SEED RESEARCH FOR IMPROVED TECHNOLOGIES

R.J. BINO[1]; H. JALINK[1]; M.O. OLUOCH[1]; S.P.C. GROOT[1]

[1]Centre for Plant Breeding and Reproduction Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands.

ABSTRACT: The production of high-quality seed is the basis for a durable a profitable agriculture. After production, seed is processed, conditioned, stored, shipped and germinated. For quality assurance, seed quality has to be controlled at all steps of the production chain. Seed functioning is accompanied by programmed transitions from cell proliferation to quiescence upon maturation and from quiescence to reinitiation of cellular metabolism upon imbibition. Despite the obvious importance of these control mechanisms, very little information is available at the molecular level concerning those elements that regulate seed germination. In the present study, the induction of cell cycle activity and the regulation of ß-tubulin expression is related to the water content and other physical properties of the seed.
Key Words: seed, quality

PESQUISA PARA O APRIMORAMENTO DE TECNOLOGIA DE SEMENTES

RESUMO: A produção de sementes de alta qualidade é a base para uma agricultura produtiva. Após a colheita, a semente é beneficiada, embalada, armazenada, transportada e semeada. Para maior segurança, tanto dos produtores como dos consumidores, a qualidade da semente deve ser mantida sob controle em todas as fases do processo de produção. O desempenho da semente é resultado de transições programadas desde a divisão celular até a quiescência, durante a maturação, e da quiescência até o reinicio do metabolismo celular, durante a embebição. Apesar da importância destes mecanismos de controle, há pouca informação disponível, a nível molecular, no que diz respeito aos elementos que regulam a germinação da semente. No presente trabalho, a indução do ciclo de atividade celular e a regulação da expressão de ß-tubulina são relacionadas ao grau de umidade e a outras propriedades físicas da semente.
Descritores: sementes, qualidade

INTRODUCTION

The success in stand establishment of a good crop is limited by adverse environmental conditions, soil borne diseases, and soil crusting. Together, these factors serve to reduce germination and seedling emergence of many crop species. The establishment of a good seedling stand is a prerequisite for improved yield and quality (Wurr & Fellows, 1983). The seed is the primary propagation material for most commercial crop production and successful seed quality management is thus vital for sustainable crop production. Although various applied and basic research programs aimed at improving seed quality are currently in use, new developments in technological advances have introduced new techniques for effective and standardised characterisation of seed quality. Physiological, biochemical, molecular, and biophysical mechanisms are currently being used to develop applied and fundamental markers essentially linked to seed quality.

PHYSIOLOGICAL MECHANISMS

The seed occupies a critical stage in the life cycle of crop plants. The success in crop stand establishment is thus largely determined by the physiological and biochemical features of the seed that can sustain the plant in the early stages of seedling growth (Bewley & Black, 1994).

Germination: in analysing the physiology of germination, population models based on empirical mathematical functions have been used to describe germination time courses (Bradford, 1995). A germination time course is the accumulation with time of terminal physiological events within a seed

population that reflects the lag periods individual seeds require from the start of imbibition to the point of radicle emergence. Growth models have been developed that can analyse and predict germination rate distributions within a given seed population.

Seed priming: various types of seed treatments, geared towards improving germination under adverse conditions have been reported. Nerson et al. (1985) showed that higher germination at low temperatures could be obtained in watermelons by seed coat splitting. Seed coat removal in melon seeds improved germination at low water potential (Dunlap, 1988) and low temperature (Edelstein et al., 1995). Seed priming has been used extensively to improve germination of many species. Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination but permits pregerminative physiological and biochemical changes to occur (Bradford, 1986; Khan, 1992; Heydecker & Coolbear, 1977). Upon rehydration, primed seeds may exhibit faster rates of germination, more uniform emergence, greater tolerance to environmental stress, and reduced dormancy in many species (Khan, 1992). The two most common types of priming treatments is osmotic and solid matrix. These priming treatments rely on the osmotic and matric property of the priming solution or media, respectively. Prehydration in water has emerged as a useful and effective priming technique that is cheaper and manageable in comparison to osmotic and matric treatments. It has been demonstrated with muskmelon (Oluoch & Welbaum, 1996a, b) and tomato (Oluoch et al., unpublished results) that an optimal priming treatment is required to achieve the best criteria in seed vigour. In some studies, priming treatments adversely affected the storage life of tomato (Alvarado & Bradford, 1988; Owen & Pill, 1994), wheat (Nath et al., 1991), and muskmelon (Oluoch & Welbaum, 1996) seeds but did not adversely affect the storage life of tomato (cv. lerica) (van Pijlen et al., 1996), carrot, and leek seeds (Dearman et al., 1987). The response of primed seeds to storage is species and variety dependant. It is thus important that for the beneficial effects of priming to be retained, new techniques and markers have to be used to monitor the priming progress.

