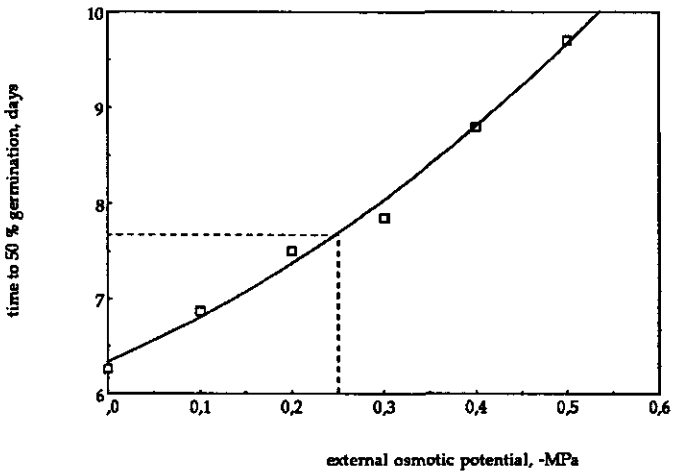


Figure 4. The time to 50 % germination of celery seeds cv. Monarch lot 9092 with density 0.98 mg.mm^{-3} when incubated in PEG solutions at 15 °C in light. Dotted line indicate the t_{50} of celery seeds cv. Monarch with density 1.15 mg.mm^{-3} (data from Fig. 1).

Effect of the external osmotic potential on embryo cell size and cell number during PEG incubation

Embryo length, and number and size of embryo cells, were also measured after different period of incubation times in a range of PEG concentrations. There were no detectable differences in cell size between the treatments. The average cell size for seeds of both density fractions at all PEG concentrations was 227±10 μm^2. (Tab. 3). The embryo cell number and embryo length were positively correlated with incubation time, but the difference in cell number and embryo length between the two fractions could not be eliminated by a difference in length of incubation period.

However, a decrease in the osmotic potential of the incubation medium strongly reduced the maximum cell number and the embryo length. Interestingly, it was observed that the mean embryo length of high density seeds incubated in -1.2 and -1.6 MPa PEG were reached in low density seeds in -1.6 and -2.0 MPa PEG, respectively. Roughly the same conclusion was valid for cell numbers.

Table 3. Embryo cell size, cell number and embryo length after PEG incubation of Celery cv. Monarch low and high density seeds at 15 °C in light, depending on length of incubation and osmotic potential of incubation medium.

osmotic potential, MPa	length of incubation period, days	cell size, μm^2		cell number		embryo length, mm	
		density, $mg.mm^{-3}$		density, $mg.mm^{-3}$		density, $mg.mm^{-3}$	
		0.98	1.15	0.98	1.15	0.98	1.15
-1.2	10	216	236	710	491	0.930	0.749
-1.2	17	238	226	684	501	0.986	0.719
-1.2	24	223	233	770	502	1.020	0.756
-1.2	31	218	224	812	567	1.030	0.798
-1.6	10	239	258	465	361	0.727	0.644
-1.6	17	-	226	-	437	0.769	0.652
-1.6	24	221	230	542	438	0.760	0.641
-1.6	31	215	214	614	513	0.812	0.708
-2.0	10	232	227	402	361	0.613	0.573
-2.0	17	223	237	476	363	0.668	0.561
-2.0	24	217	215	446	400	0.627	0.579
-2.0	31	228	217	448	391	0.656	0.564
untreated		186	181	339	314	0.458	0.430

The seed water relations during incubation in water

The effect of pericarp removal on embryo development and the effect of an externally applied osmotic potential on the t_{50} suggested an osmotic regulation of embryo growth. Therefore, the seed water relations were studied. The time course of the water uptake of true seeds of the two density fractions is shown in Fig. 5. At the end of imbibition (day 1) true seeds of low and high density seeds contained 41.3 and 38.9% water, respectively. The true seed water content was rather low when compared to the seed water content (chapter 5). It has to be realized, however, that the pericarp, that makes up about 40% of the total seed volume (chapter 3), contains about 70% water when the seed is fully imbibed. The difference of about 3% remained constant during the whole germination process. For both fractions the water content of the true seeds raised sharply after day 4 (Fig. 5).

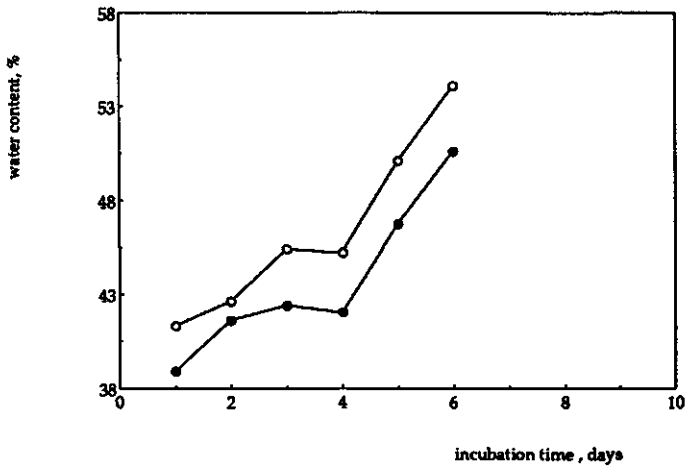


Figure 5. The course of true seed water content in seeds of celery cv. Monarch lot 9092 with density 0.98 mg.mm^{-3} (o) and 1.15 mg.mm^{-3} (•) during incubation at 15 °C in light. Intact seeds were incubated, the pericarp was removed shortly before determination of the water content.

Additional imbibition of the true seeds during 4.5 h when the pericarp was removed after 3 days incubation caused a rise in water uptake to 50.2% ± 1.5% for low density seeds and 50.1% ± 1.3% for high density seeds.

After 3 days incubation at 15 °C in light the water potential (ψ) of seeds of both fractions was 0 MPa. Thus, the seeds were in equilibrium with the water that surrounded the seed. The ψ of true seeds, however, was very negative, -1.81 and -1.97 MPa for low and high density seeds, respectively (Tab. 4). The osmotic potential (ψ_π) of true

Table 4. Water relations of low and high density seeds and true seeds. Water potential (ψ) was measured after 3 days incubation in water at 15 °C in light, either direct (seed) or immediatly after removal of the pericarp (true seed), with or without additional imbibition for 4.5 h after pericarp removal, of celery cv. Monarch. Measurement of ψ_π of intact seeds were not performed because of pericarp bound soluble factors.

	potential, MPa			
	density 0.98 mg.mm^{-3}		density 1.15 mg.mm^{-3}	
	ψ	ψ_π	ψ	ψ_π
seeds	-0.02	-	0	-
true seeds	-1.81	-2.73	-1.97	-2.90
true seeds + imbibition	-0.22	-	-0.22	-

Table 5. Measured and calculated water (ψ), osmotic (ψ_π) and pressure (ψp) potentials of true seeds, and pressure potential of the pericarp on the true seed, in low and high density seeds of celery cv. Monarch, after 3 days incubation at 15 °C in light.

seed density, mg.mm^{-3}	potential, MPa			
	$\psi_{true\ seed}$	$\psi_{\pi,true\ seed}$	$\psi_{p,true\ seed}$	$\psi_{p,pericarp}$
0.98	-1.81	-2.73	0.92	1.79
1.15	-1.97	-2.90	0.93	1.97
difference	0.16	0.17	0.01	0.18

seeds was -2.73 and -2.90 MPa for low and high density seeds, respectively. After additional imbibition of the true seeds for 4.5 h after pericarp removal the ψ raised to -0.22 MPa for both fractions.

The pressure potential in the true seed($\psi_{p,true\ seed}$) could be calculated with the formula:

$$\psi_{p,true\ seed} = \psi_{true\ seed} - \psi_\pi \quad (1)$$

The $\psi_{p,\ true\ seed}$ was 0.92 and 0.93 MPa in the low and high density seeds, respectively (Tab. 5). The ψ_p caused by the pericarp could be calculated as the diffe-

rence between the ψ of seeds and true seeds:

$$\psi_{p,pericarp} = \psi_{seed} - \psi_{true\ seed} \quad (2)$$

The $\psi_{p,pericarp}$ was 1.79 and 1.97 MPa for low and high density seeds, respectively (Tab. 5).

The seed water relations during incubation in PEG

Seed water relations were also measured after 7 days incubation in -1.2 MPa PEG at 15 °C in light. The water content of the true seeds was slightly lower in the high density seeds (Tab. 6). The water potential (ψ) of intact seeds was about equal to the osmotic potential (ψ_π) of the medium that after 7 days of incubation was decreased to -1.48 MPa (Tab. 6). The true seed had a much lower ψ, -3.20 and -3.38 MPa for low and high density seeds, respectively. The Ψ_π of the true seeds was -3.52 and -3.86 MPa for low and high density seeds , respectively. The pressure potential of the true seed ($\psi_{p,true\ seed}$) was calculated with formula (1) and was 0.32 and 0.42 MPa for low and high density seeds, respectively (Tab. 7). The ψ_p caused by the pericarp ($\psi_{p,pericarp}$) was calculated with formula (2) and was 1.72 and 1.93 MPa for low and high density seeds, respectively (Tab. 7).

