Plant & Cell Physiol. 19(2): 349–354 (1978)

Short communication

Metabolic changes induced by hydration-dehydration treatment in wheat embryos

A. Dell'Aquila, G. Savino and P. De Leo

Laboratorio del germoplasma C.N.R., Via Amendola, 165/A, 70126 Bari, Italy

(Received June 25, 1977)

Wheat embryos isolated from seeds at two viability levels treated with the hydration-dehydration technique (presoaking treatment) showed an in vivo increase of radioactive precursor incorporation into proteins, RNA and DNA and remarkable DNA-polymerase activity in their crude extracts compared to untreated germs.

Key words: Macromolecules biosynthesis — *Triticum durum* — Wheat.

Seed hydration and dehydration (presoaking treatment) improve seed germination and seedling vigour (*1*, *7–10*, *19*). The metabolic events activated by this treatment of the embryo can be devided into two phases connected with the length of imbibition (*17*). Protein and RNA syntheses occur in phase one, and phase two begins with DNA replication and terminates with the first cell division. Previous studies have demonstrated that protein synthesis can be detected within the first 30 min of imbibition (*13*), whereas RNA and DNA synthesis start later (*3*, *4*). Among the biochemical changes occurring during the early hours of germination are those related either directly or indirectly to DNA replication (*14*, *15*) and those which can be connected with the repairing of damages induced by ageing, as described by Berjack and Villiers (*2*) and Villiers (*22*, *23*).

In order to study the effect of presoaking treatment on the metabolic changes, we measured the amount of L-[U-^{14}C] leucine, (5-^{3}H) uridine and [6-^{3}H] thymidine incorporated respectively into protein, (total) RNA and DNA, in wheat embryos isolated from seeds at two viability levels with or without presoaking. The activity of DNA-polymerase was also measured in the crude extract from the same material.

Two stocks of wheat embryos (*Triticum durum* Desf. cv. Lamia) were used: the first, at 92% viability level (92% V embryos), was obtained from seeds harvested in 1975 and the second, at 60% viability level (60% V embryos), was obtained from the same seeds stored under experimental ageing conditions at 14% moisture content and at 38°C for the required time (*16*). Presoaking treatment included seed imbibition with 30% water with respect to the seed fresh weight at 20°C for 18 hr, then dehydration at 30$\pm$1°C up to the original seed water content, as described elsewhere (*6*). Next, the embryos were prepared according to Johnston and Stern (*11*).

In order to estimate the amount of L-[U-^{14}C] leucine, [5-^{3}H] uridine and [6-

Table 1 L-[*U-14C*] *leucine incorporated into TCA-alcohol-insoluble fraction at the end of the first 5 hr of imbibition by embryos isolated from wheat seeds at two viability levels with or without presoaking* (M±SD)

Embryo viability	Treatment	Radioactivity (cpm)	Increase (%)
92% V	Control	1630± 84	0
	Presoaking	2015±105	24
60% V	Control	840± 92	0
	Presoaking	1615±107	92

^{3}H] thymidine incorporated respectively into protein, (total) RNA and DNA, samples of five embryos were incubated at 20°C for the different periods given in the tables, during which active synthesis of these macromolecules occured (*17*). The incubation medium contained 0.1 ml of 1 mM sodium phosphate buffer (pH 5.5), 25 μg penicillin-G, 25 μg streptomycin sulfate, and one of the following: 0.025 μCi of L-[U-^{14}C] leucine (sp. act. 23 Ci/mmol), 0.36 μCi [5-^{3}H] uridine (sp. act. 29 Ci/mmol), or 0.025 μCi [6-^{3}H] thymidine (sp. act. 23 Ci/mmol). After incubation, the embryos were rinsed in running tap water, kept overnight in four volumes of absolute ethanol, then homogenized. The homogenate was collected on nitrocellulose filters (Millipore H.A.W.P. 0.45 μm pore diameter) and sequentially washed twice with ethanol (80%, v/v), trichloroacetic acid (5%, w/v) and finally ethanol-ether (1/1, v/v). The filters were dried and counted in PPO-POPOP-toluene scintillation medium (*5*, *18*).