Seed maturity: the production of good quality seed requires timely harvest at physiological maturity for maximal seed vigour. Seed development falls in three phases. The first phase begins with fertilisation and rapid cell division. The second phase is characterised by cell expansion and accumulation of reserve materials by the embryo and endosperm. The third phase is characterised by water loss from the seed as seeds pass into a quiescent or dormant state before metabolic systems are re-activated upon imbibition (Bradford, 1986). In many orthodox seeds, such as cereals or other grain crops, desiccation coincides with the attainment of maximal dry weight and physiological maturity (Rosenberg & Rinne, 1986). Investigations relating maize seed quality to maturity has shown that maximal germination occur at high moisture levels early in development before the seed attains physiological maturity (TeKrony & Hunter, 1995). However, determining the optimum time of harvest for seeds that develop inside fleshy fruits is more complicated because seed maturation may precede fruit maturity (Coombe, 1976). In overripe tomato fruits, a high percentage of seeds may undergo precocious germination (vivipary) while in muskmelon fruits, seeds are either dead or had very low vigour if harvest was delayed until fruits start decomposing (Welbaum, 1993; Oluoch & Welbaum, 1996b). When fruits are harvested prematurely, seeds require dry storage (afterripening), washing, or priming to improve vigour to the same level as mature seeds (Welbaum & Bradford, 1991; Oluoch & Welbaum, 1996b). Harvesting after fruit maturation and dry afterripening increased germination in various *Capsicum* species by overcoming seed dormancy (Edwards & Sundstrom, 1987). In fleshy fruits, the highest quality seed may be obtained from fruits harvested at edible maturity but before the onset of severe decomposition. Therefore, seeds must be harvested at the correct stage of development to obtain maximum viability and vigour.

BIOCHEMICAL AND MOLECULAR MECHANISMS

Biochemical and molecular markers are currently being used that can effectively monitor the efficiency of priming treatments, determine the optimum time for harvest, longevity of seeds in storage, and in general, detection of events that are closely associated with seed quality. For effective use of biochemical and molecular tools in relation to seed quality, interactive mechanisms need to be essentially linked to processes that regulate germination.

Enzymatic activity: in endospermic seeds, germination is an integrated process where the increase in embryo growth potential, endosperm softening, and germination is thought to occur concurrently. It has been hypothesised that cell wall loosening enzymes modify the wall to allow turgor-driven extension of the radicle (Cosgrove, 1993). The endosperm cell walls in most species are primarily composed of manna or galactomannan polymers and thus endo-β-mannanase, galactosidase, and mannohydrolase are thought to be the enzymes closely related to their breakdown (Halmer et al., 1975; Groot et al., 1988; Sanchez et al., 1990; Dutta et al., 1997; Dahal et al., 1997). Endo-β-mannanase has been identified as the key cell wall degrading enzyme closely linked to the breakdown of endosperm cell walls during the germination of tomato seeds (Groot et al., 1988) and pre- and post-germinative galactomannan hydrolysis in the endosperm cell walls of lettuce (Dutta et al., 1994; Dulson et al., 1988) and *Datura* spp. (Sanchez et al., 1990). The synthesis of mannanase in the lettuce endosperm for galactomannan reserve mobilisation appears to be regulated by hormones cytokinin and gibberellins and feedback inhibited by abscisic acid (ABA) (Bewley & Black, 1994). Endo-β-mannanase activity has shown potential as a marker in determining priming and germination progression in tomato and physiological maturity in muskmelon seeds. Mannanase activity increases with priming or seed development then declines gradually with prolonged priming duration or maturity (Oluoch et al., unpublished data). The characterisation and expression of enzymatic activity in many seed species show potential for use in determining seed quality.

