Effects of Temperature on Germination Rate and Mitochondrial Activity in *Beta vulgaris* L. Seeds *

SONG Song-quan[1], FREDLUND Kenneth M[2], MØLLER Ian M[2]
(1. School of Life Sciences, Sun Yat-sen (Zhongshan) University, Guangzhou 510275, China;
2. Department of Seed Physiology, Novartis Seeds AB, Box 302, S-26123 Landskrona, Sweden
3. Department of Plant Physiology, Lund University, Box 117, S-22100 Lund, Sweden;)

Abstract: Effects of different temperature on germination rate and mitochondrial activity during germination of *Beta vulgaris* L. seeds were studied. The times required for 50% germination were 7 d at 7 ℃, 3 d at 14 ℃ and 2.3 d at 20 ℃, respectively. The thermal times for 50% of germination were about 46 degree days (℃·d). The content of total proteins and activities of cytochrome oxidase (CCO) and malate dehydrogenase (MDH) in mitochondria from seeds and seedlings germinated at 7, 14 and 20 ℃ progressively increased with germination; and specific and total activities of CCO and MDH at 20 ℃ were higher than those at 7 and 14 ℃. Proteins with approximate M_w of 36 000, 35 000 and 18 800 in mitochondria from seeds and seedlings germinated at 7, 14 and 20 ℃ gradually decreased with germination, 22 000 proteins in mitochondria progressively increased at 7 ℃, and slightly increased from 0 d of germination to 4 d (14 ℃) and 3 d (20 ℃), and then declined. These results showed that germination rate of seeds, activities of CCO and MDH and lmw proteins in mitochondria were significantly affected by germination temperature.

Key words: activities of CCO and MDH; *Beta vulgaris* L. seeds; germination rate; lmw HSP22; temperature
CLC number: Q945.6 **Document code**: A **Article ID**: 0529-6579 (2002) 01-0059-05

Seed germination incorporates those events that commence with the uptake of water by the quiescent seed and terminate with the elongation of the embryonic axis[1]. Temperature affects both the capacity for germination and the rate of germination[1]. Heat energy (℃·d) as a quantitative measure of temperature requirement for germination has been reported. The thermal times required from seed sowing until 50% germination in chickpea, lentil, soybean and cowpea are 43, 24, 35 and 29.8 ℃·d, respectively[2]; in cvs Shadegani and Isfahani of taree Irani are 62.5 and 61.5 ℃·d, respectively[3].

Mitochondria is the main organelle in plant cell, they can provide energy and substrate to embryo growth during seed germination. Respiration by mature "dry" seeds (usual moisture content: 10% ~ 15%) of course is extremely low when compared with developing or germinating seeds[1]. One of the first changes upon imbibition is the resumption of respiratory activity, which can be detected within minutes. After a steep initial increase in oxygen consumption, the rate declines until the radicle penetrates the surrounding structures. At this time, another burst of respiratory activity occurs[1,4].

Lund et al[5] have found that levels of heat shock protein (HSP) 70 and chaperonin 60 do not change to any extent during stress treatments. In contrast, levels of mitochondrial HSP 22 increase dramatically during stress and decrease after the stress is relieved. The 23 000 light-stress-regulated HSP of *Chenopodium rubrum* L. is located in the mitochondria[6]. Mitochondria may be a major target for the protective effects of HSP against oxidative stress[7]. The repairs of membrane, mitochondria, DNA and protein synthesis using extant mRNAs could be earlier events during seed germination[8]. Sugar beet is drilled in late April or early May in most southern parts of Sweden, where the temperature is often below 10 ℃ for an extended period, and the crop therefore may be subject to cold stress. In this paper, we have studied the effect of different temperature on germination rate and thermal times of sugar beet seeds, and on activities of CCO and MDH in mitochondria; and the changes in soluble proteins of mitochondria.

1 Materials and methods

Sugar beet (*Beta vulgaris* L. cv. Loke) seeds were supplied by Novartis Seeds AB (Landskrona, Sweden).