DNA-polymerase activity was measured as in vitro incorporation activity of deoxythymidine triphosphate [^{3}H-TTP], using the crude extracts (*14*) obtained from either ungerminated embryos (500 mg) or germs (500 mg) imbibed with water for 18 hr. Their capacity to catalyze the in vitro ^{3}H-TTP incorporation was measured according to Mory et al. (*14*). The reaction mixtures contained in a total volume of 0.25 ml, 20 mM Tris-HCl (pH 7.6), 4.4 mM $MgCl_2$, 16 mM KCl, 0.1 mM each of dATP, dGTP and dCTP, 1 μCi of [methyl-^{3}H]TTP (sp. act. 30 Ci/mmol), 4% (w/v) glycerol; 0.4 mM 2-mercaptoethanol, 57 μg of calf thymus DNA and 300–500 μg of protein of crude extracts. The reaction was carried out at 37°C for 10 min and stopped with ice-cold TCA (20%, w/v) containing 2 mM $Na_2P_2O_4$. The precipitate was collected on Millipore filter, washed with cold 5% TCA and ethanol-ether (3/1, v/v), dried and counted as described elsewhere. The results of radioactive precursor incorporation represented the mean of four determinations, repeated in two series of experiments.

Table 2 [*5-3H*] *uridine incorporated into TCA-alcohol-insoluble fraction at the end of the first 12 hr of imbibition by five embryos isolated from wheat seeds at two viability levels with or without presoaking* (M±SD)

Embryo viability	Treatment	Radioactivity (cpm)	Increase (%)
92% V	Control	420±70	0
	Presoaking	540±45	29
60% V	Control	190±85	0
	Presoaking	415±63	118

Table 3 *[6-^3H] thymidine incorporated into TCA-alcohol-insoluble fraction at the end of the first 20 hr of imbibition by five embryos isolated from wheat seeds at two viability levels with or without presoaking* (M±SD)

Embryo viability	Treatment	Radioactivity (cpm)	Increase (%)
92% V	Control	670±41	0
	Presoaking	840±25	25
60% V	Control	480±45	0
	Presoaking	915±42	91

Protein concentration was determined according to the method of Lowry et al. (*12*), using bovine serum albumin as standard.

Effect of presoaking on L-[*U-^{14}C*] *leucine incorporation into the TCA-alcohol insoluble fraction.* Table 1 shows the amount of L-[U-^{14}C] leucine incorporated into proteins by wheat embryos isolated from seeds at the two viability levels with or without presoaking. The data show a different rate of ^{14}C-leucine incorporation between the 92% V and the 60% V embryos at the end of the first five hr of imbibition. Presoaking treatment enhanced the radioactive precursor incorporation into proteins. This enhancement was higher in the 60% V embryos (92%) than the 92% V embryos (24%).

Effect of presoaking on [5-^3H] uridine incorporation into the TCA-alcohol-insoluble fraction. Table 2 shows the amount of [5-^{3}H] uridine incorporated into (total) RNA by wheat embryos isolated from seeds at both viability levels with or without presoaking. The incorporation, measured at the end of the first 12 hr of imbibition, was highest in the 92% V germs. The presoaked embryos showed an increase of (total) RNA synthesis. This increase was much higher in the 60% V than the 92% V embryos.

Effect of presoaking on [6-^3H] thymidine incorporation into the TCA-alcohol-insoluble fraction. Table 3 shows the amount of [6-^{3}H] thymidine incorporated into DNA by wheat embryos at both viability levels, with or without presoaking. At the end of the first 20 hr of imbibition, an increase of ^{3}H-thymidine incorporation was observed in treated embryos compared to the control. Radioactive incorporation seemed to be enhanced more in the presoaked 60% V embryos than the 92% V germs.

An in vitro study of the ^3H-TTP incorporated by crude extracts derived from dry and imbibed embryos. As shown in Table 4, the capacity to catalyze in vitro ^{3}H-TTP incorporation was very low in the crude extracts obtained from dry embryos isolated from seeds at both viability levels. After 18 hr of water imbibition, a significant amount of ^{3}H-TTP incorporation into DNA was observed, it was higher in the 92% V embryos than the 60% V ones.