Table 6. Water content, ψ and ψ_π of seeds and true seeds of low and high density fractions of celery cv. Monarch incubated for 7 days in -1.2 MPa PEG at 15 °C in light. At that time the measured ψ_π of the incubation medium was -1.48 MPa.

	water content, % fw.		potentials, MPa			
	density, mg.mm^{-3}		density, mg.mm^{-3}			
	0.98	1.15	0.98		1.15	
			ψ	ψ_π	ψ	ψ_π
seed	-	-	-1.48	-	-1.45	-
true seed	37.0	35.6	-3.20	-3.52	-3.38	-3.86

Discussion

Water relations

The present results provide an explanation for the negative relationship that was found between embryo growth rate and the relative proportion of pericarp tissue in seed fractions of celery separated by fluid density grading. The larger and thicker pericarp of high density seeds (chapter 3) corresponded to a larger pressure potential

Table 7. Measured and calculated water (ψ), osmotic (ψ_π) and pressure (ψp) potentials of true seeds, and pressure potential of the pericarp on the true seed, in low and high density seeds of celery cv. Monarch, after 7 days incubation in -1.2 MPa PEG at 15 °C in light.

seed density, $mg.mm^{-3}$	potential (MPa)			
	$\psi_{true\ seed}$	$\psi_{\pi,true\ seed}$	$\psi_{p,true\ seed}$	$\psi_{p,pericarp}$
0.98	-3.20	-3.52	0.32	1.72
1.15	-3.38	-3.86	0.48	1.93
difference	0.18	0.34	0.16	0.21

of the pericarp ($\psi_{p,pericarp}$) (tab. 5). The difference in $\psi_{p,pericarp}$ was concluded from the determinations of ψ and ψ_π. Whereas the ψ of seeds of both fractions were similar, pre-incubated true seeds of the high density fraction showed directly after pericarp removal a more negative ψ and ψ_π than the low density seeds. As a consequence $\psi_{p,true\ seeds}$ were similar. Evidently, the difference in $\psi_{\pi,true\ seed}$ is caused by the difference in $\psi_{p,pericarp}$. It will be shown in chapter 7 that seeds of both fractions did not differ in the content of osmotic constituents.

Embryo growth

Embryo growth in mature celery seeds prior to visible germination occurred through both an increase in cell size and in the number of cells (Fig. 1). The increase in cell size mainly occurred during the first 3 days (Fig. 1c). It differed between the fractions. It is likely that the increase in cell size during imbibition is in the first place due to swelling of dry cells. Therefore, the different rate and degree of swelling of low and high density seeds may be partly explained by the negative relationship between seed density and the relative proportions of air and endosperm (chapter 3). Cells in low density seeds have more space to swell than cells in high density seeds. After imbibition the differences in size persisted. Thus, newly formed embryo cells reached a larger size in low density seeds. When the pericarp was removed, after 5 days of incubation cells in embryos of both fractions reached the same size (Tab. 2). Therefore, the size of newly formed cells will be a function of $\psi_{p,pericarp}$. During incubation in -1.2 MPa PEG a similar difference existed between the $\psi_{p,pericarp}$ of low and high density seeds as in water (Tabs. 5,7). However, the addition of an extra hindrance to water uptake caused a very small size of all embryo cells. At the bottomline differences did not exist anymore between the cells in the two fractions. As a consequence embryo growth in PEG only occurred through cell division. Similar observations were made in *Daucus carota* seeds during seed 'hardening', a treatment that also consists of incuba-

tion of seeds under limited water availability (Austin, Longden and Hutchinson 1969).

The rate of cell division also differed between the seed fractions (Fig. 1b). An involvement of water relations in the control of cell division can be deduced from the effects of PEG. Incubation in -1.2 MPa PEG clearly inhibited the rate of cell division (Figs. 1b,2b). A difference in the osmotic potential of the PEG medium of 0.4 MPa neutralized the difference in cell division rate (Tab. 3). Such a difference in external osmotic potential roughly coincided with the difference in $\psi_{\pi,\text{true seed}}$ of the fractions in -1.2 MPa PEG (Tab. 7). It is concluded that the different rate of cell division in low and high density seeds is at least partly a function of the different ψ_π of the seeds that resulted from the differences in morphology of the seeds. Inhibitory effects of lowering ψ_π on metabolic activities in seeds has been shown in barley aleurone layers (Jones and Armstrong 1971) and lettuce seeds (Weges 1987). The lower ψ_π in high density seeds caused a lower number of cells in the embryo after 10 to 13 days incubation than in low density seeds (Fig. 2b), most probably because mitosis is inhibited completely at that time (Hsiao 1973).

When the pericarp was removed at the start of imbibition the embryos in the true seeds still showed after 5 days incubation different number of cells (Tab. 2). Thus, the difference in cell division rate did not solely depend on water relations. Since low density seeds had a more extended ripening period on the mother plant than high density seeds (chapter 4) the biochemical mechanisms involved in cell division might be better prepared.

The moment around day 3 to 4 that the cell division rate increased in seeds incubated in water (Fig. 1b), coincided with a shift in the time course of water uptake in true seeds of both fractions (Fig. 5). A relationship between both events may have a base in the different characteristic of embryo and endosperm cells. Around day 3 to 4 endosperm hydrolysis caused the dissapearance of endosperm cells and the formation of new embryo cells.

Priming

The present experiments provide an explanation for the beneficial effect of osmotic pre-incubation or priming. After 17 days in -1.2 MPa PEG embryos grew much faster in water than without a pretreatment (Fig. 3). The higher growth rate was due to the much larger size that embryo cells of both fractions reached soon after transfer from PEG to water (Fig. 3c). Thus, although embryo cells of celery during incubation in -1.2 MPa PEG seems to become inert after 10 to 13 days when the increase in cell number stops (Fig. 2b), evidently cell enlargement is prepared enabling a quick cell expansion after transfer to water. The mechanisms involved in increased cell expansion capacity are not understood yet. It is interesting to note that also in lettuce seeds pre-incubation in PEG increased the cell wall extensibility (Weges 1987).

Studies on endosperm breakdown in celery (*Apium graveolens* L.) seeds.

Summary

Both during germination and incubation of celery seeds in PEG, embryo growth occurred at the expense of the endosperm. It was shown that cell wall hydrolysis occurred in endosperm cells at the chalazal and at the mycropylar side of the endosperm when seeds were germinated in water. Endo-ß-mannanase activity in extracts of the seeds increased during germination in water and incubation in PEG. It is concluded that endo-ß-mannanase is involved in endosperm hydrolysis. During incubation in PEG the increase in endo-ß-mannanase activity was reduced as compared to germination in water, but the reduction was less than the reduction in embryo cell division rate.

Low density seeds had a higher endo-ß-mannanase activity than high density seeds. It is concluded that endo-ß-mannanase activity and embryo cell division rate are positively correlated, both during germination in water and incubation in PEG.

The difference in endo-ß-mannanase activity between low and high density seeds was already present at the time of harvest. It was shown that umbel position and length of seed maturation period affected the endo-ß-mannanase activity in the seeds.

Introduction

During germination of celery seeds prior to radicle protrusion embryo growth occurs (chapter 6). It has to be realized that the embryo grows at the expense of the endosperm tissue that surrounds it. In the present chapter endosperm breakdown will be studied in seeds with different densities and in relation to incubation in PEG.

Jacobsen and Pressman (1979) have proposed a model for endosperm breakdown; this hypothesis is illustrated in Fig. 1. Light enters the seed through the pericarp and activates phytochrome in the embryo, which triggers the production of gibberellin. The gibberellin diffuses in the endosperm, where it causes the production of hydrolitic enzymes. Enzyme activity results in nutrients and space for the growing embryo.