1.1 Germination test

Seeds were germinated in special plastic box at 7, 14

* 收稿日期: 2001-09-01;
基金项目: 广东省自然科学基金资助项目 (001224); 中山大学和瑞典隆德大学交流基金资助项目
作者简介: 宋松泉 (1957—), 男, 博士, 副教授; E-mail: ssq@zsu.edu.cn

and 20 ℃ in the dark, respectively. A cover paper and a deionised water was carefully added along the side of the paper and left for 2 h before sowing. The pleated paper has 50 fold; 2 seeds were sown from the edge of the pleated paper per fold, i.e. 100 seeds per box. The cover paper was folded over the pleated paper after sowing. The lid was put on the box, and then the box was sealed with the Parafilm and incubated in darkness at 7, 14 and 20 ℃. Germination percentage was determined after different time of sowing. Seeds showing radicle emergence were counted as germinated.

1.2 Isolation of mitochondria

All procedures were carried out between 0 and 4 ℃. 100 seeds and/or seedlings germinated for different time were homogenized by moving the piston up and down 30 times in a precooled glass-teflon homogenizer in 35 mL ice-cold extraction medium containing 0.3 mol/L sucrose and 10 mmol/L 3-N-morpholinopropanesulfonic acid (MOPS, pH 7.2). The brei was squeezed through a 300 μm mesh nylon cloth. The filtrate was centrifuged at 1 000 g for 10 min. The supernatant was centrifuged at 41 400 g for 30 min. The pellet was suspended in extraction medium, and centrifuged at 1 480 g for 10 min, and then the supernatant was centrifuged at 41 400 g for 30 min. The pellet was resuspended in about 0.3 mL extraction medium. The volume of the resultant samples (washed mitochondria) was measured, and dimethylsulfoxide was added to the sample to make the final concentration 5%. The samples were frozen in liquid nitrogen and were stored at −80 ℃.

1.3 Enzyme assays

CCO (EC 1.3.9.1) activity was measured as in Rasmusson and Møller[9]. NAD^+-dependent MDH (EC 1.1.1.37) was measured as in Møller et al[10].

1.4 SDS-PAGE of soluble proteins in mitochondria

Samples (washed mitochondria) were mixed with a sample buffer[11] and heated at 95 ℃ for 4 min. Discontinuous SDS-polyacrylamide gels (12% separation gel, 4% stacking gel) were prepared and run according to the method of Laemmli[11] using a Mini-Protein Ⅱ electrophoresis cell (Bio-Rad).

1.5 Protein determination

Protein was determined by the method of Lowry et al.[12], with standard curves prepared using bovine serum albumin.

2 Results

2.1 Rate and thermal times of seed germination

Sugar beet seeds, which are fruits in fact, were harvested, stored and sowed in production. Water content of dried sugar beet seeds was 8.7% (fresh weight basis). The germination rate of seeds increased with increase of germination temperature and elongation of germination time (Fig. 1). The times required for 50% germination were 7 d at 7 ℃, 3 d at 14 ℃ and 2.3 d at 20 ℃, respectively; whereas, the times for final germination were 10 d at 7 ℃, 5 d at 14 ℃ and 4 d at 20 ℃, respectively (Fig. 1). The thermal times for 50% germination, however, were about 46 ℃·d at 7, 14 and 20 ℃ (data not shown). The final germination percentage of seeds at 20 ℃ was slightly higher than at 14 and 7 ℃ (Fig. 1).

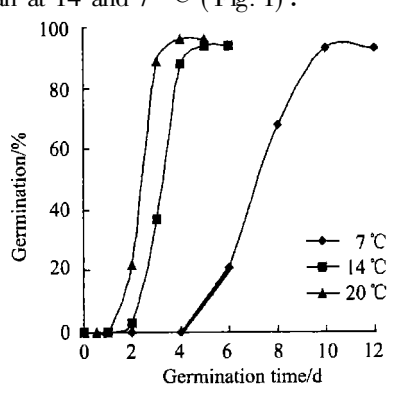

Fig. 1 Effect of different temperature on germination rate of sugar beet seeds

Seeds were germinated as indicated time and temperature. Each value is the avenage of two replicates of 100 seeds

2.2 Changes in activities and latencies of CCO and MDH

Specific and total activities of CCO in mitochondria markedly increased with seed germination, and their activities in mitochondria from seeds and/or seedlings germinated at 20 ℃ were much higher than those at 14 and 7 ℃ (Fig. 2). Specific activity of CCO at 20 ℃, for example, increased from 78.1 nmol·mg^{-1}·min^{-1} at 0 d of germination to 353.3 nmol·mg^{-1}·min^{-1} at 4 d of germination. Specific activity of CCO from seedlings and seeds germinated for 4 d at 20 ℃ was about 130% of that for 5 d at 14 ℃ and 280% of that for 10 d at 7 ℃ though their germination percentage has little difference (Fig. 2). The content of total proteins in mitochondria significantly increased with seedgermination (Fig. 3), and their contents increased by 93, 95 and 62% as germination percentage of seeds from 0 to final germination at 7, 14 and 20 ℃.

