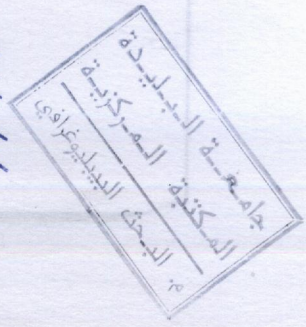

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Incubation temperature affects changes in cucumber seed proteins and mineral content

V. M. RUSSO[1] and C. L. BILES[2]

[1] United States Department of Agriculture, Agricultural Research Service, South Central Agricultural Research Service, POB 159, Lane, OK 74555
[2] Biology Department, East Central University, Ada, OK 74820

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Summary

Suboptimal soil temperatures retard germination of cucumber (*Cucumis sativus* L.) seed leading to uneven stands and reduced yield. This project was undertaken to determine how concentration of minerals (N, NO_2^-, NO_3^-, P, HPO_4^{-2}, K, SO_4^{-2}, Ca, Fe, Mg, Mn, and Na) and density of protein bands in seed of the cvs. 'Arkansas Little Leaf' and 'Earlipik 14' changed during germination and radicle elongation. Seed were incubated in distilled water in petri dishes at 13.9, 15.6 and 20°C. Seed were analyzed for mineral content and proteins prior to incubation and then at intervals up to 168 hr. At 20°C, at least 90% of seed of both cultivars germinated by 48 hr. The content of N, P, HPO_4^{-2} and SO_4^{-2} in 'Earlipik 14' seed were significantly less at 13.9 or 15.6 than at 20°C. This suggests leakage of minerals occurred at suboptimal temperatures. After 24 hr incubation at 20°C the density of the 70.1 Kda protein band in gels did not increase, while density of 37.4, 43.4, and 50 Kda protein bands increased in seed of both cultivars. At the other temperatures density of protein bands in gels increased until 120 hrs. The data suggests that low cucumber germination rates at suboptimal temperatures were related to: 1) leakage of minerals, and/or 2) the lack of formation or denaturation of proteins associated with germination and radicle elongation.

Introduction

Early and uniform establishment of cucumber (*Cucumis sativus* L.) is problematic because of reduced germination at low temperatures (Lorenz and Maynard, 1988). Yield of pickling cucumbers grown for once-over harvest is related to stand uniformity (Nienhuis *et al.*, 1983). Research on the physiological aspects of low temperature germination has been conducted (Nelson and Sharples, 1980), and breeding programs have searched to improve low temperature germination in cucumber (Nienhuis *et al.*, 1983; Wehner, 1984). Simon *et al.* (1976) suggested that denaturation of unspecified cucumber proteins was the most likely reason that seed did not germinate well at low temperatures. If this is the case, there may be problems in membrane reestablishment (Simon, 1974), which may prevent the formation of organelles or compromise the integrity of plasma membranes. Zaiter *et al.* (1994) reported that in bean (*Phaseolus vulgaris* L.) there was an in-

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crease in the synthesis of some polypeptides that probably had an essential role in the development of chilling tolerance. The proteins that are essential for commencement of radicle elongation and/or differentiation have not been identified (Kermode, 1995).

The ability of cucumber to germinate at low temperatures is, in part, cultivar dependent (Lower, 1974). Germination at low temperatures has been improved by pregermination treatment. Edwards *et al.* (1986) increased germination by fermenting seed for 4 days at 25 °C. Nelson and Sharples (1980) reported that soaking cucumber seed in fusicoccin increased germination, and fusicoccin was more effective than gibberellic acid (GA4/7, GA_3), or ethephon.

Growth and morphogenesis is dependent on the availability and activity of elements and compounds used in formation of organelles and tissues. Exposure to suboptimal temperatures may interfere with membrane formation causing minerals to leak and interfere with normal germination. This project was undertaken to record changes in proteins and minerals and relate them to germination of cucumber seed over time at optimal and suboptimal temperatures.