It is as yet unknown which hydrolitic enzymes are involved in the endosperm breakdown, and what the celery endosperm cell wall constituents are. Earl and Jones (1962) reported 23% protein, 30% oil and 7.3% ash in celery seeds; as there was no

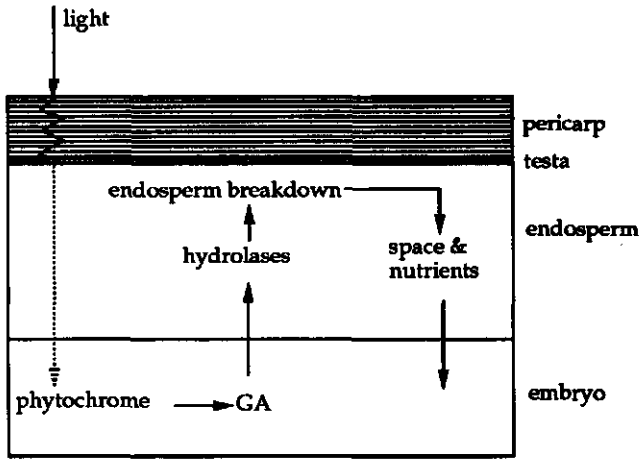


Figure 1. Proposed model of endosperm breakdown in celery seed, based on Jacobsen and Pressman (1979).

starch the remaining 40% is probably the cell wall material. Hopf and Kandler (1977) found in the related species *Carum carvi* (*Umbelliferae*) 48% polysaccharides; these polysaccharides consisted for 90.4% of mannose, 5.4% of glucose, and the remaining 4.2% were galactose, arabinose, and xylose. The mannose was characterized as a ß(1-4)-mannan. If celery contains also high amounts of mannose, the most important hydrolyzing enzyme should be endo-ß-mannanase.

Results

Embryo growth and endosperm breakdown during incubation in water

Seeds were incubated in water at 15 °C in light. Longitudinal cross sections through the embryo were made after several incubation periods, and stained with 4,6-diamidino-2-phenyl-indole (DAPI) dihydrochloride. DAPI dihydrochloride was used because the cross sections were also used to count numbers of cells (chapter 6); it reacts strongly with nucleic acids and therefore nuclei are clearly recognizable. It can be seen in Fig. 2 that in the first days of the germination process embryo growth occurred in the direction of the chalazal end of the endosperm. Growth mainly occurred in the hypocotyl, but also the cotyledons increased in length. After 4 days the embryo was about doubled in length. Radicle protrusion (Fig. 2c) in low density seeds was 6.1 days, and in high density seeds 7.8 days (chapter 5).

The DAPI stained sections also allowed some preliminary observations on endosperm breakdown.

After 4 days incubation cell hydrolysis of the endosperm cells in the mycropylar region opposite the radicle tip could be observed (Fig. 2b). A higher magnification (Fig. 3) showed that only one row of cells was hydrolyzed.

At the chalazal side the endosperm was degraded to create space for the growing embryo. Three phases in endosperm breakdown could be distinguished (Fig. 4): (1) The hydrolytic process started with the breakdown of protein bodies in the cells and, probably simultaneously, the hydrolysis of cell walls; (2) cell contents were released; (3) empty cells were further hydrolized and compressed.

At the start of the germination process seeds of low (0.98 $mg.mm^{-3}$) and high (1.15 $mg.mm^{-3}$) density fractions contained considerable endo-ß-mannanase activity (Fig. 5). In extracts of low density seeds the activity was about twice as high as in high density seeds. During the first day the activity was slightly reduced, but afterwards it rapidly increased (Fig. 5). The enzyme activity in low density seeds stayed higher than in high density seeds during the whole germination process. Thus, at any time during the germination process in water the endosperm breakdown capacity in low density seeds was potentially higher than in high density seeds.

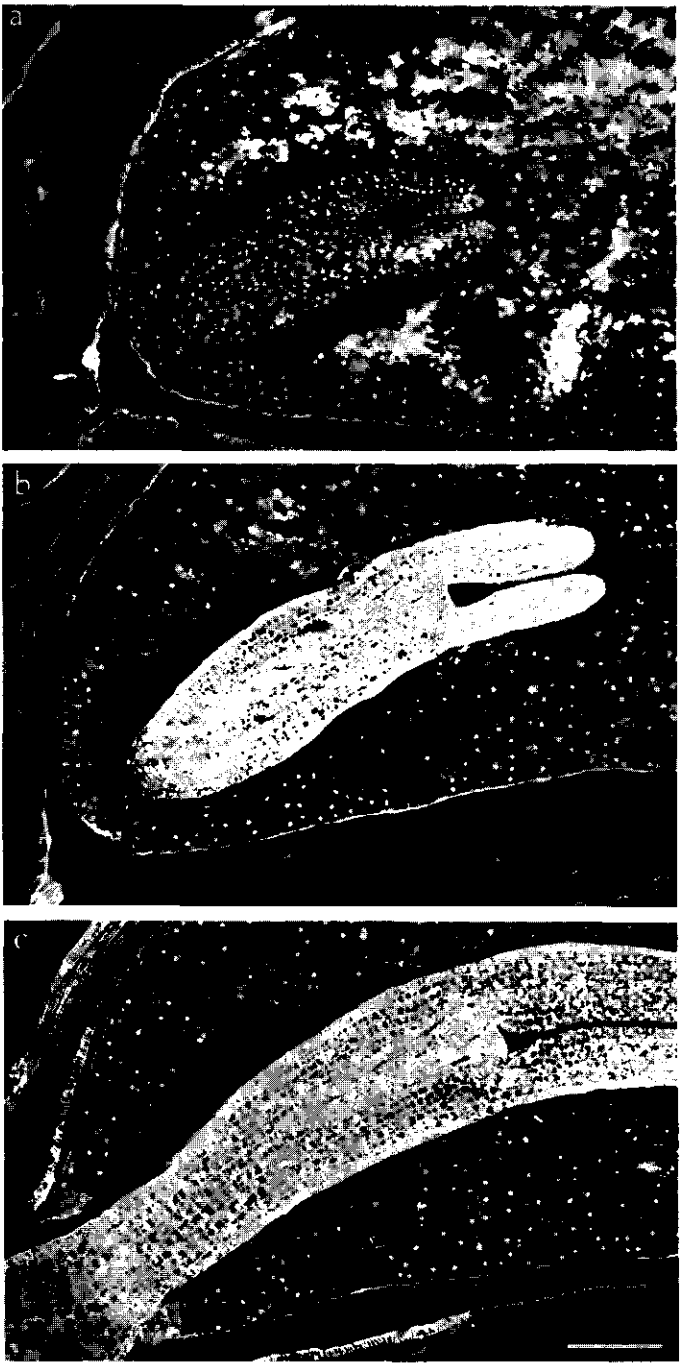
Endosperm cell wall hydrolysis will result in production of reducable sugars. The amount of reducable sugars was 40 $nmol.seed^{-1}$ after 1 day incubation and decreased to about 30 $nmol.seed^{-1}$ at the time of radicle protrusion in seeds of both fractions (Fig. 6).

Endo-ß-mannanase activity during seed development

The differences in endo-ß-mannanase activity between the two fractions at the time of harvest have to originate from the period of seed development. Therefore, endo-ß-mannanase activity during seed development was studied. Seeds of first and tertiary umbels of celery cv. Tall Utah were harvested between 35 and 84 d.a.p. Endo-ß-mannanase activity in seed of both umbels increased strongly from about 4 to 30% during this time (Fig. 7). The rate of increase in enzyme activity in seeds harvested from the tertiary umbels was higher than in seeds harvested from the primary umbels. Thus, endo-ß-mannanase activity was positively correlated with seed maturity in both umbels, and, since seed density was negatively correlated with seed maturity (chapter 4), low density seeds will have a higher activity of this enzyme than high density seeds.

Embryo growth and endosperm breakdown during incubation in PEG

During incubation of celery seeds in -1.2 MPa PEG germination is inhibited, but embryo growth occurs (chapter 6). Also in PEG, embryos grew in the direction of the chalazal end of the endosperm (Fig. 8). It was shown in chapter 6 that during growth in PEG cell size was reduced as compared to growth in water. Comparison between Figs. 2 and 8 indicates that in PEG the embryo cells are more densily stained than in


a
b
c

◀— Figure 2 A-C. Embryo growth in seeds of celery cv. Monarch lot 9092 during incubation at 15 °C in light. A. 1 day; B. 4 days; C. 7 days. Arrow on Fig. B indicates hydrolysis of endosperm cells in the mycropylar side, prior to radicle protrusion, that is seen on Fig. C. Bar is 200 µm.