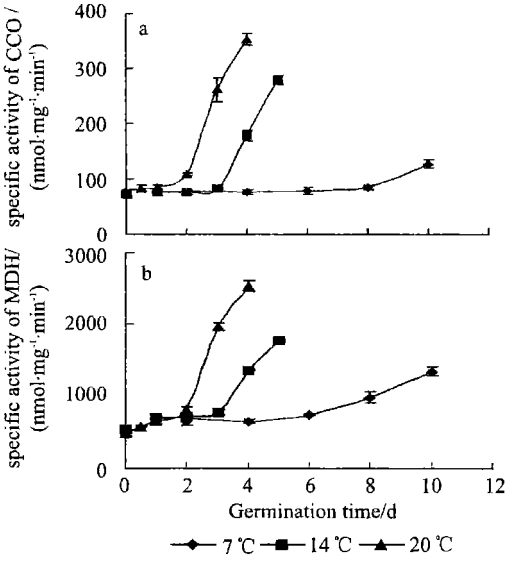

Fig 2　Changes in specific activity CCO (a) and MDH (b) in mitochondria from seeds and seedling germinated as indicated time and temperature

CCO medium contained 0.3 mol/L sucrose, 50 mmol/L Tris-acetate, 100 mmol/L KCl, 45 μmol/L reduced cytochrome c, pH 7.2; 5 μL ρ= 5% Triton X-100 and 2.5 μL washing mitochondria were added in a total volume of 1 mL. MDH medium contained 0.3 mol/L sucrose, 20 mmol/L MOPS, pH 7.2; 2 μL 100 ng/μL antimycin A, 20 μL 100 mmol/L oxaloacetate (pH 7.0), 4 μL 50 mmol/L NADH, 5 μL ρ= 5% Triton X-100, and 2.5 μL washing mitochondria were added in a total volume of 1 mL. All of three repli-cates.

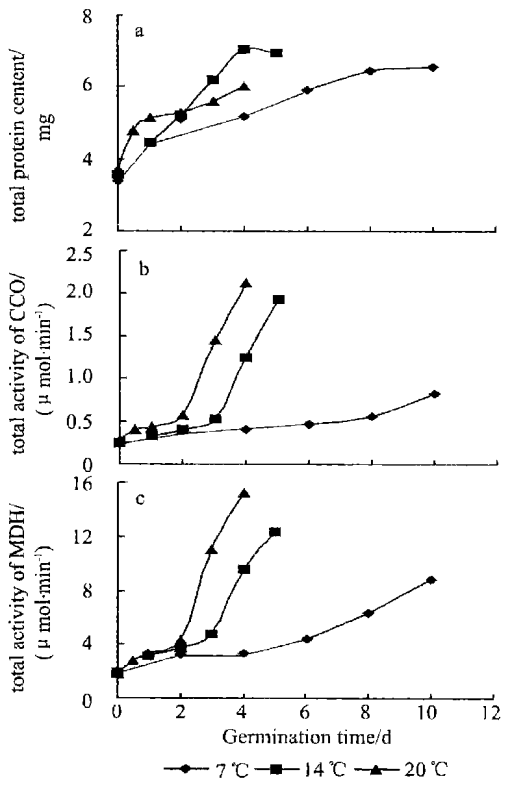

Fig. 3　Changes in content of total protein (a), total activities of CCO(b) and MDH(c) in mitochondria from 100 seeds and seedlings germinated as indicated time and temperature

respectively (Fig. 3). Specific and total activities of MDH also significantly increased with seed germination, and their activities at 20 ℃ were much higher than those at 14 and 7 ℃ (Fig. 2, 3). In addition, the specific and total activities of MDH in mitochondria at 7, 14 and 20 ℃ were much higher than those of CCO with seed germination (Fig. 2, 3).

Latency of CCO and MDH, measured as in Rasmusson and Møller[9], increased with seed germination, and the increase in latencies of CCO and MDH from seeds and seedlings germinated at 20, 14 and 7 ℃ was about the same (data not shown).