Materials and methods

Germination and preparation of tissue for analysis

Two layers of filter paper (Whatman 42, Fisher Scientific, Houston, TX) were placed in petri dishes and moistened with 20 ml of distilled water. Seed of the cucumber cultivars 'Arkansas Little Leaf' (AR 79-75) and 'Earlipik 14', purchased from Stokes Seed® (Buffalo, NY), were used. The latter is a gynoecious hybrid with 15% 'Sumter' blended as pollinater. The pollinater seed were identified with a colored coating and therefore could be removed so that effects of this genotype and the seed coating would not confound results. These cultivars were choosen because they showed syncrony of harvest and promising yields for the local conditions, and because they have the potential to be used in relay cropping systems.

Fifty untreated seed of each cultivar were placed in individual petri dishes. Seed were incubated at 13.9, 15.6, and 20 °C in the dark in a growth chamber. Seed in petri dishes were removed at each incubation time [0 (preimbibition), 12, 24, 36, 48, 60, 72, 84, 96, 120 and 168 hrs], and percent germination recorded when the radicle was at least equal to the width of the seed. Subsamples from each incubation time were prepared for mineral or protein analysis. At some sampling times there were ungerminated seed and at others there were germinated and ungerminated seed present in petri dishes. Where both were present only germinated seed were used. At incubation times and temperatures where germination was low sufficient numbers of petri dishes were used to provide tissue for analyses. Samples were oven dried (32 °C), ground in a Wiley mill and passed through a 20 mesh screen and stored at –20 °C until analyzed.

Mineral analysis

Extraction of tissues to determine levels of total Kjeldahl-N (N), total phosphorous in a Kjeldahl digest (P), NO_2^-, Ca, Fe, K, Na, Mg, and Mn was conducted according to

Jones (1981), Baker and Suhr (1982), and Jones and Case (1990). Levels of N, P, and NO_3^- were determined with an automated ion analyzer (AIA; Model AE, Lachat, Inc., Milwaukee, WI) in the flow injection analysis mode. Levels of Ca, Fe, K, Na, Mg, and Mn were determined with a double beam atomic absorption spectrophotometer (Model 902, GBC, Melbourne, Australia). The automated sampling and recording of levels of these minerals was controlled through the software in the AIA. Mineral content was reported as $mg \cdot g^{-1}$ of dry weight.

Extraction of anions was performed with the methods of Basta and Tabatabi (1985), with the exception that tissues were not ignited with alcoholic sulfonic acid prior to placement in a muffle furnace. Digested samples were passed through a 0.45 mM cellulose nitrate/cellulose acetate membrane filter (Westboro, MA). Levels of anions (NO_3^-, HPO_4^{-2}, SO_4^{-2}) were determined with the AIA in the ion chromatography mode. The eluent used in conjunction with solid phase reagent, according to the methods provided by Lachat, was 1.7 mM sodium hydrogen carbonate plus 2.2 mM sodium carbonate with a pump rate of 2 $mL \cdot min^{-1}$. All samples were compared to standard curves for all minerals, ions and compounds tested prepared according to methods for the analytical instruments.

Protein extraction and gel electrophoresis

Samples (1 g) were ground with a mortar and pestle in a mixture of 0.1 M sodium phosphate buffer, pH 7, 50 mg of polyvinylpyrrolidone, and 100 mg of sterile quartz sand. The slurry was centrifuged (5 min at 12,000 g) and the supernatant filtered twice through a 0.45 mM cellulose nitrate/cellulose acetate membrane filter. Bromophenol blue loading buffer (5X) was added to the supernatant which was then boiled for 3 min and the sample centrifuged (3 min at 12,000 g) before loading onto the gel. Each lane was loaded with 5 mg of protein sample.

Total protein was separated on 4% stacking and 12% separation SDS-PAGE slab gels (0.75 mm thick) using the Laemmli (1970) buffer system, according to the instructions provided by BioRad (BioRad, Hercules, CA). Gels were electrophoresed for 10 min at 100 V and then 200 V (constant voltage) until the bromophenol tracking dye front moved off the gel (approximately 40 min). Gel proteins were stained with colloidal brilliant blue G-250 (Neuhoff *et al.*, 1988). Density of protein bands were determined qualitatively by staining. BioRad low molecular weight protein standards (14.5 to 97.4 Kda) were used to estimate protein size.

