PHYSIOLOGICAL ANALYSIS OF METHODS TO RELIEVE DORMANCY OF LETTUCE SEEDS

FYSIOLOGISCHE ANALYSE VAN METHODEN VOOR DE VERBREKING VAN KIEMRUST VAN SLAZADEN

Promotor: dr. C.M. Karssen
hoogleraar in de fysiologie der planten

Roelf Weges

Physiological analysis of methods to relieve dormancy of lettuce seeds

Proefschrift

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 28 januari 1987
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

Druk: Krips Repro Meppel

Dit onderzoek maakte deel uit van het onderzoekprogramma van de Stichting voor Biologisch Onderzoek in Nederland (BION) als onderdeel van de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek (ZWO) en werd mogelijk gemaakt door financiële steun van de Stichting voor Technische Wetenschappen (STW).

in herinnering aan W. Heydecker
de grondlegger van de osmotische
voorbehandeling van zaden.

VOORWOORD

Een proefschrift en de openbare verdediging daarvan zijn traditiegetrouw de bekroning van het werk van een promovendus. Het voorwoord is de geëigende plaats om duidelijk te maken dat dit mogelijk gemaakt werd door de hulp van mensen die veelal achter de schermen blijven.

Studenten en stagiaires hebben ieder op hun eigen wijze een belangrijke bijdrage geleverd aan dit proefschrift. Van de in totaal 1.500.000 slazaden, die voor kiemingsexperimenten gebruikt zijn, namen zij 60% voor hun rekening. Celia de Haan, Bert Houter, Siep Koning, Leo Marcelis en Rachel Miezgiel werkten aan de waterhuishouding van de zaden. Hun tijdrovende, theoretisch moeilijke pionierswerk was nodig om uiteindelijk hoofdstuk 7 te kunnen schrijven. Het werk van Marian van Kessel en Thijs Veldhuizen aan het effect van voorbehandeling op verschillende slarassen is een onderdeel van hoofdstuk 3. Harold Beek heeft met zijn begassingsexperimenten en ademhalingsmetingen een bijdrage geleverd aan hoofdstuk 2. De grote snelheid van tellen van Rob Plomp kwam goed van pas voor de bewaarexperimenten beschreven in hoofdstuk 4.

Hulp bij het uitvoeren van chemische analyses heb ik gekregen van met name Henk Hilhorst, Elly Koot-Gronsveld en Irma Temminck. Irma voerde op nauwgezette wijze vrijwel alle kaliumgehaltebepalingen uit voor hoofdstuk 5. Hoofdstuk 6 zou niet geschreven zijn zonder de zorgvuldige activiteitsbepalingen van het enzym glutamine synthetase door Elly m.b.v. een methode, opgezet door Henk. Na lang zoeken was het een hele opluchting dat Wim Roelofsen van de Vakgroep Microbiologie de bijbehorende aminozuuranalyses zo goed bleek te kunnen uitvoeren.

Technische ondersteuning heb ik gekregen van met name Ruth van der Laan en Ben van der Swaluw. Zij verrichtten allerlei reparaties, maar bouwden ook de droogbak naar een ontwerp dat samen met Dr. Erik Knegt tot stand gekomen is. Dankzij het elektronische knutselwerk van Rienk Bouma van de Vakgroep Plantenfysiologisch Onderzoek werd de psychrometer van de Vakgroep Tuinbouw zo aangepast dat de waterpotentiaal en de osmotische potentiaal van slazaden gemeten konden worden. Gerrit van Geerenstein, Aart van Ommeren en Jan Verburg verzorgden het plantmateriaal voor de veldproeven. Siep Massalt verzorgde de fotografie.

Ik ben door het werken aan dit proefschrift nog beter gaan beseffen dat ná

het uitvoeren van experimenten een minstens even belangrijke fase komt, namelijk de juiste interpretatie van de gegevens en het onder woorden brengen van de conclusies. Mijn promotor, prof.dr. C.M. Karssen, heeft daarbij de belangrijkste rol gespeeld. Beste Kees, jouw goede vragen en jouw vermogen om hoofdzaken van bijzaken te onderscheiden waren essentieel bij de interpretatie van de resultaten. De beste herinneringen bewaar ik echter aan jouw fraaie formuleringen, die ik zo graag zelf had willen bedenken.

Het 'zaadcluboverleg' samen met Steven Groot en Henk Hilhorst was een broedplaats van goede ideeën om het fysiologisch mechanisme van de voorbehandeling te begrijpen. Goede adviezen heb ik tevens ontvangen van het wetenschappelijk personeel van de Vakgroep Plantenfysiologie.

De resultaten van het onderzoek zijn regelmatig besproken met vertegenwoordigers van met name de veredelingsbedrijven Enza Zaden, Nunhems Zaden, Royal Sluis, Rijk Zwaan, Sluis & Groot en Van den Berg. Dit was mede een uitvloeisel van de financiering door de Stichting voor Technische Wetenschappen. Behalve de leerzame discussies is het gratis ter beschikking stellen van slazaden zeer op prijs gesteld. Andere externe adviseurs waren met name dr. Lieke Kraak van het Rijks Proefstation voor Zaadonderzoek voor de bewaarexperimenten en dr. Aart van Bel van het Botanisch Laboratorium te Utrecht als begeleider vanuit de werkgemeenschap Fysiologie en Morfogenese van Planten.

De uiteindelijke vormgeving van het proefschrift heb ik te danken aan Frank Dumoulin, Allex Haasdijk en Paul van Snippenburg. Laatstgenoemden zorgden voor het prima tekenwerk. Frank, hartelijk dank voor jouw bijdrage. Dankzij je grote vaardigheid met de PC heb je in in zeer korte tijd de tekst getypt en de lay-out fijn verzorgd. De uitbeelding van kieming en kiemrust op het omslag heb ik te danken aan een goede vriendin en toeverlaat, Hanneke Mertens.

Dit proefschrift kon mede tot stand komen dankzij de bijzonder goede sfeer op de vakgroep. Bijzondere aandacht verdient hierbij Steven Groot, mijn kamergenoot, die steeds beschikbaar was voor een knetterende discussie over wetenschappelijke onderwerpen, maar evenzeer over onze politieke voorkeuren.

Ten slotte past een woord van dank aan mijn nieuwe werkgever, Royal Sluis, voor de soepele opstelling als ik tijd nodig had voor de afronding van het proefschrift.

Bovenal wil ik echter mijn ouders bedanken voor de mogelijkheid die zij mij geboden hebben om te gaan studeren en de ondersteuning bij het werken aan dit proefschrift.

INHOUDSOPGAVE

De hoofdstukken 2 t/m 7 zullen tevens in wetenschappelijke tijdschriften gepubliceerd worden.

ABSTRACT

Low maximum temperature of germination restricts the cultivation of a number of lettuce cultivars to temperate conditions. Pretreatments have been studied that increase the maximum germination temperature, which is characterized by the temperature for 50% germination (T_{50}). Pretreatment consists of pre-incubation, redesiccation and storage. Pre-incubation during 16 to 20 h at 15 °C in water increases T_{50} with 4 to 10 °C, depending on cultivar. Redesiccation does not cause visible damage to seeds, as judged from the developing seedling, provided that germination has not started during pre-incubation. In cultivars with a high original T_{50} redesiccation does not affect T_{50}. However, if the original T_{50} is low, redesiccation causes a drop of T_{50} to values close to the original T_{50} value. The effect of redesiccation is mainly determined by the final moisture content. Temperature and rate of drying are of minor importance in this respect. Pre-incubation in solutions of polyethylene glycol makes the seeds desiccation tolerant. Dehydration results in increased K^+ leakage from isolated embryos, in contrast to intact seeds that do not leak due to K^+-impermeable endosperm cells. T_{50} decreases during storage. The decrease of T_{50} and the loss of seed viability show a similar positive relationship to temperature and moisture content during storage. Besides the rise of T_{50}, pre-incubation at 15 °C also enhances the capacity of seeds to germinate at increasingly more negative osmotic potentials of the incubation medium ($\psi_{\pi e}$). The effect is characterized by the minimal $\psi_{\pi e}$ of the incubation medium for 50% germination (ψ_{50}). Pre-incubation at 30 °C has an opposite effect. The change of ψ_{50} does not correspond with a change in concentration of amino nitrogen compounds. Both actual water potential (ψ) and osmotic potential (ψ_{π}), as determined with a psychrometer, are not correlated with changing ψ_{50} values. Calculations based on the general equation of hydraulic cell growth indicate that changes in T_{50} are correlated with parallel changes in the yield threshold (Y) of turgor pressure. Changes in Y appear to occur mainly in the seed envelope.

CHAPTER 1

ALGEMENE INLEIDING

Het doel van een voorbehandeling

De moderne land- en tuinbouw stellen steeds hogere eisen aan de kiemingseigenschappen van zaden. In het meest ideale geval zouden alle zaden moeten kiemen met een grote snelheid en grote uniformiteit en zou kieming over een breed traject van milieu-omstandigheden plaats moeten vinden. Dit ideaal is echter nog slechts in enkele gewassen ook werkelijk bereikt. Het wordt daarom van belang om te weten door welke processen de kiemingseigenschappen van zaden bepaald worden. Er is ongetwijfeld sprake van een genetische component. Secundaire factoren zoals de milieu-omstandigheden tijdens de ontwikkeling, de kwaliteit van de schoning en de condities tijdens de bewaring spelen echter ook een grote rol. Om het ideaal zoveel mogelijk te benaderen, wordt in toenemende mate een voorbehandeling van de zaden in het laboratorium toegepast. Een voorbehandeling bestaat meestal uit een periode van pre-incubatie in water, in een oplossing van kiemingsstimulerende stoffen of een osmoticum bij een geschikte temperatuur, gevolgd door terugdrogen tot het oorspronkelijke zaadgewicht. Sinds Heydecker et al. (1973) wordt de pre-incubatie in osmoticum toegepast. Als osmoticum wordt vaak gebruik gemaakt van polyethyleen glycol (PEG) met een molekuulgewicht van 6000 tot 7500. Een oplossing van deze inerte stof voorkomt zichtbare kieming. Het vermogen van de PEG-oplossing om kieming te voorkomen hangt af van de osmotische potentiaal ($\psi_{\pi e}$), met als eenheid MPa of bar (1 MPa = 10 bar). $\psi_{\pi e}$ heeft een maximale waarde van nul. Een meer geconcentreerde oplossing heeft een negatieve $\psi_{\pi e}$. Heydecker (1977) noemt als voorbeeld het effect van pre-incubatie van tomatezaden in -0.5 MPa PEG bij 20 °C gedurende 7 dagen op de snelheid van kieming bij 15 °C (50% kieming na 2,2 dagen i.p.v. 7,6 dagen. Brocklehurst & Dearman (1983) toonden aan dat na pre-incubatie van zaden van verschillende cultivars van wortel en selderij in -1.0 MPa PEG gedurende 14 dagen bij 15 °C en terugdrogen de gemiddelde tijdsduur, tot het begin van de kieming bij 15 °C teruggebracht werd van resp. 5 en 15 dagen tot resp. 2 en 4 dagen, waarbij tevens de uniformiteit verbeterd bleek te zijn. De teelt van een aantal landbouwgewassen is in Alberta niet mogelijk door een te kort groeiseizoen. Bodsworth & Bewley (1981) slaagden er in om de snelheid en de uniformiteit van kieming van sojabonen, mais en sorghum bij een lage temperatuur van 10 °C te verbeteren door pre-incubatie gedurende optimale tijdsduur (1-6 dagen) in variabele $\psi_{\pi e}$

van PEG bij 10 °C. De mogelijkheden voor de teelt van deze gewassen is door voorbehandeling weliswaar toegenomen, maar de grootschalige voorbehandeling van landbouwzaden roept nog vele praktische problemen op.

Bij sla lijkt op het eerste gezicht een voorbehandeling nauwelijks nodig. Zaden van veel cultivars kiemen voor 100%, snel en uniform. Het probleem dat echter toch een voorbehandeling noodzakelijk maakt, is de vaak lage maximum-temperatuur van kieming. D.w.z. dat er bij slazaden een kiemrustprobleem bestaat (zie apart kader). Zo bereikten Valdes et al. (1985) in Calfornië niet meer dan 20% kieming bij bodemtemperaturen tussen 25 en 40 °C. Een verbetering van de kieming is van groot economisch belang, omdat in de wintermaanden in die omgeving 90% van de sla in de U.S.A. geteeld wordt en hoge bodemtemperaturen regelmatig voorkomen. Gray (1975) en Thompson et al. (1979) onderzochten de maximum-temperatuur voor 50% kieming van een groot aantal cultivars van sla. Deze maximumtemperatuur varieerde tussen 23 en 33 °C. Dit levert niet alleen problemen op bij de teelt van sla in de volle grond in warmere streken, maar ook bij de teelt in kassen in Nederland. Het probleem wordt nog versterkt doordat de slazaden, om mechanische zaai mogelijk te maken, voorzien worden van een ronde omhulling (het zgn. pilleren). Het is zeer goed voorstelbaar dat door zo'n extra laag rondom het zaad de kieming nog verder negatief beïnvloed wordt. Ten einde toch 100% kieming te bereiken, hebben enkele gespecialiseerde plantenkweekbedrijven grote klimaatruimten laten bouwen waarin de zaadpillen na uitzaai op perspotten enkele dagen voorgekiemd worden bij 12 tot 15 °C, alvorens verdere kweek onder kascondities plaatsvindt. Deze ruimte- en tijdvergende behandeling zou voorkomen kunnen worden indien de slazaden reeds voorafgaande aan het pilleringsproces afdoende voorbehandeld zouden worden in de veredelingsbedrijven. Dit spreekt des te meer omdat voorkieming in koelcellen niet in alle plantenkweekbedrijven mogelijk is en zeker niet toepasbaar is bij de teelt in de volle grond.

Voetnoot: In dit proefschrift wordt gesproken over slazaden, terwijl dit strikt genomen niet juist is. Sla vormt namelijk, evenals andere soorten in de familie Asteraceae, éénzadige droge vruchtjes (achenen). In aansluiting met de fysiologische literatuur wordt echter toch over zaden gesproken.

kiemrust van slazaden

Er bestaat veel verwarring over het begrip kiemrust (dormancy). In dit proefschrift wordt er vanuit gegaan dat kiemrust van zaden zich altijd vertaalt in het temperatuurtraject waarbij kieming mogelijk is (Karssen 1982). Verbreken van rust leidt tot een verbreden van het temperatuurtraject en induceren van rust tot een versmalling. In sla concentreert deze verandering zich met name in de maximumtemperatuur van kieming, de minimumtemperatuur die dicht bij 0 °C ligt is niet sterk aan verandering onderhevig. De remming van de kieming bij hoge temperaturen komt, behalve bij sla, bij veel andere soorten voor die afkomstig zijn uit het Middellandse Zeegebied. Dit kan beschouwd worden als een aanpassing om de soort in stand te houden, immers tijdens de droge, warme zomer zijn de omstandigheden voor een succesvolle groei en ontwikkeling ongunstig (Thompson 1973). Het is opvallend dat het in cultuur nemen van sla aan deze temperatuurafhankelijkheid van de kieming blijkbaar niets veranderd heeft. Reynolds & Thompson (1971) hebben de 'upper temperature cut-off point' geïntroduceerd om de maximale temperatuur te karakteriseren waarbij nog 50% kieming mogelijk is. In dit proefschrift wordt deze temperatuur aangeduid met T_{50}. Voor de praktijk is uiteraard de temperatuur waarbij nog 98-100% kiemt van het meeste belang. De temperatuurkarakteristiek van kieming wordt vastgesteld door kiemtesten uit te voeren bij een reeks temperaturen en de T_{50} door interpolatie vast te stellen. Uit deze curve is ook T_{100} en T_0 te schatten.

Incubatie van slazaden bij een temperatuur hoger dan T_0 maakt kieming onmogelijk (thermo-inhibition). Indien de temperatuurcurve wordt bepaald nadat de zaden enige tijd zijn geïncubeerd bij T_0 blijkt de curve die de kieming karakteriseert naar lagere waarden te zijn verschoven. M.a.w. er heeft rustinductie plaatsgevonden. Dit werd in 1954 voor het eerste door Borthwick et al. beschreven. In 1928 constateerden Borthwick & Robbins al dat pre-incubatie van slazaden bij lagere temperaturen dan T_{100} rustbreking tot gevolg heeft, d.w.z. een verschuiving van T_{max} naar hogere temperaturen. Het blijkt dat veranderingen van het rustniveau door wijziging van de pre-incubatie omstandigheden weer teniet gedaan kunnen worden. In Fig. 1 staan de zojuist beschreven veranderingen van het rustniveau in schema weergegeven.

Ook de verschuiving van de maximumtemperatuur kan onder natuurlijke om-

standigheden een overlevingsfunctie hebben. De hoge zomertemperaturen drukken de T_{max}, terwijl de lagere temperaturen van het najaar het temperatuurtraject verbreden, tot een overlapping met de actuele veldtemperatuur kieming mogelijk maakt.

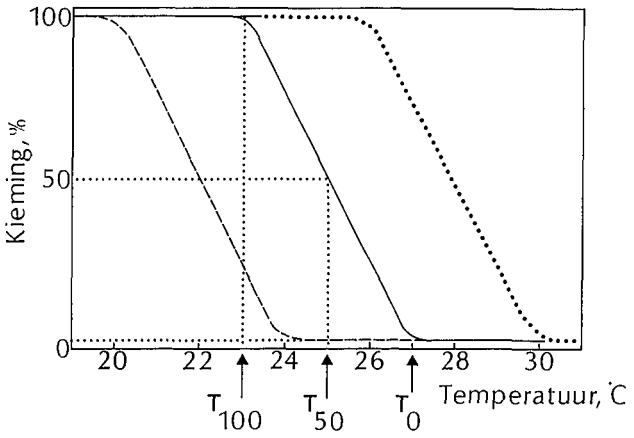

Fig. 1. Schematische voorstelling van de invloed van pre-incubatie temperatuur op de kieming van slazaden bij verschillende temperaturen. —— kieming zonder behandeling, ... kieming na rustbreking door pre-incubatie bij lage temperaturen, --- kieming na rustinductie door pre-incubatie bij hoge temperaturen.

De literatuur bevat slechts enkele voorbeelden van voorbehandelingen van slazaden met als doel om de kieming bij hoge temperaturen te verbeteren. Guedes & Cantliffe (1980) incubeerden slazaden 3 tot 12 uur bij 5 tot 25 °C in water of in een oplossing van K_3PO_4 of PEG. Na pre-incubatie werden de zaden aan de lucht of in een oven bij 32 °C gedroogd, waarna ze tot 4 maanden lang droog bewaard werden. Kretschmer (1982) en Valdes et al. (1985) pasten een dergelijk soort voorbehandeling toe, in zoverre dat Valdes et al. (1985) de zaden naderhand lieten pilleren. In alle gevallen rapporteerden de onder-

zoekers een aanzienlijke verhoging van de maximumtemperatuur. Zo konden Guedes & Cantliffe (1980) de kieming bij 35 °C verbeteren van 0 naar 80%. Valdes et al. (1985) bereikten onder de eerder genoemde extreme veldcondities en na pillering een verbetering van 20 tot 70% en Kretschmer (1982) constateerde, afhankelijk van de verschillende cultivars en de bewaarduur (tot 12 maanden bij 15 °C), een verbetering bij 30 °C van 0-20% tot 40-100%.

De voorbehandeling van slazaden lijkt in toenemende mate te worden toegepast. In de praktijk blijkt dat niet steeds het gewenste effect bereikt wordt. Bovengenoemde onderzoekers hadden eveneens met problemen te stellen. Deze waren onder andere terug te voeren op de grote verschillen tussen zaden van verschillende cultivars. Maar ook het terugdrogen na pre-incubatie leidde tot moeilijkheden doordat een groot deel van de verhoging van de maximumtemperatuur weer verloren ging (Guedes & Cantliffe 1980). De onderzoekers rapporteerden ook zeer verschillende reacties op belichting en er heerst ook onzekerheid over de meest geschikte concentratie en de aard van het osmoticum.

Er is tevens onduidelijkheid over de vraag of het gebruik van groeiregulatoren tijdens de voorbehandeling aan te raden is. Khan (1977) toonde in zijn overzichtsartikel aan dat het heel goed mogelijk is om door de toepassing van de groeiregulatoren kinetine en gibberelline (GA) tijdens de voorbehandeling de kieming van slazaden flink te stimuleren. Hij paste de regulatoren toe door de zaden gedurende enkele uren te incuberen in een oplossing van de stoffen in zuivere aceton. Het aceton werd daarna weer verwijderd door drogen aan de lucht.

Behandeling van slazaden met groeiregulatoren kan echter met name gevaarlijk zijn voor de kwaliteit van de kiemplanten, waaraan hoge eisen gesteld worden. De foto's in Khan (1977) laten zien dat uit GA voorbehandelde zaden kiemplanten ontstaan, die onnatuurlijk gestrekt waren. Bovendien toonden Hegarty & Ross (1979) aan dat de groei van de slakiemplant negatief beïnvloed werd door gibberelline en kinetine.

De toepassingsproblemen van de voorbehandeling van slazaden worden mede veroorzaakt door het 'trial and error' karakter van het onderzoek. In het betrekkelijk geringe aantal publicaties dat aan de voorbehandeling van zaden gewijd is, ontbreekt vrijwel steeds de fysiologische analyse. Het hoofddoel van deze studie is daarom een bijdrage te leveren aan de fysiologische kennis van de processen die betrokken zijn bij de voorbehandeling van slazaden. Op grond van die achtergrondkennis zal de huidige praktijk kritisch worden be-

keken en zullen adviezen worden opgesteld om in de toekomst fouten te voorkomen.

Variabelen in de voorbehandeling

Een voorbehandeling bestaat uit drie achtereenvolgende onderdelen: pre-incubatie, terugdroging en bewaring. Uit Guedes & Cantliffe (1980) blijkt dat een aantal factoren gevarieerd kan worden tijdens de verschillende onderdelen. In eerste instantie is het onderzoek gericht op het analyseren van deze factoren.

Tijdens de pre-incubatie kunnen gevarieerd worden: temperatuur, tijdsduur, type en concentratie osmoticum, de hoeveelheid licht en de lichtkleur en de concentratie zuurstof. Omdat de literatuur en eigen oriënterende proeven een sterke indicatie gaven dat allerlei chemische toevoegingen aan het incubatiemedium, zoals stikstofhoudende verbindingen en groeiregulatoren, afwijkende kiemplanten tot gevolg hebben, zijn dergelijke toevoegingen niet nader bestudeerd. PEG wordt beschouwd als het meest inerte osmoticum. De zeer grote moleculen (M = 6000-7500) kunnen niet doordringen in cellen. Osmotica, zoals diverse zouten en mannitol, kunnen dit wel (Carpita et al. 1979d). Daarom is in dit onderzoek - om mogelijke beschadiging van het embryo te voorkomen - gekozen voor PEG als osmoticum. Osmotica worden toegepast om celstrekking tijdens de groei van het embryo te voorkomen (Heydecker & Coolbear 1977). Echter langdurige vertraging van de kieming is ook de belangrijkste voorwaarde voor het ontstaan van secundaire kiemrust (Karssen 1982). Het doel van pre-incubatie is juist tegenovergesteld, de kieming bij hoge temperaturen moet immers verbeterd worden.

In hoofdstuk 2 staat daarom de rol van PEG tijdens pre-incubatie centraal. De temperatuur tijdens pre-incubatie in PEG is gevarieerd omdat de temperatuur bepaalt of rustbreking of juist rustinductie plaatsvindt (Totterdell & Roberts 1979). Er is nog geen overeenstemming over het werkingsmechanisme van PEG. Enerzijds wordt betwijfeld of er wel voldoende zuurstof in de PEG-oplossing kan doordringen (Mexal et al. 1975), terwijl zuurstof essentieel is voor veranderingen van het rustniveau (Karssen 1980/81). Anderzijds blijkt PEG veel toegepast te worden om het vochtgehalte van zaden te verminderen (Ibrahim & Roberts 1983a).

Terugdrogen van de zaden na pre-incubatie tot een laag vochtgehalte is noodzakelijk om bewaring en zaaien mogelijk te maken. Verschillende factoren kunnen tijdens het drogen gevarieerd worden: tijdsduur, temperatuur, windsnelheid en de relatieve vochtigheid van de lucht. Vanzelfsprekend mag het kiemproces tijdens pre-incubatie niet zo ver voortgeschreden zijn dat beschadiging van het embryo optreedt tijdens het drogen. Echter voordat beschadiging van het embryo optreedt, blijkt er een negatief effect van terugdrogen op de kieming bij hoge temperaturen te zijn (Guedes & Cantliffe 1980). Brocklehurst & Dearman (1983) constateerden bij zaden van wortelen, uien en selderij, dat droging bij 15 °C een minder negatief effect had dan droging bij 30 °C.

Gray (1975) en Thompson et al. (1979) constateerden al dat er grote verschillen in kiemrustniveau bestaan tussen cultivars en verschillende partijen zaden van dezelfde cultivar. Het ligt voor de hand dat zulke verschillen tevens tot uiting komen in het effect van pre-incubatie en terugdrogen op het kiemrustniveau. Daarom wordt in hoofdstuk 3 beschreven hoe verschillend cultivars en partijen van die cultivars reageren op terugdrogen. Er wordt tevens beschreven hoe de omstandigheden tijdens pre-incubatie aangepast kunnen worden om ondanks terugdrogen toch nog voldoende kieming te bereiken bij hoge temperaturen. In hoofdstuk 5 komt de rol van de genoemde factoren tijdens het terugdrogen op de kieming aan de orde. Bovendien wordt aandacht besteed aan het fysiologische mechanisme dat ten grondslag ligt aan het terugdroogeffect.

Na pre-incubatie en terugdrogen moeten de zaden enige tijd bewaard kunnen worden. Het uitgebreide onderzoek van Ellis & Roberts (1980a,b, 1981) heeft aangetoond dat het doodgaan van zaden tijdens bewaring volgens een voorspelbaar patroon verloopt, dat afhankelijk is van de temperatuur en het vochtgehalte van de zaden in de aanwezigheid van voldoende zuurstof. Dezelfde onderzoekers voorspelden dat onder invloed van pre-incubatie zaden sneller zouden doodgaan tijdens bewaring dan zaden zonder pre-incubatie. Dit zou een ernstige handicap zijn voor de praktische toepasbaarheid van de voorbehandeling. Guedes & Cantliffe (1980) en Kretschmer (1982) toonden echter aan dat slazaden na pre-incubatie en terugdroging in ieder geval gedurende enige maanden bewaard konden worden zonder dat het vermogen om bij hoge temperatuur te kiemen daalde, hetgeen inhoudt dat ook de 'viability' behouden bleef. Verlies van 'viability' wordt meestal bepaald door het aantal zaden te tellen dat geen worteltje met een minimale lengte van 2 mm kan produceren. Er kan ook

gekeken worden of de ontwikkeling van het totale kiemplantje geen abnormaliteiten vertoont. Volgens dezelfde onderzoekers zou kiemrust niet gecorreleerd zijn met 'viability'. In hoofdstuk 4 wordt de invloed van de bewaring bij verschillende temperaturen van gepre-incubeerde slazaden, die tot verschillende vochtgehaltes werden teruggedroogd, op de terugval van de maximumtemperatuur vergeleken met de invloed op het verlies van 'viability'.

Het zou voor de hand hebben gelegen om na pre-incubatie, terugdrogen en bewaren ook het effect van pilleren op de kieming bij hoge temperaturen te onderzoeken. Pilleren is echter alleen mogelijk bij particuliere ondernemingen. Dit heeft de experimenten zodanig beperkt dat ze niet voor publicatie in aanmerking komen.

Fysiologische analyse

Zoals hierboven werd aangeduid wil dit proefschrift vooral een bijdrage leveren aan de fysiologische analyse van de voorbehandeling van slazaden, om daardoor een betere basis te vormen voor de ontwikkeling van deze methoden. In de hoofdstukken 5, 6 en 7 komen een aantal aspecten van deze fysiologische analyse aan de orde.

In hoofdstuk 5 wordt aandacht besteed aan de fysiologische mechanismen die het effect van terugdrogen op de maximumtemperatuur zouden kunnen verklaren. In het algemeen wordt er in de literatuur vanuit gegaan dat drogen van zaden de membraanstructuur van cellen beschadigd, waardoor met name de cellen aan het oppervlak van de zaden iets van hun inhoud verliezen (Bewley 1979). Lekkage van electrolyten en andere celbestanddelen is een veel voorkomend verschijnsel gedurende de eerste uren van de imbibitie in water van zaden van vele soorten (Bewley & Black 1978). Geïsoleerde embryo's van de erwt vertoonden opnieuw lekkage wanneer ze na pre-incubatie en terugdrogen opnieuw werden geïncubeerd (Simon & Harun 1972). Intacte zaden vertonen aanzienlijk minder lekkage (Simon 1984). In hoofstuk 5 is onderzocht of de lekkage van K^+-ionen onder invloed van terugdrogen van gepre-incubeerde zaden gecorreleerd kan worden met de daling in de maximumtemperatuur van de zaden.