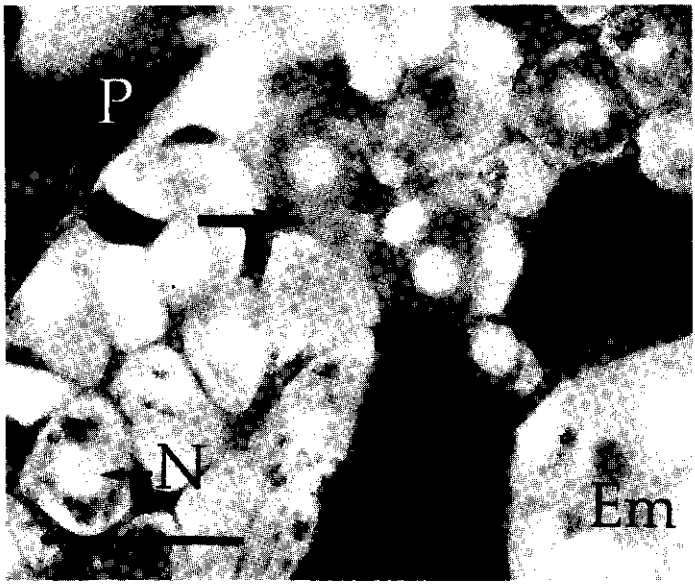


Figure 3. Endosperm cells at the mycropylar side, opposite the radicle tip in seeds of celery cv. Monarch lot 9092 after 4 days incubation at 15 °C in light. This figure is a magnification of figure 2b. Note the small area of cell hydrolysis, indicated with the arrow. P: pericarp, Em: embryo radicle, N: nucleus. Bar is 50 µm.

water, probably because of the abscence of vacuoles.

During incubation in PEG, endo-ß-mannanase activity in the two density fractions increased to levels comparable to those found in seeds germinating in water (compare Figs.9 and 5). In both fractions, the rate of increase in enzyme activity was about 60% of the rate of increase during germination. Untill 13 days after the start of the incubation in PEG the enzyme activity in low density seeds was higher than in high density seeds. There was no change in enzyme activity when the seeds were incubated at -1.6 MPa instead of -1.2 MPa.

The reducable sugar content of low density seeds incubated in -1.2 MPa slowly decreased from about 40 to 30 nmol.seed^{-1}; reducable sugar content in high density seeds was about 10% higher at any time during incubation in PEG (Fig. 10).

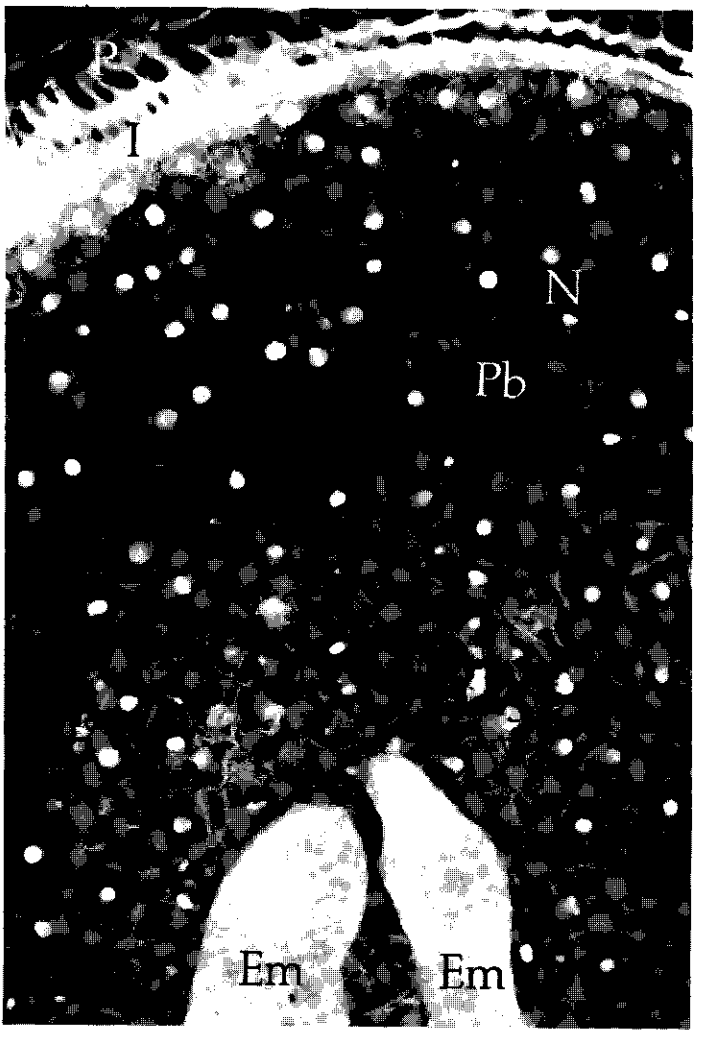


Figure 4. Stages in endosperm breakdown during incubation of celery seeds cv. Monarch lot 9092 at 15 °C in light. Note the breakdown of protein bodies (1), the dissapearance of cell contents (2), and cell wall hydrolysis (3). P: pericarp, I: testa, Em: embryo cotyledons, N: nucleus, Pb: protein bodies. Bar is 100 µm.

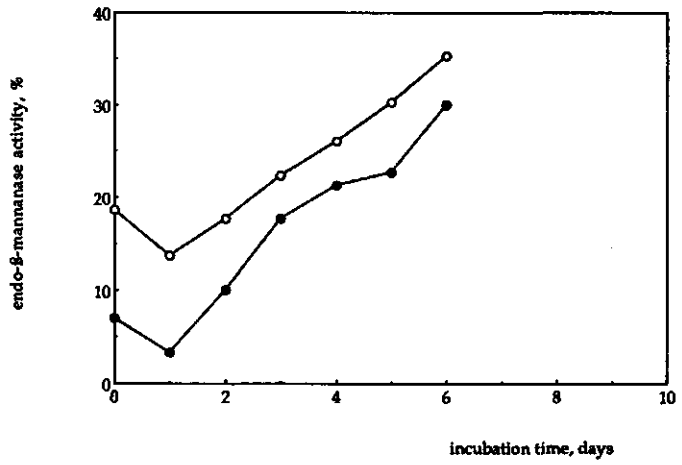


Figure 5. Endo-ß-mannanase activity in celery seeds cv. Monarch lot 9092 with density 0.98 mg.mm^{-3} (o) or 1.15 mg.mm^{-3} (•) during incubation in water at 15 °C in light. Activity is expressed as percentage decrease in flow time of assay-mixture as compared to control mixtures with boiled enzyme extracts.

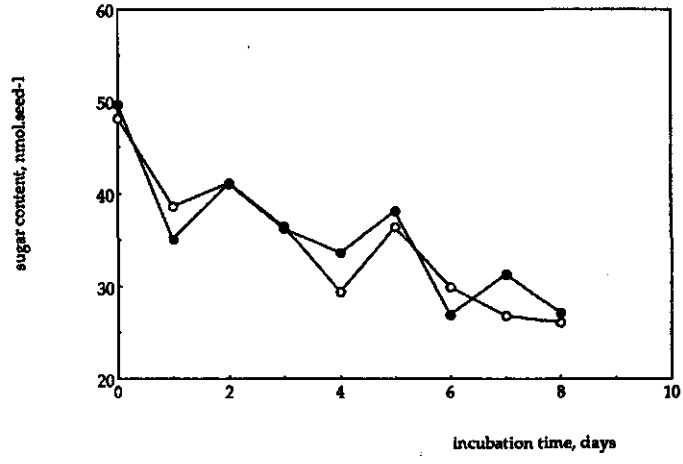


Figure 6. Reducable sugar content in celery seeds cv. Monarch lot 9092 with density 0.98 mg.mm^{-3} (o) or 1.15 mg.mm^{-3} (•), expressed as nmol glucose equivalents, during incubation in water at 15 °C in light.

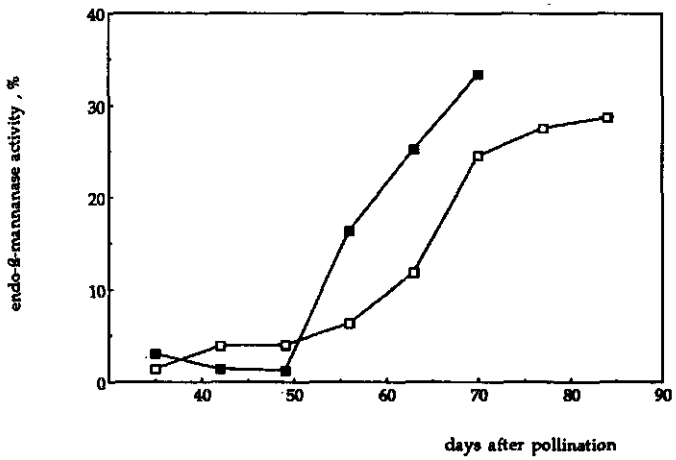


Figure 7. Endo-ß-mannanase activity in celery seeds of cv. Tall Utah harvested at different times after polination from primary (□) and tertiary (■) umbels.