Statistical analysis was performed on germination and mineral content data. The experimental design was a split-plot, with temperature as the main plot and cultivar as the split, arranged in a randomized complete block with three replications. Data were analyzed with procedures in SAS (Ver. 6.1, SAS, Cary, NC).

Results

Germination

Germination trends in both cultivars were similar (Figure 1A, B). Approximately 90% of seed incubated at 20°C germinated, with at least 80% of germination occurring be-

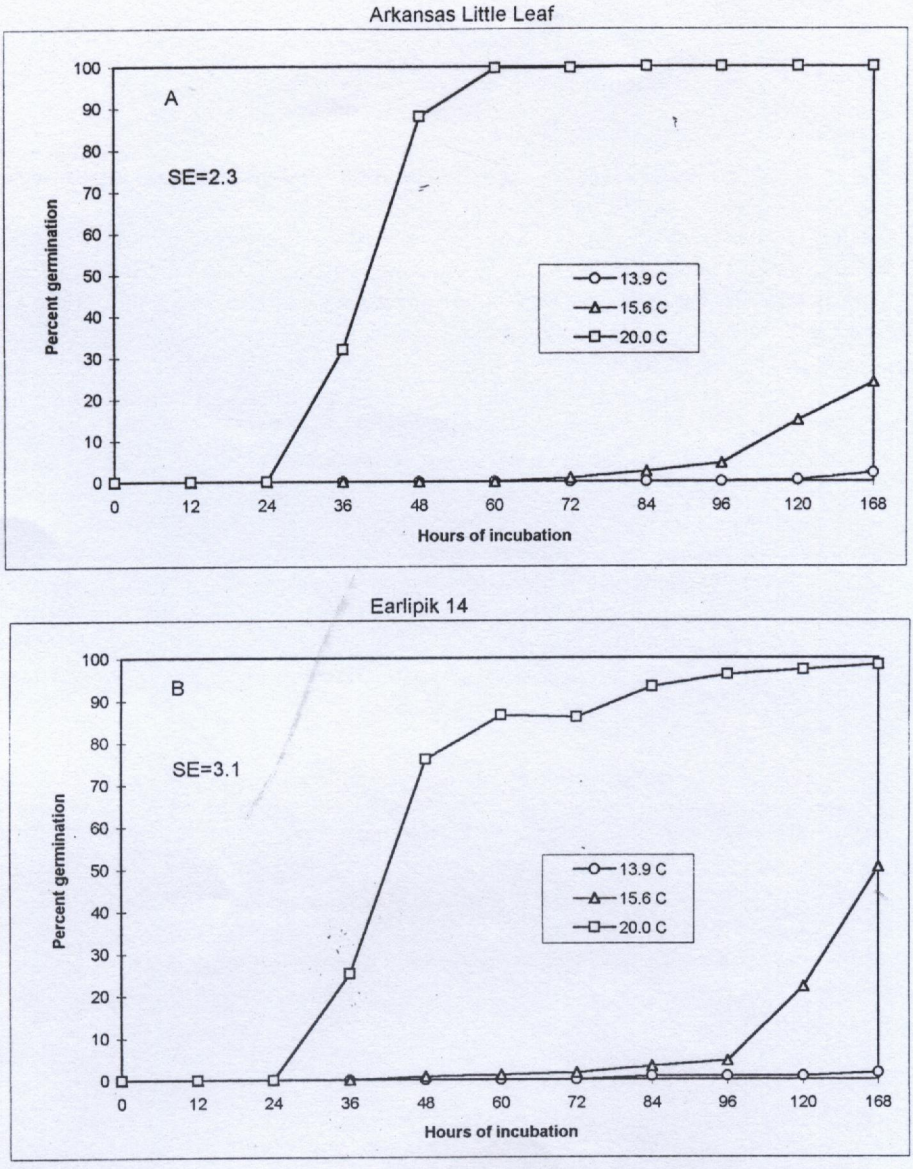

Figure 1. Average germination of seed of cucumber cultivars incubated at 13.9, 15.6, and 23 °C. Verticle bars represent standard error.

tween 36 and 48 hr. For seed incubated at 15.6 °C measurable germination began by 48 hr, but no more than 50% of seed germinated by 168 hr. No more than 2% of seed incubated at 13.9 °C germinated.