De centrale vraag van dit proefschrift is welk fysiologisch mechanisme ten grondslag ligt aan de verhoging c.q. de verlaging van de maximumtemperatuur van kieming onder invloed van een voorbehandeling bij lage respectievelijk

hoge temperatuur. Of met andere woorden: hoe kiemrust verandert tijdens een voorbehandeling. Het onderzoek naar kiemrustmechanismen heeft zich enerzijds sterk gericht op een verklaring via veranderingen in de stofwisseling van het zaad en anderzijds op een verandering in het hormoongehalte van zaden (Bewley & Black 1982). Voor beide theorieën bestaat echter onvoldoende bewijsvoering. Zelfs wanneer de primaire verandering, die door een temperatuurvoorbehandeling optreedt, gelocaliseerd is in hetzij de stofwisseling, hetzij de hormoonhuishouding, dan blijft het nog van belang te weten op welke manier deze veranderingen tot een betere of slechtere kieming leiden. In dit verband dient vermeld te worden dat kiemingsstimulerende factoren zoals licht en de groeiregulatoren gibberelline, kinetine en ethyleen slazaden het vermogen verschaffen om in een osmoticum met een lagere (méér negatieve) $\psi_{\pi e}$ te kiemen, terwijl factoren die de kieming remmen, zoals hoge temperaturen en abscisinezuur, het omgekeerde effect hebben (Carpita et al. 1979, Hegarty & Ross 1978, Reynolds 1975, Negm & Smith 1978, Takeba & Matsubara 1979). Dit zijn even zovele aanwijzingen dat kiemrustverschijnselen zich uiten in een verandering van de waterhuishouding van de zaden.

Kieming is irreversibele groei van het embryo. Voor groei is celstrekking voldoende (Haber & Luippold 1960). Celstrekking vindt plaats door wateropname en watertransport en is per definitie alleen mogelijk indien er een verschil in waterpotentiaal (ψ) bestaat. ψ heeft een negatieve waarde met een maximum van nul MPa. ψ van droge zaden kan zeer sterk negatieve waarden bereiken (tot -400 MPa, Shaykewich 1973), waardoor een snelle wateropname mogelijk is. ψ wordt bepaald door een aantal componenten:

$$\psi = \psi_{\pi} + \psi_{p} + \psi_{m} \qquad (1)$$

Er wordt vanuit gegaan dat de matrixpotentiaal (ψ_m) met name verantwoordelijk is voor de zeer negatieve ψ in droge zaden, maar na afloop van de imbibitie in water verwaarloosd mag worden. Als ψ_m verwaarloosbaar is, wordt ψ bepaald door de som van de osmotische potentiaal (ψ_π) en de turgordruk (ψ_p). ψ_π in het zaad wordt, evenals de al eerder geïntroduceerde $\psi_{\pi e}$ van een oplossing, bepaald door de hoeveelheid opgeloste stoffen. Een toename van het gehalte opgeloste stoffen zou zowel ψ_π als ψ laten dalen, mits ψ_p gelijk blijft. Dus zou wateropname en groei van het embryo mogelijk worden. Takeba (1980a) meende dat ψ_π de bepalende factor is voor de kieming van slazaden. Hij was in staat het gehalte aminozuren in het embryo en de kieming te manipu-

leren met behulp van rustbrekende en rustinducerende factoren. In hoofdstuk 6 wordt daarom geanalyseerd of kiemrustveranderingen van slazaden gecorreleerd zijn met veranderingen in het aminozuurgehalte van slazaden. Daarnaast is de aktiviteit van het enzym glutaminesynthetase onderzocht, omdat dit enzym volgens Takeba (1983a,b) voor een groot deel verantwoordelijk was voor de ophoping van de aminozuren. Het is bij dit onderzoek van groot belang om exact te kunnen bepalen wanneer de groei van het embryo precies begint. Zichtbaar waarneembare kieming is daarvoor een te laat moment. Daarom werd in navolging van Schopfer et al. (1979) het criterium van de droogschade aan het embryo ingevoerd, dat het begin van groei ongeveer 6 uur eerder aantoont.

Carpita et al. (1979b) meenden dat de drijvende kracht voor de groei van sla-embryo's onder invloed van belichting wordt bepaald door zowel een daling van ψ_π als een daling van ψ_p. Een protonenpomp zou verantwoordelijk zijn voor zowel een stijging van het gehalte K^+ ionen als 'cell wall loosening'. Schopfer & Plachy (1985) vonden bij de ABA-gereguleerde kieming van koolzaad echter een daling van het gehalte opgeloste stoffen, waardoor ψ_π steeg in plaats van daalde. Volgens deze onderzoekers is alleen ψ_p de regulerende factor voor kieming. De grootste verdienste van Schopfer & Plachy (1985) is hun aanpassing van de theorie over de waterhuishouding van celgroei aan de kieming van zaden. Zij legden een verband tussen de wateropname van zaden, $\psi_{\pi e}$ van het osmoticum waarin de zaden geïncubeerd worden, ψ_π en de minimale turgor (Y) die nodig is voor wateropname. Het is mogelijk $\psi_{\pi e}$ dusdanig te kiezen dat slechts 50% van de zaden in staat is te kiemen ($\psi_{\pi e} = \psi_{50}$). Dit betekent dat tevens van 50% van de zaden de wateropname nul is, immers alleen indien kieming optreedt zal het watergehalte stijgen. Bij een wateropname van nul geldt:

$$\psi_{50} = \psi_\pi + Y \qquad (2)$$

Het is mogelijk ψ_π te meten met behulp vanm een psychrometer. Dit maakt het mogelijk Y te berekenen uit ψ_{50} en ψ_π. In hoofdstuk 7 wordt beschreven hoe de pre-incubatie van slazaden van verschillende cultivars invloed heeft op de bovengenoemde parameters van de waterhuishouding. Dit proefschrift wordt ten slotte besloten met een samenvatting.

CHAPTER 2

THE ADVANTAGES AND DISADVANTAGES OF THE USE OF POLYETHYLENE GLYCOL DURING PRE-INCUBATION OF LETTUCE SEEDS

Abstract

Pre-incubation of lettuce seeds is directed to the shift of the maximum temperature of germination to higher temperatures. It is studied whether pre-incubation has to occur in osmotic solutions or can evenly proceed in water. In seeds of cv. Musette the maximum temperature for 50% germination (T_{50}) shifts during pre-incubation for 24 h in water or 40 h in -0.5 MPa polyethylene glycol (PEG) at 15 °C from 25 to more than 32 °C. However, only after pre-incubation in PEG, seeds are resistant to subsequent redesiccation to a moisture content of 5.5%. When at 15 °C pre-incubation in -1.0 and -1.5 MPa PEG is prolonged, alleviation of dormancy changes into the induction of secondary dormancy. At 30 °C PEG inhibits the induction of secondary dormancy. The effect of PEG is not due to interference with oxygen availability. At both 15 °C and 30 °C PEG decreases the water saturation during imbibition, which correlates with reduced respiration rate. Therefore, changes in dormancy appear to depend on active, O_2-requiring processes. The practical application of the results is discussed.

Introduction

Pretreatment of seeds has been developed during the last decades to improve the germination characteristics of seeds. In lettuce the objective of seed pretreatment is in particular the improvement of germination at higher temperatures. Restriction of germination at higher temperatures is an expression of dormancy. Reynolds & Thompson (1971) proposed the maximum temperature at which 50% of the seeds germinate (T_{50}) as a suitable parameter for dormancy. As early as 1928 Borthwick & Robbins (1928) showed that T_{50} can be increased by pre-incubation at low temperatures. The opposite effect was obtained by pre-incubation of the seeds at high temperatures (Borthwick et al. 1954).

The pretreatments which have been developed to increase T_{50} of lettuce seeds often took the form of a pre-incubation at low (negative value) osmotic potentials at a suitable low temperature, followed by redesiccation and storage. Lettuce seeds have been pre-incubated in various osmotica consisting of solutions of salts, sugars or polyethylene glycol (PEG) (Khan 1977, Guedes

& Cantliffe 1980). In general PEG is regarded as the most inert osmoticum because it does not penetrate into the seeds (Heydecker & Coolbear 1977). It was supposed that osmotic solutions allowed the normal occurrence of all germination processes except for the final growth process which is characterized by cell elongation and thus depends on extra water uptake. It was thought to be essential that the inhibition of germination in osmotica permitted a more prolonged activity of the growth preparing processes than in water. It has to be realized, however, that prolonged inhibition of germination is also a good condition for the induction of secondary dormancy (Karssen 1982). Therefore, incorrect timing of pre-incubation might have a negative effect on the treatment.

Also other reasons make a critical re-appraisal of the osmotic pretreatment necessary. It has been shown for instance that the osmotic potentials which are normally applied not only inhibited the water uptake during growth, but also during imbibition. Ibrahim et al. (1983b) showed that at such reduced seed moisture contents respiration was reduced.

Reduced respiration might be caused by a decrease of oxygen availability. In PEG -6000 oxygen solubility is only half that in water and oxygen mobility is only 10%, which depresses relative oxygen availability to not more than 5% (Mexal et al. 1975). Free availability of oxygen has been shown essential for changes in dormancy to occur (Karssen 1980/81).

It is the aim of this chapter to study whether such disadvantageous effects of PEG occur indeed during pre-incubation of lettuce seeds and whether they interfere with the effectiveness of the pre-incubation.

Abbreviations - PEG, polyethylene glycol; T_{50}, maximum temperature which permits 50% germination.

Material and methods

Seeds of lettuce cv. Musette 82889 were obtained from Enza Zaden, Enkhuizen and of cv. Capitan (31722) from Sluis & Groot, Enkhuizen. The seeds were stored dry at 2 to 5 °C until use in the experiments which were performed in 1984 and 1985.

Triplicates of 50 seeds were sown in 5 cm Petri dishes on one layer of

filter paper (Schleicher & Schüll No. 595) moistened with 1.5 ml distilled water, or a solution of PEG. Osmotic potentials of PEG solutions were calculated according to Michel (1983). After pre-incubation seeds were rinsed with 100 ml of distilled water, surface dried by suction in a Büchner funnel and transferred to fresh filter paper in another Petri dish. Thereafter, the seeds were either used for determination of moisture content or they were transferred to germination conditions after moistening the filter paper with 1.5 ml distilled water or PEG solution, with or without a preceding redesiccation treatment. Redesiccation occurred in the hygrostat above saturated salt solutions or silicagel, as described in more detail in chapter 5. Surface dried seeds were placed in Petri dishes in the hygrostat until the moisture content of the seeds was in equilibrium with the relative humidity of the atmosphere (mostly 24 h). The moisture content of the seeds was determined by weighing ($\pm$ 0.1 mg) about 100 mg seeds in little vials before and after oven drying at 130 °C during 1.5 h. Germination occurred in darkness at different temperatures which were realized in cooled incubators (Gallenkamp, Crawley, U.K., T$\pm$1 °C). Germination was counted after 2 days of incubation at the germination conditions. All manipulations were conducted in dim green light obtained by filtering irradiation from one green fluorescent tube (Philips TL 40W/17) through 2 layers of yellow no. 46 and 2 layers of blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

Standard pre-incubation occurred in air, except for one experiment where different O_2 concentrations were realized in 500 ml beakers, each placed inversely in a 9 cm Petri dish and water locked. Three 5 cm Petri dishes were piled up in each beaker. A small hole covered with a rubber septum enabled addition of pure N_2 and O_2 to the required O_2 concentration. The O_2 concentration was checked by gas chromatography.

Concentrations of CO_2, O_2 and N_2 in the atmosphere of the respiring seeds was measured with a gaschromatograph according to Hoekstra & Bruinsma (1975), connected to an electronic integrator (Spectra Physics, SP 4100). Four replicate samples of pre-incubated seeds of a known dry weight were placed on one layer of filter paper in 1.0 ml distilled water or -1.5 MPa PEG in 6.0 ml vessels fitted with microflex valves to avoid gas leakage for another 2 h at 15 °C or 30 °C. Following pre-incubation the seeds (four replicates) were placed in these vessels for 2 h at 15 °C or 30 °C on a filter paper disc in 1.0 ml H_2O or -1.5 MPa PEG.

Results

The first part of this study was performed with a batch of lettuce seeds cv. Musette which germinated for 100% at a maximum temperature of 20 °C, while germination stopped at 26-28 °C (Fig. 1).

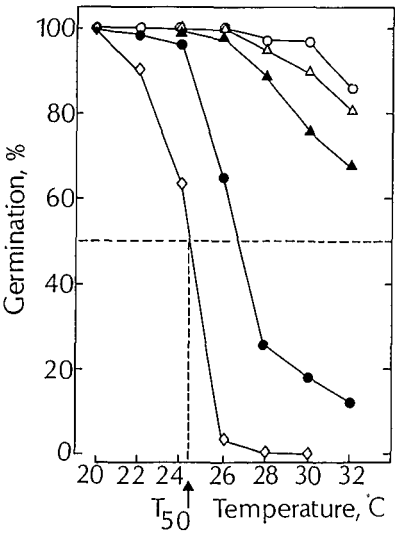

Fig. 1. Influence of pretreatment on germination of lettuce seeds cv. Musette at different temperatures. Seeds were not pretreated (◇), pre-incubated at 15 °C during 24 h in water (circles) or 40 h in -0.5 MPa PEG (triangles) and subsequently redesiccated to 5.5% moisture content (closed symbols) or directly (open symbols) transferred to water at the indicated temperature. The temperature at which 50% of the seeds germinated is indicated with T_{50}.

The T_{50} of untreated seeds (24.5 °C) was increased to values above 32 °C by pre-incubation during 24 h at 15 °C in water. Pre-incubation in -0.5 MPa PEG during 40 h had a similar effect. Therefore, it could be questioned whether pre-incubation in PEG was strictly required. For seeds of this cultivar, however, redesiccation of pre-incubated seeds revealed that pre-incubation in PEG had a clear advantage. While redesiccation to 5.5% moisture content caused after pre-incubation in water a decrease of T_{50} to 26.5 °C, it hardly affected the T_{50} of PEG-incubated seeds (Fig. 1). Thus, pre-incubation in PEG caused a

greater resistance to redesiccation. The redrying occurred in this study to one moisture content only, effects of different degrees of water loss are described in chapter 5.

A possible explanation for the redesiccation resistance of the PEG-incubated seeds could be that pre-incubation in PEG lasted longer than in water. The maximal length of pre-incubation depended on the moment redesiccation caused visible and irreversible damage to the seeds. Damage was recorded on the seedlings that developed from pre-incubated and redried seeds during a second incubation for 48 h at 15 °C in water (Table 1). In chapter 6 it is shown that the moment damage occurred due to redesiccation preceded the moment of visible germination by 3 h.

Table 1. Influence of the osmotic potential of the pre-incubation medium and the length of the pre-incubation period on the percentage lettuce seeds cv. Musette that after redrying developed damaged seedlings during renewed incubation in water. The data represent the mean ± SD of different experiments in which desiccation occurred to various moisture content ranging from 5 to 10%.

osmotic potential, MPa					
0		-0.5		-1.0	
duration hours	damage %	duration hours	damage %	duration days	damage %
20	0	32	0	4	0
24	1 ± 1	40	1 ± 1	5	2 ± 1
28	19 ± 20	48	10 ± 11	7	23 ± 23
32	61 ± 32	72	96 ± 2	9	73 ± 31

A first disadvantage of a pre-incubation in PEG is shown in Fig. 2. Seeds were pre-incubated in PEG solutions at different osmotic potentials and during different periods of time at 15 °C. Germination was recorded in water at 28 °C (A) or 30 °C (B), temperatures at which untreated seeds did not germinate (Fig. 1). Evidently, pre-incubation in PEG retarded the process of dormancy breaking. When the pre-incubation period was very prolonged alleviation of

dormancy even turned into its opposite, the induction of secondary dormancy. Consequently, pre-incubation in -1.5 MPa PEG never caused full germination at 30 °C (Fig. 2B).

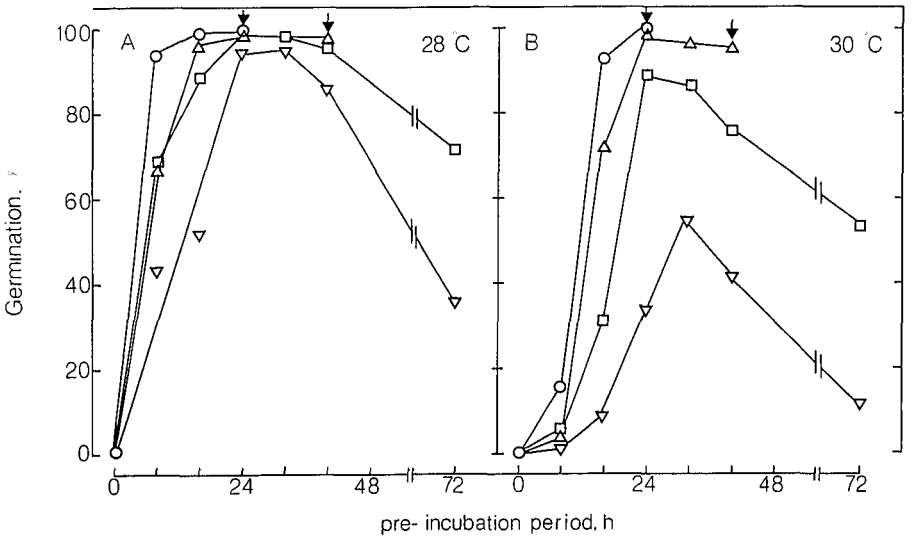

Fig. 2. Influence of the length of pre-incubation at 15 °C and the *incubation medium on germination in water at 28 °C (A) and 30 °C (B)* of lettuce seeds cv. Musette. Seeds were pre-incubated in water (○) or PEG at osmotic potentials of -0.5 (△), -1.0 (□) or -1.5 (▽) MPa. The arrows denote the maximum length of pre-incubation, since the first drying damage occurred at that moment.

It was studied whether the effects of PEG were caused by an inhibition of oxygen diffusion. The experiments were performed with seeds of the lettuce cv. Capitan, which had a slightly higher T_{50} than seeds of cv. Musette. Due to the higher T_{50}, germination was tested at 32 °C. Pre-incubation occurred in distilled water or -1.5 MPa PEG at different oxygen levels (Fig. 3). In air, the alleviation of dormancy in Capitan seeds was also retarded by PEG. In the range of 5.6 to 100% the O_2 level did not cause much differences. Therefore, limitation of oxygen diffusion during incubation in PEG could be ruled out as explanation for the retarded dormancy breaking. Oxygen levels became critical both in water and PEG at 2 and 0% indicating energy dependency of dormancy release.

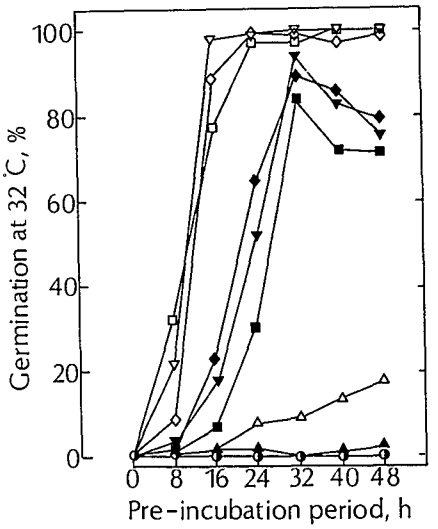

Fig. 3. Influence of oxygen level during pre-incubation at 15 °C in water (open symbols) or -1.5 MPa PEG (closed symbols) on subsequent germination of lettuce seeds cv. Capitan at 32 °C. During pre-incubation oxygen level was 0% (○,●), 2% (△,▲), 5.6% (◇,◆), 20.1% (▽,▼) or 100% (□,■).

It was also studied whether lower levels of water saturation at the end of imbibition interfered with the ability to change dormancy. Seeds of cv. Capitan were pre-incubated in water or -1.5 MPa PEG at either 15 °C, to break dormancy, or 30 °C, to induce dormancy. Germination was tested at 32 °C or 24 °C, respectively. The moisture content at the end of imbibition in PEG was lower at both pre-incubation temperatures (Fig. 4). The experiment also clearly showed that PEG not only retarded the breaking of dormancy at 15 °C (Fig. 4A), but also the induction of secondary dormancy at 30 °C (Fig. 4B). Whereas 48 h at 30 °C in water was sufficient to reduce germination at 24 °C to 30%, a similar pre-incubation in -1.5 MPa PEG hardly affected subsequent germination at 24 °C. This result seemed at first view contradictory to the development of secondary dormancy in -1.5 MPa PEG at 15 °C (Fig. 2). The explanation is that during pre-incubation at 15 °C the effect of PEG on induction of secondary dormancy could not be compared to water because seeds germinated in water. The latter did not occur at 30 °C.

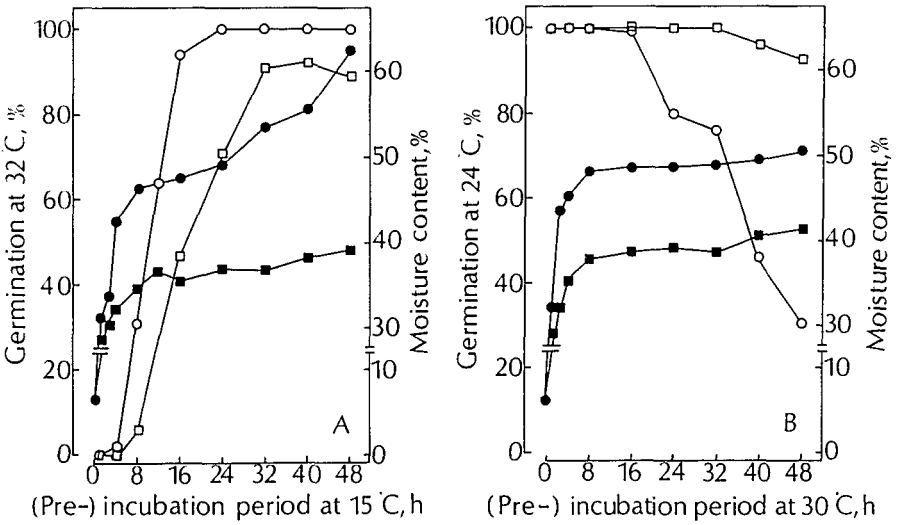

Fig. 4. Influence of pre-incubation in water or PEG on dormancy and moisture content of lettuce seeds cv. Capitan. A. To alleviate dormancy seeds were pre-incubated during the indicated period at 15 °C in water (circles) or -1.5 MPa PEG (squares) and thereafter germinated in water at 32 °C (*open* symbols) or used for determination of moisture content (closed symbols). B. To induce dormancy seeds were pre-incubated likewise but pre-incubation occurred at 30 °C and germination at 24 °C.

Incubation in PEG also strongly influenced energy metabolism (Fig. 5). At 30 °C O_2 uptake and CO_2 release rose during the early phase of incubation, both in water and PEG, to similar values. Thereafter, the respiratory activity stayed constant during incubation in water, but slowly decreased in PEG. Thus, induction of secondary dormancy at 30 °C correlated with the maintenance of high respiratory activity. Unfortunately at 15 °C some data on O_2 uptake are missing after certain incubation times due to technical failure. Nevertheless the same trend was seen as at 30 °C. Incubation in PEG inhibited both the uptake of O_2 and the release of CO_2 from the seeds. The values for RQ varied between 0.5 and 0.6.

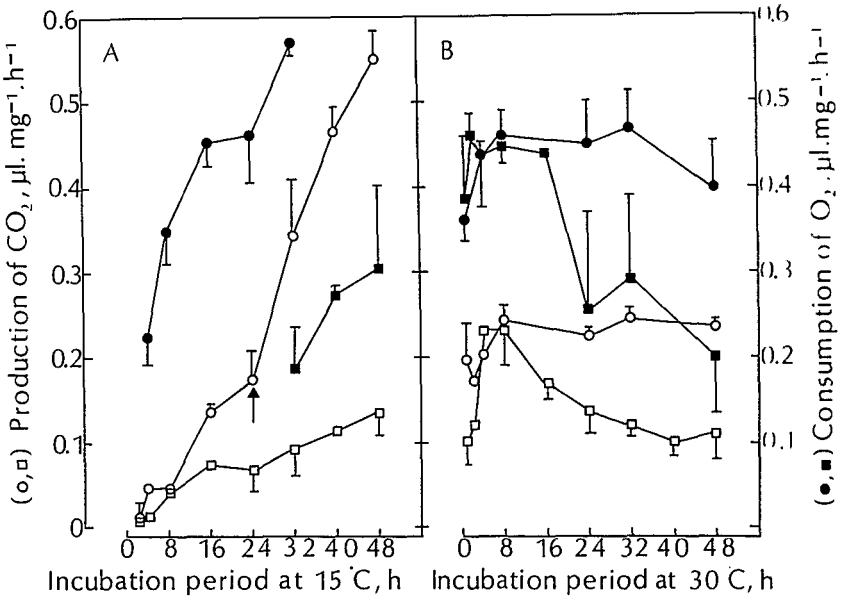

Fig. 5. Influence of incubation in water (○) or -1.5 MPa PEG (□) at 15 °C (A) or 30 °C (B) on consumption of O_2 (closed symbols) and production of CO_2 (open symbols) by lettuce seeds cv. Capitan. CO_2 and O_2 were measured during 2 h after the indicated incubation periods. The arrow denotes the first moment desiccation damage appeared due to the start of germination.

Discussion

The present study has clearly shown that pre-incubation of lettuce seeds in PEG solutions with low osmotic potentials inhibited not only the moment of visible germination but also some processes preceding growth, which were related to the alleviation and induction of dormancy (Figs. 2, 4). Kahn (1960) found similar effects of prolonged pre-incubation in mannitol on lettuce seeds cv. Grand Rapids. The retardation of dormancy induction is most clearly shown at 30 °C where germination in water did not occur (Fig. 4B). A change from alleviation to induction of dormancy, as seen in our experiments during pre-incubation in -1.0 and -1.5 MPa PEG at 15 °C, has also been observed during

prolonged inhibition of germination due to other means by Totterdell & Roberts (1979) in Rumex obtusifolius, Cone & Spruit (1983) in Arabidopsis thaliana and Hilhorst & Karssen (1986) in Sisymbrium officinale. Evidently, at intermediate temperatures like 15 °C alleviation of dormancy is followed by re-induction. At lower and higher temperatures alleviation or induction, respectively, dominate.

This study has also shown that the effect of PEG during incubation of lettuce seeds is not due to interference with oxygen availability. Nevertheless, oxygen availability is essential for both alleviation of dormancy (Fig. 3) and induction of secondary dormancy in lettuce and other species (Karssen 1980/81). The effect of PEG must be due to the decrease of the moisture content of the seeds (Fig. 4), which by unknown mechanisms caused a decrease of respiration (Fig. 5). Ibrahim & Roberts (1983) described a similar reduction of respiration in lettuce seeds of low moisture content. They also showed that at high temperatures the initial respiration rate was much higher than at lower temperatures (Fig. 5B). The RQ of 0.5 - 0.6 is in accordance with the high lipid content of lettuce seeds. Pre-incubation in PEG did not change the RQ value. It is concluded that changes in dormancy depend on active processes requiring sufficient O_2. Similar conclusions were reached by Vidaver & Hsiao (1975).

With respect to the application of seed pretreatment in horticultural practice the present results indicate that pre-incubation in PEG instead of water is mainly required to obtain desiccation tolerance of the seeds. A good result of the pretreatment depend, however, strongly on the length of the pre-incubation and the PEG concentration chosen. In general, it is advised to use short pre-incubation times and low concentrations of PEG. It will be shown in chapter 3 that cultivars with a much higher T_{50} than cv. Musette are desiccation tolerant after pre-incubation in water and, therefore, hardly require pre-incubation in PEG. In chapter 7 the physiological mechanisms underlying low temperature pre-incubation are studied in more detail.

CHAPTER 3

DIFFERENT REACTIONS OF LETTUCE CULTIVARS TO SEED PRETREATMENT

Abstract

Studies on the maximum temperature at which 50% of seeds germinate (T_{50}) show that seed dormancy in lettuce varies largely between cultivars and between batches of an individual cultivar. Values of T_{50} are found between 15 and 30 °C. Pre-incubation in water at 15 °C during 16 to 20 h increases T_{50} with 3.5 to 9 °C. Red light increases the effect of the pre-incubation only slightly in most cultivars, except cv. Grand Rapids. Subsequent redesiccation to a moisture content of 4% hardly decreases T_{50} in some cultivars, whereas in others T_{50} drops to values close to those of untreated seeds.

Germination of a batch of seeds with a T_{50} of 15 °C can be improved to 27.5 °C by pre-incubation at 10 °C in -0.5 MPa PEG during 3 days. Pre-incubation in PEG prevents moreover for the greater part the decrease of T_{50} due to redesiccation. Unfortunately, a pre-incubation temperature of 2 °C, which results in even better alleviation of dormancy, is less likely since it causes vernalization. It is concluded that dormancy levels of seeds determine which method of pre-incubation has to be used.

Introduction

Pretreatment of lettuce seeds has been shown a good method to improve germination at high temperatures (Guedes & Cantliffe 1980). In chapter 2 it was confirmed that pre-incubation in water increased T_{50}, the maximum temperature at which 50% of the seeds germinate. It was also shown that redesiccation of pre-incubated seeds caused a decrease of T_{50}, which could be prevented by pre-incubation in polyethylene glycol (PEG). The experiments described in chapter 2 were performed with two cultivars only. Since it is known that the germination characteristics of different lettuce cultivars vary largely, in particular with respect to T_{50} (Bekendam 1972, Gray 1975, Thompson et al. 1979), it was decided to extend the experiments to a larger group of cultivars. Also different batches of one cultivar were compared because Harrington & Thompson (1952) showed that T_{50} also depended on the environmental conditions during seed development.

Khan (1977) showed that osmotic pretreatment combined with the application

of the growth regulators gibberellin and cytokinin raised T_{50} to 40 °C. However, the photographs showed that seeds treated with gibberellin produced abnormal, stretched seedlings. The present study will concentrate on methods to raise T_{50} by means of light, optimal temperature and osmoticum.