Cell cycle events: the expression and quantification of the regulation of cell cycle events has emerged as an important and promising process in characterising seed quality. The cellular and molecular events during seed germination occur in a sequence of activated processes within the cell nucleus. These processes are mainly DNA synthesis and cell division and are termed cell cycle events. The cell cycle starts at mitosis (M) phase when chromosomes are in the two stranded configuration (2C). This is followed by the G1 (Gap 1) phase which is the period during normal cell growth, then the DNA synthesis (S phase) stage which results in the doubling of the chromosome to a 4C configuration but without cell division. Finally, the second growth period (G2) phase occur before mitosis (Bewley & Black, 1994). Flow cytometry has opened possibilities for quantifying cell cycle events and for the estimation of nuclear DNA content in seeds. By incorporating DNA-specific fluorescent dyes, nuclear DNA contents expressed as C values have been quantified in tomato (Bino et al., 1992; Liu et al., 1994) and pepper (Lanteri et al., 1993) seeds during germination and priming. The maintenance of DNA integrity during cell division and differentiation is of critical importance to normal seed germinative processes. The characterisation of DNA damage by use of flow cytometry has opened possibilities for its use in seed quality analysis.

As cells undergo division and differentiation, microtubules assemble, disassemble and rearrange into new configurations that are dependant on the interactions with microtubule associated proteins (Goddard et al., 1994). These proteins are mainly α- and β-tubulin polypeptides. The expression of β-tubulin during germination and priming has shown promise as a parameter for determining seed quality. Through the use of two-dimensional PAGE, immuno-cytochemistry and western blotting techniques, de Castro et al. (1995) showed that β-tubulin accumulates in tomato seeds during germination and priming and the expression precedes visible germination.

Chlorophyll fluorescence: a new method has been developed for the assessment of maturity and quality of seeds. The method is based on measuring nondestructively the relative amount of chlorophyll a of individual seeds. In general, during maturation the amount of chlorophyll in the seed and seed coat decreases. The quality of seeds is directly related to their maturity status. For instance, cabbage (*Brassica oleracea* L.) seeds mature in a dry silique as part of a determinate inflorescence with flowers of various ages located on side branches. A seed lot may therefore include seeds of varying maturation stages. The differences in maturity of individual seeds may become even larger, due to adverse weather conditions preceding the moment of harvest, or because of plant to plant differences due to variations in soil conditions. The newly developed method uses the unique property of chlorophyll that it shows fluorescence when it is excited by light of the proper wavelength. Using this property and a combination of red lasers to excite the chlorophyll and narrow bandwidth filters to filter out the fluorescence, the chlorophyll in seeds can be determined with a much

higher sensitivity and selectivity than by conventional methods like colour or reflection spectrum measurements. We use chlorophyll fluorescence to show the differences in relative amounts of chlorophyll of individual seeds in a seed lot. Based on the magnitude of the chlorophyll fluorescence signal we have sorted seeds of a number of crops (cabbage, carrot, sugar beet) and flower seeds (Impatiens, Pelargonium, Cyclamen persicum) in various classes of maturity and have linked the chlorophyll signal to seed performance. Seeds from the high CF class germinated at a much lower percentage and resulted in a much lower amount of normal seedlings as compared to the seeds with low and medium CF values. Apparently, the quality of the seeds was inversely related to the amount of chlorophyll in the seed coat. The differences in quality between the three CF classes became even more evident in the controlled deterioration stress test. The results show that chlorophyll fluorescence can be used to improve the quality of seeds by removing less mature seeds and indicate the possibility to use chlorophyll as a new non-destructive marker for the physiological maturity of seeds. CF of seeds can also be used to analyse seed lots with respect to their maturity distribution and as a new tool to study stress physiology. Advantages of the CF method for determining seed maturity and seed quality are the very high sensitivity, the method being fully non-destructive and the very high speed at which the fluorescence is generated and measured.