Table 1. Mineral content in seed of two cucumber cultivars incubated at three temperatures.[X]

°C	N	P	HPO_4^{-2}	K	SO_4^{-2}	Ca	Mg	Na
	mg·g–l dry weight							
Arkansas Little Leaf								
13.9	43.63a[Y]	9.83a	0.72a	5.50a	0.51a	1.14a	7.47a	2.06a
15.6	42.43a	9.68a	0.76a	5.52a	0.61a	1.15a	6.94a	2.15a
20.0	42.98a	9.70a	0.75a	5.51a	0.64a	0.64a	5.63a	2.03a
Earlipik 14								
13.9	42.57b	10.55b	0.595	5.64b	0.30b	0.63a	4.80a	1.89a
15.6	43.86b	10.82b	0.67a	5.37b	0.40a	0.58a	4.54a	1.76a
20.0	47.78a	11.39a	0.G5a	6.69a	0.39a	0.41a	4.49a	1.56a

[X] Values are pooled because there were no differences over sampling times from 0 to 168 hrs.
[Y] Mean separation by Duncan's Multiple Range Test, $p \leq 0.05$.

Mineral analysis

There was no NO_2^-, NO_3^-, or Mn detected in either cultivar. There was less than 0.1 $mg \cdot g^{-1}$ of Fe in each cultivar, and these levels were not affected by treatment. Six to 8% of P in both cultivars was in the form of HPO_2^{-2}. For each cultivar there were no interactions between temperature and sampling time affecting mineral concentrations. Incubation temperature affected content of some minerals only in 'Earlipik 14' (Table 1). Concentrations of N, P, and K were significantly higher for seed germinated at 20°C than at the other temperatures. For seed incubated at 15.6 and 20°C, HPO_4^{-3} and SO_4^{-2} concentrations were the same and greater than those for seed incubated at 13.9°C.

Levels of N were positively correlated with P levels in both cultivars ['Arkansas Little Leaf' ($r = 0.93$, $p = 0.0001$); 'Earlipik 14' ($r = 0.80$, $p = 0.0001$)]. Concentrations of P and K were positively correlated for 'Earlipik 14' [$r = 0.60$, $p = 0.019$]. In addition, concentrations of HPO_4^{-2} and SO_4^{-2} in 'Arkansas Little Leaf' were positively correlated [$r = 0.60$, $p = 0.0001$].

Electrophoretic protein separation

The distribution of proteins in both cultivars was similar. For seed incubated at 13.9 and 15.6°C, concentrations of a 70.1 Kda protein began increasing at 72 hrs. At 120 hr, 37.4, 43.4, and 50 Kda proteins were present (Figure 2A, B). For seed incubated at 20°C, beyond 12 hr, the concentration of the 70.1 Kda protein was constant. At 30 hr, concentrations of the 37.4, 43.4, and 50 Kda proteins began to increase (Figure 2 C).

Discussion

In sweet corn, poor seed vigor is consistent with high leachate conductivity (Styer and Cantliffe, 1983), which is indicative of damaged membranes (DeLouche and Baskin,