Abbreviations - PEG, polyethylene glycol; T_{50}, maximum temperature for 50% germination.

Material and methods

Seeds of lettuce were obtained from various sources. Two harvests of cv. Musette were obtained from Enza Zaden, Enkhuizen. Batch 82985 and batch 82889 were harvested from plants grown at different circumstances. Batch 82889 is indicated as $Musette_{25}$ since T_{50} appeared to be about 25 °C. Many experiments were performed with batch 82985. During the experimental period T_{50} of this batch changed from 15 to 20 °C at normal storage conditions. Seeds of cvs. Capitan and Palmyran were obtained from Sluis & Groot Research, Enkhuizen; cv. Marcia from Royal Sluis, Enkhuizen; cv. Mariska from Nunhem's Zaden, Haelen; cv. Ravel from Rijk Zwaan, De Lier; and cvs. Grand Rapids and Montello from Ferry Morse Seed Co., Mountain View, U.S.A. The seeds were stored at 2 to 5 °C until use, the experiments were performed in 1984, 1985 and 1986.

The conditions during pretreatment and germination were similar to chapter 2, except that in some experiments seeds received a saturating 5 minutes dose of red light from 6 red fluorescent tubes (Philips TL 20W/15) filtered by 3 mm plexiglas (red 501, Röhm & Haas, Darmstadt, G.F.R.), the light intensity at seed level being 250 $\mu W.cm^{-2}$.

For determination of the date of harvest and flowering, pre-incubated seeds were transferred on May 24, 1985 to a growth cabinet at 20 °C with a diurnal cycle of 16 h light and 8 h darkness. On May 28 the seedlings were transplanted to potting compost in a glasshouse at 25 °C. On June 18 two replicated of 5 plants each were planted outdoors. Development was observed until the first flowers appeared.

Results

Lettuce seeds cv. Musette$_{25}$ and cv. Ravel differed in their ability to germinate at a range of temperatures, T_{50} values being 25 and 29 °C respectively (Fig. 1).

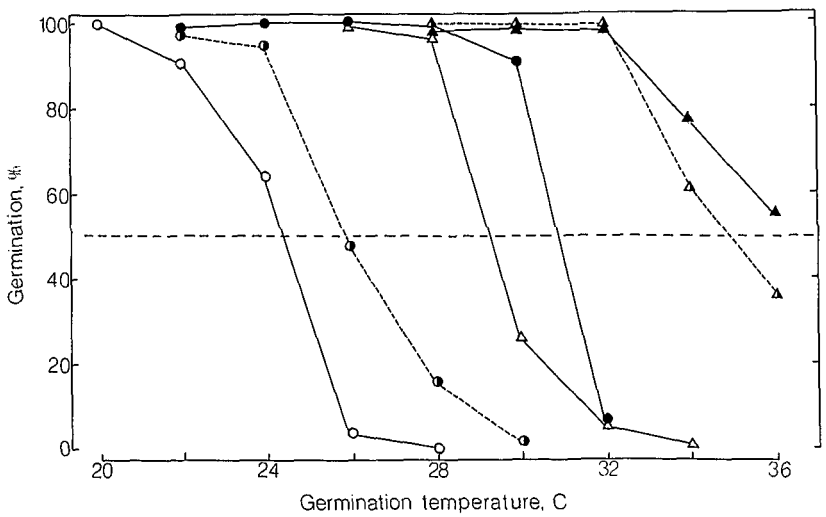

Fig. 1. Influence of pretreatment on the germination of lettuce seeds cvs. Musette$_{25}$ (circles) and Ravel (triangles) at different temperatures. Seeds were either sown directly at the indicated temperatures (open symbols) or pre-incubated in water at 15 °C during 20 h (Musette) or 16 h (Ravel), without (closed symbols) or with subsequent redesiccation to a moisture content of 4.2% (Musette) or 4.7% (Ravel).

After pre-incubation the difference in T_{50} remained, T_{50} of Musette increased due to 20 h at 15 °C in water to 31 °C and T_{50} of Ravel was 36.5 °C at the end of 16 h at 15 °C. However, seeds of the two cultivars differed in their reaction to subsequent redesiccation. The reduction in T_{50} was 5.5 and 1.5 °C, respectively.

It appeared that Musette and Ravel both represented different groups of lettuce cultivars (Fig. 2). The cultivars Capitan, Mariska, Montello and Grand

Rapids lost like Ravel 10 to 20% of the increase of T_{50} due to redesiccation to low moisture content. The other cultivars lost like Musette 50 to 90% of the increase of T_{50}. Seeds of five cultivars were shortly irradiated with red light at the end of pre-incubation. Red light increased T_{50} in Grand Rapids to much higher values than in the cvs. Musette, Marcia, Palmyran and Montello. In none of the cultivars red irradiation changed the reduction of T_{50} by redesiccation. Fig. 2 also depicts the influence of pretreatment on another batch of cv. Musette than the $Musette_{25}$ seeds which were described in Fig. 1. The seeds of this seed batch were extremely dormant. At the beginning of the experimental period they showed a T_{50} of 15 °C ($Musette_{15}$). During dry storage at 2 to 5 °C T_{50} increased in the course of a year to 20 °C ($Musette_{20}$). Due to our standard pre-incubation at 15 °C in water T_{50} reached values varying between 23 and 26 °C. However, redesiccation caused a dramatic reduction of T_{50}. Altogether a very unsatisfactory result of a pretreatment. Therefore it was tried whether changes in the pre-incubation could improve the result.

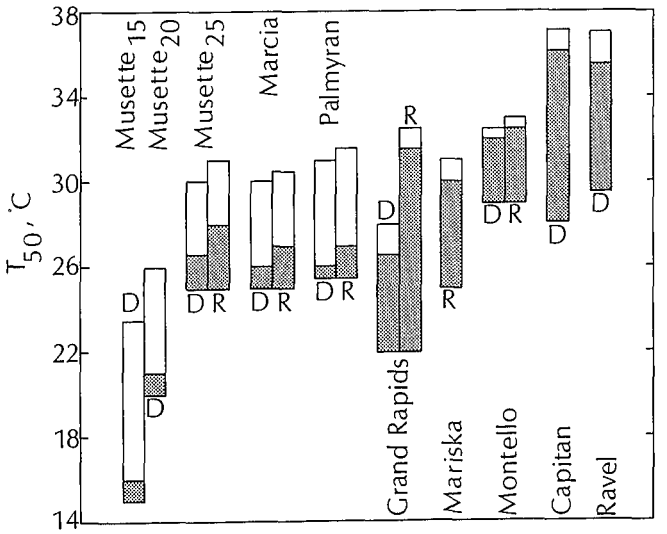

Fig. 2. Influence of pretreatment on the T_{50} of lettuce seeds of different cultivars. T_{50} was determined on seeds that were either sown directly at a range of temperatures (bottom lines of vertical bars) or on seeds that had been pre-incubated in water at 15 °C during 16 or 20 h without (top line of bars) or with subsequent redesiccation to moisture contents between 3.6 and 4.5% (middle line in bars). Seeds were kept in darkness during pre-incubation (D) or were irradiated during 5 min at the end of pre-incubation with red light (R). From cv. Musette 3 batches were tested with different T_{50} (15, 20 or 25 °C)(see text for origin of batches).

Since the normal pre-incubation temperature of 15 °C was equal to the T_{50} of the seeds, a better effect was expected of lower pre-incubation temperatures. Indeed, pre-incubation at 10 and 2 °C in water resulted in much higher T_{50} values than a 15 °C pre-incubation (Table 1). Pre-incubations were extended to the moment that desiccation damage started to occur. After redesiccation part of the improvement remained.

Table 1. Influence of different pre-incubation conditions and redesiccation on T_{50} of lettuce seeds cv. Musette$_{15}$. Seeds were pre-incubated in water or -0.5 MPa PEG at different temperatures for various periods of time and thereafter either redesiccated to a moisture content of 4.1% or not dried.

pre-incubation conditions	T_{50}, °C not dried	dried
none	15	-
water, 20 h 15 °C	23.5	16.0
water, 40 h 10 °C	26.5	21.0
water, 5 d 2 °C	>34	25.0
PEG -0.5 MPa, 40 h 15 °C	25.5	21.5
PEG -0.5 MPa, 72 h 10 °C	27.5	23.5
PEG -0.5 MPa, 17 d 2 °C	>34	28.0

As was shown in chapter 2 pre-incubation in -0.5 MPa PEG only slightly improved the rise of T_{50}, it prevented, however, partly the fall of T_{50} due to redesiccation. Is therefore a pre-incubation during 17 days at 2 °C in -0.5 MPa PEG followed by redesiccation the best advisable pretreatment of such deeply dormant seeds? If the effect on plant growth is taken into account, it evidently is not. It is shown in Table 2 that pre-incubation at 2 °C in water and PEG caused an earlier bolting of the plants. Obviously, the seeds were vernalized particularly during the prolonged pre-incubation periods at 2 °C.

Table 2. Influence of pre-incubation of lettuce seeds cv. Musette on *bolting and flower date. Seeds were pre-incubated in water or -0.5* MPa PEG at different temperature for various periods of time. Seedlings were transplanted to potting compost in a glasshouse on May 28, 1985, and planted outdoors on June 18. On August 2 plants from untreated seeds were harvest ripe, a firm compact head was formed, indicated with 0. Plants from other treatments were compared and bolting was indicated with + or ++.

pre-incubation conditions	bolting on Aug. 2	first flower on (date)
untreated	0	Oct. 3
water, 20 h 15 °C	0	Oct. 4
-0.5 MPa PEG, 40 h 15 °C	0	Oct. 3
water, 40 h 10 °C	0	Oct. 3
-0.5 MPa PEG, 72 h 10 °C	0	Oct. 3
water, 7 d 2 °C	+	Sept. 28
-0.5 MPa PEG, 14 d 2 °C	++	Sept. 10
water, 14 d 2 °C	++	Sept. 10
-0.5 MPa PEG, 28 d 2°C	++	Sept. 6

Discussion

This chapter has confirmed that large variation exists in the T_{50} values of lettuce seeds of different cultivars and of batches of the same cultivar. The differences between cultivars point to the attribution of genetic factor(s) in the regulation of dormancy. A study on the inheritance of dormancy in four lettuce cultivars (Eenink 1981) concluded that one chromosomal gene was responsible for the greater part of the genetic variation in dormancy between the cultivars. An effect of such environmental factors is emphasized by the variation in T_{50} of different batches of cv. Musette. As well conditions during development of the seeds ($Musette_{25}$ compared to M_{20} and M_{15}) as conditions during storage (M_{20} compared to M_{15}) played a role. The knowledge about the breaking of dormancy during dry storage is minimal (Bewley & Black 1982). It certainly does not consist of deterioration since T_{50} increased instead of decreased. Deterioration of seeds due to storage is described in

chapter 4.

From a practical point of view it is desirable that lettuce seeds germinate at a broad range of temperatures to secure application in different climatic regions. The present results show that seeds with a high T_{50} are already close to the ideal germination capacity while others are far removed. Therefore, the requirements that are made of a pretreatment differ strongly in dependence of the degree of dormancy. In general, the present results support the rule that the higher the T_{50} the simpler the pretreatment can be. Seeds of cv. Ravel for instance which germinate without pretreatment already for 100% up to 28 °C (T_{50} = 29.5 °C) have only to be pre-incubated in water for 16 h at 15 °C to reach a T_{50} of 37 °C. This result is hardly affected by redesiccation (Figs. 1, 2).

At the other extreme side of the spectrum seeds of $Musette_{15}$ required a more complicated pretreatment to raise T_{50} of 15 °C to more suitable values. Pre-incubation in water at 15 °C proved to be insufficient (Table 1). A change to lower temperatures showed better results, but meets with the complication of vernalization when seeds are pre-incubated at 2 °C. Vernalization in the seed stage was shown before by Prince (1980). Also Cantliffe et al. (1981) found an earlier maturity of plants after pretreatment of lettuce seeds. Consequently 10 °C was left as most suitable pre-incubation temperature. Water had to be replaced by PEG to avoid a strong reduction of T_{50} by redesiccation. But nevertheless the T_{50} of seeds redried to 4% moisture content reached not higher than 23.5 °C (Table 1). It will be shown in chapter 5 that when more moisture is left in the seeds at the end of redrying T_{50} is less reduced. Such treatment, however, interferes negatively with storage of the seeds (chapter 4). In conclusion, pretreatment of $Musette_{15}$ seeds cannot meet the requirements normally asked for. One might consider the application of growth regulators like ethylene, cytokinins, gibberellins or thioureum. Preliminary results (not shown) indicated indeed a higher T_{50}. However, seedling quality was unsatisfactory.

Satisfactory results could be obtained with $Musette_{25}$ (chapter 2) in particular after pre-incubation in PEG. Therefore, attempts to improve dormancy characteristics of lettuce seeds may apart from the development of better pretreatments also be directed to the improvement of seed production and seed storage. However, above all breeding programs have to pay attention to dormancy characteristics of new lines.

CHAPTER 4

ENHANCED REDUCTION OF GERMINATION AND VIABILITY DURING STORAGE OF PRETREATED LETTUCE SEEDS

Abstract

The maximum temperature for 50% germination (T_{50}) of lettuce seeds cv. Mariska is raised by pre-incubation at 15 °C in -0.25 MPa PEG. Redesiccation of pre-incubated seeds causes only a slight decrease of T_{50}. Storage of pretreated seeds decreases T_{50} particularly at higher temperatures and moisture contents. Prolonged pre-incubation enhances the sensitivity to storage conditions. The storage time required to reduce germination at a certain test temperature to 50% (G_{50}) is introduced as an analogue to p_{50}, the storage time required for viability to fall to 50%. Both log G_{50} and log p_{50} decrease linear to log moisture content of the seeds, the storage temperature influences the distance between the curves. Values of G_{50} and p_{50}, estimated at 22 °C and 20 °C, respectively, and obtained after similar storage conditions show a linear relationship. It is concluded that storage-induced dormancy and loss of viability are governed by the same basic processes. Predictability of changes in germination capacity and viability of pretreated lettuce seeds is discussed.

Introduction

The germination of seeds of several lettuce cultivars is inhibited at temperatures exceeding 23 °C (chapters 2, 3; Gray 1975). Osmotic pretreatment of the seeds at lower temperatures has been shown an effective method to raise the upper temperature limit of germination (chapters 2, 3; Guedes & Cantliffe 1980), which is referred to as an alleviation of dormancy (Karssen 1982). Evidently, it is of great practical importance that during storage of pretreated seeds dormancy is not re-induced. In general, studies on seed storage have been concentrated on changes in viability, little is known about the effect of storage on dormancy of pretreated seeds. It is the aim of the present study to investigate the effect of different storage conditions on the upper temperature limit of germination in pretreated lettuce seeds.

Ellis & Roberts (1980a) provided the theoretical framework for the influence of storage on viability. The first equation describes the seed survival curve in terms of the viability (v, probit percentage viability) to be expected after a given storage period (p, days)

$$v = K_i - p/\sigma \qquad (1)$$

where σ is the standard deviation of the frequency distribution of seed deaths in time, and K_i is an entity described as the initial theoretical percentage viability which is unique to each seed lot (Ellis & Roberts 1980b). The differences between seed lots do not affect the value of σ . In contrast, the storage environment has no effect on K_i but affects σ according to the equation

$$\log \sigma = K_E - C_W \log m - C_H t - C_Q t^2 \qquad (2)$$

which estimates the value of σ to be expected in an environment where seeds have a moisture content of m (percent, fresh weight) and a temperature t (°C) and where K_E, C_W, C_H, and C_Q are constants whose values are common to all seed lots of a species. Eqns (1) and (2) may be combined as follows:

$$v = K_i - p / 10^{K_E - C_W \log m - C_H t - C_Q t^2} \qquad (3)$$

Provided that the values of the species constants and the seed lot constant K_i are known, eqn (3) renders the percentage viability to be expected for that seed lot after any time when stored at various temperatures and moisture contents. It has been expected that pretreatment of a seed lot will influence the value of K_i (Ellis & Roberts 1980b), but it has not been determined yet. Therefore, this study will also investigate the influence of pretreatment on the percentage viability as predicted by eqn. (3) in different storage environments. The effects of storage conditions on dormancy and viability will be compared.

Abbreviations: - G_{50}, the storage time at which germination is reduced to 50%; p_{50}, the storage time at which viability is reduced to 50%; m, moisture content of seeds; T_{50}, the maximum temperature for 50% germination; PEG, polyethylene glycol.

Material and methods

Seeds of lettuce (Lactuca sativa L.) cv. Mariska, harvested in 1983, were obtained from Nunhems Zaden, Haelen, The Netherlands. The seeds had an initial

moisture content of 6.7% and were stored dry at 2-5 °C until use. Experiments were performed in 1985 and 1986.

Seeds were pre-incubated during 2 h or 16 h at 15 °C. It occurred in portions of 25 g seeds in 250 ml PEG solution (-0.25 MPa) in 500 ml flasks. Osmotic potential of the PEG solution was calculated according to Michel (1983). To ensure sufficient oxygen availability to the seeds the flasks were gently shaken in a water bath kept at 15 °C. After pre-incubation during 2 or 16 h seeds were rinsed several times with 500 ml of distilled water to remove the PEG. For redesiccation the seeds were spread on nylon wire-netting in a climate cabinet. Different moisture content of the seeds was reached by varying the relative humidity (r.h.) of the cabinet.

Desiccation occurred during 24 h at 20 °C, starting during the 8 h period of fluorescent light in the climate cabinet. The cabinet was on a 8 h light/16 h dark regime since it was also used for viability tests. The seeds were then left to equilibrate in an air-tight container for another 24 h at 2 °C, after which the actual moisture content was determined by weighing ($\pm$ 0.1 mg) about 200 mg seeds in little vials before and after oven drying at 130 °C during 1.5 hours. For each storage treatment seeds were divided into 8 to 10 sub-samples of about 1 g. Each sub-sample was stored in a glass vial 13 mm in diameter and 55 mm long. The vials were sealed with a tightly fitting plastic lid. Finally, the vials were sealed in a polyethylene-lined aluminium foil packet and transferred to either cooled incubators (Gallenkamp, Crawley, U.K., T$\pm$1 °C) at 2, 10, 15, 22, 30 or 37 °C, or to a water bath maintained at 55 $\pm$ 0.5 °C.

At appropriate intervals vials were withdrawn from the storage treatments and seeds were removed for germination tests. Two kinds of germination tests were performed. For dormancy testing, triplicates of 50 seeds were incubated in 5 cm Petri dishes on one layer of filter paper (Schleicher & Schüll No. 595) moistened with 1.5 ml distilled water. Germination occurred in darkness at different temperatures which were realized in incubators. Germination, defined as the ability of the seed to produce a radicle of more than 2 mm long, was counted after 2 days. For viability testing, triplicates of 50 seeds were incubated for 3 days at 10 °C in darkness to break dormancy and thereafter for 7 days at 20 °C in 8 h fluorescent light and 16 h darkness a day. The seeds were sown under a transparent cover on 'seed test thick' filter paper (Schut, Heelsum, The Netherlands, 0.5 mm thick), which was kept wet by a filter paper bridge hanging in distilled water. After 7 days abnormalities in

seedling growth were recorded (ISTA, 1976). In one experiment seeds received a 5 minutes dose of red light from 6 red fluorescent tubes (Philips TL 20W/15) filtered by 3 mm plexiglass (red 501, Röhm & Haas, Darmstadt, G.F.R.), the light intensity at seed level being 250 $\mu W.cm^{-2}$.

Results

The standard pretreatment involved pre-incubation in -0.25 MPa PEG at 15 °C during 16 h followed by redesiccation to various moisture contents. Control seeds were pre-incubated during 2 h and then redesiccated to ascertain a similar moisture content in pre-incubated and control seeds at the beginning of storage. The effects of 2 h and 16 h pre-incubation on the maximum temperature of germination were compared to untreated seeds (Fig. 1).

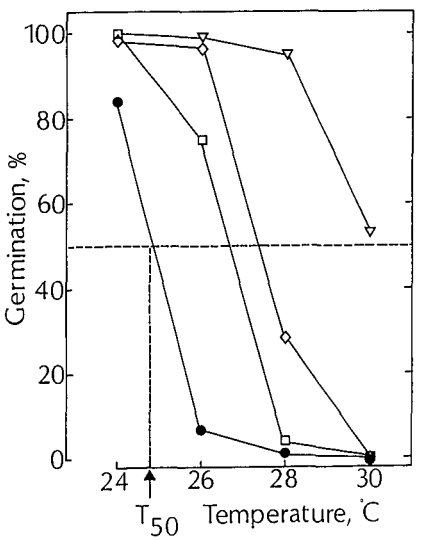

Fig. 1. Influence of light and pretreatment on germination of lettuce seeds cv. Mariska at various temperatures. Seeds were either sown directly at the indicate temperatures in darkness (●) or with 5 min red light 1 h after start of imbibition (□), or pre-incubated at 15 °C in -0.25 MPa PEG in darkness during 2 h (◇) or 16 h (▽) and redesiccated to 9.6% moisture content during 24 h in a climate cabinet at a 8 h light/16 h dark regime.

When seeds of cv. Mariska were directly sown at the different test temperatures in darkness 50% germination occurred at 25 °C (T_{50}). However, since a 8 h photoperiod was part of the redesiccation period of pre-incubated seeds, seeds that had been irradiated with red light were a better comparison for the control seeds. Indeed, the T_{50} values of untreated seeds irradiated during 5 min with red light came very close to control seeds that had been pre-incubated during 2 h and redried, being 26.6 and 27.3 °C, respectively (Fig. 1). Pre-incubation during 16 h and redesiccation to 9.6% moisture resulted in a T_{50} of 30 °C. It has been shown before (chapter 3) that redrying of pre-incubated seeds caused in seeds of cv. Mariska only a slight reduction of the beneficial effect of a pre-incubation. The degree to which the seeds were redried was insignificant in this cultivar (data not shown).

During storage of seeds pre-incubated for 16 h at 15 °C and dried to 9.7% moisture content T_{50} decreased (Fig. 2). A decrease of T_{50} was also observed after pre-incubation during 2 h, desiccation to other moisture contents or storage at other temperatures. Plotting of T_{50} against storage time showed that the T_{50} of pretreated seeds decreased faster than those of the 2 h control, despite the higher initial T_{50} value (Fig. 3).

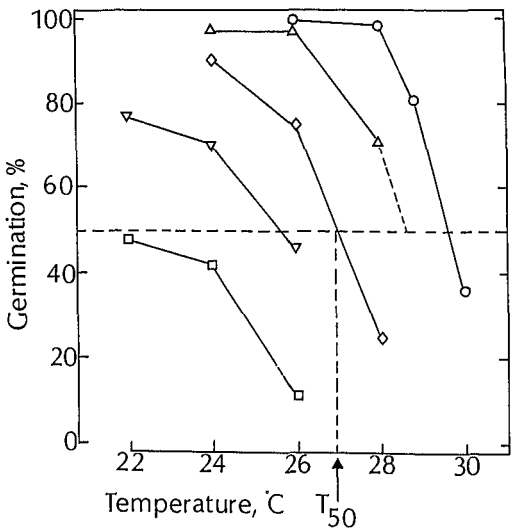

Fig. 2. Influence of storage time of pretreated lettuce seeds cv. Mariska on germination at various temperatures. The seeds were pre-incubated during 16 h at 15 °C in -0.25 MPa PEG in darkness, re-desiccated to 9.7% moisture content of and stored at 22 °C during 0 (○), 34 (△), 62 (◇), 84 (▽) or 102 (□) days. The estimation of T_{50} is indicated.

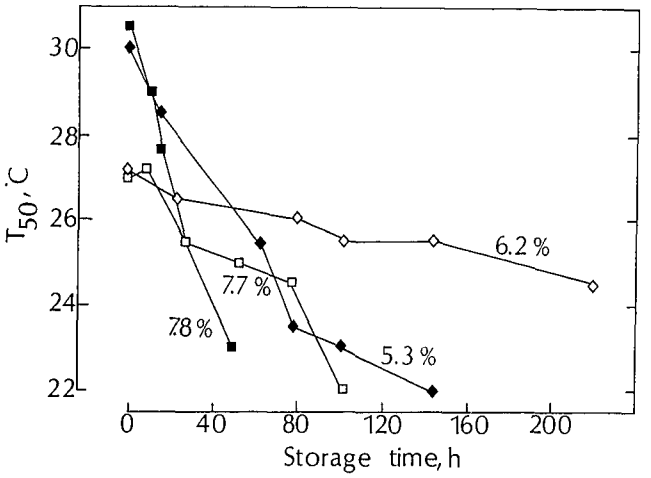

Fig. 3. Influence of pretreatment of lettuce seeds cv. Mariska on the decrease of T_{50} during storage. The seeds were pre-incubated at 15 °C in PEG (-0.25 MPa) during 2 h (open symbols) or 16 h (closed symbols), redesiccated to the moisture content indicated beside the curves and stored at 55 °C. The T_{50} values were determined as indicated in Fig. 2.

The decrease of T_{50} was retarded when the seeds had a lower moisture content. To compare different storage conditions it was convenient to know after what storage time germination was reduced to 50% (G_{50}). The determination of G_{50} is depicted in Fig. 4. It is shown that at a certain moisture content G_{50} depended both on storage time and temperature during germination. G_{50} was introduced as an analogue to p_{50}, the storage time taken for viability to fall to 50%. The following equation can be derived from Eqn (3), if it is realized that probit 50% equals zero:

$$\log p_{50} = \log (K_i) + K_E - C_W \log m - C_H t - C_Q t^2 \quad (4)$$

Eqn (4) predicts that the relationship between $\log p_{50}$ and $\log m$ (moisture content) is linear. Substituting G_{50} for p_{50} renders the possibility to analyze the influence of moisture content and storage temperature on the decrease

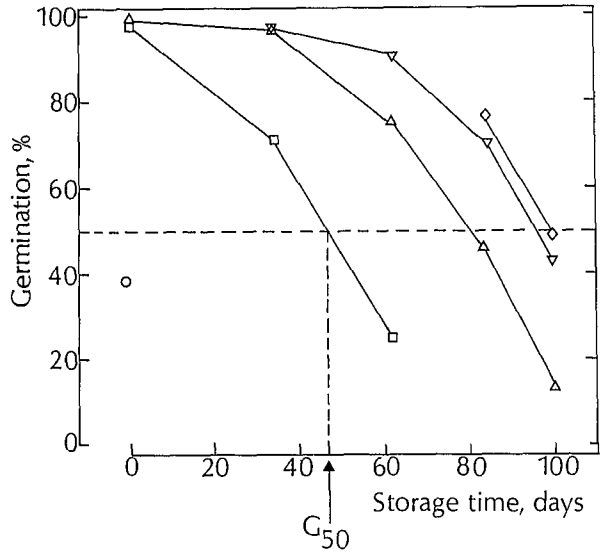

Fig. 4. Reduction of germination at different temperatures as function of storage time seeds were pre-incubated during 16 h at 15 °C in -0.25 MPa PEG in darkness, redesiccated to 9.7% moisture content and stored for different periods of time at 22 °C. Germination of stored seeds was tested at 22 (◇), 24 (▽), 26 (△), 28 (□) or 30 °C (○). The estimation of G_{50} is indicated.

of T_{50}. In Fig. 5 log G_{50} data obtained at a germination temperature of 26 °C with seeds pretreated for 2 h or 16 h were plotted against log moisture content during storage. Although these data did not completely fit with linear curves we concluded provisionally to a linear relationship between log G_{50} and log m. In accordance with Eqn (4) the temperature (t) during storage influenced the distance between the curves (Fig. 5). The slopes of the curves depended on the species constant C_W (Eqn 4) and was, indeed, seen to be independent of storage temperature. Only one point, indicated with an arithmetic, was in contrast with this conclusion. At the storage temperatures 30 °C and 37 °C a pre-incubation of 16 h resulted in lower G_{50} values than a 2 h control treatment. At 55 °C such a difference was not seen.

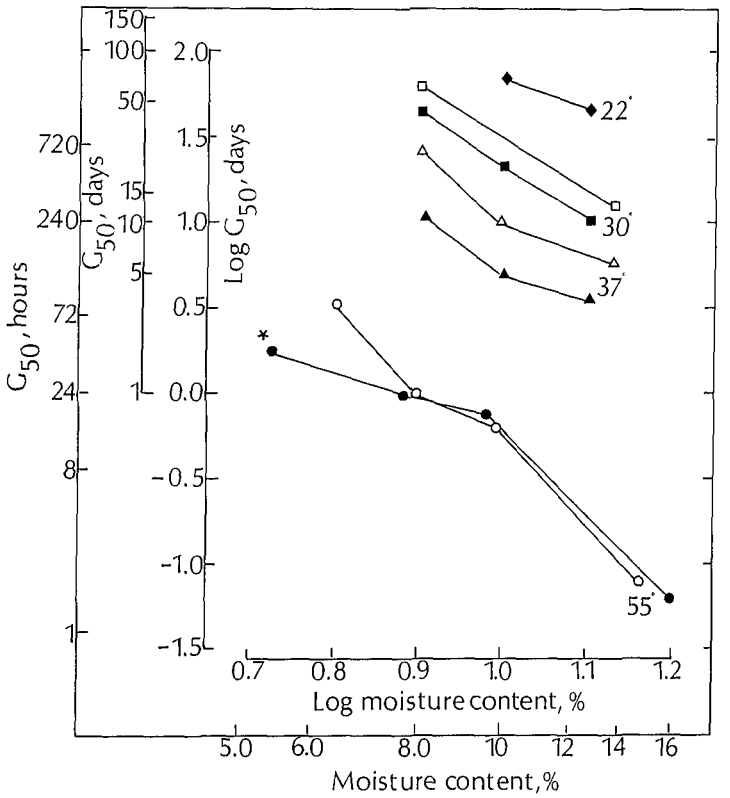

Fig. 5. Influence of pretreatment of lettuce seeds cv. Mariska on the relationship between seed moisture content and the storage time taken for germination at 26 °C to fall to 50% (G_{50}). Seeds were pre-incubated during 2 h (open symbols) or 16 h (closed symbols) at 15 °C in -0.25 MPa PEG in darkness, redesiccated to different moisture content at 22 °C, stored at 22 °C (◇ ◆), 30 °C (□ ■), 37 °C (△ ▲), or 55 °C (○ ●) and germinated at 26 °C.