2.3　SDS-PAGE analysis of mitochondrial protein

Coomassie blue-stained SDS-PAGE gel showed that mitochondrial proteins markedly changed with seed germination at different temperature. Proteins with approximate M_w of 36 000, 35 000 and 18 800 in mitochondria from seeds and seedlings germinated at 7 ℃ gradually decreased with germination, 22 000 proteins progressively increased, and 38 600 proteins did not changed (Fig. 4a). The Changes in mitochondrial proteins were the similar as seeds germinated at 14 and 20 ℃ (Fig. 4b, 4c). The proteins with approximate M_w of 38 600, 36 000, 35 000 and 18 800 gradually decreased with germination; 22 000 proteins, however, progressively increased from 0 d of germination to 4 d (14 ℃) and 3 d (20 ℃), and then declined (Fig. 4b, 4c).

3　Discussion

Germination commences with the uptake of water by the dry seed and is completed when a part of the embryo, usually the radicle, extends to penetrate the surrouding structures. Although all seeds of a species may germinate over a fairly wide range of temperature, the time needed for the maximum germination percentage to be reached varies with the temperature; that is to say, the rate of germination is temperature dependent[1]. The rate and percentage of germination of sugar beet seeds at 20 ℃ were higher than at 7 and 14 ℃ (Fig. 1). Rapid germination of seeds in production is of importance, because it permits crop establishment before soil borne pathogens and pests may affect the seedling, and it permits the root access wet soil ahead of the drying front. The thermal times for 50% germination of sugar beet seeds were about 46 ℃·d, the time of sowing could be selected before practical application using this data.

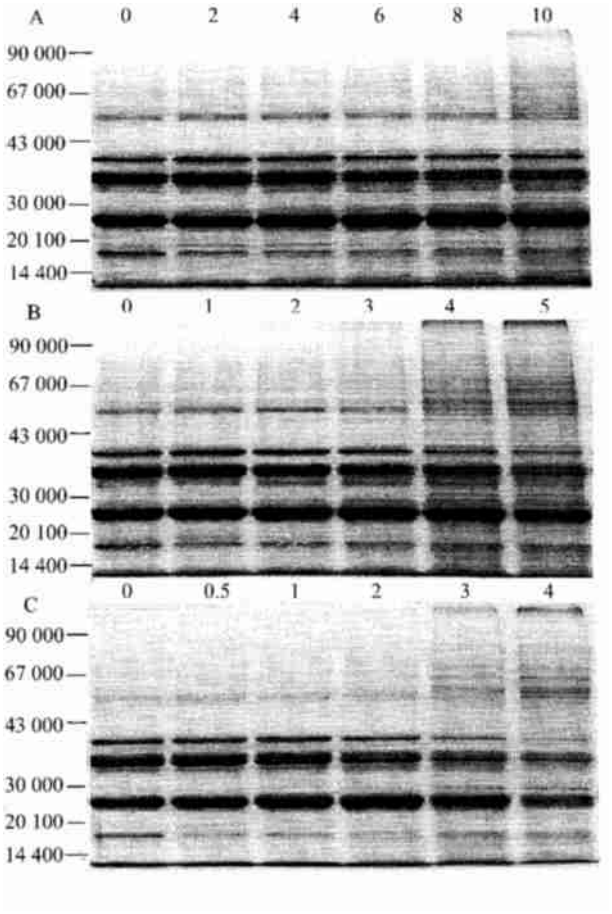

Fig. 4 SDS-PAGE analysis of mitochondrial proteins
Seeds were germinated for indicated time at 7, 14, and 20 ℃ respectively, and then mitochondria were immediately isolated. The panel a (7 ℃), b (14 ℃) and c (20 ℃) are Coomassie blue-stained SDS-PAGE gel loaded with 20 μg of mitochondrial protein per lane.

CCO, which is a marker enzyme of mitochondria[13], is the terminal complex of the electron transport chain, and contains two cytochromes (cytochrome a and cytochrome a_3) and two atoms of copper (Cu_A and Cu_B). The reaction catalyzed by CCO is the four-electron reduction of O_2[14]. MDH catalyses the oxidation of malate to oxaloacetate. Oxaloacetate is a necessary substrate for citric acid cycle. The citric acid cycle also produces a wealth of primary and secondary metabolites (carbon skeletons). The supply of carbon skeletons may be more important than the supply of ATP during active cell division[14]. The content of total proteins and specific and total activities of CCO and MDH in mitochondria markedly increased with seed germination (Fig. 2, 3). These results showed that activities of CCO and MDH strongly correlated with progress and temperature of seed germination, and that the recovery of mitochondria function was important for seed germination and seedling establishment. A decline in cytochrome oxidase activity occurs in parallel with accumulation of mitochondrial damage and viability loss[15].