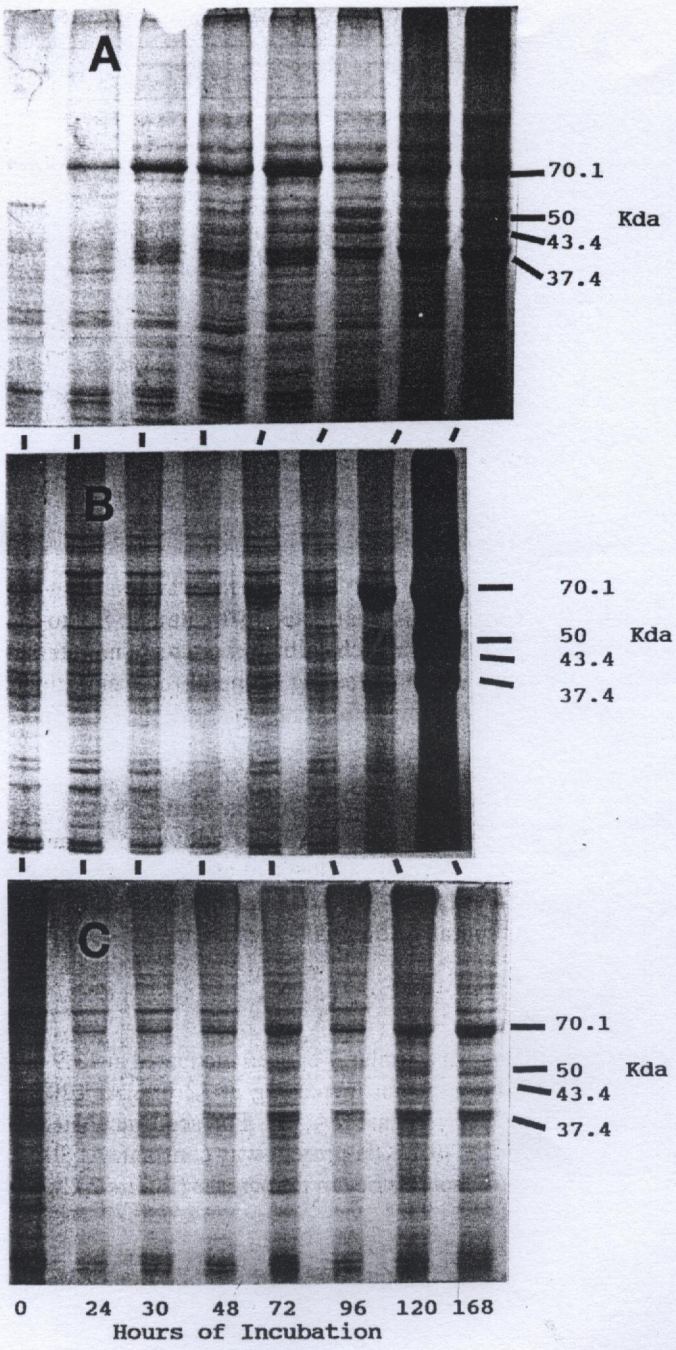

Figure 2. SDS-PAGE gels of proteins from cucumber seeds of cvs. 'Arkansas Little Leaf' and 'Earlipik 14' incubated for up to 168 hrs. Figure 2A for seeds incubated at 20°C; Figure 2B for seeds incubated at 15.6°C; and Figure 2C for seed at 13.9°C. Protein weights in Kda are provided to the right of representative bands.

1973; Waters and Blanchette, 1983). Wann (1986) suggested that leakage of soluble protein was due to defective membranes in seed of the *shrunken2* sweet corn genotype. Germination failure is more likely when leakage from seed occurs under cold conditions (Tatum, 1954). Bramlage *et al.* (1978) suggested that cold water imbibition may interfere with membrane reorganization.

Since there were no differences in levels of minerals over time it is inferred that preimbibed seed contain all the elements that were measured. Higher levels of minerals were found in 'Earlipik 14' seed incubated at 20°C than at other temperatures. It is inferred that minerals had leaked from seed incubated at the lower temperatures. This may be because membranes were disrupted, and agrees with data from sweet corn (DeLouche and Baskin, 1973; Waters and Blanchette, 1983). However, this was not the case for the cv. 'Arkansas Little Leaf' where incubation at various temperatures did not affect mineral content. This suggests that there is opportunity for genetic manipulation to improve membrane stability and germination of cucumber at suboptimal temperatures. Even in this cultivar, which did not appear to exhibit leakage, germination at 13.9 and 15.6°C was well below 50%. This suggests that leakage of minerals from seed is only one factor involved in initiating and sustaining germination. That there was no difference in mineral levels, and germination was affected by temperature suggest that other factors are also involved in germination.