In analogy to Figs. 4 and 5 we also determined the influence of storage conditions on p_{50}. 'Normal' germination was used as criterion for viability (Ellis & Roberts 1981). Germination was called 'normal' when during 7 days at 20 °C in a diurnal 8 h light/16 h dark regime seedlings developed without any visible abnormality in any part, except for minor ones allowed under ISTA rules (1985).

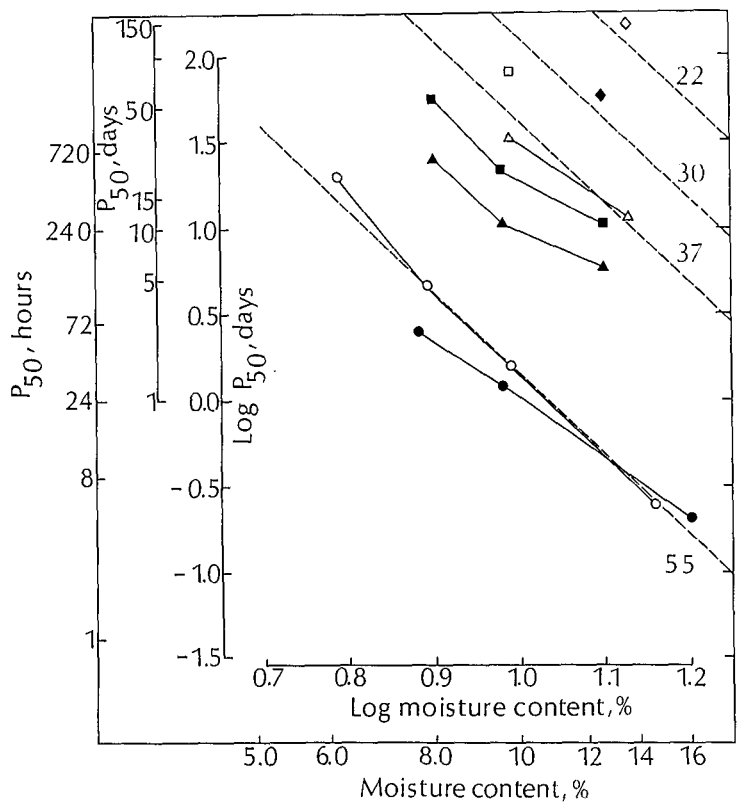

Fig. 6. Influence of pretreatment of lettuce seeds cv. Mariska on the relationship between seed moisture content and the storage time taken for the number of normal seedlings to fall to 50% (p_{50}). See for conditions of pre-incubation, redesiccation and storage Fig. 5. 'Normal' germination was determined after 7 days at 20 °C in 8 h light/16 h dark regime, preceded by 3 days at 10 °C in darkness. See text for explanation of broken lines.

Values of p_{50} were determined at the same storage conditions as G_{50}, and accordingly log p_{50} was plotted against log m (Fig. 6). It was attempted to fit these data with Eqn 4. Values for the four species constants of lettuce seeds were determined by Kraak & Vos (1987): K_E = 8.218, C_W = 4.797, C_H = 0.0489 and C_Q = 0.000365. Unfortunately our data were insufficiently detailed

to allow a reliable calculation of K_i for pre-incubations during 2 and 16 h. Therefore, K_i was estimated. It is shown in Fig. 6 that the p_{50} values of seeds that had been pre-incubated during 2 h fitted reasonably well with curves calculated with a K_i value of 3.5 (broken lines). Such a value of K_i pointed theoretically to an initial viability of 99.9%. When the seeds were pre-incubated during 16 h the values of p_{50} at the storage temperatures 37, 30, and 22 °C shifted for roughly the same distance to lower values of log p_{50}. At a storage temperature of 55 °C such a shift did not occur.

Finally, we studied the relationship between the changes in p_{50} and G_{50} during storage. Comparison of the data in Figs. 5 and 6 learned that at similar storage temperatures G_{50} was lower than p_{50}. It has to be realized however, that G_{50} was determined at 26 °C and p_{50} at 20 °C. Therefore, G_{50} values were also determined at 22 °C, 24 °C, and 28 °C and plotted against log p_{50} (Fig. 7).

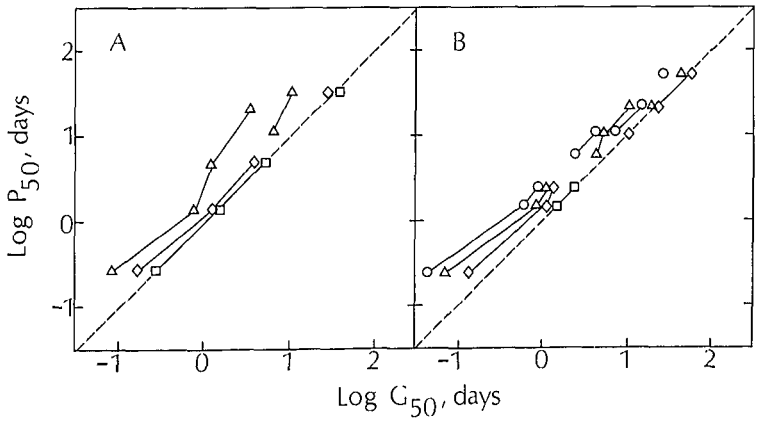

Fig. 7. Relationship between log p_{50} and log G_{50} of pretreated and stored lettuce seeds cv. Mariska. The curves were constructed by plotting values of p_{50} (Fig. 6) to values of G_{50} (Fig. 5) obtained after the same storage condition. p_{50} was always determined at 20 °C, G_{50} at either 22 °C (□), 24 °C (◇), 26 °C (△) or 28 °C (○). Values obtained at one germination temperature and at the same storage temperature are connected. Pre-incubation occurred during 2 h (A) or 16 h (B) at 15 °C in -0.25 MPa PEG, in darkness.

Evidently, log G_{50} and log p_{50} showed a linear relationship for both pre-incubation periods, and all test-temperatures of G_{50}. At the test-temperatures 28 °C and 26 °C G_{50} was always lower than p_{50}, at 22 °C G_{50} equaled p_{50} at all storage conditions whereas 24 °C took a position between these two extremes. This observation means that for this seed lot at 22 °C p_{50} and G_{50} will depend on the same values of the species constants and the seedlot constant K_i. Since we could predict p_{50} for seeds that had been pre-incubated during 2 h (Fig. 6) it can also be done for the G_{50} at 22 °C.

Discussion

The present data clearly showed that during storage of pretreated lettuce seeds the beneficial effects of the pretreatment were gradually lost, dormancy was re-induced (Fig. 2). High temperatures and moisture contents stimulated the decrease of T_{50} (Figs. 3, 5). Interestingly, the loss of viability of the pretreated seeds was enhanced by the same storage conditions (Fig. 6). The decrease of the parameters G_{50} and p_{50} showed a linear relationship (Fig. 7). Therefore, it is strongly suggested by the present results that the storage-induced induction of dormancy and loss of viability of pretreated lettuce seeds are governed by the same basic processes. In contrast, Ellis and Roberts (1981) concluded from experiments with 6 rice cultivars, that the release of dormancy during storage was not related to the loss of viability. A relationship to the induction of dormancy was not studied, however. In general, induction of dormancy is regarded as a reversible process and the loss of viability as irreversible. It will be shown in chapter 5 that T_{50} of lettuce seeds cv. Musette subsequently can be increased by pre-incubation at 15 °C, decreased by desiccation and increased again by renewed incubation at lower temperatures.

Our present experiments do not permit conclusions about the nature of the processes that cause the storage-induced induction of dormancy and loss of viability. Further analyses of the mechanisms that regulate the changes in T_{50} (chapters 5, 6, 7) will be of some help, however.

The possibility to predict the decrease of G_{50} during storage of pretreated lettuce seeds would be of great practical use. Unfortunately, our present data are insufficiently detailed to prove that the distribution of dormancy in-

duction (Fig. 5) had the same shape as the survival curves (Fig. 6). Additional experiments e.g. in the form of the accelerated ageing test (Ellis & Roberts 1981) are required to obtain firm data on the seedlot constant K_i. Knowledge of K_i in combination with the species constant of lettuce (Kraak & Vos 1987) might enable the prediction of dormancy induction during storage of pretreated lettuce seeds.

Our data clearly show that prolonged pre-incubation of lettuce seeds made them more vulnerable to storage conditions (Figs. 3, 5, 6). Thus, pretreated seeds have to be stored even more careful, at lower temperature and lower moisture content, than untreated seeds to retain high quality.

CHAPTER 5

THE INFLUENCE OF REDESICCATION ON DORMANCY AND K^+ LEAKAGE OF PRE-INCUBATED LETTUCE SEEDS

Abstract

The maximum temperature at which 50% of lettuce seeds cv. Musette germinates (T_{50}) shifts to higher values by pre-incubation in water at 15 °C. When pre-incubated seeds are redried to moisture contents below 10% the greater part of the rise in T_{50} is lost again. A method is developed to redry seeds to different moisture content above various saturated salt solutions establishing a range of relative humidities. The decrease of T_{50} is mainly determined by the relative humidity at which seeds are redried, whereas the rate of redesiccation and the temperature during drying are of minor importance. The changes in T_{50} are reversible, they express changes in the level of dormancy. Measurements of K^+ leakage show that freshly imbibed seeds loose during the first hours of imbibition K^+ from the fruit- and seed wall. Pre-incubated seeds, with or without desiccation, do not loose K^+ in water following pre-incubation. The seeds contain a K^+ impermeable layer in the seed envelope, which is most probably formed by the endosperm cells. It prevents both K^+ leakage from the embryo and K^+ uptake into the embryo. Dehydration of isolated embryos increases K^+ leakage, particularly at low moisture levels. It is concluded that the capacity to germinate at higher temperatures is somehow fixed in hydrated ultrastructures, being possibly membranes.

Introduction

Pretreatment of seeds directed to improvement of germination characteristics is frequently more applied in horticulture. Priming of seeds mostly involves a period of pre-incubation in water or some osmotic solution, sometimes enriched with promotive compounds, at lower temperatures varying from 2 to 20 °C (Heydecker & Coolbear 1977). In lettuce, pretreatment of seeds is required to raise the upper temperature limit of germination, which in many cultivars is too low to guarantee cultivation at a wide range of culture conditions.

Following pre-incubation seeds have to be dried back to permit seed handling and storage. Unfortunately, the beneficial effects of pre-incubation are often antagonized by the redesiccation process (Guedes & Cantliffe 1980).

Drying at 15 °C was shown to be less deleterious than at 30 °C in seeds of celery, carrot and onion (Brocklehurst & Dearman 1983).

It is suggested that drying causes disruption of membranes since dry seeds leak solutes into liquid medium (Bewley 1979). Pre-incubated pea embryos leak after redesiccation in a similar way as embryos from untreated seeds (Simon & Harun 1972). It is the aim of the present study to investigate the effect of redesiccation on pre-incubated lettuce seeds in more detail. Seeds will be dried at different rates and to different final moisture levels. The effect of redesiccation on membrane integrity will be tested by measurement of K^+ *leakage and* K^+ *uptake*.

Abbreviations: - r.h., relative humidity; T_{50}, maximum temperature of germination.

Material and methods

Seed material and germination conditions

Seeds of lettuce cv. Musette were obtained from Enza Zaden, Enkhuizen, The Netherlands. Most experiments were performed with batch 82889; for one experiment we used seed lot 82985. The seeds were harvested in 1982 and were stored dry at 5 °C. Experiments were performed in 1984, 1985, and 1986. Triplicates of 50 seeds were sown in 5 cm Petri dishes on one layer of filter paper (Schleicher & Schüll No. 595) moistened with 1.5 ml distilled water. Pre-incubation occurred at 15 °C in darkness. After pre-incubation seeds were surface-dried by suction in a Büchner funnel and transferred to fresh filter paper in another Petri dish. Thereafter, the seeds were transferred to germination conditions after moistening the filter paper with 1.5 ml distilled water, with or without a preceding redesiccation treatment. Germination occurred in all experiments in darkness at different temperatures which were realized in cooled incubators (Gallenkamp, Crawley, U.K., $T \pm 1$ °C). Germination was counted after 2 days of incubation at the germination conditions. All manipulations were conducted in dim green light obtained by filtering irradia-

tion from one green fluorescent tube (Philips TL 40W/17) through 2 layers of yellow no. 46 and 2 layers of blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

Drying conditions

Seeds were dried back in a hygrostat based on the principle that a saturated salt solution is in equilibrium with a known relative humidity (r.h.) (Fig. 1). Surface-dried seeds in Petri dishes were placed in the hygrostat until the moisture content of the seeds was in equilibrium with the r.h. of the atmosphere. The hygrostat was used in all experiments except for one in which the influence of temperature on the drying process was tested. Drying in the latter experiment was performed in a climate cabinet (Heraeus Vötsch) set at different temperatures and r.h., in fluorescent light. A Rotronic hygroscope GT-L (± 2% r.h., Rotronic AG, Zürich, Switzerland) was used to measure

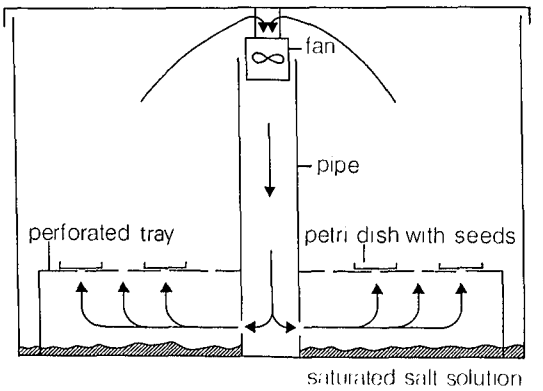

Fig. 1. Scheme of the hygrostat. The hygrostat consists of a plastic container (65 x 45 x 40 cm), filled with 2.5 l saturated salt solution. The Petri dishes with seeds were placed on top of a perforated and inverted tray. The air in the hygrostat was moved, by means of a fan, through a plastic pipe with small holes just above the salt solution.

the r.h. above the salt solutions. Water content of the seeds was determined by weighing (± 0.1 mg) 50, 100 or 200 mg seeds in little vials before and after oven drying at 130 °C during 1.5 h. The different amounts of seeds in the vials resulted in determination of moisture content with a precision of ± 0.4, 0.2 and 0.1%, respectively.

Potassium leakage

The amount of K^+ ions was measured with a flame photometer (Eppendorf). Leakage of K^+ from 50 seeds was measured in 0.5 ml samples from the 1.5 ml incubation medium in the Petri dish. Total K^+ content of 20 seeds was measured by extraction of K^+ from the surface-dried seeds, which were punctured with a needle through the envelope, to ensure complete extraction of K^+ ions. Extraction occurred by 5 ml mixture of 0.02 M HCl, 0.03 M CsCl and 0.14 M oxalic acid. In some experiments K^+ leakage of isolated embryos was measured. Embryos were isolated from the seeds as careful as possible after pre-incubation for at least 5 hours with two dissecting pens according to Halmer & Bewley (1978).

Results

The maximum temperature at which lettuce seeds cv. Musette germinated varied between 20 °C and 28 °C (Fig. 2), 50% of the seeds germinated at 24.5 °C (T_{50}). A pre-incubation in darkness during 16 h at 15 °C in water increased T_{50} to 31 °C, subsequent redesiccation of the seeds to a moisture content of 4.5% decreased T_{50} again to 26 °C. However, when the seeds were redried to 13% moisture the beneficial effect of pre-incubation on T_{50} was maintained. The relationship between the moisture content that remained after redesiccation and the change in germination capacity at higher temperatures was analyzed in more detail. Pre-incubated seeds were redried during 24 h above 5 different saturated salt solutions or dry silica gel, i.e. at different relative humidity (r.h.), and thereafter set to germinate at 28 °C. A temperature of 28 °C was chosen because pre-incubation increased germination at that temperature from 0 to 100% (Fig. 2). Fig. 3 shows the germination at 28 °C as a function of the final moisture content of the seeds after redrying at different r.h.

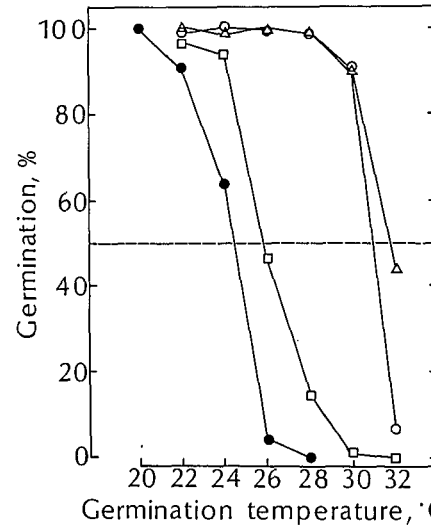

Fig. 2. Influence of pre-incubation and desiccation on germination of lettuce seeds cv. Musette at different temperatures. Seeds were germinated untreated (●) or after 20 h pre-incubation in water at 15 °C (○). Desiccation occurred at 25 °C during 24 h to a moisture content of 4.5% (□) or 13.1% (△).

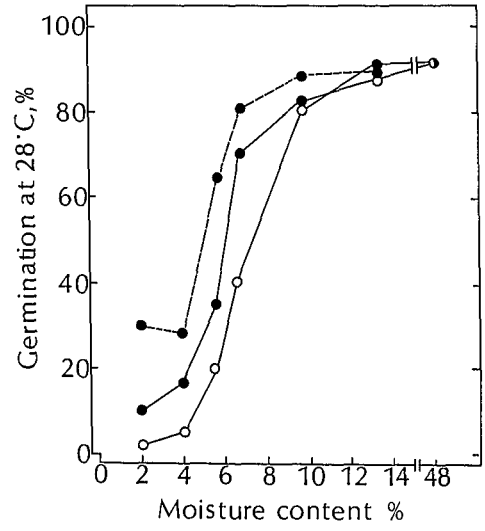

Fig. 3. Influence of desiccation of pre-incubated lettuce seeds cv. Musette to various moisture content in open (○) or closed (●) Petri dishes on germination at 28 °C. Seeds were pre-incubated during 16 h at 15 °C in water, desiccation occurred during 24 h at 25 °C in the hygrostat, subsequent germination was in water. In the treatment indicated with the broken line pre-incubated seeds were desiccated in closed Petri dishes, remoistened to a moisture content of 20% during 8 h in 95% r.h. at 25 °C and subsequently incubated at 28 °C.

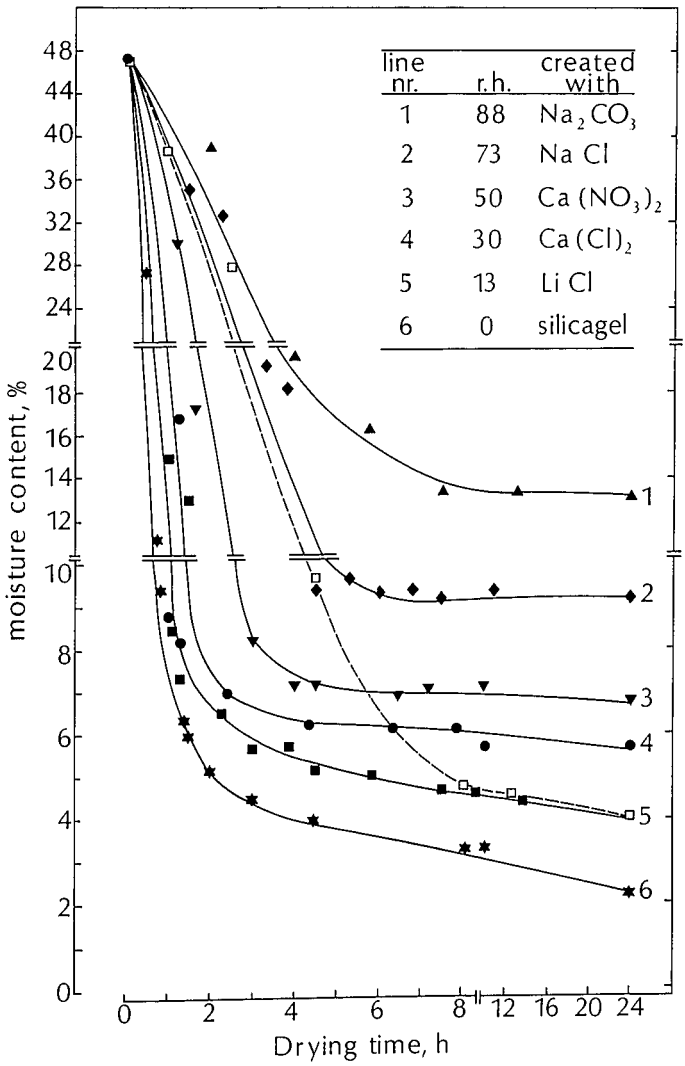

Fig. 4. Influence of relative humidity on desiccation rate of lettuce seeds cv. Musette. Different relative humidities were created in the hygrostat with the use of different saturated salt solutions and dry silica gel, desiccation occurred at 25 °C in open (closed symbols) or closed Petri dishes (open symbols, broken line).

A moisture content of 10% seemed to be critical. Redrying below that level caused an increasing loss of germination at 28 °C. However, it has to be realized that at decreasing r.h. also the rate of desiccation was influenced (Fig. 4). The influence of desiccation rate on the one side and the influence of final moisture content at the other side could be discriminated by comparing desiccation at a certain r.h. in either open or closed Petri dishes. These treatments only differed in desiccation rate, final moisture content was unaffected. It was observed that at a r.h. of 13%, realized above saturated LiCl, closure of the Petri dishes caused a strong reduction of the desiccation rate (Fig. 4). Nevertheless, the reduction of germination at 28 °C was at maximum 20% less in an open than in a closed dish (Fig. 3). Therefore, it was concluded that the final moisture content after redesiccation was the primary factor for the reduction of germination capacity of the seeds. The desiccation rate was of minor importance.

When after desiccation the seeds were remoistened to a moisture content of 20% before transfer to imbibition conditions at 28 °C, germination was somewhat improved but the seeds never reached the germination capacity they showed before redrying (Fig. 3). Complete reversal of the redesiccation effect did occur when the seeds were incubated again at 15 °C for 16 h (Fig. 5). This second stimulation of germination at higher temperatures could again be antagonized by redesiccation. After the latter treatment a few seedlings showed signs of external damage (discoloured roots). Such damages were never observed at any of the preceding treatments. To prevent desiccation damage to seedlings it was essential that the pre-incubation at 15 °C did not last for more than 24 h (Fig. 6). At that moment growth was about to begin, making the seeds much more vulnerable to desiccation. In our experiments we could simply prevent such drying damage because 16 to 20 h at 15 °C was already sufficient to stimulate full germination at 28 °C (Fig. 6). It seemed that during a second period at 15 °C, which followed after an intermittent redesiccation, the critical time of incubation was somewhat shorter. Consequently damage occurred in a few seedlings after 16 h (Fig. 5).

The effect of temperature during desiccation was also tested. Pre-incubated seeds, in this experiment at 2 in stead of 15 °C, were redried at different r.h. and different temperatures. Both conditions were established in a climate cabinet, during 24 h. It was demonstrated that the desiccation temperature was not relevant to the change in germination at 30 °C (Tab. 1).

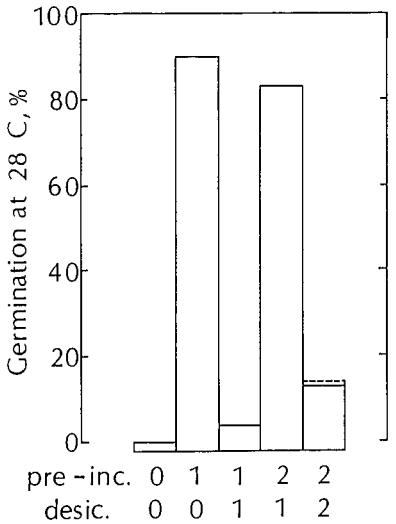

Fig. 5. Germination of lettuce seeds cv. Musette at 28 °C in water under the influence of various cycles of pre-incubation (16 h 15 °C) and desiccation to 5% moisture content (6 h 13% r.h. at 25 °C). The broken line indicated the number of damaged seedlings following two periods of pre-incubation and desiccation.

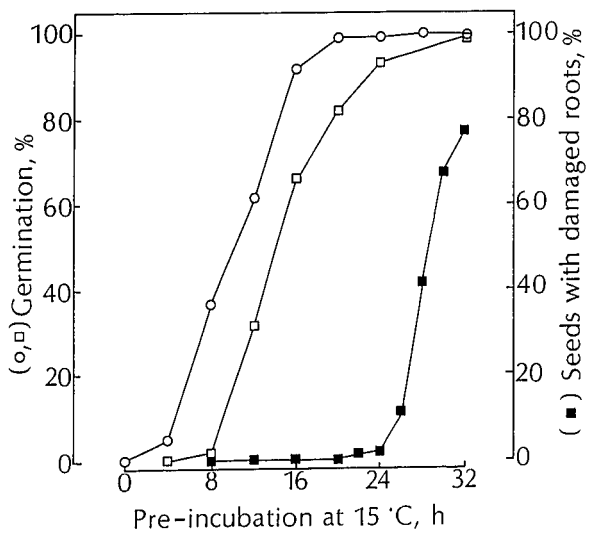

Fig. 6. Influence of pre-incubation at 15 °C on the germination of non-desiccated lettuce seeds cv. Musette at 28 °C (O) and 30 °C (□) and on the number of seedlings with damaged roots that occurred during germination at 30 °C (■) following desiccation to 10% moisture content.

Table 1. The influence of temperature and relative humidity during drying of pre-incubated lettuce seeds cv. Musette in a climate cabinet on germination at 30 °C in water. Pre-incubation occurred during 72 h at 2 °C in water.

	Germination, %		
	relative humidity, %		
temperature, °C	30	60	90
10 °C	1	57	92
20 °C	11	70	99
30 °C	1	73	91

Effects of desiccation on seeds and plants are often associated with changes in membrane properties. Therefore, the K^+ leakage and K^+ uptake of pre-incubated and redried seeds was measured. Dry seeds contained about 8.8 $\mu g\ K^+.seed^{-1}$ (Tab. 2). After 16 h of incubation at 15 °C 0.9 $\mu g\ K^+.seed^{-1}$ had leaked into the incubation medium. To observe leakage in more detail during the course of the pre-incubation period, seeds were removed at certain moments from the pre-incubation medium and subsequently incubated for an extra 2 h at 15 °C in fresh water, K^+ was measured in the latter medium. The major part of the K^+ ions leaked from the seeds during the first hours of imbibition. From the 5th hour onwards seeds lost only an extra 0.1 $\mu g.seed^{-1}$ during the 2 h measurement period (Fig. 7A). The leakage did not increase when visible germination started. Naked embryos and envelopes, which were isolated just before the beginning of the measurements, lost a little more K^+ than intact seeds, in particular after 5 h of pre-incubation. It is supposed that isolation of the seed parts damaged some cells, the more so in partly imbibed seeds.

Redesiccation of seeds or seed parts to a moisture content of 4% prior to the measurement period hardly increased K^+ loss from intact seeds during the first 24 h (Fig. 7B). After 32 h of pre-incubation, when drying caused visible damage to rootlets (Fig. 6), K^+ leakage from seeds increased drastically (Fig. 7B). Thus, apart from damaged cells, membranes in intact seeds seemed to be desiccation tolerant. This conclusion was also supported by the measurements

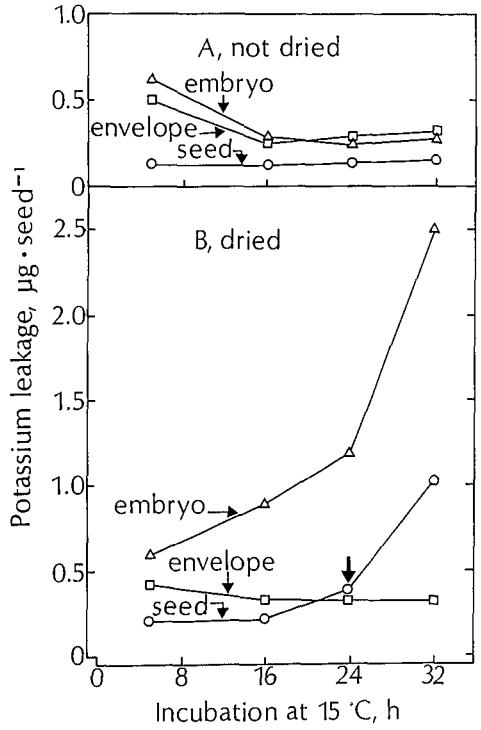

Fig. 7. Influence of pre-incubation at 15 °C on subsequent leakage of K^+ from embryos, envelopes and intact lettuce seeds cv. Musette. Embryos were isolated from the seeds after the indicated pre-incubation period. K^+ content of fresh incubation medium was measured after 2 h, K^+ leakage was measured without (A) or with (B) a preceding desiccation of seeds or seed parts to 4% moisture content (24 h 13% r.h. 25 °C). The arrow indicates the moment first desiccation damage occurred in intact seeds.

of K^+ content (Tab. 2). Two incubation periods of 16 h at 15 °C were separated by a desiccation to 5% moisture. Desiccation and a second incubation did not change the K^+ content of both seeds and embryos significantly. It is suggested by these data that intact seeds do not leak during the second incubation, because the envelope had lost nearly all K^+ during the first period and extra K^+ loss from the embryo was prevented by the presence of K^+ impermeable endosperm cells around the embryo. When intact seeds were incubated in KCl solutions, K^+ ions hardly penetrated into the embryos, such in contrast to the incubation of isolated embryos (Tab. 3).