HSPs are thought to act as molecular chaperones and to prevent further aggregation of partially folded/denatured proteins or to promote the correct folding of the target protein[16]. Work by Lee et al.[17] has shown that two cytosolic lmw HSPs from pea can enhance refolding and prevent aggregation of artificially denatured proteins in an ATP-independent manner. Antibodies against HSP 22 from *Zea mays* L., which is a gift from Thomas E. Elthon (School of Biological Sciences and the Center for Biotechnology, University of Nebraska, Nebraska, USA), could recognize the 22 000 proteins from mitochondria of sugar beet seeds and seedlings (data not shown). 22 000 proteins in mitochondria from sugar beet seeds could be a constitutive protein (Fig. 4). Work by Bettey and Finch-Savage[18] and Bettey et al[18,19] has showed that HSP 17.6 accumulated in the latter stages of *Brassica* seed development and was present in mature dry seeds and in imbibed seeds. Proteins with approximate M_w of 36 000, 35 000 and 18 800 in mitochondria from seeds and seedlings germinated at 7, 14 and 20 ℃ gradually decreased with germination (Fig. 4). 22 000 proteins in mitochondria progressively increased at 7 ℃ (Fig. 4a), and increased from 0 d of germination to 4 d (14 ℃) and 3 d (20 ℃), and then declined (Fig. 4b, 4c). These results showed that change in proteins of mitochondria (Fig. 4) was in close relationship with temperature and rate of seed germination (Fig. 1), and activities of CCO and MDH in mitochondria (Fig. 2, 3).

The mitochondrial low molecular weight (lmw) HSP could protect NADH: ubiquinone oxido-reductase (complex Ⅰ), and consequently electron transport from complex Ⅰ to cytochrom c: O_2 oxido-reductase (complex Ⅳ) in submitochondrial vesicles; and addition of purified lmw HSP to submitochondrial vesicles lacking this HSP increased complex Ⅰ electron transport rates 100% in submitochondrial vesicles assayed at high temperature[20]. Lmw HSP in the chloroplast could protect photosynthetic electron transport during heat stress[21]. Therefore, the further studies on relationship between lmw HSPs and germination rate under different temperature, especially in stress temperature, are very important.

Acknowledgements: We are grateful to Christina Nilsson (Department of Plant Physiology, Lund University) for excellent technical assistance, to Professor T. E. Elthon (School of Biological Sciences and the Center for Biotechnology, University of Nebraska) for donating the HSP 22 antibody.

References:

[1] BEWLEY J D, BLACK M. Seeds. Physiology of development and germination [M]. 2nd edition. New York: Plenum Press, 1994.

[2] COVELL S, ELLIS R H, SUMMERFIELD R J. The influence of temperature on seed germination rate in grain legumes. Ⅰ. A comparison of chickpea, lentil, soybean and cowpea at constant temperatures [J]. J Exp Bot, 1986, 37: 705—715.

[3] RAMIN A A. The influence of temperature on germination of taree Irani (*Allium ampeloprasum* L. spp *iranicum* W.) [J]. Seed Sci Technol, 1997, 25: 419—426.

[4] BOTHA F C, POTGIETER G P, BOTHA A M. Respiratory metabolism and gene expression during seed germination [J]. J Plant Growth Reg, 1992, 11: 211—224.

[5] LUND A A, BLUM P H, BHATTRAMAKKI D, et al. Heat-stress response of maize mitochondria [J]. Plant Physiol, 1998, 116: 1097—1110.

[6] DEBEL K, SIERRALTA W D, BRAUN H P, et al. The 23-kDa light-stress-regulated heat-shock protein of *Chenopodium rubrum* L is located in the mitochondria [J]. Planta, 1997, 201: 326—333.

[7] POLLA B S, KANTENGWA S, FRANCOIS D, et al. Mitochondria are selective targets for the protective effects of heat shock against oxidative injury [J]. Pro Nat Aca Sci USA, 1996, 93: 6458—6463.

[8] BEWLEY J D. Seed germination and dormancy [J]. Plant Cell, 1997, 9: 1055—1066.