Genetic purity: several studies in recent years have demonstrated that seedlots of high quality may vary in their ability to germinate with resultant differences in seedling establishment and a decrease in crop yield (Bray, 1997). The stresses encountered during pre- and post-harvest storage has some influence in the variation in vigour of otherwise uniform seedlots. *In situ* hybridisation methods for characterising temporal and spatial regulation of seed proteins have shown expression patterns that are closely linked to seed quality (Chandler et al., 1983). The development of transgenic mutant plants has also facilitated the localisation of important regulatory proteins that characterise the expression of seed quality genes. Studies on hormone biosynthesis and hormonal response in mutant plants have added a new approach to the study of molecular processes that regulate seed germination. Gibberellic (GA) and abscisic acid (ABA) deficient tomato mutants have been developed. The characterisation and expression of the key biochemical and molecular processes (e.g. hydrolytic enzymes) that are regulated by GA and ABA have shown potential for use as markers in seed quality analysis.

BIOPHYSICAL MECHANISMS

The time from sowing to seedling establishment is thus a crucial phase in crop production and can have a major impact on final yield and postharvest seed quality (Wurr and Fellows, 1983). Seed germination is a triphasic process which begins with the rapid initial water uptake (phase I), a plateau phase with little change in water content (phase II), and an increase in water content that coincides with radicle growth (phase III) (Bradford, 1986). The length of phase three is important since germination is considered complete when embryo growth is initiated. The soil environment on the other hand is never conducive for rapid germination and seedling growth. Adverse temperatures, soil crusting, limited or excess water, salinity, and disease pathogens all combine to create a stressful environment for germination and seedling emergence. Water relations are central to understanding the adaptive strategy that allows the seed to germinate and emerge under adverse conditions.

Water relations: the expression and quantification of biophysical mechanisms closely related to seed germination processes has generated fundamental parameters for understanding the influence of physiological processes on seed vigour. Using the moisture release curves approach, psychrometric measurements have demonstrated in lettuce (Bradford, 1986) and muskmelon (Oluoch, 1996) that primed seeds have a lower water potential (ψ) and a higher turgor potential (ψ_p) than nonprimed seeds. The effect is correlated to the improvement in germination rates after priming. It has been suggested that during priming, seeds may accumulate solutes that are retained through drying and rehydration. The hydrostatic turgor pressure of the embryonic axes may increase through the accumulation of osmotic solutes allowing the radicle to exceed the yield threshold of the tissues surrounding the radicle (Bradford, 1986, 1995; Thanos, 1984).

Germination modelling: in many species, the endosperm tissue completely envelops the embryo and restrains the germination process by presenting

a physical barrier which restricts radicle emergence (Weges, 1987; Bradford, 1995). The reduction in yield threshold during germination in these seeds involve enzymatic degradation of endosperm tissues that form a physical barrier to radicle growth (Ni & Bradford, 1993). Scanning electron microscopy has revealed images that offer evidence of enzymatic degradation of the endosperm tissue during seed germination. Intracellular cracks were observed in the micropylar endosperm tissue of muskmelon seeds during germination and culminated with the eventual rupture of the endosperm in coincidence with radicle emergence (Welbaum et al., 1995). The mechanical resistance of the endosperm tissue surrounding the radicle depicts the force and energy the radicle requires to puncture the barrier tissue for germination to occur. The use of an Instron Universal Testing Machine has shown significant promise in quantifying this resistance. Instron measurements have revealed that the strength of the endosperm tissue declines during germination in pepper (Watkins & Cantliffe, 1983), lettuce (Tao & Khan, 1979), *Datura* spp. (Sanchez et al., 1986), tomato (Groot & Karssen, 1987), and muskmelon (Welbaum et al., 1995; Wilson et al., 1994) seeds. Instron measurements have also offered evidence for priming induced hydrolysis of the endosperm tissue of muskmelon seeds (Oluoch & Welbaum, 1996a; Wilson et al., 1994).