Table 2. K^+ content of intact (or parts of) lettuce seeds cv. Musette and leakage from intact seeds after various cycles of pre-incubation (16 h 15 °C) (Inc.) and desiccation (6 h 13% r.h. 25 °C) (Des). Embryos could not be isolated from dry seeds. Leakage was measured in the incubation medium at the end of the pre-incubation period.

	K^+ content, $\mu g.seed(part)^{-1}$			
Pre-incubation conditions	seed	embryo	envelope	leakage from seeds
control	8.8 ± 0.2			
Inc.	8.2 ± 0.4	7.3 ± 0.2	0.3 ± 0.0	0.9 ± 0.1
Inc. + Des.	8.0 ± 1.0			
Inc. + Des. + Inc.	7.9 ± 0.4	7.8 ± 0.4	0.1 ± 0.0	0.0

Table 3. K^+ uptake of lettuce seeds and embryos cv. Musette (82985) from KCl solutions. K^+ content was measured in extracts of 20 intact seeds or parts of seeds that had been pre-incubated during 20 h at 15 °C, were rinsed with 100 ml water and surface dried. Isolated embryos were incubated for 18 h following 6 h imbibition of the intact seed and dissection of the envelope.

	K^+ content, $\mu g.seed(part)^{-1}$		
	KCl, mM		
	0	10	25
Incubation of seeds			
seed	5.5 ± 0.4	6.5 ± 0.1	7.3 ± 0.4
embryo	6.0 ± 0.5	6.0 ± 0.2	5.9 ± 0.2
envelope	0.0	0.3 ± 0.1	0.6 ± 0.0
Incubation of embryos			
embryo	5.6 ± 0.3	6.6 ± 0.2	7.1 ± 0.4

Removal of the envelopes before desiccation also caused an increase of K^+ leakage (Fig. 7B), the effect being stronger after longer pre-incubation.

Thus, embryo cells are not desiccation tolerant. The effect of different degrees of desiccation was tested (Tab. 4). Drying to 4 and 6% moisture caused about twofold stronger K^+ leakage than less severe water stress. Remoistening of the embryos to 20% after 8 h at 4% prevented the extra los of K^+ during the measurement period. Again, K^+ leakage from intact seeds was not affected by dehydration.

Table 4. Influence of desiccation to various moisture content of pre-incubated lettuce seeds cv. Musette on the K^+ leakage from embryos or intact seeds. Seeds were pre-incubated during 16 h at 15 °C in water. Thereafter embryos were isolated. Intact seeds or isolated embryos were desiccated to the indicated moisture content in the hygrostat during 24 h at 25 °C and in one treatment remoistened from 4 to 20% moisture content. After desiccation seeds were surface dried and incubated during 8 h in distilled water at 25 °C. K^+ was measured in the incubation medium and expressed as $\mu g.seed^{-1}$) or as the percentage of total K^+ in the seeds (8.9 µg) or embryos (7.9 µg).

	K^+ leakage						
	moisture content, %						
	47	20	9	7	5	4	4→20
intact seed, $\mu g.seed^{-1}$	0.15	0.03	0.09	0.06	0.11	0.11	0.11
%	1.7	0.3	1.0	0.7	1.2	1.2	1.2
embryo, $\mu g.seed^{-1}$	0.89	0.82	0.82	0.69	1.28	1.44	0.65
%	11	10	10	8.7	16	18	8.3

Discussion

In a large number of species the level of seed dormancy expressed itself most clearly in the width of the temperature range of germination (Karssen 1982). Breaking of dormancy consists of a widening of the range and induction of dormancy means a narrowing. Therefore, the increase of the T_{50} of lettuce seed germination by pre-incubation at lower temperatures (Fig. 2) can best be described as breaking of dormancy and the opposite change, induced by re-desiccation, as induction of secondary dormancy. The reversibility of the

changes of germination capacity at 28 °C (Fig. 5) support the previous conclusion, because reversibility is a common feature of dormancy. Moreover, it excludes the alternative explanation that redesiccation acts through an irreversible damaging of the seeds.

The present study indicates that the result of dormancy breaking is somehow fixed in hydrated ultrastructures. Redesiccation of seeds to moisture levels below 10% re-induced dormancy that just had been alleviated during a period of pre-incubation (Fig. 3). It has often been suggested that the effect of desiccation depends on changes in membrane permeability. Measurements of K^+ leakage learns about membrane integrity. The present data on K^+ leakage from lettuce seeds are somewhat confusing, however. Whereas the increased K^+ leakage from isolated embryos at lower moisture levels (Tab. 4) showed some correlation to the stronger fall in germination capacity (Fig. 3), the K^+ leakage from intact seeds was not influenced at all by a redesiccation treatment (Fig. 7B). It is concluded that a permeation barrier in the endosperm cells that is desiccation tolerant, most probably prevents K^+ leakage from the embryo inside the intact seed. Endosperm impermeability for leucine was shown in lettuce seeds by Klein et al. (1971). The presence of this permeation barrier prevents us from drawing conclusions about membrane changes that occur in the embryo inside the envelope. It is evident, however, that if embryo cells are also desiccation intolerant inside the envelope, desiccation induced changes in membrane structure did not lead to the loss of essential compounds from the embryo and, therefore, must be reversible. The function of the envelope in lettuce reflects to a certain extent the role of the seed coat in pea seeds that protects embryos from rapid imbibition, prevents extensive leakage from the embryo and stops damage to membranes by low temperature treatment (Simon 1984).

Pre-incubation of lettuce seeds is used as a practical means to improve their germination characteristics. Before seeds can be shipped and stored, redesiccation is required. The present data indicate that great care has to be taken during the drying process. The final moisture level of the seeds is the most important factor that determines the T_{50}, the drying rate is of minor importance (Figs. 3, 4). Similar conclusions were reached by Guedes & Cantliffe (1980) for lettuce and by Brocklehurst & Dearman (1983) for onion, carrot and celery. The temperature during the drying process is also of minor importance (Tab. 1). It has to be pointed out that not all cultivars of

lettuce are as sensitive to redesiccation as the cultivar Musette. Parallel studies with seeds of different cultivars have shown that desiccation can also be without an effect on T_{50} (chapter 3).

CHAPTER 6

INDEPENDENCY OF GERMINATION AND DORMANCY ON SOLUBLE AMINO NITROGEN METABOLISM IN LETTUCE SEEDS

Abstract

At the moment germination ends and seedling growth begins lettuce seeds cv. Musette become desiccation intolerant: damaged rootlets appear when desiccated seeds are re-imbibed. At 15 °C this moment occurs 3 h before visible germination. It precedes a rise in the content of soluble amino nitrogen compounds, amino acids and in the activity of glutamine synthetase (GS). At 30 °C germination only occurs when seeds are pre-incubated at lower temperature. Pre-incubation during 24 h at 15 °C in water causes full germination at 30 °C. Osmotic concentrations of polyethylene glycol (PEG) enable prolonged pre-incubation which delays dormancy breaking and even turns it at osmotic potentials of -1.0 MPa and below into its opposite the induction of secondary dormancy. During pre-incubation rises in amino nitrogen compounds and GS activity occur, but, particularly at 2 °C, much later than the increase in the capacity to germinate at 30 °C. It is concluded that changes in the level of dormancy in lettuce seeds occur independently of the stimulation of soluble amino nitrogen metabolism. The latter stimulation is also not strictly bound to the start of visible germination. Germination is less sensitive to methionine sulfoximine, a specific inhibitor of GS, than the enzyme activity.

Introduction

Imbibition of non-dormant lettuce seeds starts a chain of events that culminates in extra water uptake, cell elongation and protrusion of the radicle through surrounding layers. Dormant seeds need some stimulatory action like irradiation with light, application of growth regulators or pre-incubation at low temperature to start germination. To understand dormancy it is important to know which processes are induced by such stimulatory actions and in particular, which are the causes and which the results of the start of germination. Takeba (1980a,b) demonstrated in the axes of lettuce seeds the accumulation of free amino acids during the first 24 h of incubation at 18 °C. The accumulation was affected by red light, growth regulators and temperature (Takeba 1980c), and it accounted quantitatively for the increase of the growth potential of the axes (Takeba 1980b). In osmotically inhibited axes the accumulation was even larger than in water (Takeba 1980c,d). It was concluded that the accumulation of amino acids and in particular glutamine (Gln) and

glutamic acid (Glu)(Takeba 1980a), was the cause and not the result of germination (Takeba 1980c,d). Carpita et al. (1979b) and Leung et al. (1979) reached the opposite conclusion: increases in soluble amino nitrogen compounds and other hydrolytic products occurred only as a result of growth. Carpita et al. (1979a) proposed that the red light-induced increase in the capacity of lettuce embryos to germinate in solutions with increasingly more negative osmotic potentials involved changes in both the osmotic and the pressure potentials of the cells. The decrease of the osmotic potential correlated with a shift in the concentration of K^+, Na^+ and possible inorganic cations from the cotyledons to the axes (Carpita et al. 1979b).

It is the aim of the present study to investigate a possible relationship between changes in the dormancy level of lettuce seeds cv. Musette and the soluble amino nitrogen compounds. Dormancy in lettuce seeds always refers to states in which germination is limited to a certain range of temperatures. Alleviation or induction of dormancy involves widening or narrowing of this range (Karssen 1982). Pre-incubation of lettuce seeds in osmotica at a low temperature is an effective method to raise the upper temperature limit (Guedes & Cantliffe 1980, chapters 2,3) Effects on dormancy levels will be separated from processes resulting from stimulated germination by a more precise determination of the moment visible germination start.

Abbreviations: - Arg, arginine; Asn, Asparagine; Gln, glutamine; GS, glutamine synthetase; MSO, methionine sulfoximine; PEG, polyethylene glycol; ψ_π , osmotic potential.

Material and methods

Seed material and germination conditions

A seed lot of lettuce cv. Musette was obtained from Enza Zaden, Enkhuizen, The Netherlands. The seeds were harvested in 1982 and were stored dry at 5 °C. Triplicates of 50 seeds were pre-incubated in 5 cm Petri dishes on one layer of filter paper (Schleicher & Schüll No. 595) moistened with 1.5 ml distilled water or polyethylene glycol (PEG) solution. Osmotic potentials of PEG-solu-

tions were calculated according to Michel (1983). After pre-incubation seeds were rinsed with 100 ml of distilled water and transferred to filter paper in a fresh Petri dish. Thereafter, seeds were used either for chemical analysis or transferred to germination conditions after moistening the filter paper with 1.5 ml distilled water. In some treatments seeds were dried back prior to germination in a hygrostat based on the principle that a saturated salt solution is in equilibrium with a known relative humidity (see chapter 5). Surface-dried seeds in Petri dishes were placed in the hygrostat until the moisture content of the seeds was in equilibrium with the relative humidity of the atmosphere (mostly 24 h). The moisture content of the seeds was determined by weighing ($\pm$ 0.1 mg) about 200 mg seeds in little vials before and after oven drying at 130 °C during 1.5 h. Germination occurred in all experiments in darkness at different temperatures which were realized in cooled incubators (Gallenkamp, Crawley, U.K., T $\pm$ 1 °C). Germination was counted after 2 days of incubation at the germination conditions. All manipulations were conducted in dim green light obtained by filtering irradiation from one green fluorescent tube (Philips TL 40W/17) through 2 layers of yellow no. 436 and 2 layers of blue no. 62 cinemoid filters (Strand Electric, London, U.K.).

Chemical analysis

After pre-incubation, rinsing and surface drying 200 seeds (240 mg) were homogenized in a mortar in 4 ml 0.05 M tris/HCl buffer (pH 7.0) containing 1 mM $MgSO_4$ and 10 mM 2-mercaptoethanol on ice. The extract was centrifuged for 5 min at 2400 x g. The supernatant was used for determination of soluble amino nitrogen and GS activity. Before determination of soluble amino nitrogen trichloroacetic acid (final concentration 5%) was used to precipitate protein. The ninhydrin method was used modified form Yemm & Cocking (1955). After centrifugation 0.1 ml of the supernatant was mixed with 1.0 ml H_2O, 0.5 ml NaAc/HAC buffer (1 M) pH 5.2, 50 µL 0.01 M KCN and 0.5 ml ninhydrin 3% in methylcellosolve. After heating for 20 min in boiling water the mixture was cooled in ice and after addition of 5 ml ice cold isopropanol/H_2O (1/1) the absorbance was measured at 570 nm. An amino acid analyzer Biotronic LC 6000 E (physiological program) was used to analyze the compositions of the free amino acids. Sulfo salicylic acid (final concentration 2%) was used to precipitate the protein instead of trichloroacetic acid.

Glutamine synthetase (GS) was assayed according to Takeba (1983a) with modifications according to Stadtman et al. (1979) as follows: the reaction mixture (0.5 ml) contained 162 mM imidazole-HCl buffer pH 6.8, 160 mM L-Gln, 0.4 mM sodium-ADP, 21 mM potassium arsenate, 42 mM hydroxylamine-HCl and 0.4 mM $MnCl_2$. The enzyme extract (0.1 ml) was mixed with 0.5 ml assay mixture and incubated for 30 min at 37 °C. After addition of stop mixture and centrifugation the absorbance was measured at 540 nm. All analyses, except amino acid composition, were at least done in duplicate.

Results

The maximum temperature for full germination of lettuce seeds cv. Musette is about 20 °C (chapters 2, 3). At 30 °C in darkness germination did not occur without a pre-incubation at lower temperatures (Fig. 1A). To reach full germination at 30 °C, a pre-incubation in water had to last 24 h at 15 °C or 4 d at 2 °C. Pre-incubation at 15 °C in osmotic solutions of PEG delayed the stimulative effect on subsequent germination at 30 °C. In -1.0 and -1.5 MPa the maximal stimulation was not reached anymore. During prolonged pre-incubation at these osmotic potentials the stimulatory effect even turned into its opposite, secondary dormancy developed.

The most common way to determined the moment at which germination ends and seedling growth begins is the observation of the protrusion of the radicle through the surrounding layers. However, growth may start before that moment. Schopfer et al. (1979) showed that this can be detected by desiccation of the seeds. Seeds that begin to grow will become desiccation intolerant: damaged rootlets will develop during renewed incubation. Comparison with visible germination revealed that the desiccation method traced the end of germination about 3 h earlier than the naked eye (Fig. 2). The former method was not only more precise, it was also easier to apply since damaged rootlets could be better observed than the small crack in the fruit wall and the protruding tiny rootlet. The arrows in Fig. 1B indicate the start of germination determined via the desiccation method.

Dry seeds contained 36.9 μ mol free amino acids·(1000 seeds)$^{-1}$ (Fig. 3A). Analysis showed that 89% of this pool consisted of 5 amino acids only: arginine (Arg) 55%, asparagine (Asn) 19%, aspartic acid (Asp) 4%, glutamic acid (Glu) 8% and glutamine (Gln) 4%. During pre-incubation at 15 °C in water the

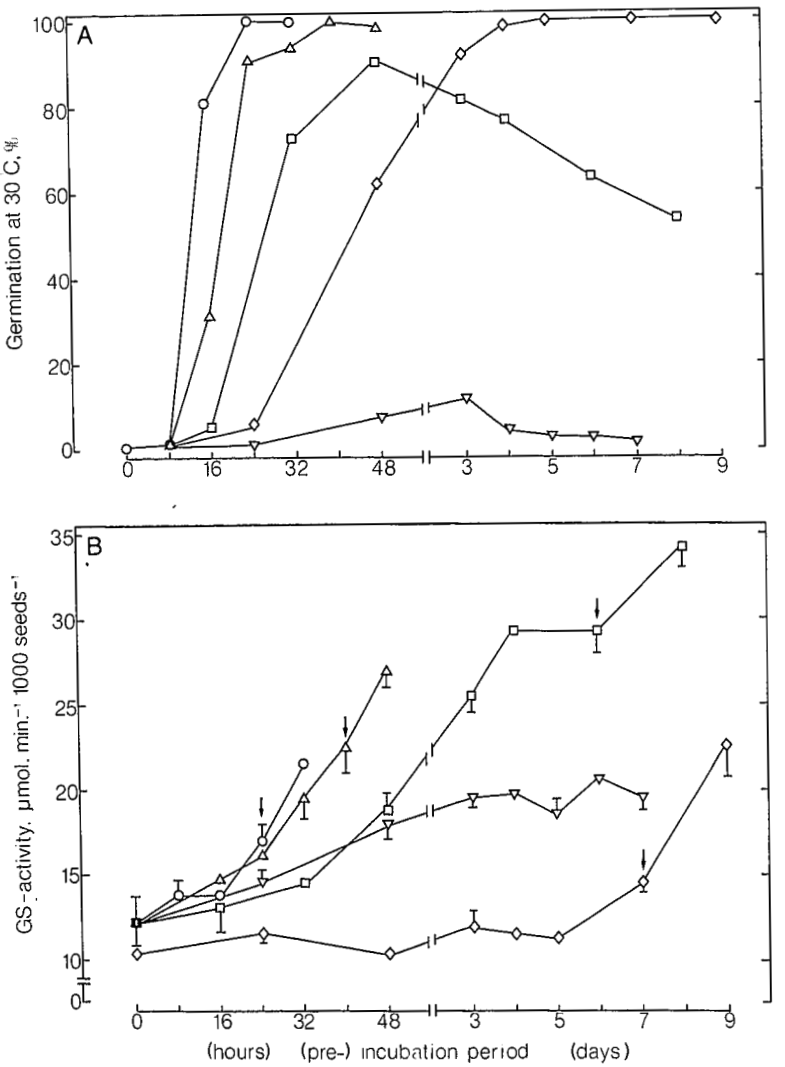

Fig. 1. The influence of pre-incubation of lettuce seeds cv. Musette at 15 °C in water (○), PEG -0.5 MPa (△), -1.0 MPa (□) or -1.5 MPa (▽) or at 2 °C in water (◇) on (A) subsequent germination in water at 30 °C or (B) activity of GS during pre-incubation. Arrows indicate the moment the seeds became desiccation intolerant. Vertical bars indicate SD.

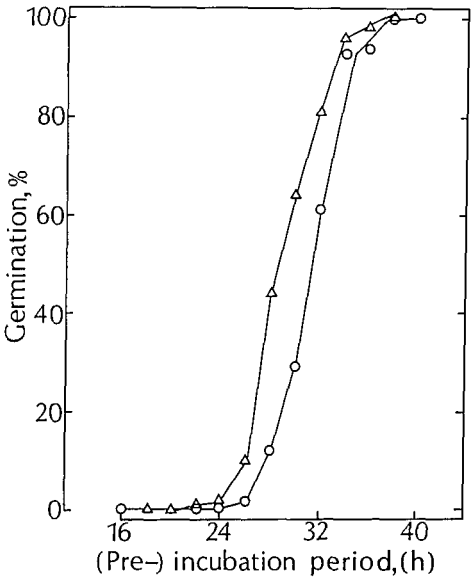

Fig. 2. Comparison of two methods for the determination of the moment of germination. Germination was determined by observation of visible roots during incubation at 15 °C (○), or by the number of damaged roots which appeared when the seeds following incubation at 15 °C were desiccated to a moisture content of 10% and subsequently re-incubated at 30 °C (△).

total content rose to 57 μmol·(1000 seeds)$^{-1}$. This increase could be attributed for 73% to an accumulation of Asp (16%), Glu (24%) and Gln (33%) (Fig. 3B). The level of Arg and Asn did not change or tended to decrease (Fig. 3A). Measurement of the total soluble nitrogen content in the seeds showed values that were 44% higher than the amino acid content (Fig. 4). Obviously, other compounds with free amino groups different from amino acids were present in the seeds. The increase in both contents during incubation at 15 °C showed a good correlation (r^2 = 0.992). The same was true for the increase in the total soluble nitrogen and the activity of GS (r^2 = 0.989). Since GS activity could be determined most accurately the present study will concentrate on the enzyme activity. The pattern of change in GS activity in the whole seed corresponded with that in the embryo, the cotyledons and the axes (Fig. 5). Thus, there was no need to study certain seed parts separately.

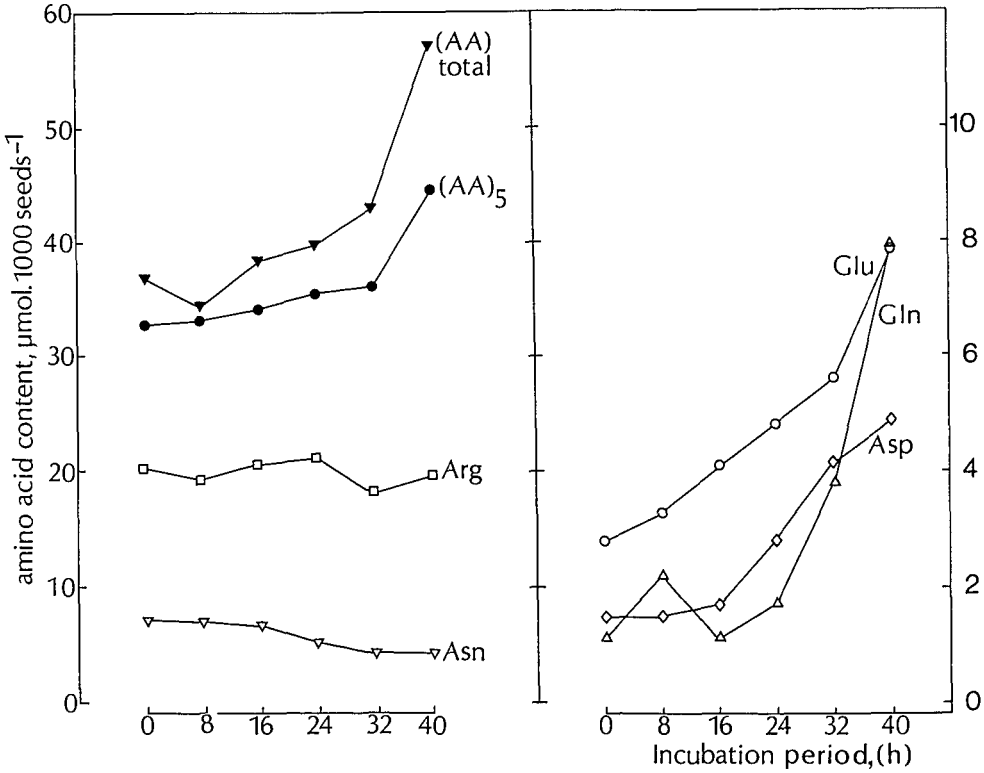

Fig. 3. The content of the most abundant amino acids during incubation of lettuce seeds cv. Musette at 15 °C in water; $(AA)_5$ (●) is the sum of Arg (□), Asn (▽), Asp (◇), Glu (○), and Gln (△). The difference between the total amino acid content $(AA)_{total}$ and $(AA)_5$ was caused by about 12 minor amino acids.

The increase of the different parameters of soluble amino nitrogen metabolism mainly occurred after the start of germination as determined with the desiccation method (Fig. 4). The significance of GS activity, i.e. of Gln germination was proven by experiments with a specific inhibitor of GA activity for methionine sulfoximine (MSO). When seeds were incubated in a 1 mM solution of this inhibitor, Gln was absent in the seeds after 24 h (data not shown). MSO was tested at 24 °C, a temperature that neither alleviated nor induced dormancy. MSO inhibited both GS activity and germination (Fig. 6). Germination was less sensitive to MSO than the enzyme activity.

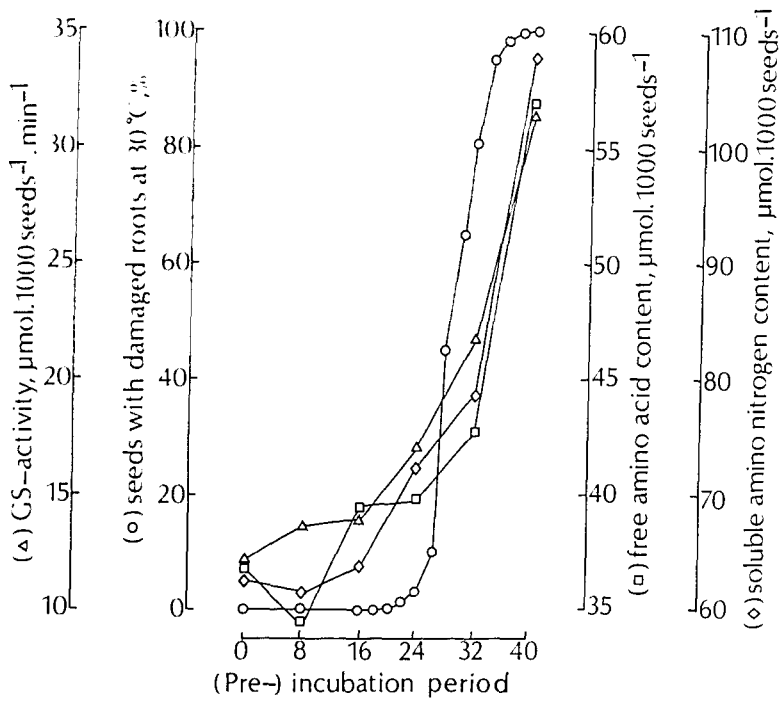

Fig. 4. Comparison between the moment lettuce seeds cv. Musette became desiccation intolerant during incubation in water at 15 °C (○) and different parameters of soluble amino nitrogen metabolism: free amino acid content (□), soluble amino nitrogen content (◇) and GS activity (▵). Note the different scale and intercept values.

The results presented in Fig. 1A,B showed that the alleviation of dormancy certainly did not coincide with the stimulation of the soluble amino nitrogen metabolism. In particular during a pre-incubation at 2 °C in water the germination capacity at 30 °C rose several days before the increase of GS activity. Also during incubation at 15 °C the germination capacity at 30 °C rose prior to the GS activity. During pre-incubation in water the main rise in enzyme activity occurred after the start of germination. During incubation in PEG, however, the delay of germination enabled GS activity to rise to considerable values. This is most clearly seen at -1.0 MPa, but it also occurred in -1.5 MPa where germination was completely inhibited.

The stimulative effect of a pre-incubation at 15 °C on the germination at 30 °C is reduced when the seeds are dehydrated at the transfer between the two incubation periods (Tab. 1). In contrast to germination at 30 °C GS activity was not reduced by the dehydration treatment.

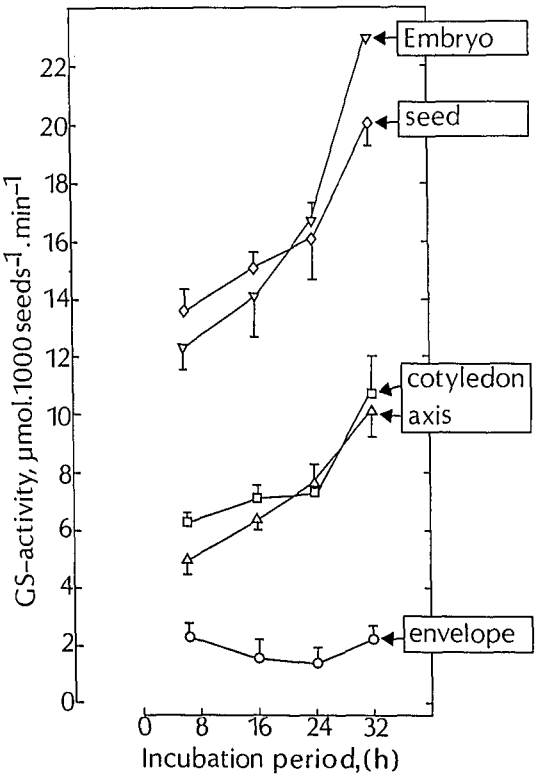

Fig. 5. The GS activity in the envelope (○), the axis (△), the cotyledons (□), the embryo (▽) and intact lettuce seeds cv. Musette (◇) during incubation in water at 15 °C.

When lettuce seeds were directly sown at 30 °C dormancy was strengthened, as could be seen at a subsequent germination experiment at 24 °C (Fig. 7). Also in this experiment the change in GS activity did not correspond to the level of dormancy. Germination at 24 °C of not pretreated seeds was lower than in the experiment with MSO (Fig. 6) where seeds had to be punctured to enable the penetration of MSO.

Table 1. The influence of desiccation to 5% moisture content on GS activity and germination at 30 °C after pre-incubation in water at 15 °C.