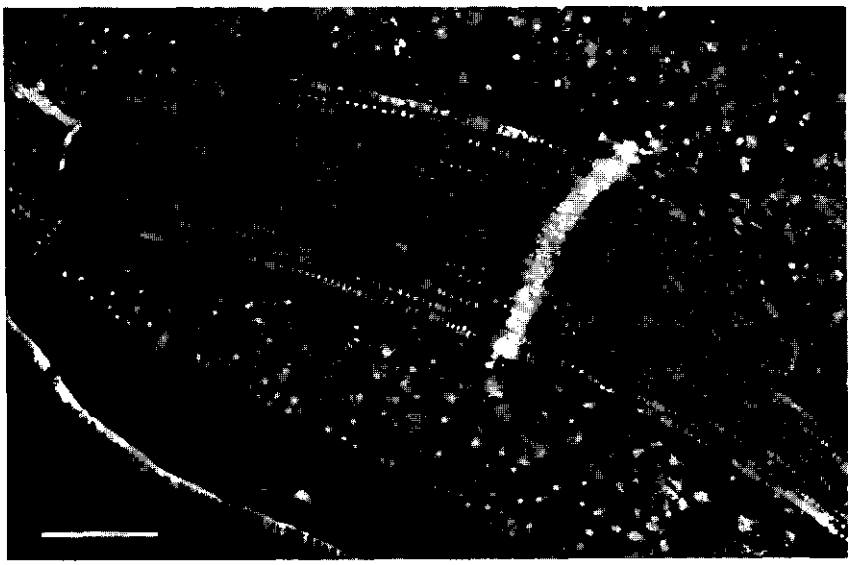


Figure 8. Embryo in seed of celery cv. Monarch lot 9092 after incubation in -1.2 MPa PEG at 15 °C in light for 17 days. Bar is 200 µm.

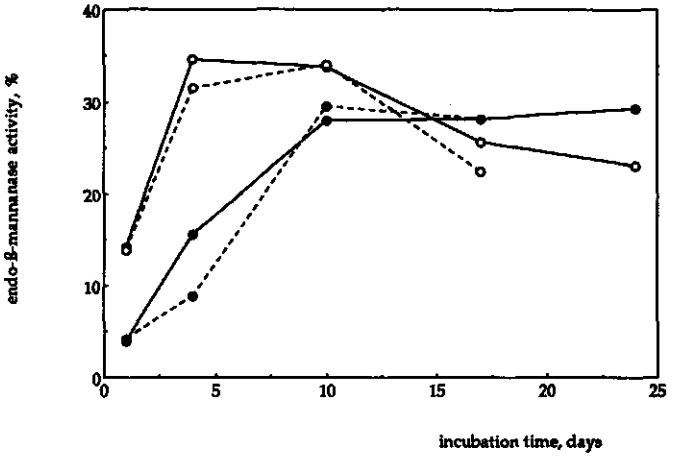


Figure 9. Endo-ß-mannanase activity in celery seeds cv. Monarch lot 9092 with density 0.98 $mg.mm^{-3}$ (○) or 1.15 $mg.mm^{-3}$ (●) during incubation in -1.2 MPa PEG (continued lines) or -1.6 MPa (dotted lines), at 15 °C in light.

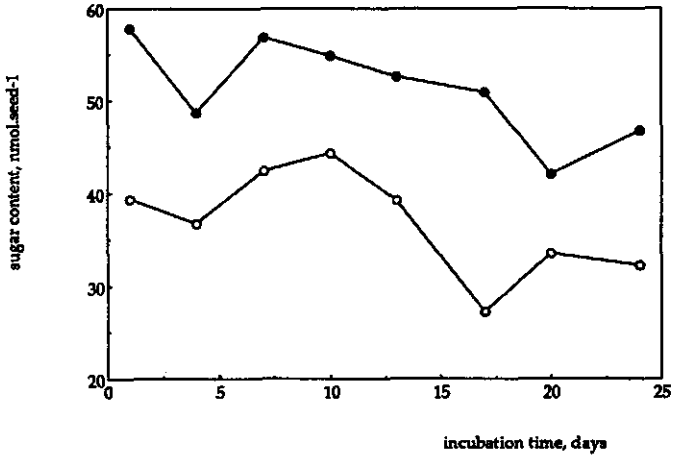


Figure 10. Reducable sugar content in celery seeds cv. Monarch lot 9092 with density 0.98 $mg.mm^{-3}$ (○) or 1.15 $mg.mm^{-3}$ (●), expressed as nmol glucose equivalents, during incubation in -1.2 MPa PEG at 15 °C in light.

Discussion

Endo-ß-mannanase activity and endosperm breakdown

The present results show that both during germination in water and incubation in PEG of celery seeds, embryo growth occurred at the expense of the endosperm. Since cell wall hydrolysis is part of the endosperm breakdown process and the endosperm cell walls probably consist of galactomannans, and since endo-ß-mannanase activity increased during incubation in water and PEG, this hydrolytic enzyme may be involved in the endosperm breakdown process. Endo-ß-mannanase is not the only enzyme necessary for the hydrolysis of endosperm cell walls in celery seeds. Galactomannans consists of a ß-D-(1-4) linked backbone of ß-D-mannopyranosyl residues having side stubs linked α-D-(1-6), and consisting of single α-D-galactopyranosyl groups. Three enzymes are required for the galactomannan breakdown: α-D-galactosidase, for removal of the (1-6) α-D-galactose side chains, ß-D-mannanase, for fission of the (1-4) ß-D-mannan backbone into oligosaccharides, and ß-D-mannosidase, for complete hydrolysis of the D-manno-oligosaccharides to D-mannose (Dea and Morrisson 1975). It has been shown for several species containing galactomannans as storage tissue that these three enzymes are indeed active during germination (Seiler 1977, Reid, Davies and Meier 1977, Groot, Kieliszewska-Rokicka, Vermeer and Karssen 1988).

The higher activity of endo-ß-mannanase in low as compared to high density seeds (Figs. 5 and 9) is in good agreement with the higher embryo cell division rate both in water and PEG (chapter 6). However, the amount of reducable sugars formed during germination was equal in both fractions (Fig. 6), which suggests that the higher endo-ß-mannanase activity in low density seeds was followed by a higher sugar consumption. It is as yet unclear why the reducable sugar content in high density seeds during incubation in PEG was higher than in low density seeds.

During incubation in PEG the rate of increase in endo-ß-mannanase activity was 60% of this rate during germination in water. Spyropoulos and Reid (1988) have shown that both production and activity of endosperm-degrading enzymes are negatively affected by water stress in *Trigonella foenum-carum* L., and Jones and Armstrong (1971) found evidence for osmotic inhibition of enzyme production necessary for endosperm breakdown in germinating barley seeds. Interestingly, the cell division rate during PEG incubation was only about 27% of this rate during germination in water (chapter 6). Thus, the rate of endosperm hydrolysis during PEG incubation may be higher than necessary for the embryo growth. The relatively high hydrolytic activity during incubation in PEG may lead to an excess of space inside the endosperm. In the experimental work described in this and the previous chapter weakening was observed in seeds that were incubated in PEG, as compared to seeds incubated in water. When seeds were incubated in water after incubation in PEG, the rapid increase in embryo cell size (chapter 6) may be not only related to an increase in embryo cell wall extensibility, but also to this excessive space.

Endosperm breakdown at the mycropylar side of the endosperm, observed in

seeds of both fractions after 4 days of incubation in water, may cause the shift in the direction of embryo growth that leads to protrusion (chapter 6). This shift was marked by a slight retardation of embryo growth - although at the chalazal end of the endosperm there was still enough space for continued embryo growth - and a strong increase in true seed water content. Hydrolysis of these endosperm cells may be a prerequisite for radicle protrusion, as Groot and Karssen (1987) have shown for tomato seeds. Haigh (1988) has shown that in tomato the endosperm cells opposing the radicle tip are smaller than all other endosperm cells. A similar situation occurs in celery (Jacobsen and Pressman 1979).

Endo-ß-mannanase activity during seed development

The present results showed that during celery cv. Tall Utah seed development the endo-ß-mannanase activity strongly increased from 35 to about 77 d.a.p. (Fig. 7). This suggests that during seed development the same mechanism of embryo growth occurs as in mature seeds. It is assumed that during seed development the embryo grows from the globular to the torpedo stage, as is the case with the related species *Daucus carota* (Halperin 1966).

Interestingly, the umbel position had an influence on the rate of increase in enzyme activity. It is as yet not known what factor causes this difference. However, it is concluded that seeds of later in season formed umbels require a shorter ripening time to build up a sufficient amount of hydrolytic enzymes. Nevertheless, the mean seed quality of the total harvested seed lot will increase if the time of harvest is postponed as long as possible.

Comparison of increase in endo-ß-mannanase activity (Fig. 7) and decrease in seed density (chapter 4 Fig. 2) during seed development shows that seed fractions separated with fluid density grading, as we have used in our studies, will be different in their enzyme content when the difference in density is large enough. It can be concluded that seeds with density 1.15 $mg.mm^{-3}$ are certainly not mature and have a low enzyme content when compared to seeds with density 0.98 $mg.mm^{-3}$.