Image analysis: image analysis has emerged as a practical technological approach for assessing individual characteristics of seeds in a quantifiable process using criteria such as distinctness, homogeneity, and stability. Image analysis is an automated technique where images are recorded, processed, and analysed with the aid of a computer. The computer image is a matrix of points where each point is expressed as a certain value (e.g. brightness or colour).The application of arithmetical operations leads to recognition of objects in the image. Image analysis is as good as manual assessment but increases objectivity, efficiency, and reproducibility. Image analysis has been used for variety testing of several species (Vooren & Van der Heijden, 1993; Van der Heijden et al., 1996). The quantification and discrimination of the characteristic height and diameter by image analysis has made possible its use for the granting of plant breeders rights (UPOV, 1976). In seed quality analysis, image analysis is currently being used to characterise seedlots by size and shape and to quantify the free space in the embryo of poor quality seed lots.

CONCLUSION

A successful seed quality management program requires an integrated approach that incorporates advanced technological applications for efficient management strategies. This strategy envisions the use of modern physiological, biochemical, molecular, and biophysical research techniques so as to generate useful information that can be used as reliable markers for seed quality analysis or for breeding tools. The incorporation of seed research with new advancements in molecular biology and physics holds the key to the understanding and integration of multiple mechanisms that can lead to improved seed germination, better stand establishment, and higher crop yield.

REFERENCES

ALVARADO, A.D.; BRADFORD, K.J. Priming and storage of tomato (*Lycopersicon lycopersicum*) seeds: I. Effects of storage temperature on germination rate and viability. **Seed Science and Technology**, v.16, p.601-612, 1988.

BEWLEY, J.D.; BLACK, M. **Seeds**: physiology of development and germination. New York: Plenum Press, 1994. 445p.

BINO, R.J.; DE VRIES, J.N.; KRAAK, H.L.; VAN PIJLEN J.G. Flow cytometric determination of nuclear replication stages in tomato seeds during priming and germination. **Annals of Botany,** v.69, p.231-236, 1992.

BRADFORD, K.J. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. **HortScience, v.**21, p.1105-1112, 1986.

BRADFORD, K.J. Water relations in seed germination. In: Kigel, J.; Galili, G. (Ed.) **Seed development and germination**. New York: Marcel Dekker, 1995. p.351-395.

BRAY, C. M. Stress, protein biosynthesis and loss of vigour and viability in cereal seed. In: ELLIS, R.H.; BLACK, M.; MURDOCH, A.J.; HONG, T.D. (Ed.) **Basic and applied aspects of seed biology**. s.l.: s.ed., 1997. p.437-449.

CHANDLER, P.M.; HIGGINS, T.J.V.; RANDALL, P.J.; SPENCER, D. Regulation of legumin levels in developing pea seeds under conditions of sulfur deficiency: rates of legumin synthesis and levels of legumin mRNA. **Plant Physiology,** v.71, p.47-54, 1983.

COOMBE, B.G. The development of fleshy fruits. **Annual Review of Plant Physiology,** v.27, p.507-528, 1976.

COSGROVE, D.J. How do plant cell walls extend. **Plant Physiology,** v.102, p.1-6, 1993.

DAHAL, P.; NEVINS, D.J.; BRADFORD, K.J. Relationship of endo-(β-D-mannanase activity and cell wall hydrolysis in tomato endosperm to germination rates. **Plant Physiology,** v.113, p.1243-1252, 1997.

DEARMAN, J.; BROCKELHURST, P.A.; DREW, R.L.K. Effects of osmotic priming and aging on the germination and emergence of carrot and leek seed. **Annals Applied Biology,** v.111, p.717-722, 1987.

DE CASTRO, R.D.; ZHENG, X.; BERGERVOET, J.H.W.; DE VOS, C.H.R.; BINO, R.J. (β-tubulin accumulation and DNA replication in imbibing tomato seeds. **Plant Physiology,** v.109, p.499-504, 1995.

DULSON, J.; BEWLEY, J.D.; JOHNSTON, R.N. Abscisic acid is an endogenous inhibitor in the regulation of mannanase production by isolated lettuce (*Lactuca sativa* cv. Grand rapids) endosperm. **Plant Physiology,** v.87, p.660-665, 1988.

DUNLAP, J.R. Effects of temperature and water potential on the germination of muskmelon cultivars. **Annals Applied Biology,** v.112, p.187-194, 1988.