Duration of pre-incubation (h)	GS activity µmol.1000 seeds^{-1}.min^{-1} not dried	dried	Germination % at 30 °C not dried	dried
0	11.1 ± 0.04	-	0	0
8	13.4 ± 0.16	-	2	0
16	14.1 ± 0.57	13.2 ± 1.30	81	5
24	17.0 ± 1.30	17.0 ± 1.60	100	18

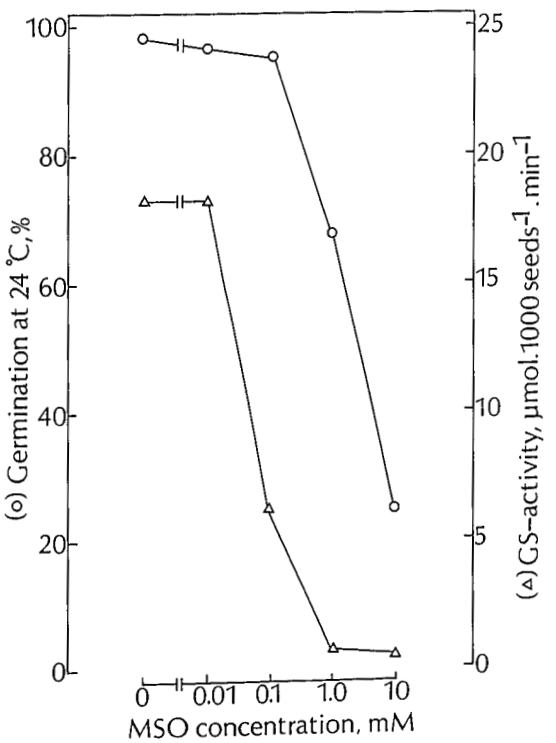

Fig. 6. Germination (O) and GS activity (Δ) of lettuce seeds cv. Musette during incubation in MSO at 24 °C. Seeds were punctured with a dissecting needle 2 hours after imbibition to improve penetration of the inhibitor. Enzyme activity was measured 24 h after imbibition, germination was scored 24 h later.

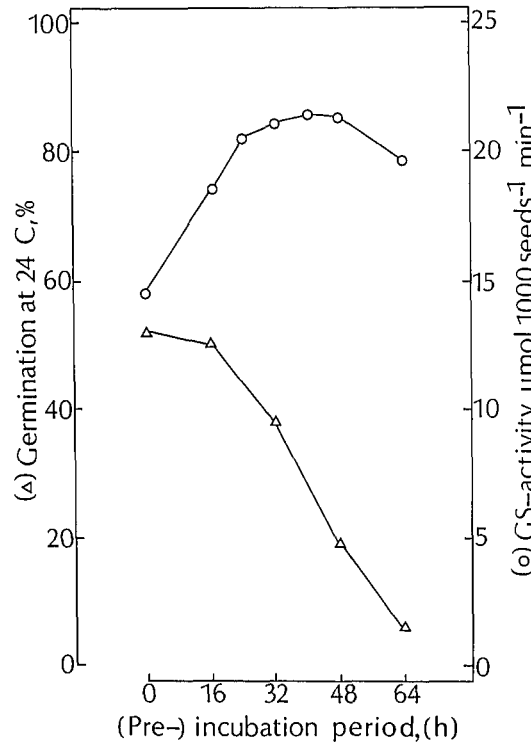

Fig. 7. The influence of pre-incubation in -0.5 MPa PEG at 30 °C on GS activity (O) and germination (Δ) in water at 24 °C of lettuce seeds cv. Musette.

Discussion

The present study has clearly shown that at 15 °C the stimulation of the level of soluble amino nitrogen compounds and of the GS activity coincided with the start of visible germination (Fig. 2, 4), but occurred much later than the stimulation of the germination capacity at 30 °C (Fig. 1). Also several other experiments (Figs. 1, 6, Tab. 1) supported the general conclusion that changes in the level of dormancy of lettuce seeds occurred independently of the stimulation of soluble amino nitrogen metabolism. Our conclusion differs strongly from the studies of Takeba (1980a,b,c,d) who suggested that the accumulation of amino acids was the cause of stimulated germination by light, growth regulators or low temperatures. A first reason for this controversy might be that germination, according to our definition

started 3 h earlier than visible germination. The latter criterium is the most common one and was used by Takeba (1980a,b,c,d). The major reason for the controversy is that in our studies effects on dormancy and germination could be better separated. Pre-incubation at 2 °C in water or at 15 °C in PEG enabled alleviation of dormancy but inhibited germination and therefore allowed sharper conclusions

The pre-incubation at 15 °C in PEG solution revealed that although the stimulation of amino nitrogen metabolism is not correlated to alleviation of dormancy it may nevertheless occur prior to the start of visible germination. Also Takeba (1980c,v) and Thanos (1984) showed that the accumulation of soluble amino nitrogen compounds continued during osmotic incubation. A stimulation of enzyme activity during osmotic pretreatment has also been observed by Khan et al. (1978). In the present experiments the concentration of soluble amino nitrogen compounds increased during incubation in -1.0 MPa PEG with about 20 nmol.seed^{-1} (chapter 7). If it was assumed that 1 µmol N compound is undissociated and therefore has a similar effect on ψ_π as 1 µmol mannitol, calculations according to Michel (1983) learned that ψ_π decreased with -0.06 MPa during 5 days in -1.0 MPa PEG. This change was not restricted to the axis only, but seemed to be similar in all parts of the embryo (Fig. 5). It has to be studied whether this change can explain the extra effect that a pre-incubation in PEG had on e.g. the desiccation tolerance of seeds (chapter 2).

The present study showed that osmotic pretreatment may also have less preferable effects. At 15 °C incubation in PEG clearly inhibited alleviation of dormancy (Fig. 1A). Since alleviation of primary dormancy often occurs at the same temperatures as induction of secondary dormancy (Totterdell & Roberts 1979(, the latter process obviously became dominant after a certain period of time (Fig. 1A), thus turning the beneficial effect of pre-incubation into its opposite.

Our study was restricted to soluble amino nitrogen compounds that form 0.5% of total seed dry weight. Also sugars did not accumulate before visible germination (Halmer et al. 1978). Besides GS also other enzymes play an important role in the biosynthesis of amino acids in germinating seeds (Miflin & Lea 1977). GS is probably the major enzyme for formation of Gln from Glu and NH_4^+ since application of MSO totally removed Gln from the seeds. The abundance of Arg and Asn in the dry seed (Fig. 3) agreed with the common finding that these two compounds are often used for storage in plants (Miflin & Lea 1977).

The specific increase of the content of Asp, Glu and Gln during germination (Fig. 3, Takeba 1980a) agreed with the common pathways for the utilization of storage N-compounds.

This study leaves the question open how dormancy of lettuce seeds is broken and germination is stimulated. Correlation to a decreased osmotic potential seems unlikely, however. Schopfer & Plachy (1985) underlined the crucial function of changes in cell wall extensibility for the increase of the growth potential of seeds. We will extend our studies to these parameters (chapter 7).

CHAPTER 7

GERMINATION- AND DORMANCY-RELATED CHANGES IN THE WATER RELATIONS OF LETTUCE SEEDS

Abstract

Pre-incubation of lettuce seeds cv. Capitan during 24 h at 15 °C in water, in darkness, shifts both the maximum temperature for 50% germination upwards, from 26 to 36 °C, and the minimum external osmotic potential for 50% germination (ψ_{50}) downwards from -0.8 to -1.3 MPa. Pre-incubation at 30 °C has an opposite effect, T_{50} decreases and ψ_{50} increases, while at 24 °C hardly any change occurs. The changes in both parameters show a linear relationship. Such a relationship is also found when T_{50} and ψ_{50} are compared of cvs. Capitan, Ravel and 2 batches of cv. Musette with a range of T_{50} values. In half seeds, containing the radicle, T_{50} of all cultivars shifts to temperatures above 36 °C and ψ_{50} decreases considerably. $\Delta\psi_{50}$ ($\psi_{50\ intact}-\psi_{50\ half}$) is 0.29 MPa for Ravel but 0.93 MPa for Musette. It is concluded that lack of extensibility in endosperm cell walls is the major cause of low T_{50}. Pre-incubation at low temperature brings T_{50}, ψ_{50} and $\Delta\psi_{50}$ of Musette seeds close to untreated Ravel seeds.

Psychrometric measurements of the actual water potential (ψ) and the osmotic potential (ψ_π) of Capitan seeds show that both parameters do not change in the period between the end of imbibition and the start of visible growth and are not affected by temperature. ψ always equals zero and ψ_π has a value of -0.9 MPa. Thus, pre-incubation in water causes not an accumulation of osmotic compounds. Based on the general equation of extension growth inhibition of germination is regarded as a steady state growth rate of zero. Without growth ψ_{50} equals ψ_π + Y, where Y is the yield threshold of turgor pressure for cell extension. Y was calculated from measured ψ_{50} and ψ_π after correction of ψ_π for the decrease at ψ_{50}. The effect of temperature on germination correlates with Y. Alleviation of dormancy at 15 °C correlates with a decrease of Y and induction of dormancy at 30 °C with an increase of Y.

Introduction

Germination processes in seeds ultimately lead to the start of cell expansion which is followed by the protrusion of the expanding embryo through the surrounding layers and the beginning of visible growth. Taking into

account the central role of cell expansion it might be expected that germination is controlled via the regulation of the water relations of a seed. It has indeed been shown that germination-stimulating factors, like light and the growth regulators gibberellins, cytokinins and ethylene, enable seeds or isolated embryonic axes to expand in much more negative osmotic potentials of the incubation medium ($\psi_{\pi e}$)(Carpita et al. 1979c, Hegarty & Ross 1979, Negm & Smith 1978, Takeba & Matsubara 1979). Germination inhibiting factors like high temperatures and abscisic acid cause the opposite effect (Carpita et al. 1979c, Reynolds 1975, Takeba & Matsubara 1979). The changed reaction to $\psi_{\pi e}$ which has often been described as a change in the growth potential (GP) of the seed, which has often been identified erroneously with a change in the actual waterpotential of the seeds (ψ)(for references see Schopfer & Plachy 1985). Nabors and Lang (1971), however, demonstrated that in light-treated embryos of lettuce the critical $\psi_{\pi e}$ was 7 bar lower than in the dark control, although the actual ψ was close to zero in both light and darkness. It has been thought that the increase in the capacity of lettuce seeds to overcome osmotic stress was due to a combination of decreased ψ_{π} of the axes caused by a shift of cations (probably K^+ and Na^+) from cotyledons to axes, and an increased cell wall loosening by H^+ secretion (Carpita et al. 1979b). Also the accumulation of free amino acids in the rootlet tip was held responsible for the change (Takeba 1980a).

It is the general aim of our studies on lettuce seeds to localize the site of action of temperature pretreatments that change the dormancy of the seeds. We characterize dormancy by the value of the upper temperature limit of germination, indicated by T_{50}, the maximum temperature for 50% germination. Preincubation at temperatures below or above the actual T_{50} of a seedlot shifted the parameter to higher or lower values, respectively (see chapters 2 and 3). It is our attempt to separate the causes and results of these changes in germination capacity. This approach showed us before that seeds of the lettuce cultivar Musette developed during pre-incubation at 15 °C the capacity to germinate at 30 °C much earlier than the rise in soluble amino nitrogen compounds (chapter 6). Therefore, it seems unlikely that a change in ψ_{π} of the seed is the direct cause of the change in germination capacity. In the present study we will further analyze the physical parameters that govern the water relations of seeds. First, we study the relationship between changes in T_{50} and the critical $\psi_{\pi e}$ that still allows 50% germination. Second, the

effects of temperature during incubation at various temperatures on ψ and ψ_π will be measured by psychrometry.

The theoretical framework of the present experiments is based on the study of Schopfer & Plachy (1985). Their experiments demonstrated that during visible germination expanding rootlets of Brassica napus seeds showed the characteristics of steady state growth, that can be described with the equation (Lockhart 1965) :

$$\frac{dV}{dt} = \frac{Lm}{L+m} (\Delta\psi_\pi - Y) \qquad (1)$$

where $\frac{dV}{dt}$ ($m^3.s^{-1}$) is the rate of volumetric growth, m ($m^3.s^{-1}.MPa^{-1}$) is a rate coefficient attributed to cell wall extensibility, L ($m^3.s^{-1}.MPa^{-1}$) is the water conductivity of the tissue, $\Delta\psi_\pi$ (MPa) is the difference in osmotic potential of medium ($\psi_{\pi e}$) and cell (ψ_π) and Y (MPa) is a yield threshold (minimum turgor required for wall expansion). The analysis of Lockhart is based on the fact that during steady state growth the rate of water influx

$$\frac{dV}{dt} = L\,\Delta\psi \text{ for } \psi_e - \psi \geqq 0 \qquad (2)$$

must equal the rate of irreversible volumetric expansion of the cell walls

$$\frac{dV}{dt} = m\,(\psi_p - Y) \text{ for } \psi_p - Y \geqq 0 \qquad (3)$$

where $\Delta\psi$ (MPa) is the difference in water potential of the medium ($\psi_e = \psi_{\pi e}$) and the cell ($\psi = \psi_\pi + \psi_p$) and ψ_p (MPa) is the turgor pressure.

When equation (1) is rewritten as

$$\frac{dV}{dt} = \frac{Lm}{L+m} [\,\psi_{\pi e} - (\psi_\pi + Y)] \qquad (4)$$

it is seen that the value of $\psi_{\pi e}$ that causes a steady state water uptake (is growth) rate of zero equals ($\psi_\pi + Y$). Schopfer & Plachy (1985) signified the latter expression as GP, which has therefore a negative value. In so far as zero growth coincides with inhibition of visible germination, the determination of $\psi_{\pi e50}$ (the minimum osmotic potential of the medium that allows 50% germination, for short ψ_{50}), in combination with the psychrometric measurement of ψ_π of the seeds, enabled calculation of Y. The germination experiments provided no means to determine m, L and $\frac{dV}{dt}$.

Direct measurement of ψ and ψ_π opened a second way to judge whether water uptake during germination is controlled by changes in ψ_π or ψ_p according to

$$\psi = \psi_\pi + \psi_p + \psi_m \qquad (5)$$

where ψ_m (MPa) is the matrixpotential that approaches zero at the end of imbibition.

Abbreviations

PEG - Polyethylene glycol
T_{50} - Maximum temperature for 50% germination (°C)
ψ - Water potential (MPa)
ψ_π - Osmotic potential (MPa)
$\psi_{\pi e}$ - Osmotic potential of the external medium (MPa)
ψ_{50} - Minimum $\psi_{\pi e}$ for 50% germination (MPa)
ψ_m - Matrix potential (MPa)
ψ_p - Pressure potential (MPa)
Y - Yield threshold of ψ_p for irreversible growth (MPa)
m - Cell wall extensibility ($m^3.s^{-1}.MPa^{-1}$)
L - Water conductivity ($m^3.s^{-1}.MPa$)
GP - Growth potential (MPa)

Material and Methods

Seed material and germination conditions

Seeds of lettuce (Lactuca sativa L.) were obtained from Dutch plant breeding firms: cv. Capitan (A06438/031722) from Rijk Zwaan, De Lier in 1983 and two batches of cv. Musette from Enza Zaden, Enkhuizen. Batch 82985 (indicated as $Musette_{20}$) and batch 82889 ($Musette_{25}$) were harvested from plants grown at different environmental conditions, the number indicates T_{50} in darkness. The seeds were stored at 5 °C until use. The experiments were performed in 1985 and 1986. Triplicates of 50 seeds were sown in 5 cm Petri dishes on one layer

of filter paper (Schleicher & Schüll No. 595) moistened with 1.5 ml distilled water. After pre-incubation seeds were surface-dried by suction on filter paper in a Büchner funnel and transferred to fresh filter paper in another Petri dish. Thereafter seeds were either directly used for determination of moisture content, ψ or ψ_π, or the seeds were transferred to germination conditions after moistening the filter paper with 1.5 ml distilled water or polyethylene glycol (PEG) solution, with or without a preceding redesiccation treatment. Redesiccation occurred in the hygrostat above saturated salt solutions as described in chapter 5. Surface-dried seeds in Petri dishes were placed in the hygrostat until the moisture content of the seeds was in equilibrium with the relative humidity of the atmosphere (24 h). The moisture content of the seeds was determined by weighing ($\pm$ 0.1 mg) 100 mg seeds in little vials before and after oven drying at 130 °C during 1.5 h.

In some germination experiments half seeds were used. The top half of the seeds, consisting of part of the envelope and the cotyledons, was removed with a razor blade. These manipulations were conducted in a humid chamber (98% r.h.) to prevent evaporation of the seeds.

PEG was obtained from Serva, Heidelberg, F.R.G. Osmotic potential of PEG solutions was calculated according to Michel (1983). Germination occurred in all experiments in darkness at different temperatures which were realized in cooled incubators (Gallenkamp, Crawley, U.K. T$\pm$1 °C). During incubation in water germination was counted after 2 days, when incubation occurred in PEG solutions germination was counted regularly, the interval depending on temperature and osmotic potential. A refractometer was used to check the osmotic potential of the PEG solution ($\psi_{\pi e}$). When $\psi_{\pi e}$ had decreased more than 0.1 MPa the PEG solution was refreshed. Counting of germination during incubation in PEG solutions proceeded until no seeds had germinated between two subsequent counts.

All manipulations were conducted in dim green light obtained by filtering irradiation from one green fluorescent tube (Philips TL 40W/17) through 2 layers of yellow no. 46 and 2 layers of blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

The amount of solutes in the seeds can be calculated from the values of ψ_π and moisture content. The absolute amount of water in seeds was calculated from moisture content on fresh weight basis and dry weight of the seeds (Capitan 1 seed = 1.071 mg, Musette_{25} 1 seed = 1.026 mg). If it was assumed

that the cells only contained mannitol the amount of mannitol necessary to reach the measured value of ψ_π could be calculated according to Michel (1983).

Determination of ψ and ψ_π

Water potential (ψ) of lettuce seeds was determined with a thermocouple psychrometer. A C_{52} sample chamber (Wescor, Logan, U.S.A.) was adapted for connection to a NT-3 Nanovoltmeter (Decagon, Pullman, U.S.A.). The sample chamber was placed in a humid chamber (95-100% r.h., 25 °C), to prevent evaporation during manipulations with the seeds. Samples of 10 surface dry seeds were introduced in the measuring chamber (2.3 mm deep, 7.2 mm diameter). Water vapour equilibration was reached after about 0.5 h). For measurements, readings were taken 0.5, 1.0 and 1.5 h after closing the measuring chamber and averaged. The highest value in µV of the instrument was read after a standardized cooling time of 20 s.

Osmotic potential (ψ_π) of the seeds was determined by the same procedure using samples of 10 seeds which were frozen in liquid nitrogen in small plastic vials and thawed for 5 min at 25 °C. The seeds were homogenized before the measurement by means of crushing the seeds in the measuring chamber with a copper rod. Water vapour equilibration was reached after about 10 min. For measurements, readings were taken 10 min, 20 min, and 30 min after closing the measuring chamber and averaged. The value after 30 min was always about 0.2 µV higher than the value obtained after 10 min equilibration, probably due to enzymic reactions taking place in the seeds.

The instrument was calibrated with PEG solutions, the ψ_π of the solutions was calculated according to Michel (1983). The regression line describing the relation between $\psi_{\pi e}$ and µV was described by : y µV = 0.39 + 0.31 x .

Results

Effect of pre-incubation temperature on T_{50} and ψ_{50}

During pre-incubation in water at 15 °C seeds of lettuce cv. Capitan developed the capacities to germinate both in water at increasingly higher temperatures (Fig. 1A) and, at 24 °C, at decreasingly lower (i.e. more negative) osmotic potentials (Fig. 1B). The characterization of the effects by T_{50} and ψ_{50} is shown in Fig. 1A,B.

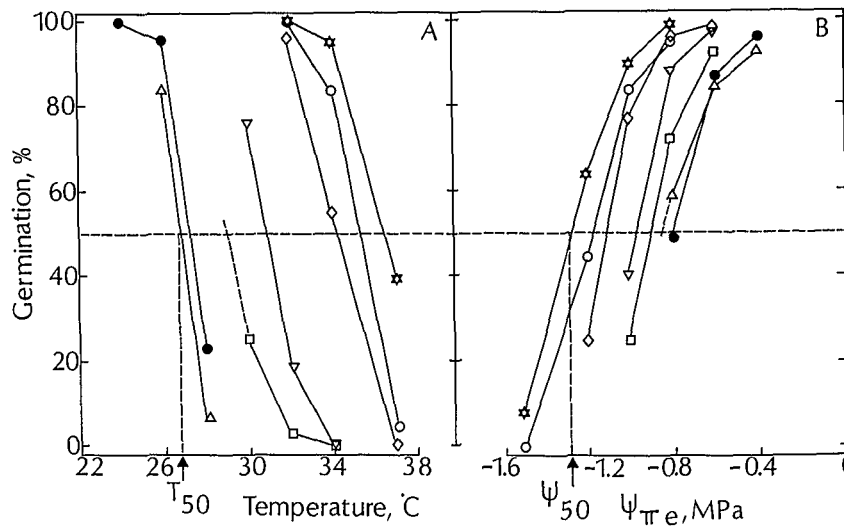

Fig. 1. Influence of the length of pre-incubation period at 15 °C in water and darkness on subsequent germination of lettuce seeds cv. Capitan at various temperatures (A) or in PEG solutions with various osmotic potentials ($\psi_{\pi e}$) at 24 °C (B). Seeds were pre-incubated during 2 h (△), 8 h (□), 12 h (▽) 16 h (◇), 20 h (○) or 24 h (✡), surface dried and subsequently incubated at the germination temperature (A)or in the PEG solution (B). The closed circles represent the untreated control seeds. The temperature (A) and the osmotic potential (B) at which 50% of the seeds germinated for one specific treatment is indicated.

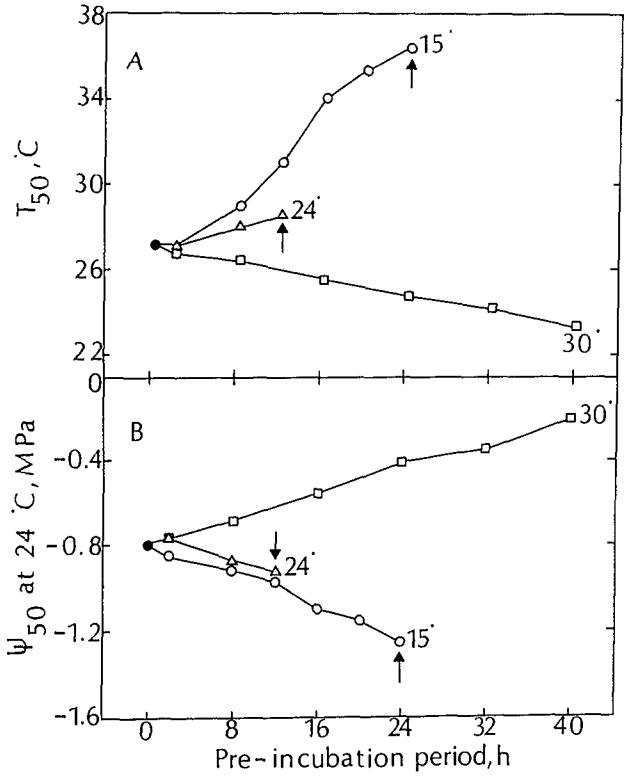

Fig. 2. Influence of length and temperature of pre-incubation in water on T_{50} (A) and ψ_{50} (B) of lettuce seeds cv. Capitan. Pre-incubation temperatures were 15 °C (O), 24 °C (Δ) and 30 °C (□), ψ_{50} was determined at 24 °C. See Fig. 1 for determination of T_{50} and ψ_{50}. The arrow indicates the moment during pre-incubation at which the first damaged seeds appeared following redesiccation.

It has to be realized that as a consequence of lower ψ_{50} the germination test took considerably more time (data not shown). Seeds were also pre-incubated at 24 and 30 °C, temperatures just below and above 27 °C, which is the T_{50} of untreated seeds (Fig. 2). Since pre-incubation occurred in water, the maximum length of incubation at 15 and 24 °C was limited since at those temperatures the seeds approached the beginning of growth after 24 and 12 h, respectively (arrows).

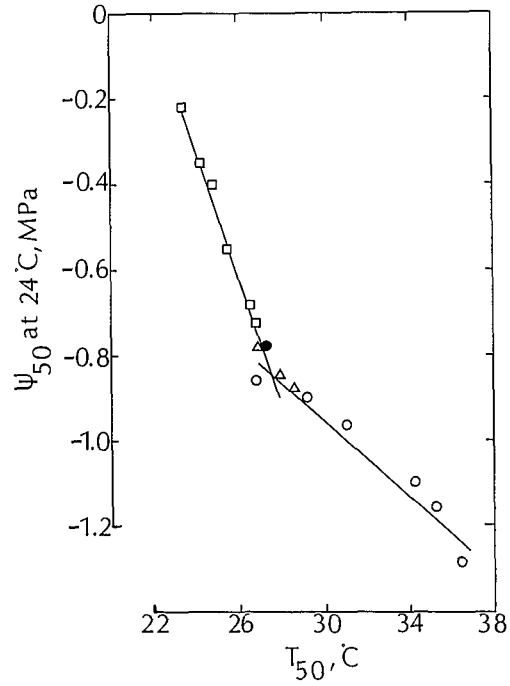

Fig. 3. Relationship between T_{50} and ψ_{50} of lettuce seeds cv. Capitan as influenced by pre-incubation at 15 °C (O), 24 °C (△) and 30 °C (□). The closed circle represents the untreated control. Data are derived from Fig. 2.

At 30 °C germination did not occur. In our studies the moment the seeds became damaged by desiccation was taken as criterion for the beginning of growth instead of the first sign of visible germination (Chapter 6).

Pre-incubation at 30 °C lowered T_{50} and increased ψ_{50} and therefore, evidently acted opposite to 15 °C (Fig. 2A,B). Pre-incubation at 24 °C had an intermediate position. Comparison between Figs. 2A and 2B indicated that the effects of the pre-incubation temperature on T_{50} and ψ_{50} were clearly related. Plotting of T_{50} against ψ_{50} obtained after the same pre-incubation showed a linear relationship between the two parameters (Fig. 3) that differed, however, for the dormancy breaking at 15 °C and the dormancy induction at 30 °C. Interestingly, the values for the untreated seeds and for the 24 °C

pre-incubation were roughly at the break of the curve. A linear relationship between the values of T_{50} and ψ_{50} was also found when the parameters were determined in untreated seeds of 3 different cultivars, including two different harvests of the cultivar Musette (Fig. 4). Seeds of cv. Ravel showed high T_{50} and low ψ_{50}, whereas cv. $Musette_{20}$ showed the opposite. It is also shown in Fig. 4 that pre-incubation in water at temperatures below 15 °C improved the germination characteristics of Musette seeds in such a way that they became close to Capitan and Ravel seeds.

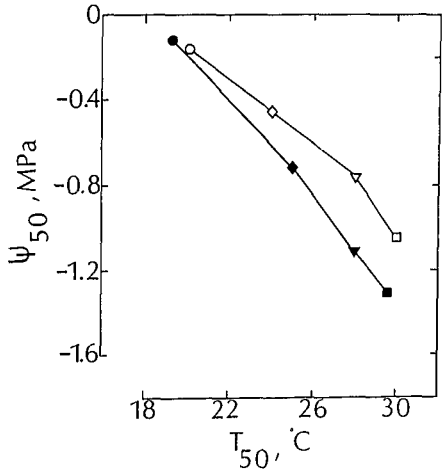

Fig. 4. Relationship between T_{50} and ψ_{50} of lettuce seeds of different cultivars (closed symbols) or of cv. $Musette_{20}$ pre-incubated at different temperatures (*open symbols*). Prior to the determination of T_{50} and ψ_{50} (at 20 °C) seeds of the cultivars $Musette_{20}$ (○ ●), $Musette_{25}$ (◇ ◆), Capitan (▽ ▼) and Ravel (□ ■) were pre-incubated 2 h at 15 °C in water and redried to 10% moisture content, seeds of cv. $Musette_{20}$ were moreover pre-incubated during 20 h at 15 °C (◇), 40 h at 10 °C (▽) and 5 days at 2 °C (□) and redried.

Similar experiments were performed with the radicle part of half seeds, i.e. in fact with the embryonic axes, since the envelope lost its restraining effect after preparation. In half seeds the differences between the cultivars disappeared for the greater part. T_{50} values rose to such high values (around 38 °C, not shown) that precise determination was hardly possible. ψ_{50} in all

seedlots dropped to values between -1.0 and -1.5 MPa (Table 1). This means that $\Delta\psi_{50}$ (ψ_{50} intact seeds - ψ_{50} half seeds) of Ravel seeds was only 0.29 MPa but in Musette seeds 0.93 MPa. Pre-incubation also narrowed this gap, 5 d at 2 °C reduced $\Delta\psi_{50}$ of $Musette_{20}$ to 0.48 MPa. These data strongly suggest that cultivar differences in lettuce depend at least for a considerable part on differences in the restraint of the envelope. Pre-incubation seems to decrease this restraint.