Seed germination and priming of celery (*Apium graveolens* L.) cultivars.

Summary

Mean seed density and the mean volume of seed parts varied greatly between seeds of 8 different cultivars of celery. The seed density was not correlated with the time to 50% germination (t_{50}). The seed density was negatively correlated with the volume of air that was present in the seeds. The air volume was negatively correlated with the t_{50}. It was argued that the differences in the relative proportion of seed parts may affect the germination rate in a similar manner as in genetically identical but physiologically different seeds originating from one seed lot.

Endo-ß-mannanase activity was not correlated with any other parameter, but reducable sugar content was negatively correlated with all seed parts. The t_{50} was correlated positively with the sugar content. It was concluded that the amount of reducable sugars indicated the efficiency of the turnover of nutrients from the endosperm to the embryo.

In all cultivars, the reduction in t_{50} found after a priming treatment was 66%. This reduction was not correlated to the increase in embryo length due to the priming, that varied greatly between the cultivars. Increase in embryo length was solely due to an increase in embryo cell number.

It was concluded that genetic differences that exist between cultivars with respect to germination rate and the effect of a priming treatment, may cause similar physiological differences as exist between genetically identical seeds with different density.

Introduction

When seed germination is slow, seed priming offers good perspectives to improve seed quality (Heydecker 1974, Heydecker et al. 1973). Osmotic priming of seeds was first described by Levitt and Hamm (1943) and again by Ells (1963). The treatment consists of an incubation of the seeds during a certain period of time (for celery usually 14 days) at a certain temperature (for celery usually 15 °C) in an osmoticum of -1.0 to -1.5 MPa, made up of salt or polyethylene glycol with a molecular weight of 6000 (PEG 6000), dissolved in water.

Because of slow and irregular germination, priming of celery seeds has often been

studied (Salter and Darby 1976, Khan et al. 1978, Darby et al. 1979, Haarman 1980, Khan et al. 1980/81, Nakamura et al. 1982, Brocklehurst and Dearman 1983, Brocklehurst et al. 1982, Cantliffe et al. 1987, Globerson and Feder 1987, Tanne and Cantliffe 1987). Commercial application of priming treatments to celery seeds, however, is limited by the variable reaction of different cultivars and seed lots to a standard treatment (Heydecker and Coolbear 1977, Brocklehurst and Dearman 1983,1984, Cantliffe et al. 1987, Globerson and Feder 1987).

It was shown in this thesis that fluid density grading can separate within one seed lot seeds with different density (chapter 3). Density correlated positively and negatively with a range of morphological and physiological differences (chapters 3, 5, 6, 7). Low density seeds germinated quicker than high density seeds. A pre-incubation in PEG substantially improved the germination rate in seeds of both fractions but the reaction of high density seeds was more pronounced than of low density seeds (chapter 6). It was shown in chapter 4 that density decreased during seed maturation on the mother plant. Therefore, low density seeds were more mature than high density seeds.

It is hypothesized that differences between seed lots of a cultivar, and differences between cultivars, might be explained by the same mechanisms as the differences originating from progressing seed maturity. In this study we will compare germination rate and embryo growth during and after seed priming in seeds of different cultivars and seed lots. The relation of germination and growth to structural and physiological parameters will be studied.

Results and discussion

Germination rate

Optimal conditions for germination of celery seeds are alternating temperatures (15/20 °C) and light (chapter 3). However, the present experiments were performed at a constant temperature (15 °C) in light, because the results had to be comparable to the experiments reported of in the previous chapters. The germination percentage at 15 °C in light was more than 95% of the percentage at optimum conditions for all cultivars used in this chapter, exept cv. Alba, (Tab. 1).

In Tab. 2 structural and physiological parameters, and the t_{50} of the 10 seed lots are presented. The seed lots in this table were tabulated in order of increasing mean seed density, that ranged from 1.043 (cv. Monarch 9081) to 1.191 $mg.mm^{-3}$ (cv. Elio).

The mean seed density of different seed lots and cultivars did not correlate with the t_{50} (Tab. 2 last column), in contrast to the positive correlation between seed density and t_{50} when different density fractions from one seed lot were compared (chapter 3).

In the previous chapters it was shown that low density seeds contained more air and had a lower pericarp volume than high density seeds. Therefore, the endosperm could swell more upon imbibition and the ψ_π in the seed became less negative. The ψ_π correlated to embryo cell size, and, therefore, low density seeds had a higher embryo

Table 1. Germination percentages at optimal conditions (15/20 °C in light) and at suboptimal temperature (15 °C in light) of ten cultivars of celery, used in this study.

cultivar	germination, %	
	15/20 °C in light	15 °C in light
Monarch 9081	97.3	97.4
Tall Utah 10421	92.0	92.2
Monarch 9092	96.2	93.9
Iram	96.1	95.3
Arvi	85.4	80.1
Alba	85.1	71.2
Celebrity	97.3	93.7
Tall Utah 601501	97.4	95.1
Selfira	93.9	91.6
Elio	97.8	96.4

growth rate and a lower t_{50}. It has to be realized that in those studies density fractions were used that had a similar endosperm volume. The cultivars in the present study differ strongly in seed and endosperm volumes (Tab. 2). For the determination of endosperm volume it was assumed that the density of endosperm and pericarp were 1.214 and 1.48 $mg.mm^{-3}$, respectively, as was determined for the cvs. Monarch, Selfira and Tall Utah in chapter 3. The volume of seed parts varied strongly between the ten seed lots. Endosperm volume varied between 0.109 (cv. Celebrity) and 0.250 mm^3 (cv. Iram), pericarp volume between 0.090 (cv. Selfira) and 0.248 mm^3 (cv. Monarch 9092), and air volume between 0.029 (cv. Selfira) and 0.127 mm^3 (cv. Monarch 9081) (Tab. 2).

Regression analysis showed that seed density was mainly a function of the air volume (av) present in the seeds (Tab. 3). Interestingly, the air volume was also the only structural parameter that correlated significantly with the t_{50} (Tab. 3), although the correlation coefficient was much lower than for the density fractions from one seed lot (chapter 3). It has to be realized that a cultivar with a larger air volume has in general also larger endosperm and pericarp volumes (Tab. 2). It is shown that all three seed parts will somehow affect the water imbibing capacity of the endosperm. In the present study the endosperm moisture content, determined after 3 days of incubation in water, correlated negatively with all seed structure parameters, in particular with air volume (Tabs 2 and 3). It indicates that the air volume as such is not critical for water uptake in the different seed lots and cultivars, as it was for the density fractions originating from one seed lot, that showed a positive correlation between air volume and water uptake (chapter 6).

Table 2. Structural and physiological parameters and t_{50} of seeds of celery cultivars. Endosperm moisture content was determined after 3 days incubation at 15 °C in light. t_{50} was determined at 15 °C in light when seeds were incubated in water at 15 °C. The other parameters were determined at dry seeds.

cultivar	density, $mg.mm^{-3}$	weight, mg			volume, mm^3			
		endosp.	peric.	seed	endosp.	peric.	air	seed
Monarch 9081	1.043	0.276	0.316	0.592	0.227	0.214	0.127	0.568
Tall Utah 10421	1.046	0.183	0.182	0.365	0.151	0.123	0.075	0.349
Monarch 9092	1.065	0.296	0.367	0.663	0.244	0.248	0.131	0.623
Iram	1.079	0.304	0.355	0.658	0.250	0.240	0.120	0.610
Arvi	1.096	0.283	0.257	0.540	0.233	0.174	0.086	0.493
Alba	1.102	0.270	0.358	0.628	0.222	0.242	0.106	0.570
Celebrity	1.104	0.132	0.143	0.275	0.109	0.096	0.044	0.249
Tall Utah 601501	1.141	0.249	0.262	0.511	0.205	0.177	0.066	0.448
Selfira	1.168	0.152	0.133	0.285	0.125	0.090	0.029	0.244
Elio	1.191	0.267	0.202	0.469	0.220	0.136	0.038	0.394

cultivar	endosperm moisture content, %	endosperm content, mg^{-1}		embryo length, mm	t_{50}, days
		endo-ß-mannanase, %	sugar, nmol		
Monarch 9081	39.9	27.6	111	0.369	6.1
Tall Utah 10421	45.5	9.9	209	0.307	7.7
Monarch 9092	45.7	34.7	131	0.442	7.1
Iram	45.0	1.8	162	0.475	8.8
Arvi	40.1	18.0	137	0.434	10.2
Alba	45.5	23.9	190	0.437	9.7
Celebrity	53.4	17.1	371	0.213	10.8
Tall Utah 601501	53.3	35.7	149	0.423	7.7
Selfira	50.7	6.2	259	0.249	9.7
Elio	50.4	4.3	114	0.347	9.2

The present results still indicate that the proportion of seed parts in seeds of different cultivars may have the same effect on the t_{50} as was analyzed in the previous chapters for density fractions from one seed lot.