DUTTA, S.; BRADFORD, K.J.; NEVINS, D.J. Cell wall autohydrolysis in isolated endosperm of lettuce (*Lactuca sativa* L.). **Plant Physiology**, v.104, p.623-628, 1994.

DUTTA, S.; BRADFORD, K.J.; NEVINS, D.J. Endo-(β-mannanase activity present in cell wall extracts of lettuce endosperm prior to radicle emergence. **Plant Physiology**, v.113, p.155-161, 1997.

EDELSTEIN, M., CORBINEAU, F.; KIGEL, J.; NERSON, H. Seed coat structure and oxygen availability control low-temperature germination of melon (*Cucumis melo*) seeds. **Physiologia Plantarum**, v.93, p.451-456, 1995.

EDWARDS, R.L.; SUNDSTROM, F.J. Afteripening and harvesting effects on tabasco pepper seed germination performance. **HortScience**, v.22, p.473-475, 1987.

GODDARD, R.H.; WICK, S.M.; SILFLOW, C.D.; SNUSTAD, D.P. Micro-tubule components of the plant cell cytoskeleton. **Plant Physiology**, v.104, p.1-6, 1994.

GROOT, S.P.C.; KIELISZEWSKA-ROKICKA, B.; VERMEER, E.; KARSSEN, M. Gibberellin- induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. **Planta**, v.174, p.500-504, 1988.

GROOT, S.P.C.; KARSSEN, M. Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellins deficient mutants. **Planta**, v.171, p.525-531, 1987.

HALMER, P.; BEWLEY, J.D.; THORPE, T.A. Enzyme to breakdown lettuce endopserm cell wall during gibberellin- and light-induced germination. **Nature**, v.258, p.716-718, 1975.

HEYDECKER, W.; COOLBEAR, P. Seed treatments for improved performance. Survey and attempted prognosis. **Seed Science and Technology**, v.5, p.353-425, 1977.

KHAN, A.A. Preplant physiological seed conditioning. **Horticultural Reviews**, v.14, p.131-181, 1992.

LANTERI, S.; KRAAK, H.L.; DE VOS, C.H.R.; BINO, R.J. Effects of osmotic preconditioning on nuclear replication activity in seeds of pepper (C*apsicum annuum*). **Physiologia Plantarum**, v.89, p.433-440, 1993.

LIU, Y.; BERGERVOET, J.H.W.; DE VOS, C.H.R.; HILHORST, H.W.M.; KRAAK, H.L.; KARSSEN, C.M.; BINO, R.J. Nuclear replication activities during imbibition of abscisic acid- and gibberellin-deficient tomato (*Lycopersicon esculentum* Mill.) seeds. **Planta**, v.194, p.368-373, 1994.

NATH. S., COOLBEAR, P.; HAMPTON, J.G. Hydration-dehydration treatments to protect or repair stored 'karamu' wheat seeds. **Crop Science,** v.31, p.822-826, 1991.

NERSON, H.; PARIS, H.S.; KARCHI, Z.; SACHS, M. Seed treatments for improved germination of tetraploid watermelon. **HortScience**, v.20, p.897-899, 1985.

NI, B-R.; BRADFORD, K.J. Germination and dormancy of abscisic acid-and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds. Sensitivity of germination to abscisic acid, gibberellin,and water potential. **Plant Physiology**, v.101, p.607-617, 1993.

OLUOCH, M.O. Effects of priming and development on vigor and longevity of muskmelon (*Cucumis melo* L.) seeds. Blacksburg, 1996. Thesis (Ph.D.)- Virginia Polytechnic Institute and State University.

OLUOCH, M.O.; WELBAUM, G.E. Viability and vigor of osmotically primed muskmelon seeds after nine years of storage. **Journal of the American Society for Horticultural Science,** v.121, p.408-413, 1996a.

OLUOCH, M.O.; WELBAUM, G.E. Effect of postharvest washing and post-storage priming on viability and vigour of six year old muskmelon (*Cucumis melo* L.) seeds from eight stages of development. **Seed Science and Technology**, v.24, p.195-209, 1996b.