Table 1. Comparison of the ψ_{50} of intact seeds and the radicle part of halved seeds of different lettuce cultivars (a) and of cv. Musette pre-incubated at different temperatures (b). Prior to the determination of ψ_{50} at 20 °C seeds of the different cultivars were pre-incubated during 2 h at 15 °C in water and redried to 10% moisture content; seeds of cv. $Musette_{20}$ were also pre-incubated at 10° and 2° C for different times and redried. $\Delta\psi_{50} = \psi_{50}$ intact $- \psi_{50}$ halved.

a. pre-incubation 2 h at 15 °C

cultivars	osmotic potential, MPa		
	ψ_{50} intact	ψ_{50} halved	$\Delta\psi_{50}$
$Musette_{20}$	-0.05	-0.98	0.93
$Musette_{25}$	-0.70	-1.32	0.62
Capitan	-1.08	-1.46	0.38
Ravel	-1.24	-1.53	0.29

b. pre-incubation of $Musette_{20}$

conditions	osmotic potential, MPa		
	ψ_{50} intact	ψ_{50} halved	$\Delta\psi_{50}$
2 h 15 °C	-0.17	-1.15	0.98
20 h 15 °C	-0.44	-1.34	0.90
40 h 10 °C	-0.73	-1.38	0.65
5 d 2 °C	-1.02	-1.50	0.48

Effect of pre-incubation temperature on moisture content, ψ and ψ_π

The increase in moisture content and the changes in ψ and ψ_π were followed at the pre-incubation temperatures 15°, 24°, and 30 °C. The water uptake of the seeds during incubation showed the well-known triphasic pattern of (1) imbibition phase with a high rate of water uptake, (2) the equilibrium phase and (3) the growth phase, characterized by renewed water uptake (Fig. 5A). Temperature influenced all phases. An increase of the incubation temperature enhanced the rate of imbibition increased the saturation level in the second phase and since temperature determined the moment of germination, it also influenced the transition to phase 3. Direct psychrometric measurements showed that ψ increased during imbibition from very low values in the dry seed to an equilibrium value of zero (Fig. 5B).

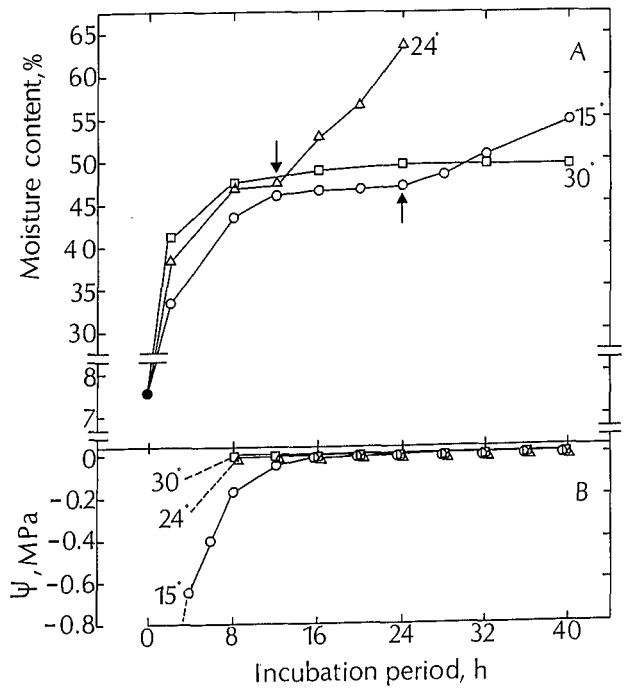

Fig. 5. Moisture content (A) and water potential (ψ)(B) of lettuce seeds cv. Capitan during incubation at 15 °C (O), 24 °C (Δ) or 30 °C (□) in water. SD of moisture content measurements was smaller than 1%. See for arrow Fig. 2.

The effect of the pre-incubation temperature on imbibition was translated into a similar effect on the change of ψ. Thus, it appeared that at the end of imbibition when ψ_m and ψ approached zero, ψ_π and ψ_p were at equilibrium (Eq. 5). ψ remained zero, when, at 15 ° and 24 °C, germination started. Obviously small changes in ψ which had to anticipate cell expansion, were immediately compensated for by extra water uptake. Water uptake must be so fast that no measurable gradient in ψ could be detected.

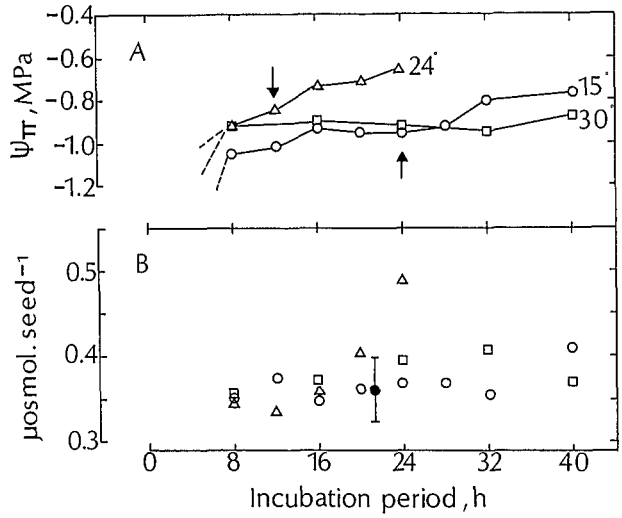

Fig. 6. Osmotic potential (A) and amount of solutes (B) of lettuce seeds cv. Capitan during incubation at 15 °C (○), 24 °C (△) or 30 °C (□) in water. The solutes were calculated from ψ_π and the moisture content (Fig. 5A). The point at 24 h and 24 °C was not used for the calculation of the mean (closed circle). See for arrow Fig. 2. The standard deviation of the mean is indicated.

Direct measurements showed that ψ_π stayed at all three temperatures at a stable level of about -0.9 MPa from the end of imbibition till the start of germination (Fig. 6A). As soon as at 15 °C and 24 °C germination occurred ψ_π began to increase to less negative values. From data on ψ_π and the moisture content (Fig. 5A) the amount of solutes was calculated, assuming that mannitol was the osmotic active compound (Fig. 6B). Except for the point of 24 h at 24 °C all calculated values were not significantly different. These calculations demonstrated that the increase in ψ_π at 15° and 24 °C at the beginning of germination, was not accompanied by a decrease in osmotic constituents and,

therefore, had to be solely due to extra water uptake during the start of growth. Because ψ remained zero (Fig. 5B), the pattern of change in ψ_p (not shown) will be an exact mirror image of the changes in ψ_π (Fig. 6A) and, thus, ψ_p will decrease following the beginning of growth. Thus, psychrometric measurement of ψ and ψ_π made clear that at none of the pre-incubation temperatures a build-up of osmotic constituents occurred prior to the start of growth.

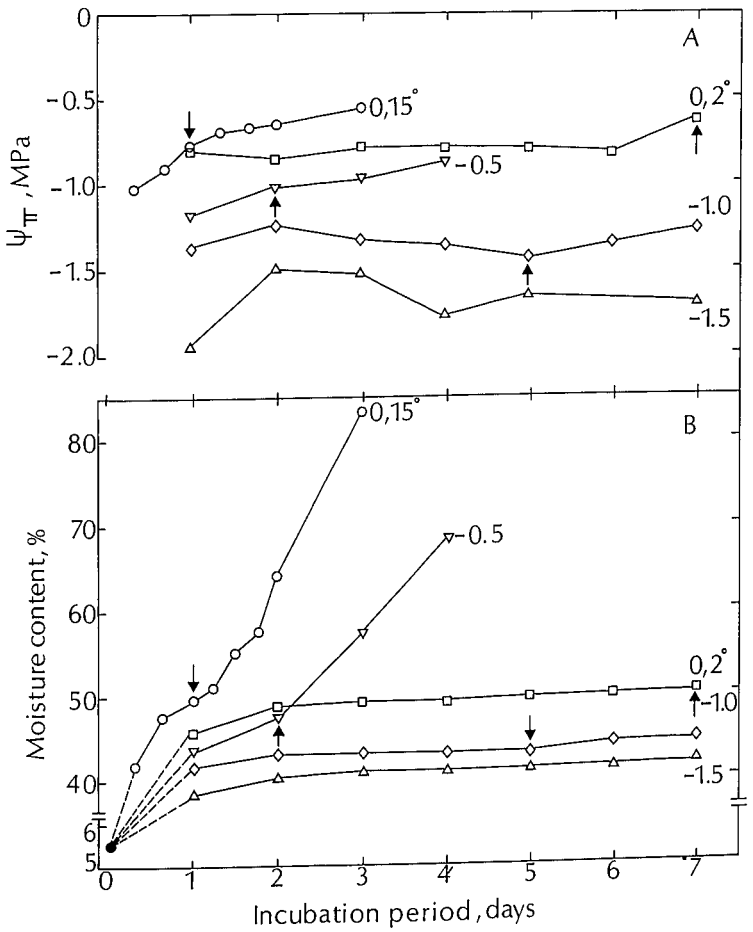

Fig. 7. Influence of $\psi_{\pi e}$ and temperature on ψ_π (A) and moisture content (B) of lettuce seeds cv. Musette$_{25}$. Seeds were incubated at 2 °C (□) or 15 °C (other symbols) in water (○ □), -0.5 MPa (▽), -1.0 MPa (◇) or -1.5 MPa PEG (△). See for arrow Fig. 2.

It has been argued above that in the situation of zero growth, i.e. in the absence of visible germination, $\psi_{\pi e} = \psi_{\pi} + Y$ (Eq. 4). Hence, changes in Y during pre-incubation at different temperatures could be calculated from the data on ψ_{50} and ψ_{π}. However, such a calculation is only allowed when ψ_{π} is unaffected by $\psi_{\pi e}$. Measurements of both moisture content and ψ_{π} at 15 °C in water and PEG solutions of seeds of cv. Musette proved that this assumption was incorrect (Fig. 7). In lower $\psi_{\pi e}$, ψ_{π} of the seeds clearly decreased (Fig. 7A) and the moisture content rose to lower maximum levels (Fig. 7B). These differences were reached at the end of imbibition and were maintained during a substantial number of days unless germination occurred. The mean values of both ψ_{π} and moisture content during the equilibrium phase were plotted versus $\psi_{\pi e}$ (Fig. 8). How to explain the effect of $\psi_{\pi e}$ on ψ_{π} ?

(1) Because the lines in Fig. 7 stayed at a constant level for prolonged periods of time, it is unlikely that ψ_{π} decreased due to an active stress-

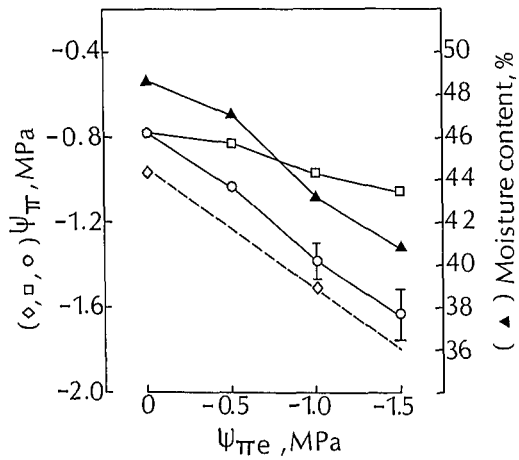

Fig. 8. Influence of $\psi_{\pi e}$ on ψ_{π} and moisture content of lettuce seeds cv. Musette$_{25}$ and Capitan. The data for ψ_{π} (○) and moisture content (▲) of cv. Musette$_{25}$ are mean values of the data shown in Fig. 7. The calculated effect of the lower moisture content on ψ_{π} is shown in the curve with the open squares. The broken line (◇) contain data on cv. Capitan, it is based on measurements at two $\psi_{\pi e}$ values and on parallelism with the Musette curve.

induced accumulation of newly formed osmotic solutes; (2) The decrease of ψ_π could also be caused by a passive increase of the concentration of solutes due to reduced water uptake at lower values of $\psi_{\pi e}$. The curve in Fig. 8 that is based on this assumption proved that such an effect indeed partly explained the decrease of ψ_π ; (3) In addition, we think that the decrease of $\psi_{\pi e}$ also caused a renewed decrease of ψ_m, because also cell walls and other structures will loose water. Data on ψ_π for the cultivar Capitan were partly measured and partly derived by parallelism to the Musette curve (Fig. 8).

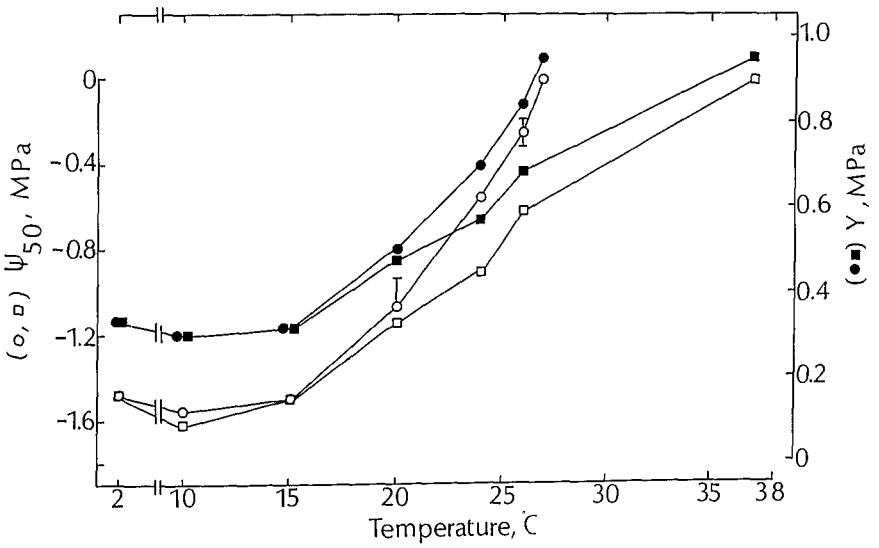

Fig. 9. Influence of pre-incubation at 15 °C in water during 2 h (○ ●) or 16 h (□, ■) on the relationship between ψ_{50} (open symbols) and Y (closed symbols) and the temperature at which ψ_{50} was determined. Y was calculated from the ψ_{50} data shown in this Fig. and ψ_π data corrected according to Fig. 8.

The application of Eq. 4 also depended on the temperature during germination which strongly influenced ψ_{50} (Fig. 9) but did not affect ψ_π (Fig. 6). Therefore, Y showed a similar relationship to temperature as ψ_{50}. For the calculation of Y the values of ψ_π were corrected according to Fig. 8. The curve for the seeds that had been pretreated for 2 h at 15 °C ('untreated control')(Fig. 9) indicated that at 27 °C ψ_{50} was zero, i.e. 50% germination could only occur in water, in other words T_{50} = 27 °C. When ψ_{50} equals zero,

$Y = -\psi_\pi$ (Eq. 4) and with an actual ψ of zero (Fig. 5) $Y = \psi_p$ (Eq. 5). Thus, the effective turgor (ψ_p - Y)(Eq. 3) approached zero and germination could not occur in water. At lower temperatures Y decreased and, therefore, in spite of unchanged ψ_π and ψ_p the effective turgor increased. As a consequence seeds germinated in water and also at increasingly lower osmotic potentials. It is concluded that the essential factor determining the relationship between temperature and germination is Y. At 15 °C and lower temperatures Y reached its minimum level.

Pre-incubation at 15 °C during 16 h decreased ψ_{50} at higher temperatures (Fig. 9). Because ψ_π was not influenced by such a pre-incubation (Fig. 6) it was calculated that the essential change was a decrease of Y. Obviously, the lower Y at 15 °C was remembered by the seeds after transfer to higher temperatures. It is shown in Fig. 10 that the change in Y depended on the length and the temperature of the pre-incubation. Y was calculated from the data on ψ_{50} in Fig. 2 and on ψ_π corrected according to Fig. 8. It is shown that Y depended in a similar way on the pre-incubation temperature as ψ_{50} (Fig. 2B) and reverse to T_{50} (Fig. 2A), therefore, Y and T_{50} will relate on a similar linear way as shown for ψ_{50} and T_{50} in Fig. 3. It is concluded that alleviation of dormancy at 15 °C correlated with a decrease of Y and induction of dormancy at 30 °C with an increase of Y.

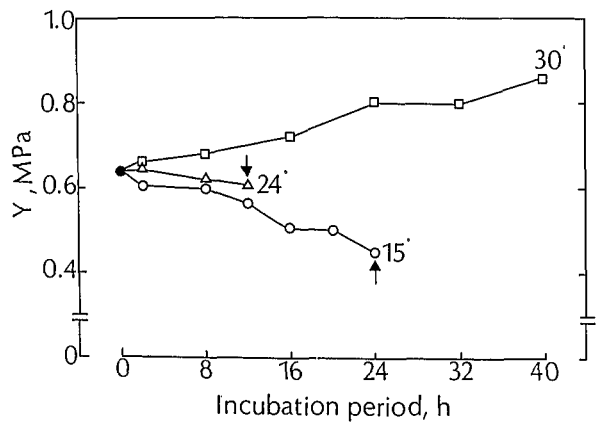

Fig. 10. Influence of length and temperature of pre-incubation in water on Y of lettuce seeds cv. Capitan. Pre-incubation temperatures were 15 °C (O), 24 °C (Δ) and 30 °C (□), Y was calculated from data on ψ_{50}, determined at 24 °C as shown in Fig. 2B and on ψ_π corrected according to Fig. 8.

Discussion

In lettuce seeds the critical factor determining the start of visible germination is the yield threshold Y of the turgor pressure. The effect of temperature on germination correlated with Y (Fig. 9) and pre-incubation changed Y to either lower or higher values (Fig. 10) in linear relationship to T_{50}. Our conclusions are based on the Lockhart equation of extension growth (Eq. 4). We regarded inhibition of germination as a steady state growth rate of zero. In contrast to Schopfer & Plachy (1985) and Carpita et al. (1979a) we did not perform actual measurements of growth rate.

The present experiments did not permit the determination of the rate coefficients L and m. However, it is unlikely that the yes or no response that is studied in germination will depend on rate constants. It is the more unlikely that L limited start of growth since ψ could never be distinguished from zero (Fig. 5B) indicating a very fast water uptake. Schopfer & Plachy (1985) concluded to a very close linkage of Y and m. Therefore, temperature may influence Y in concert with m. Lastly environmental effects on cell growth have been explained by changes in ε, the elastic coefficient of the cell wall that regulates the increase of ψ_p when the cell accumulates water (Dainty 1976). Also this factor seems not relevant to our experiments since ψ_p did not change during pre-incubation.

The changes in ψ_{50} and Y were the only effects of pre-incubation that clearly correlated with changes in T_{50}. A lack of correlation was observed for accumulation of amino nitrogen compounds (chapter 6) and for the changes of ψ_π (Fig. 6). Thus, the growth of embryo cells at the start of germination is regulated as any plant cell by the effective turgor (ψ_p-Y), where Y is the regulable factor, and probably m and not by a decrease of ψ_π as suggested by Takeba (1980a). In fact ψ_π increased during germination (Fig. 6, Schopfer & Plachy 1985), probably because extra uptake of water preceded osmotic adjustment. It was shown before (chapter 6) that accumulation of soluble amino nitrogen compounds only occurred when pre-incubation in PEG was prolonged. It was not related to changes in dormancy.

The comparison between intact and half seeds suggested that pre-incubation induced changes in both embryo and endosperm, the latter being the only living tissue in the envelope. The most effective pre-incubation of 5 days at 2 °C [T_{50} was increased from 20 °C to 30 °C (Fig. 4)], induced in intact seeds a

change in ψ_{50} twice as large as in halved seeds (Table 1b). These data and the comparison between seeds of different cultivars (Table 1a) supported the general conclusion that higher T_{50} values correlated with small $\Delta\psi_{50}$. Thus, the low extensibility of endosperm cell walls seems the major cause of low T_{50}, embryo cells played a secondary role. Therefore, studies with isolated lettuce embryos only (Carpita et al. 1979a, b, c) seems less conclusive about the regulation of dormancy than the present comparison of intact and halved seeds. Nevertheless, those studies produced valuable information about the effect of light and growth regulators on growth of embryos.

The mechanism of cell wall loosening in seeds has not been studied yet. Our data suggest that the process must be reversible because T_{50} can be shifted in both directions by temperature, dehydration and storage (see chapters 2, 3, 4 and 5). Because of reversibility relevant cell wall changes must be different from the hydrolysis of cell wall galactomannans in lettuce endosperm that occurs after start of germination (Bewley et al. 1983).

Most likely temperature does not influence cell wall extensibility directly. It has been shown in different tissues that applied growth regulators influence Y and m (see Cleland 1986 for references). Red light and growth regulators decreased ψ_{50} in isolated lettuce embryos (Carpita et al. 1979 a, c). Temperature effects on hormone levels in seed have been postulated for several decades but have hardly been demonstrated experimentally (Bewley & Black 1982). Besides hormone levels temperature may also affect hormone sensitivity. Recently studies with GA-deficient mutants of Arabidopsis thaliana showed a shift in the requirement for exogenous GA_{4+7} to lower or higher concentrations by pre-incubation in low and high temperatures, respectively (Karssen, in press).

In parallel studies Groot & Karssen (1985) have shown in our laboratory that in tomato seeds weakening of endosperm resistance is a prerequisite for germination. Studies with GA-deficient mutants showed an absolute dependence on GAs. GAs are most likely transported from embryo to endosperm. Endosperm resistance was measured directly by an Instron technique. Unfortunately, lettuce seeds are not fitted to measure endosperm strength at the relevant place of normal protrusion. Therefore, direct evidence for changes in endosperm cells is missing. Georghiou et al. (1983) have shown, however, that prior to germination changes occur in the endosperm cells directly opposite the radicle tip.

CHAPTER 8

SAMENVATTING

De aard van de voorbehandeling

Kiemrust beperkt de kieming van slazaden tot temperaturen beneden een bepaald maximum. De maximale temperatuur waarbij 50% van de zaden nog kan kiemen wordt aangeduid met T_{50}. Dit is een goede maat voor het niveau van kiemrust. De T_{50} van veel cultivars van sla ligt rond 25 °C. Voor de volle-grondsteelt in warmere gebieden en voor de kasteelt in Nederland vormt deze lage T_{50} een probleem. Door een voorbehandeling van de zaden in het laboratorium vóór het uitzaaien is de kiemrust van slazaden te breken, d.w.z. T_{50} te verhogen. Een voorbehandeling bestaat uit drie onderdelen: (1) pre-incubatie van de zaden in water of een osmoticum bij een geschikte temperatuur om T_{50} te verhogen, (2) terugdrogen van de zaden tot een laag vochtgehalte om bewaring en zaaien mogelijk te maken, en (3) bewaring tot het moment van zaaien. Een voorbehandeling van slazaden wordt in toenemende mate toegepast, echter in de praktijk blijkt voorbehandeling nogal eens problemen op te leveren. Helaas zijn er echter zeer weinig studies gepubliceerd over de aard van dergelijke voorbehandelingen. Dit onderzoek stelde zich daarom tot doel een fysiologische analyse van de voorbehandeling uit te voeren, gericht op het oplossen van de hierbij optredende problemen. Hierbij werd tot doel gesteld om enerzijds de factoren aandacht te geven die tijdens de verschillende onderdelen van de voorbehandeling van invloed zijn op het kiemrustniveau en om anderzijds de fysiologische processen die de basis vormen van kiemrust te onderzoeken. Behalve een omschrijving van de probleemstelling bevat hoofdstuk 1 de inleiding tot de verschillende hoofdstukken.

Gebruik van osmoticum tijdens pre-incubatie

Hoofdstuk 2 behandelt de voordelen en de nadelen van de toepassing van polyethyleen glycol (PEG) als osmoticum tijdens pre-incubatie. Pre-incubatie in water bij 15 °C verhoogde de T_{50} van slazaden cv. Musette van 25 °C tot boven 32 °C, echter door terugdrogen tot een vochtgehalte van 5.6% ging een groot deel van deze stijging weer verloren (T_{50} = 26.5 °C). Indien terugdrogen plaatsvond voor het begin van de kieming, raakte het embryo niet zichtbaar beschadigd. Pre-incubatie in PEG (-0.5 MPa) had het grote voordeel dat deze

terugval van T_{50} door drogen grotendeels voorkomen kon worden. Door pre-incubatie in PEG werd, naarmate de osmotische potentiaal van het incubatie-medium ($\psi_{\pi e}$) lager was, het moment van zichtbare kieming steeds meer uit-gesteld, waardoor de pre-incubatie langer kon worden volgehouden zonder dat beschadiging van de embryo's optrad door het terugdrogen.

Een nadeel van de toepassing van PEG was dat tijdens een langdurige pre-incubatie bij een $\psi_{\pi e}$ van -1.0 MPa of -1.5 MPa de rustbreking trager verliep en T_{50} zelfs wat begon te dalen: er werd secundaire kiemrust geïnduceerd. Dit vertragend effect van PEG kon niet geweten worden aan een tragere zuurstof-diffusie, want in zuivere O_2 verliepen rustbreking en rustinductie zoals in lucht. Pas bij een zuurstofconcentratie lager dan 5.6% werd rustbreking zowel in water als in PEG geremd.

Tijdens pre-incubatie bij 30 °C trad alleen rustinductie op, die eveneens door PEG werd geremd. De opname van O_2 en de produktie van CO_2 door de zaden tijdens rustbreking en rustinductie werden ook door PEG geremd. De conclusie luidt dat PEG door verlaging van het vochtgehalte van de zaden aan het einde van de imbibitie het metabolisme van de zaden remt en daardoor veranderingen van rustniveau vertraagt.

Terugdrogen

Het hiervoor n.a.v. hoofdstuk 2 reeds genoemde negatieve effect van terug-drogen op de T_{50} van zaden na de pre-incubatie staat nader beschreven in hoofdstuk 3 en in hoofdstuk 5. In laatstgenoemd hoofdstuk zijn de factoren onderzocht die het effect van terugdrogen beïnvloeden. Zowel de droogsnelheid als de temperatuur tijdens het drogen, bleken van marginaal belang. De daling van T_{50} werd vooral bepaald door het vochtgehalte dat bereikt werd aan het einde van het droogproces. In slazaden cv. Musette, die een uitgangs T_{50} van 25 °C vertoonden, bleef T_{50} alleen op het niveau van 31 °C dat bereikt werd door de pre-incubatie wanneer ze niet verder teruggedroogd werden dan een vochtgehalte van 10% . Na terugdrogen tot 4.5% vochtgehalte daalde T_{50} echter tot 26 °C. Omdat deze daling van T_{50} teniet gedaan kon worden door hernieuwde pre-incubatie bij 15 °C, werd geconcludeerd dat terugdrogen niet tot een on-herstelbare schade leidde, maar reversibel het kiemrustniveau van slazaden beïnvloedde.

In hoofdstuk 3 wordt beschreven hoe verschillende cultivars en partijen zaden van dezelfde cultivar reageren op pre-incubatie en terugdrogen. De T_{50} van verschillende partijen slazaden varieerde van 15 tot 30 °C. Door pre-incubatie gedurende 16 tot 20 uur bij 15 °C werd T_{50} met 3.5 tot 9°C verhoogd. Terugdrogen tot een vochtgehalte van 3.5 tot 4% veroorzaakte bij de ene groep cultivars (Musette, Palmyran, Marcia) een verlaging van T_{50} met 50 tot 90% van de aanvankelijk stijging, terwijl bij de andere groep cultivars (Grand Rapids, Montello, Mariska, Capitan, Ravel) de T_{50} slechts 10 tot 20% daalde.

Door belichting van zaden van de verschillende partijen aan het einde van de pre-incubatie bleek T_{50} meestal maar 1 tot 1,5 °C hoger te zijn dan na pre-incubatie in het donker. Bij Grand Rapids veroorzaakte eenzelfde belichting echter een verhoging van de T_{50} met 5 °C.

De experimenten die beschreven staan in hoofdstuk 3 tonen ook aan dat de aard van de voorbehandeling van een partij slazaden sterk afhangt van de uitgangs T_{50}. Aan de ene zijde van het spectrum van mogelijkheden staat bijvoorbeeld een cultivar als Ravel, waarvan de zaden zonder voorbehandeling reeds voor 50% kiemen bij 28 °C. Pre-incubatie in water bij 15 °C was voldoende om de T_{50} tot 37 °C te verhogen, een effect dat na terugdrogen gehandhaafd bleef. Het andere extreem wordt gevormd door een bepaalde partij van de cultivar Musette, die een uitgangs T_{50} vertoonde van 15 °C. Pre-incubatie in water bij 15 °C was volstrekt onvoldoende. Voorbehandelen bij 10 °C en zeker bij 2 °C verbeterde het effect. Bij 2 °C bleek echter vernalisatie op te treden. Daardoor blijft 10 °C als meest geschikte pre-incubatie temperatuur over. Voorbehandelen in -0.5 MPa PEG zorgde voor een zeker behoud van de winst in T_{50}, het eindresultaat van 23,5 °C was echter toch niet voldoende. Zoals hierboven beschreven werd (hoofdstuk 2), werden er met een andere partij van dezelfde cultivar Musette wèl bevredigende resultaten geboekt bij voorbehandelen in PEG.

Conclusies over pre-incubatie en terugdrogen

De algemene conclusie ten aanzien van de aard van de voorbehandeling is dat een eenheidsrecept niet bestaat. Als uitgangspunt geldt de conclusie van Heydecker & Coolbear (1977) "geen voorbehandeling is de beste voorbehandeling". Indien toch nodig, dient een voorbehandeling zo simpel mogelijk te zijn

omdat elke toevoeging risico's in zich draagt, zoals in hoofdstuk 2 werd aangetoond voor het osmoticum PEG. Een lage uitgangs T_{50} blijkt echter samen te gaan met een snelle terugval van de T_{50} door terugdrogen. In die gevallen is toepassing van PEG zeer nuttig. De risico's, zoals hierboven genoemd, zijn te voorkomen door de concentratie PEG zo laag mogelijk te kiezen en de pre-incubatietijd zo kort mogelijk te houden. In dit onderzoek is weinig aandacht besteed aan de toediening van groeiregulatoren aan het pre-incubatiemedium. Ze lijken echter pas in aanmerking te komen als voorbehandeling in PEG en voorzichtig terugdrogen niet voldoende zijn, omdat in het bijzonder de toediening van groeiregulatoren risico's van kiemplantschade inhoudt. Een roodbelichting tijdens de voorbehandeling had slechts een gering positief effect.