Table 3. Correlation matrix of seed structural and physiological parameters, and t_{50}, based on data in table 2. The minus sign indicates a negative correlation. Asterix indicate significant correlation ($p < 0.05$).

	regression coefficient								
	density	ew	pw	sw	ev	pv	av	sv	t50
density		0.22-	0.47-	0.37-	0.22-	0.47-	0.77-*	0.48-	0.49
moisture	0.66*	0.59-*	0.55-*	0.59-*	0.59-*	0.56-*	0.73-*	0.64-*	0.33
endo-ß-mann.	0.30-	0.26	0.44	0.38	0.26	0.44	0.43	0.40	0.46
reducable sugar	0.09	0.87-*	0.62-*	0.75-*	0.87-*	0.62-*	0.52-	0.72-*	0.60-*
embryo length	0.25-	0.93*	0.89*	0.94*	0.93*	0.89*	0.73*	0.91*	0.33-
t50	0.49	0.40-	0.45-	0.45-	0.40-	0.45-	0.57-*	0.49-	

The endo-ß-mannanase activity in the 10 seed lots was also analyzed. The results were expressed per mg endosperm to exclude the influence of differences in endosperm volume. The endo-ß-mannanase activity in dry seeds varied strongly between 1.8 (cv. Iram) and 35.7%.mg endosperm^{-1} (cv. Tall Utah 601501). There was no significant correlation between endo-ß-mannanase activity and one of the other seed parameters (Tab. 3). Endosperm breakdown is a prerequisite for embryo growth, but hydrolytic activity may be higher than the subsequent consumption of nutrients, resulting in the accumulation of reducable sugars. Seeds of different density fractions originating from one seed lot all contained similar reducable sugar content but differed in hydrolytic activity. Therefore, the higher hydrolytic activity in low density seeds must be correlated with an increased consumption of the nutrients (chapter 7), which in turn might be the reason for the higher embryo cell division rate during incubation in water and PEG of those seeds (chapter 6).

The endoperm sugar content in the 10 seed lots of the present study varied from 111 (cv. Monarch 9081) to 371 nmol.mg endosperm^{-1} (cv. Celebrity); it was negatively correlated with almost all seed structure parameters. This suggests that cultivars with larger seeds may have a less efficient metabolism leading to slower embryo growth. Indeed, the endosperm reducable sugar content was negatively correlated with the t_{50} (Tab. 3), although the regression coefficient is rather low. It is concluded that the reducable sugar content may be an indication of the efficiency of the turnover of nutrients

from the endosperm to the growing embryo.

The length of the embryo in the 10 seed lots varied from 0.21 (cv. Celebrity) to 0.47 mm (cv. Iram) (Tab. 2) and was correlated with all seed structure parameters (Tab. 3), which indicates that larger seeds have longer embryos. The embryo length was not correlated with the t_{50}. Most probably, in cultivars with large seeds, the increase in embryo length before germination can occur has to be larger than in small seeded cultivars.

It is concluded that the differences in t_{50} between seeds of genetically different cultivars are caused by similar factors as in genetically identical but physiologically different seeds derived from one seed lot. It has to be reminded that besides genetic differences the seed lots used in this study most probably also differed in seed maturity. However, the present results provide parameters that can be used in plant breeding to improve celery seed quality.

Effect of priming on embryo length, and subsequent t_{50}

Seeds of all cultivars were incubated in -1.5 MPa for 14 days at 15 °C in light. At the start of the priming treatment, the embryo length varied from 20 to 40% of the length of the endosperm (Fig 1). After the treatment embryo length varied from 50 to 80%. There was no correlation between the embryo length before and after the priming treatment.

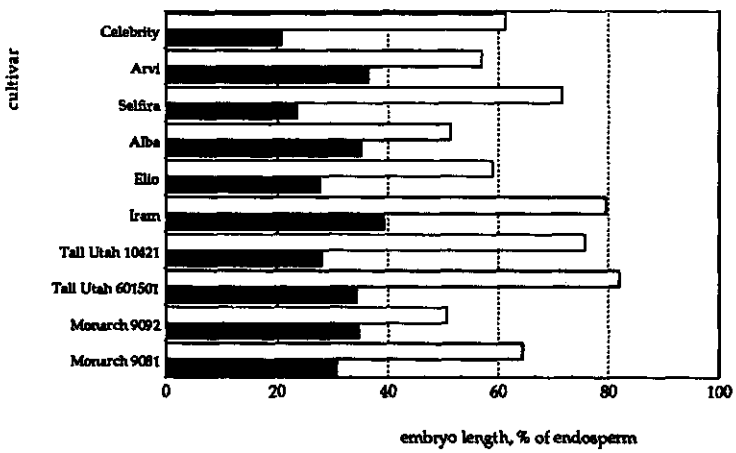


Figure 1. Embryo length in untreated (■) and PEG-incubated (□) seeds of 10 cultivars of celery as % of the length of the endosperm. Seeds were incubated for 14 days in -1.5 MPa PEG at 15 °C in light.

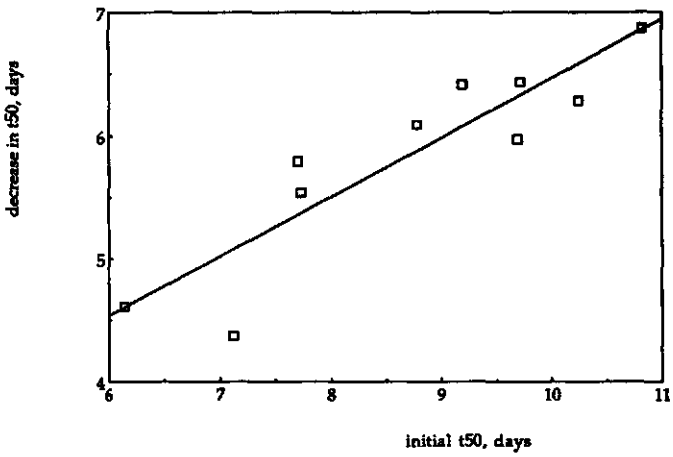


Figure 2. Decrease in t_{50} of seeds of 10 cultivars of celery, when germinated in water at 15 °C in light, after a 14 day incubation in -1.5 MPa PEG at 15 °C, related to the t_{50} of these cultivars without the treatment in PEG.

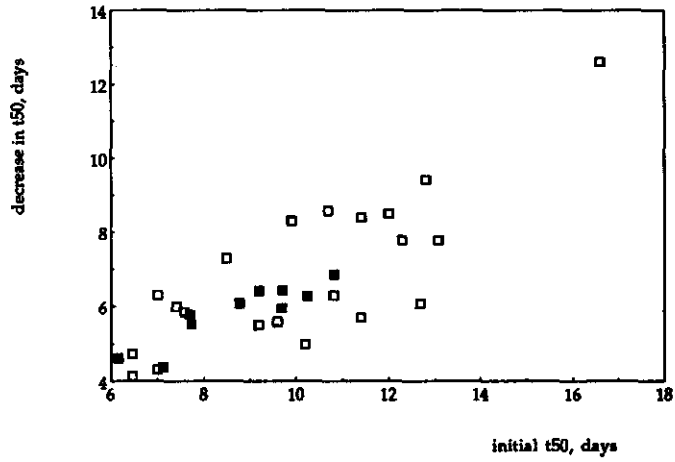


Figure 3. Decrease t_{50} of seeds of 10 cultivars of celery used in the present study (■), compared to literature data (□),when germinated in water at 15 °C in light, after a 14 day incubation in -1.5 MPa PEG at 15 °C, related to the t_{50} of these cultivars before the treatment. Literature data are obtained from Brocklehurst and Dearman (1983,1984), Haarman (1980), Khan et al. (1978), Khan et al. (1980/81), Nakamura et al. (1982), and Singh et al. (1985). If the seeds were dried after the PEG incubation the presented values of t_{50} were corrected with 1 day for imbibition.

In a second experiment, the embryo cell size and number on longitudinal cross sections of the embryos were determined after priming for 17 days in -1.2 MPa PEG. The mean cell size in all cultivars was 237 ± 23 µm, without a correllation with embryo length (R=0.19). The number of cells per embryo varied between 487 and 872 (712 ± 127) and was highly correlated with embryo length (R=0.88). It is concluded that the differences in embryo length that occurred during priming treatments of the seed lots were due to the different number of cells that were formed and not to differences in cell size.