[9] RASMUSSON A G, MØLLER I M. NADP-utilizing enzymes in the matrix of plant mitochondria [J]. Plant Physiol, 1991, 94: 1012—1018.

[10] MØLLER I M, LIDEN A C, ERICSON I, et al. Isolation of submitochondrial particles with different polarities [J]. Methods of Enzymology, 1987, 148: 442—453.

[11] LAEMMLI U K. Cleavage of structural proteins during assembly of the head of bacteriophage T_4 [J]. Nature, 1970, 227: 680—685.

[12] LOWRY O H, ROSEBROUGH N J, FARR A L, et al. Protein measurement with the Folin phenol reagent [J]. J Bio Chem, 1951, 193: 265—275.

[13] STRUGLICS A, FREDLUND K M, RASMUSSON G, et al. The presence of a short redox chain in the membrane of intact potato tuber peroxisomes and the association of malate dehydrogenase with the peroxisomal membrane [J]. Physiol Plant, 1993, 88: 19—28.

[14] DOUCE R. Mitochondria in higher plants. Structure, function, and biogenesis [M]. New York: Academic press Inc, 1985: 45—203.

[15] SMITH M T, BERJAK P. Deteriorative changes associated with the loss of viability of stored desiccation-tolerance and desiccation-sensitivity seeds // KIGEL J, et al eds. Seed development and germination [M]. New York: Marcel Dekker Inc, 1995: 701—746.

[16] LANDRY S J, GIERASCH L M. Polypeptide interactions with molecular chaperones and their relationship to *in vivo* protein folding [J]. Ann Rev Biophysic Biomole Str, 1994, 23: 645—669.

[17] LEE G J, POKALA N, VIERLING E. Structure and *in vivo* molecular chaperone activity of cytosolic small heat shock proteins from pea [J]. J Bio Chem, 1995, 270: 10432—10438.

[18] BETTEY M, FINCH-SAVAGE W E. Stress protein content of mature Brassica seeds and their germination performance [J]. Seed Sci Res, 1998, 8: 347—355.

[19] BETTEY M, SINNIAH U R, FINCH-SAVAGE W E, et al. Irrigation and seed quality development in rapid-cycling Brassica: accumulation of stress proteins [J]. Ann of Bot, 1998, 82: 657—663.

[20] DOWNS C A, HECKATHORN S A. The mitochondrial small heat-shock protein protects NADH: ubiquinone oxidoreductase of the electron transport chain during heat stress in plants [J]. FEBS Letters, 1998, 430: 246—250.

[21] HECKATHORN S A, DOWNS C A, SHARKEY T D, et al. The small, methionine-rich chloroplast heat-shock protein protects photosystem Ⅱ electron transport during heat stress [J]. Plant Physiol, 1998, 116: 439—444.

温度对甜菜种子萌发速率和线粒体活性的影响

宋松泉[1], FREDLUND K M[2], MØLLER I M[3]

(1. 中山大学生命科学学院, 广东 广州 510275;

3. Department of Plant Physiology, Lund University, Box 117, S-221 00 Lund, Sweden;

2. Department of Seed Physiology, Novartis Seeds AB, Box 302, S-261 23 Landskrona, Sweden)

摘　要：研究了不同温度对甜菜种子萌发速率和线粒体活性的影响。在 7, 14 和 20 ℃中甜菜种子萌发 50%需要的时间分别是 7, 3 和 2.3 d; 萌发 50%的热时间大约是 46 ℃·d。在 7, 14 和 20 ℃萌发的种子和幼苗的线粒体中, 总蛋白含量以及细胞色素氧化酶 (CCO) 和苹果酸脱氢酶 (MDH) 的活性随着萌发进程增加; 相对分子质量大约为 18 800, 35 000 和 36 000 的蛋白质随着萌发进程降低; 相对分子质量大约为 22 000 的蛋白质在 7 ℃中逐渐增加, 在 14 和 20 ℃中则先少量增加然后下降。20 ℃中萌发的种子和幼苗的线粒体的 CCO 和 MDH 的比活性和总活性比在 7 和 14 ℃中高。这些结果表明, 萌发温度显著地影响种子的萌发速率、线粒体的 CCO 和 MDH 活性以及较小相对分子质量热休克蛋白 22。

关键词：CCO 和 MDH 活性; 甜菜种子; 萌发速率; 小相对分子质量热休克蛋白 22; 温度

中图分类号：Q945.6