De vergelijking van de verschillende cultivars in hoofdstuk 3 toonde aan dat de noodzaak van een voorbehandeling d.m.v. veredeling voorkomen zou kunnen worden.

Bewaring

Hoofdstuk 4 handelt over de invloed van bewaring bij verschillende temperaturen op de kieming van slazaden cv. Mariska. Na pre-incubatie gedurende 2 uur of 16 h bij 15 °C in -0.25 MPa PEG werden de zaden teruggedroogd tot verschillende vochtgehalten. T_{50} bereikte waarden van resp. 27 en 30 °C. Terugdrogen tot verschillende vochtgehalten beïnvloedde de T_{50}-waarden van deze cultivar nauwelijks. Bewaring resulteerde echter wel in een daling van T_{50}, in het bijzonder wanneer bewaard werd bij hoge temperaturen en hoge vochtgehalten. Na 16 h pre-incubatie waren de zaden gevoeliger voor de bewaaromstandigheden dan na 2 uur.

Om verschillende bewaaromstandigheden met elkaar te kunnen vergelijken werd de term G_{50} geïntroduceerd. G_{50} is de bewaarduur die nodig is om de kieming in het donker, bij een bepaalde testtemperatuur (22, 24, 26 of 28 °C), te laten dalen tot 50%. Dit in analogie met de term p_{50}: de bewaarduur totdat de 'viability' tot 50% gedaald is. Als criterium voor 'viability' werd genomen: de ontwikkeling van een kiemplant zonder enig zichtbare afwijking gedurende 7 dagen bij 20 °C in een dag/nacht ritme van 8 uur licht/16 uur donker. Er bleek een negatief lineair verband te bestaan tussen log G_{50} en log p_{50} enerzijds en log vochtgehalte anderzijds. De bewaartemperatuur beïnvloedde de onderlinge

afstand van de lijnen. Bovendien bleek er een lineair verband te bestaan tussen de waarden van G_{50}, die bepaald waren bij 28, 26, 24 en 22 °C, enerzijds en p_{50}, bepaald bij 20 °C, anderzijds. De waarden van G_{50} bepaald bij 22 °C waren zelfs vrijwel gelijk aan de waarden van p_{50}. De conclusie luidt daarom dat tijdens bewaring dezelfde processen ten grondslag liggen aan de inductie van kiemrust als aan het verlies van viability.

De fysiologische analyse van de voorbehandeling

In dit proefschrift krijgen vooral de processen aandacht die ten grondslag liggen aan veranderingen van het kiemrustniveau t.g.v. voorbehandeling, om zodoende een betere basis te creëren voor de ontwikkeling van voorbehandelingen. In de hoofdstukken 5, 6 en 7 worden een aantal aspecten van de fysiologische analyse behandeld.

De analyse van het terugdroogeffect

Omdat het effect van drogen op levend weefsel altijd in verband gebracht wordt met de integriteit van celmembranen wordt in hoofdstuk 5 het onderzoek beschreven naar het verband tussen de lekkage van kaliumionen (K^+) en de T_{50} van slazaden. Na imbibitie van zaden van cv. Musette in water bleek $\pm$ 10% van het K^+ gehalte van het zaad in het incubatiemedium te lekken. Na pre-incubatie, zowel met als zonder terugdrogen, trad geen hernieuwde lekkage op. Uit naakte embryo's van slazaden bleken wel meer K^+ ionen te lekken naarmate ze tot een lager vochtgehalte teruggedroogd werden. Dit verschil in lekkage van zaden en embryo's is te wijten aan een K^+-impermeabele laag in het omhulsel van slazaden, waarschijnlijk het endosperm, die zowel de K^+ lekkage uit als de K^+ opname door het embryo remt.

De K^+ lek uit intacte zaden is daarom waarschijnlijk afkomstig uit de wandstructuren buiten het endosperm. De algemene conclusie is dat op de een of andere wijze het resultaat van rustbreking vastgelegd wordt in gehydrateerde ultrastructuren. Het ligt voor de hand om te veronderstellen dat deze ultrastructuren, waarschijnlijk membranen, beschadigd worden door drogen beneden

10% vochtgehalte. Echter door de K^+-impermeabele laag kan niet vastgesteld worden of het embryo binnen het omhulsel eveneens gaat lekken t.g.v. drogen. Het reversibele karakter van kiemrust wijst er op dat er door drogen in ieder geval geen verlies van essentiële componenten optreedt. Indien er eventueel toch (niet waarneembare) lek optreedt binnen het embryo, zou het daarna immers de uitgelekte componenten weer kunnen opnemen.

Het mechanisme van de rustverandering

Enkele voorgaande publikaties over de aard van de rustbreking in slazaden bevatten de suggestie dat er voorafgaande aan de kieming in het embryo een ophoping van aminozuren zou plaatsvinden. Door de stijging van het gehalte aminozuren zou de osmotische potentiaal (ψ_π) dalen (meer negatief worden), waardoor de waterpotentiaal (ψ) zou dalen en extra wateropname mogelijk zou worden. Het toetsen van deze hypothese vormde het uitgangspunt voor het onderzoek beschreven in hoofdstuk 6. Als criterium voor de beëindiging van kieming en de start van kiemplantgroei werd genomen de gevoeligheid van het embryo voor beschadiging door drogen. Tijdens incubatie bij 15 °C bleken slazaden cv. Musette al 3 uur vóór het moment dat het worteltje zichtbaar werd gevoelig te worden voor drogen. De toename van het gehalte oplosbare aminostikstof, het gehalte aminozuren en de activiteit van het daarbij belangrijke enzym glutamine synthetase (GS) kwam na het het moment waarop de kiemplantgroei begon.

Door variabele omstandigheden te kiezen tijdens pre-incubatie konden veranderingen in het kiemrustniveau gescheiden worden van de stijging van GS-aktiviteit. In het bijzonder tijdens pre-incubatie bij 2 °C in water steeg de capaciteit van de zaden om te kiemen bij 30 °C aanzienlijk eerder dan de activiteit van GS. Zoals hierboven al werd vermeld n.a.v. hoofdstuk 2 treedt er tijdens langdurige pre-incubatie in -1.0 en -1.5 MPa PEG bij 15 °C na aanvankelijke rustbreking weer rustinductie op. De activiteit van GS correleerde in geen enkel opzicht met deze kiemrustwisselingen. De conclusie luidt dat veranderingen van het rustniveau van slazaden onafhankelijk plaatsvinden van de stimulering van het oplosbaar aminostikstof metabolisme.

Na de conclusie uit hoofdstuk 6, dat oplosbaar aminostikstof niet verantwoordelijk kan zijn voor de volgens de hypothese veronderstelde daling van ψ_π lag het voor de hand om op zoek te gaan naar de factor die wèl de extra

wateropname tijdens kiemplantgroei zou kunnen verklaren. In hoofdstuk 7 wordt dit onderzoek beschreven.

In eerste instantie is een verband gelegd tussen kiemrustveranderingen en de waterhuishouding. Daarvoor is behalve T_{50} ook ψ_{50} bepaald. ψ_{50} is de $\psi_{\pi e}$ van het incubatiemedium waarbij nog juist 50% van de zaden in staat is om te kiemen. Pre-incubatie van slazaden cv. Capitan bij 15 °C gedurende 0 tot 24 uur had behalve een stijging van T_{50} van 26 naar 36 °C ook een daling van ψ_{50} van -0.8 naar -1.3 MPa tot gevolg. ψ_{50} werd bepaald bij 24 °C. Pre-incubatie bij 30 °C had daarentegen het tegenovergestelde effect: een daling van T_{50} en een stijging van ψ_{50}. Pre-incubatie bij 24 °C ten slotte had nauwelijks effect op T_{50} en ψ_{50}. Er bleek een lineaire relatie te bestaan tussen de veranderingen van T_{50} en ψ_{50}. Het voor de kiemrustverandering essentiële proces staat dus onmiskenbaar in relatie tot de waterhuishouding van het zaad.

Vergelijking van slazaden van cv. Capitan met die van cv. Ravel en twee verschillende partijen van cv. Musette met verschillende waarden van T_{50} leverde eveneens een negatief lineair verband op tussen T_{50} en ψ_{50}-waarden. Voor alle partijen werden T_{50} en ψ_{50} ook bepaald voor zaden waarvan het bovenste deel van de cotylen was verwijderd. In deze voornamelijk uit de embryonale as bestaande zaadhelften vielen de verschillen tussen de zaadpartijen t.a.v. T_{50} en ψ_{50} vrijwel geheel weg. T_{50} steeg steeds tot waarden hoger dan 36 °C en de ψ_{50} van de zaden van Musette met de laagste uitgangs-T_{50} daalde met 0.93 MPa, terwijl dit in zaden van cv. Ravel slechts 0.29 MPa was. De conclusie luidt dat de verschillen in kiemrustniveau dus voor een aanzienlijk deel toe te schrijven zijn aan verschillen in de weerstand van het omhulsel. Door pre-incubatie van zaden van een partij van cv. Musette bij optimale omstandigheden voor rustbreking verkleinde deze weerstand kennelijk, want het verschil in ψ_{50} tussen heel en half zaad verkleinde van 0.98 tot 0.48 MPa.

Direkte meting van ψ en ψ_{π} tijdens incubatie van slazaden cv. Capitan bij 15, 24 en 30 °C toonde aan dat deze parameters niet meetbaar veranderden tussen het einde van imbibitie en de start van groei van de kiemplant. De waarde van ψ bleek niet meetbaar te verschillen van 0 MPa, terwijl ψ_{π} tijdens deze periode een waarde had van -0.9 MPa. Dus tijdens de incubatie in water vóór de start van groei treedt er geen ophoping van osmotisch actieve stoffen op. Na de start van groei bij 15 °C en 24 °C bleek ψ_{π} te stijgen door opname van water (de celinhoud werd dus verdund), terwijl de waarde van ψ

niet meetbaar veranderde.

Volgens de Lockart-vergelijking van de hydraulische celgroei kan ψ_{50} gelijk gesteld worden aan $\psi_{\pi} + Y$, wanneer de groei nul is, waarbij Y de grenswaarde is van de turgor (ψ_p) *die nodig is voor irreversibele groei.*

Bij een niet veranderende waarde van ψ_{π} vertalen veranderingen van Y zich rechtstreeks in veranderingen van ψ_{50}. De essentiële veranderingen die tijdens pre-incubatie bij lage temperatuur plaatsvinden, blijken dus te bestaan uit een verlaging van Y. Inductie van rust daarentegen wordt veroorzaakt door een verhoging van Y.

Y en ψ_{50} zijn niet alleen afhankelijk van de pre-incubatie temperatuur, maar zij worden ook sterk beïnvloed door de temperatuur waarbij ψ_{50} bepaald wordt. Bij stijging van de *temperatuur neemt* ψ_{50} *af,* Y juist toe. Kieming is onmogelijk indien Y dezelfde waarde heeft als ψ_p en omdat ψ_p evenals ψ_{π} niet door de temperatuur beïnvloed worden, is Y de bepalende factor. Pre-incubatie bij 30 °C verhoogt Y, waardoor erop volgende kieming alleen bij lagere temperaturen dan voorheen mogelijk is. Rustbreking heeft echter het omgekeerde effect: Y wordt verlaagd, waardoor erop volgende kieming bij hogere temperaturen mogelijk is.

LITERATUURLIJST

Bekendam, J. 1972. Problemen van kiemrust en kwaliteit van slazaad in verband met precisiezaai. - Zaadbelangen 26: 266-268.

Bewley, J.D. 1979. Physiological aspects of desiccation tolerance. - Ann. Rev. Plant Physiol. 30: 195-238.

— & Black, M. 1978. Physiology and Biochemistry of seeds (Vol. 1). Development, germination and growth. - Springer-Verlag, Berlin, ISBN 3-540-08274-3.

— & Black, M. 1982. Physiology and Biochemistry of seeds (Vol. 2). Viability, dormancy and environmental control. - Springer-Verlag, Berlin, ISBN 3-540-11656-7.

— , Leung, D.W.M. & Ouelette, F.B. 1983. The cooperative role of endo- β-mannanase, β-mannosidase and α-galactosidase in the mobilization of endosperm cell wall hemicellulose of germinated lettuce seed. - Rec. Adv. Phytochem. 17: 137-152.

Bodsworth, S. & Bewley, J.D. 1981. Osmotic priming of seeds of crops species with PEG as a means of enhancing early and synchronous germination at cool temperatures. - Can. J. Bot. 59: 672-676.

Borthwick, H.A., Hendricks, S.B., Toole, E.H. & Toole, V.K. 1954. Action of light on lettuce seed germination. - Bot. Gaz. 115: 205-225.

— & Robbins, W.W. 1928. Lettuce seed and its germination. - Hilgardia 3: 275-305.

Brocklehurst, P.A. & Dearman, J. 1983. Interactions between seed priming treatments and nine seed lots of carrot, celery and onion. I. Laboratory germination. - Ann. Appl. Biol. 102: 577-584.

Cantliffe, D.J., Shuler, K.D. & Guedes, A.C. 1981. Overcoming seed thermodormancy in a heat sensitive romaine lettuce by seed priming. - HortScience 16: 196-198.

Carpita, N.C., Nabors, M.W., Ross, C.W. & Petretic, N.L. 1979a. The growth physics and water relations of red-light-induced germination in lettuce seeds. III Changes in the osmotic and pressure potential in red- and far-red-treated embryonic axes. - Planta 144: 217-224.

— , Nabors, M.W., Ross, C.W. & Petretic, N.L. 1979b. The growth physics and water relations of red light-induced germination in lettuce seeds. IV

Biochemical changes in the embryonic axes of red- and far-red treated seeds. - Planta 144: 225-233.

— , Ross, C.W. & Nabors, M.W. 1979c. The influence of plant growth regulators on the growth of the embryonic axes of red- and far-red-treated lettuce seeds. - Planta 145: 511-516.

— , Sabularse, D., Montezinos, D. & Delmer, D.P. 1979d. Determination of the pore size of cell walls of living plant cells. - Science 205: 1144-1146.

Cleland, R.E. 1986. The role of hormones in wall loosening and plant growth. - Aust. J. Plant Physiol. 13: 93-103.

Cone, J.W. & Spruit, C.J.P. 1983. Imbibition conditions and seed dormancy of Arabidopsis thaliana. - Physiol. Plant. 59: 416-420.

Dainty, J. 1976. Water relations of plant cells. In Encyclopedia of Plant Physiology, New Series, Vol. 2, Part A (U. Lüttge & M. Pittman, eds) pp. 12-35. Springer-Verlag Berlin. ISBN 3-540-07452.

Eenink, A.H. 1981. Research on the inheritance of seed dormancy in lettuce (Lactuca sativa L.) and selection for non-dormancy. - Euphytica 30: 371-380.

Ellis, R.H. & Roberts, E.H. 1980a. Improved equations for the prediction of seed longevity. - Ann. Bot. 45: 13-30.

— & Roberts, E.H. 1980b. The influence of temperature and moisture on seed viability period in barley (Hordeum distichum L.). - Ann. Bot. 45: 31-37.

— & Roberts, E.H. 1981. The quantification of ageing and survival in orthodox seeds. - Seed Science Technol. 9: 373-409.

Georghiou, K., Psaras, G. & Mitrakos, K. 1983. Lettuce endosperm structural changes during germination under different light, temperature, and hydration conditions. - Bot. Gaz. 144: 207-211.

Gray, D. 1975. Effects of temperature on the germination and emergence of lettuce (Lactuca sativa L.) varieties. - J. Hort. Sci. 50: 349-361.

Groot, S.P.C. & Karssen, C.M. 1985. Germination behaviour of gibberellin-deficient mutants of tomato. In Book of Abstracts, International workshop 'Control Processes in Seeds' Aug. 18-23, 1985, Wageningen.

Guedes, A.C. & Cantliffe, D.J. 1980. Germination of lettuce seeds at high temperature after seed priming. - J. Amer. Soc. Hort. Sci. 105: 777-781.

Haber, A.H. & Luippold, H.J. 1960. Separation of mechanisms initiating cell division and cell expansion in lettuce seed germination. - Plant Physiol. 35: 168-173.

Halmer, P., Bewley, J.D. & Thorpe, T.A. 1978. Degradation of the endosperm cell walls of Lactuca sativa L., cv. Grand Rapids. Timing of mobilization of soluble sugars, lipid and phytate. - Planta 139: 1-8.

Harrington, J.F. & Thompson, R.C. 1952. Effect of variety and area of production on subsequent germination of lettuce seed at high temperatures. - Proc. Am. Soc. Hort. Sci. 59: 445-450.

Hegarty, T.W. & Ross, H.A. 1979. Effects of light and growth regulators on germination and radicle growth of lettuce seed held under high temperature stress and water stress. - New Phytol. 82: 49-57.

Heydecker, W. 1977. Stress and seed germination: an agronomic view. - In The Physiology and Biochemistry of Seed Dormancy and Germination (A.A. Khan, ed.), pp. 237-282. North Holland, Amsterdam. ISBN 0-7204-0643-9.

— & Coolbear, P. 1977. Seed treatments for improved performance - survey and attempted prognosis. - Seed Sci. Technol. 5: 353-425.

— , Higgins, J. & Gulliver, R.L. 1973. Accelerated germination by osmotic seed treatment. - Nature 246: 42-44.

Hilhorst, H.W.M., Smitt, A.I. & Karssen, C.M. 1986. Gibberellin-biosynthesis and -sensitivity mediated stimulation of seed germination of Sisymbrium officinale by red light and nitrate. - Physiol. Plant. 67: 285-290.

Hoekstra, F.A. & Bruinsma, J. 1975. Respiration and vitality of binucleate and trinucleate pollen. - Physiol. Plant. 34: 221-225.

Ibrahim, A.E. & Roberts, E.H. 1983a. Viability of lettuce seeds. I Survival in hermetic storage. - J. Exp. Bot. 34: 620-630.

— , Roberts, E.H. & Murdoch, A.J. 1983b. Viability of lettuce seeds. II Survival and oxygen uptake in osmotically controlled storage. - J. Exp. Bot. 34: 631-640.

International Seed Testing Association. 1985. International rules for seed testing 1985. - Seed Sci. Technol. 13: 299-355.

Kahn, A. 1960. Analysis of "dark osmotic inhibition" of germination of lettuce seeds. - Plant Physiol. 35: 1-7.

Karssen, C.M. 1980/81. Environmental conditions and endogenous mechanisms involved in secondary dormancy of seeds. - Isr. J. Bot. 29: 45-64.

— , 1982. Seasonal patterns of dormancy in weed seeds. In The Physiology and Biochemistry of Seed Development, Dormancy and Germination (A.A. Khan, ed.), pp. 243-270. Elsevier, Amsterdam. ISBN 0-444-80423-4.

Khan, A.A. 1977. Preconditioning, germination and performance of seeds. - In

The Physiology and Biochemistry of Seed Dormancy and Germination (A.A. Khan, ed.), pp. 283-316. North Holland, Amsterdam. ISBN 0-7204-0643-9.

— , Tao, K.L., Knypl, J.S., Borkowska, B. & Powell, L.E. 1978. Osmotic conditioning of seeds: physiological and biochemical changes. - Acta Hortic. 83: 267-278.

Klein, S., Negbi, M., Witzum, A. & Rothberg, L. 1971. The role of the endosperm in uptake and distribution of exogenous leucine in germinating lettuce seeds. - New Phytol. 70: 143-147.

Kraak, H.L. & Vos, J. 1987. Seed viability constants for lettuce. - Ann. Bot.: in press.

Kretschmer, M. 1982 Extension of the temperature tolerance of Lactuca achenes with PEG and red light. - Gartenbauwissenschaft 47: 152-157.

Leung, D.W.M., Reid, J.S.G. & Bewley, J.D. 1979. Degradation of the endosperm cell walls of Lactuca sativa L. cv. Grand Rapids in relation to the mobilization of proteins and the production of hydrolytic enzymes in the axes, cotyledons and endosperm. - Planta 146: 335-341.

Lockhart, J.A. 1965. Analysis of irreversible plant cell elongation. - J. Theor. Biol. 8: 264-275.

Mexal, J., Fisher, J.T., Osteryoung, J. & Patrick Reid, C.P. 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. - Plant Physiol. 55: 20-24.

Michel, B.E. 1983. Evaluation of the water potentials of solutions of polyethylene glycol (8000) both in the absence and presence of other solutes. - Plant Physiol. 72: 66-70.

Miflin, B.J. Lea, P.J. 1977. Amino acid metabolism. - Ann. Rev. Plant Physiol. 28: 299-329.

Nabors, M.W. & Lang, A. 1971. The growth physics and water relations of red-light induced-germination in lettuce seeds. II Embryos germinating in water. - Planta 101: 26-42.

Negm, F.B. & Smith, O.E. 1978. Effects of ethylene and carbon dioxide on the germination of osmotically inhibited lettuce seed. - Plant Physiol. 62: 473-476.

Prince, S.D. 1980. Vernalization and seed production in lettuce. - In Seed Production (P.D. Hebblethwaite, ed.) pp. 485-499. Butterworths, London.

Reynolds, T. 1975. Characterization of osmotic restraints on lettuce fruit

germination. - Ann. Bot. 39: 791-796.

— , & Thompson, P.A. 1971. Characterization of the high temperature inhibition of germination of lettuce (Lactuca sativa). - Physiol. Plant. 24: 544-547.

— , & Thompson, P.A. 1973. Effects of kinetin, gibberellins and (±) abscisic acid on the germination of lettuce (Lactuca sativa L.). - Physiol. Plant. 28: 516-522.

Schopfer, P., Bajracharaya, D. & Plachy, C. 1979. Control of seed germination by abscisic acid. I Time course of action in Sinapis alba. - Plant Physiol. 64: 822-827.

— & Plachy, C. 1985. Control of seed germination by abscisic acid. III Effect on embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in Brassica napus L. - Plant Physiol. 77: 676-686.

Shaykewich, C.F. 1973. Proposed method for measuring swelling pressure of seeds prior to germination. - J. Exp. Bot. 24: 1056-1061.

Simon, E.W. 1984. Early events in germination. In Seed Physiology Vol. 2 Germination and Reserve Mobilization (D.R. Murray, ed.) pp. 77-115. Academic Press, Australia. ISBN 0-12511902.

— & Harun, R.M.R. 1972. Leakage during seed imbibition. - J. Exp. Bot. 23: 1076-1085.

Stadtman, E.R., Smyrniotis, P.Z., Davis, J.N., Wittenberger, M.E. 1979. Enzymic procedures for determining the average state of adenylylation of Escherichia coli glutamine synthetase. - Anal. Biochem. 95: 275-285.

Takeba, G. 1980a. Changes revealed by a tracer technique in the amino acid metabolism of thermodormant and nondormant New York lettuce seeds. - Plant Cell Physiol. 21: 1627-1638.

— 1980b. Accumulation of free amino acids in the tips of non-thermodormant embryonic axes accounts for the increase in the growth potential of New York lettuce seeds. - Plant Cell Physiol. 21: 1639-1644.

— 1980c. Effects of temperature, red light and hormones on the accumulation of free amino acids in osmotically growth-inhibited embryonic axes of New York lettuce seeds. Plant Cell Physiol. 21: 1645-1649.

— 1980d. Phytochrome-mediated accumulation of free amino acids in embryonic axes of New York lettuce seeds. - Plant Cell Physiol. 21: 1651-1656.

— 1983a. Rapid decrease in the glutamine synthetase activity during im-

bibition of thermodormant New York lettuce seeds. - Plant Cell Physiol. 24: 1469-1476.

— & Matsubara, S. 1979. Measurement of growth potential of the embryo in New York lettuce seed under various combinations of temperature, red light and hormones. - Plant Cell Physiol. 20: 51-61.

Thanos, C. 1984. Phytochrome-mediated accumulation of free amino acids in radicles of germinating water melon seeds. Physiol. Plant. 60: 422-426.

Thompson, P.A. 1973. Geographic adaptation of seeds. In Seed Ecology (W. Heydecker, ed.) pp. 31-58. Proc. 19th Easter School in Agricultural Science, University of Nottingham. Butterworths, London.

— , Cox, S.A. & Sanderson, R.H. 1979. Characterization of the germination response to temperature of lettuce achenes. - Ann. Bot. 43: 319-334.

Totterdell, S. & Roberts, E.H. (1979). Effects of low temperatures on the loss of innate dormancy and the development of induced dormancy in seeds of Rumex obtusifolius L. - Plant, Cell and Environ. 2: 131-137.

Valdes, V.M., Bradford, K.J. & Mayberry, K.S. 1985. Alleviation of thermodormancy in coated lettuce seeds by seed priming. - HortScience 20: 1112-1114.

Vidaver, W. & Hsiao, A.I. 1975. Secondary dormancy in light-sensitive lettuce seeds incubated anaerobically or at elevated temperature. - Can. J. Bot. 53: 2557-2560.

Yemm, E.W. & Cocking, E.C. 1955. The determination of amino acids with ninhydrin. - Analyst. 80: 209-213.

Curriculum vitae

Op 27 augustus 1957 ben ik te Slochteren geboren. Van 1969 tot 1976 bezocht ik de Scholengemeenschap Westerkwartier-Noordenveld in Leek. Na het aldaar behalen van het diploma H.A.V.O. en V.W.O. begon ik aansluitend met de studie aan de Landbouw Universiteit Wageningen. In 1983 behaalde ik het ingenieurs-diploma met lof met als doctoraalvakken plantenveredeling, taxonomie van cultuurgewassen en -begeleiders en plantenfysiologie. Van juni 1983 tot juni 1986 ben ik als promotie-assistent werkzaam geweest op de vakgroep Plantenfysiologie van de Landbouw Universiteit Wageningen. Dit proefschrift is het resultaat van het verrichte onderzoek. Sinds september 1986 ben ik in dienst van Royal Sluis in Enkhuizen.

Roelf Weges

STELLINGEN

1. Het ideaal van de zaadtechnologie is het overbodig maken van voorbehandeling.
   Heydecker, W. & Coolbear, P. 1977. Seed Sci. Technol. 5: 353-425.
   Dit proefschrift.

2. De conclusie van Ellis & Roberts, dat tijdens de bewaring van rijst het verlies van kiemkracht losstaat van kiemrust, geldt niet voor sla.
   Ellis, R.H. & Robers, E.H. 1981. Seed Sci. Technol. 9: 373-409.
   Dit proefschrift.

3. Takeba beweert ten onrechte dat een daling van de osmotische potentiaal, ten gevolge van de ophoping van aminozuren, vooraf gaat aan het begin van de groei van het embryo van slazaden.
   Takeba, G. 1980. Plant Cell Physiol. 21: 1645-1649.
   Dit proefschrift.

4. Ten onrechte wordt door Bewley & Black de actuele waterpotentiaal van het zaad gelijk verondersteld aan de maximale osmotische potentiaal van het medium waarin het zaad nog juist kan kiemen.
   Bewley, J.D. & Black, M. 1985. Plenum Press, New York, pp. 224.
   Schopfer, P. & Plachy, C. 1985. Plant Physiol. 77: 676-686.
   Dit proefschrift.

5. Kiemrust van slazaden is rechtstreeks gecorreleerd met de minimale turgor waarbij groei van het embryo mogelijk is.
   Dit proefschrift.

6. Het lijkt onjuist dat het 1-aminocyclopropane-1-carboxylic acid (ACC) de enige mobiele stimulans is bij de door bestuiving versnelde bloemveroudering.
   Halevy, A.H., Whitehead, C.S. & Kofranek, A.M. 1984. Plant Physiol. 75: 1090-1093.
   Hoekstra, F.A. & Weges, R. 1986. Plant Physiol 80: 403-408.

7. De verschillende soortnamen voor Lactuca sativa L. (cultuursla) en Lactuca serriola L. (kompassla) zijn niet voldoende onderbouwd met morfologische, anatomische en genetische verschillen.
Linnaeus, C. 1753. Species Plantarum 2: 795.
Linnaeus, C. 1756. Centuria II Plantarum: 29.
Vries, I.M. de & Jarvis, C.E. 1987. Taxon, in press.

8. Het fysio-specifieke karakter van de resistentie van aardbei tegen Phytophthora fragariae Hickman (roodwortelrot) is in tegenspraak met de opvatting van kwantitatieve, partieel dominante overerving en is gebaseerd op een gebrekkig inzicht in de genetica van dit allo-octoploïde gewas.
Scott, D.H., Draper, A.D. & Galetta, G.J. 1985. Plant Breeding Reviews II: 195-214.

9. Ter voorkoming in de maatschappij van zowel ongerechtvaardigde angsten als ongerechtvaardigde verwachtingen dienen wetenschappers zich meer in te spannen om hun werk te verantwoorden.

10. In tegenstelling tot wat vaak gedacht wordt, kan op de praktijk gericht onderzoek nieuwe inzichten verschaffen op fundamenteel wetenschappelijk terrein.

11. De taak van studentenpastores om levensbeschouwelijke vragen aan de orde te stellen aan de universiteiten wordt des te belangrijker, naarmate de studie meer marktgericht is en er meer bezuinigd wordt op de studentenvoorzieningen.

12. Theologen dienen er rekening mee te houden dat ten gevolge van de voortgaande secularisatie hun taal in toenemende mate onbegrijpelijk wordt.

13. De fysieke stress, veroorzaakt door hardlopen, is een goede therapie voor de gevolgen van de psychische stress, veroorzaakt door het promotie-assistentschap.

Roelf Weges, Physiological analysis of methods to relieve dormancy of lettuce seeds. Wageningen,28 januari 1987.