In both cultivars there were changes in protein expression over time. These data suggest that the 70.1 Kda protein is involved in processes affecting germination. At 20°C other proteins may be important in cucumber germination, and subsequent development of the radicle. Density of the bands in gels of some of the proteins found here began increasing before germination and continued to increase as radicle elongation continued.

Simon *et al.* (1976) found that lowered cucumber germination due to low-temperatures was: 1) not due to imbibition of cold water or loss of membrane integrity, and 2) most likely due to denaturing of proteins which may cause inactivation of enzymes. If this is the case the orderly association of proteins into mitochondria or ribosomes may be prevented. These organelles provide the energy for, and formation of, newly synthesized mRNAs that are required for commencement of radicle elongation and further development (Lalonde and Bewley, 1986; Datta *et al.*, 1987). The data reported here suggest that low germination rates at suboptimal temperatures are due to: 1) leakage of minerals, and/or 2) the lack of formation or denaturation of proteins of specific weights. The effect of the former does not appear to be uniform for both cultivars. The latter appears to support the importance of proteins in activities affecting germination as proposed by Simon *et al.* (1976). In addition proteins of specific Kda weights were found that appear to be important in germination.

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Survival of dry and ultra-dry seeds of carrot, groundnut, lettuce, oilseed rape, and onion during five years' hermetic storage at two temperatures

R. H. ELLIS[1], T. D. HONG[1], D. ASTLEY[2], A. E. PINNEGAR[2] and H. L. KRAAK[3]

[1] Department of Agriculture, The University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, UK
[2] Genetic Resources Unit, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK
[3] Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Droevendaalsesteeg 1, P.O. Box 16, 6700 AA Wageningen, The Netherlands

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Summary

Since 1974 the advice for genetic resources conservation by long-term seed storage has been to store seeds dry at 5 ± 1% moisture content at a temperature of –18 °C or less. Investigations were carried out to determine whether or not hermetic storage at lower moisture contents (those in equilibrium with about 10% r.h. at 20 °C) would provide better seed survival, particularly in circumstances where refrigeration cannot be provided. Seeds of carrot, groundnut, lettuce, oilseed rape, and onion were stored hermetically for five years in four environments (dry or ultra-dry seed storage moisture contents combined factorially with temperatures of 20 °C or –20 °C), replicated at two or three sites, and viability (assessed by ability to germinate normally in standard germination tests) estimated at six-monthly intervals. No loss in seed viability was detected during this period in any of these species at –20 °C with either moisture content. Significant loss in viability occurred at 20 °C, however, and was more rapid in dry (5.5–6.8% moisture content) than ultra-dry (2.0–3.7% moisture content) seeds. The results of this five-year study at each of the three sites confirm that storage at –20 °C rather than 20 °C is beneficial to seed survival, and that hermetic storage at 20 °C of seeds first dried at 20 °C to moisture contents in equilibrium with about 10% r.h. provides greater longevity than 5.5–6.8% moisture content in these five species.

Introduction

The International Board for Plant Genetic Resources (IBPGR) was founded in 1974. One of its first acts was to recommend hermetic storage at temperatures of –18 °C or less with 5±1% seed moisture content (fresh weight basis, f. wt) for the long-term storage of orthodox seeds (Roberts, 1973) for genetic resources conservation (IBPGR, 1976). Refrigerated stores (or the power to run them) are not always available, however. Moreover, the variation in water status among seeds of contrasting species at the same moisture content has considerable effect on longevity (Priestley, 1986; Roberts and Ellis, 1989). This helps to explain why the Food and Agricultural Organization of the United Nations (FAO) and the International Plant Genetic Resources Institute (IPGRI) have recently widened the range of moisture contents recommended for long-term storage to 3–7% depending upon species (FAO/IPGRI, 1994). The long-term