OWEN, P.L.; PILL, W.G. Germination of osmotically primed asparagus and tomato seeds after storage up to three months. **Journal of the American Society for Horticultural Science**, v.119, p.636-641, 1994.

ROSENBURG, L.A.; RINNE, R.W. Moisture loss as a prerequisite for seedling growth in soybean seeds (*Glycine max* (L.) Merr.). **Journal of Experimental Botany**, v.43, p.307-317, 1986.

SANCHEZ, R.A.; DE MIGUEL, L.; MECURI, O. Phytochrome control of cellulase activity in Datura ferox embryos and its relationship with germination. **Journal of Experimental Botany,** v.37, p.1574-1580, 1986.

SANCHEZ, R.A.; SUNNEL, L.A.; LABAVITCH, J.M.; BONNER, B.A. Changes in the micropylar region of the endosperm before radicle protrusion in the seeds of two Datura species. **Plant Physiology**, v.83, p.90-118, 1990.

TAO, K.; KHAN, A.A. Changes in the strength of lettuce endosperm during germination. **Plant Physiology,** v.62, p.126-128, 1979.

TEKRONY, D.M.; HUNTER, J.L. Effect of seed maturation and genotype on seed vigor in maize. **Crop Science**, v.35, p.857-862, 1995.

THANOS, C. Phytochrome-mediated accumulation of free amino acids in radicles of germinating watermelon seeds. **Physiologia Plantarum**, v.60, p.422-426, 1984.

UPOV. **Guidelines for the conduct of tests for distinctness, homogeneity and stability**: onion (*Allium cepa* L.) TG/46/3. Geneva: International Union for the protection of New Varieties of Plants, 1976.

VAN DER HEIJDEN, G.W.A.M.; VOSSEPOEL, A.M.; POLDER, G. Measuring onion cultivars with image analysis using inflection points. **Euphytica**, v.87, p.19-31, 1996.

VAN PIJLEN, J. G.; GROOT, S.P.C.; KRAAK, H.L.; BERGERVOET, J.H.W.; BINO, R.J. Effects of pre-storage hydration treatments on germination performance, moisture content, DNA synthesis and controlled deterioriation tolerance of tomato (*Lycopersicon esculentum* Mill.) seeds. **Seed Science Research,** v.6, p.57-63, 1996.

VOOREN, J.G.; VAN DER HEIJDEN, G.W.A. Measuring the size of french beans with image analysis. **Plant Varieties and Seeds,** v.6, p.47-53, 1993.

WATKINS, J.T.; CANTLIFFE, D.J. Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperature. **Plant Physiology,** v.72, p.146-150, 1983.

WEGES, R. Physiological analysis of methods to relieve dormancy of lettuce seeds. Wageningen, 1987. Thesis (Ph.D.) - Agricultural University.

WELBAUM, G.E. Water relations of seed development and germination in muskmelon (*Cucumis melo* L.): VIII. Development of osmotically distended seeds. **Journal of Experimental Botany**, v.44, p.1245-1252, 1993.

WELBAUM, G.E.; BRADFORD, K.J. Water relations of seed development and germination in muskmelon (*Cucumis melo* L.): VI. Influence of priming on germination responses to temperature and water potential during seed development. **Journal of Experimental Botany**, v.42, p.393-399, 1991.

WELBAUM, G.E.; MUTHUI, W.J.; WILSON, J.H.; GRAYSON, R.L.; FELL, R.D. Weakening of muskmelon perisperm envelope tissue during germination. **Journal of Experimental Botany**, v.46, p.391-400, 1995.

WILSON, J.H.; WELBAUM, G.E.; OLUOCH, M.O. Punture test to evaluate the effects of priming on seed germination. In: MEETING OF THE AMERICAN SOCIETY FOR AGRICULTURAL ENGINEERS, Winter, Atlanta, 1994. **Proceedings.** St. Joseph: ASAE, 1994. (Paper no 946511).

WURR, D.; FELLOWS, J. The effect of the time of seedling emergence of crisp lettuce on the time of maturity and head weight at maturity. **Journal Horticultural Science**, v.58, p.561-566, 1983.

Recebido para publicação em 10/07/98
Aceito para publicação em 03/08/98