Mean germination times of the 10 seed lots were measured before and after the priming treatment in -1.5 MPa. There was a correlation between the decrease in t_{50} and the initial t_{50} (Fig. 2). The mean reduction in t_{50} of all cultivars was around 66%. Therefore, the benefit of priming in absolute terms was larger for cultivars with a high initial t_{50}. Literature data shows that this is generally found in celery seed priming studies (Fig. 3). When seeds of one seed lot but with different density were primed, this reduction was also found (chapter 6). Therefore, it is most likely that also with respect to the reaction on a priming treatment, genetic differences that exist between cultivars cause similar physiological differences as occurred between genetically identical seeds with different density.

Samenvatting

Selderijzaad kiemt traag en onregelmatig, een eigenschap die in de moderne, sterk gemechaniseerde, tuinbouw zeer onwelkom is. De kieming verloopt traag omdat het embryo in het zaad moet groeien tot ongeveer twee maal de oorspronkelijke lengte voordat kieming mogelijk wordt. De centrale vraagstelling in het beschreven onderzoek was welke factoren de embryogroei in selderij zaad beïnvloeden.

In het **eerste hoofdstuk** werd een algemene introductie gegeven tot het beschreven onderzoek.

In het **tweede hoofdstuk** werd beschreven welke materialen en methoden gebruikt zijn.

In het **derde hoofdstuk** werd een techniek beschreven waarmee zaden uit één partij maar met verschillende kiemsnelheid van elkaar gescheiden kunnen worden. De techniek is gebaseerd op verschillen in het soortelijk gewicht van de zaden. De techniek is zeer waardevol gebleken voor dit onderzoek omdat fysiologisch verschillende maar genetisch uniforme zaden verkregen konden worden. Er werd aangetoond dat de kiemsnelheid afhangt van de embryo groeisnelheid, voorafgaande aan de kieming. Zaden met een hoog soortelijk gewicht hadden een lagere embryo groeisnelheid dan zaden met een laag soortelijk gewicht. Het soortelijk gewicht van selderij zaden werd bepaald door de relatieve bijdragen van pericarp, lucht en endosperm (zie voor de zaadbouw figuur 1 op bladzij 12) aan het totale volume. Zaden met een hoog soortelijk gewicht bevatten minder lucht en meer pericarp dan zaden met een laag soortelijk gewicht.

In het **vierde hoofstuk** werd nagegaan hoe de verschillen in soortelijk gewicht van zaden uit een partij ontstaan. Hiervoor is gedurende drie jaar selderij zaadteelt uitgevoerd van verschillende cultivars. Selderij heeft een zeer gecompliceerde bloeistructuur, vooral omdat er zeer veel schermen zijn. De schermen kunnen worden ingedeeld in vier categoriën, die elkaar opvolgen in de bloei, en bij de gelijktijdige oogst dus zaden met een verschillende afrijpingstijd opleveren. Het onderzoek liet zien dat de vroegst bloeiende schermen zaden gaven met het laagste soortelijk gewicht. De laatst bloeiende schermen vormden zaden met het hoogste soortelijk gewicht. Er werd aangetoond dat de verschillen in soortelijk gewicht van zaden uit een partij geheel toegeschreven kunnen worden aan verschillen in de lengte van zaadontwikkelings- en afrijpingsperiode.

In het **vijfde hoofdstuk** werden enkele in de literatuur beschreven mechanismen van beïnvloeding van de embryogroei tijdens kieming door de pericarp onderzocht. Er werd geconcludeerd dat de pericarp geen invloed had op de wateropnamesnelheid

aan het begin van het kiemproces. Hoewel de pericarp coumarinen bevat en aan deze stoffen een sterk kiemremmende werking wordt toegeschreven, bleek dat deze stoffen geen invloed hadden op de embryogroei. Het sterkste argument voor deze conclusie was de waarneming dat deze stoffen het endosperm niet binnendringen. Het is ook mogelijk dat deze stoffen een invloed hebben op de embryogroei doordat ze zuurstof fixeren, zodat het embryo een tekort aan zuurstof krijgt. Experimenten lieten echter zien dat ook dit mechanisme geen rol speelde.

In het **zesde hoofdstuk** werd de embryo groei van zaden met een verschillende soortelijk gewicht tijdens kieming, incubatie in een osmoticum, en tijdens kieming ná incubatie in het osmoticum, op microscopisch niveau gevolgd. Embryos uit zaden met een laag soortelijk gewicht hadden een hogere celdelingssnelheid, en de cellen werden ook groter, dan in embryos van zaden met een hoog soortelijk gewicht. De verschillen in celgrootte konden worden gecorreleerd met verschillen in de druk potentiaal van de pericarp in het intacte zaad. Verwijdering van het pericarp gaf een toename in het vochtgehalte, een toename in de osmotische potentiaal, en een toename in de celgrootte, waarbij geen verschillen meer aangetoont konden worden tussen zaden met verschillend soortelijk gewicht. Verwijdering van de pericarp had geen effect op de verschillen in embryo celdelingssnelheid. Geconcludeerd werd dat de relatieve volumes van endosperm, lucht en pericarp in het zaad, bepalend zijn voor het vermogen van het zaad om water op te nemen, en daarom bepalend zijn voor de celgrootte.

In een osmoticum, met een potentiaal van -1.2 MPa, is kieming niet meer mogelijk. Desondanks bleek het embryo wel te groeien tijdens de incubatie. In zaden met een laag soortelijk gewicht groeide het embryo sneller en werd ook groter dan in zaden met een hoog soortelijk gewicht. De embryolengte werd bepaald door het aantal gevormde cellen in het embryo. De embryolengte kon verkleind worden door de osmotische potentiaal van het behandelingsmedium te verlagen (meer negatief te maken). Ook tijdens PEG-incubatie was er eenzelde verschil in osmotische potentiaal in het zaad aanwezig als tijdens kieming. Er werd geconcludeerd dat zowel de osmotische potentiaal van het behandeligsmedium als het soortelijk gewicht van het zaad een invloed hadden op de osmotische potentiaal in het zaad, en beargumenteerd werd dat dat bepalend was voor de embryo groeisnelheid en de embryolengte na de behandeling.

Bij kieming in water na incubatie in het osmoticum nam de embryolengte sneller toe dan op grond van de lengte na behandeling verwacht kon worden. Deze snellere groei werd veroorzaakt doordat de embryocellen groter werden. Bij zaden met een hoog soortelijk gewicht was de celgrootte relatief sterker toegenomen. De reductie in de tijd nodig voor 50% kieming (t_{50}) na de behandeling was voor beide fracties gelijk: ongeveer 66%.

In het **zevende hoofdstuk** werd de endospermafbraak bestudeerd. Op basis van literatuurgegevens werd besloten de activiteit van het enzym endo-ß-mannanase te meten. Dit enzym is deels verantwoordelijk voor de afbraak van de celwanden van de endospermcellen, zodat ruimte en nutriënten voor het groeiende embryo beschikbaar

komen. Uit dit onderzoek bleek dat zaden met een laag soortelijk gewicht een hogere hydrolytische activiteit hadden dan zaden met een hoog soortelijk gewicht. Aangenomen werd dat het endosperm dus sneller afgebroken werd en meer ruimte en nutriënten beschikbaar kwamen voor het embryo. Dit verklaard de hogere celdelingssnelheid in embryos in zaden met een laag soortelijk gewicht.

Tijdens incubatie in een osmoticum was de toename in hydrolytische aktiviteit wat langzamer, maar bereikte eenzelfde niveau als tijdens kieming. De hydrolytische aktiviteit was minder geremd dan de celdeling in het embryo. Er werd gesuggereerd dat tijdens incubatie in osmotische oplossingen het endosperm slechts gedeeltelijk wordt afgebroken.

Er werd, tenslotte, aangetoond dat tijdens de zaadontwikkeling aan de moederplant de endo-ß-mannanase activiteit in het zaad toenam. Daarom hebben zaden met een laag soortelijk gewicht, die immers een langere afrijpingsperiode hebben gehad, meer hydrolytische aktiviteit dan zaden met een hoog soortelijk gewicht.

In het **achtste hoofdstuk** werden de fysische en fysiologische parameters uit het hierboven beschreven onderzoek gebruikt om het kiemgedrag van, en het effect van standaard behandelingen in een osmotische oplossing op, verschillende cultivars te verklaren. Het luchtvolume in de zaden en de hoeveelheid suiker in het endosperm correleerden met de t_{50}. Er werd beargumenteerd dat de genetische verschillen tussen de cultivars minstens voor een deel op dezelfde wijze tot uiting komen als de fysiologische verschillen tussen zaden met hoog en laag soortelijk gewicht.

Het effect van een standaard behandeling was voor alle cultivars gelijk: een reductie van de t_{50} met ongeveer 66%. Langzaam kiemende cultivars en partijen profiteren dus meer van de behandeling, maar de snelst kiemende partijen blijven ook na de behandeling sneller kiemen.

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Curriculum vitae

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