Table 4 *[Methyl-^3H]-TTP incorporated into DNA by crude extracts obtained from wheat embryos at two viability levels with or without imbibition* (M±SD)

Embryo viability	Radioactivity (cpm/mg protein/10 min)	
	0 hr	18 hr
92% V	280±31	4994±285
60% V	180±28	2665±165

Table 5 *[Methyl-^3H]-TTP incorporated into DNA by crude extracts obtained from dry embryos isolated from wheat seeds at two viability levels with or without presoaking* (M±SD)

Embryo viability	Radioactivity (cpm/mg protein/10 min)	
	Control	Presoaking
92% V	240±25	4000±327
60% V	178±15	1335±150

The same kind of experiments were also performed using crude extracts obtained from ungerminated germs isolated from seeds at both viability levels with or without presoaking. As shown in Table 5, the presoaked 92% V and 60% V embryos showed a marked ^3H-TTP incorporation into DNA which was extremely low in untreated germs.

Our results show that presoaking of wheat seeds induced, in isolated embryos, an increase of radioactive precursor incorporation into proteins, (total) RNA and DNA during the early hours of imbibition[1]. This may have been caused by the preservation of several metabolic processes activated during the hydration stage of the treatment and not by the enhancement of the precursor availability in the presoaked seed. In fact, if during the imbibition, the mobilization of metabolic reserves from endosperm to embryo did induce an increase of the precursor pools at biosynthetic sites, thus diluting the amount of cold precursor, this would have reduced the stimulating effect of presoaking. Among the biochemical changes occurring early in embryo imbibition, are those connected with DNA synthesis (*14*, *15*). Table 4 shows that the DNA-polymerase activity, very low in ungerminated germs, increased at the end of 18 hr of imbibition. The enhancement of this enzymatic activity was evident also after the dehydration of the embryos (Table 5). Our previous studies (*20*) showed that the respiratory and dehydrogenase activities, which increased during the early hours of imbibition, were preserved after dehydration over some weeks of dry seed storage. Also, Vidauer et al. (*21*) reported that phytochrome activation, induced in lettuce seeds under red-light condition during imbibition, occurred in these seeds after one year of storage.

Also, these results clearly show that the increases in incorporation activities were highest in embryos isolated from treated seeds at low viability level. Presoaking treatment of aged seeds may have caused several metabolic changes responsible for the "repairing processes" of the biochemical damages in embryo cells. This hypothesis was also proposed by Berjack and Villiers (*2*) and Villiers (*22*, *23*) in relation to the restoration of the functionality of cell structure in seeds which had lost their viability during storage.

We conclude that this metabolic improvement effect induced by presoaking can benefit aged embryos in addition to the preservation of activated metabolic processes occurring in high viability seed germs.

[1] Other experiments also confirmed these findings when the ratios of incorporation dpm versus the total uptake dpm were compared between the untreated and treated seeds, even when the total uptake was higher in the latter.

This research was partially performed under the Italian National Research Council project "Biology of Reproduction".

References

(*1*) Austin, R. B., P. G. Longden and J. Hutchinson: Some effects of "hardening" carrot seed. *Ann. Bot.* 33: 883–895 (1969).

(*2*) Berjack, P. and J. A. Villiers: Ageing in plant embryos II. Age induced damage and its repair during early germination. *New Phytol.* 71: 135–145 (1972).

(*3*) Chen, D. and D. J. Osborne: Ribosomal genes and DNA replication in germinating wheat embryos. *Nature* 225: 336–340 (1970).

(*4*) Chen, D., G. Schultz and E. Katachalski: Early ribosomal RNA transcription and appearance of cytoplasmatic ribosomes during germination of the wheat embryo. *Nature New Biology* 231: 69–72 (1968).

(*5*) De Leo, P., G. Dalessandro, A. De Santis and O. Arrigoni: Metabolic responses to lycorine in plants. *Plant & Cell Physiol.* 14: 487–496 (1973).

(*6*) De Leo, P., G. Savino, I. Scarascia and G. Carella: E' possibile migliorare il vigore dei semi? *Annali della Facoltà* di *Agraria dell'Università di Bari* 28: 567–573 (1976).

(*7*) Hegarty, T. W.: The possibility of increasing field establishment by seed hardening. *Hort. Res.* 10: 59–64 (1970).

(*8*) Henckel, P. A.: Physiology of plants under drought. *Ann. Rev. Plant Physiol.* 15: 363–386 (1964).

(*9*) Heydecker, W.: Vigour. In *Viability of Seeds.* Edited by E. Roberts, p. 209–253. Chapman and Hall, London, 1972.

(*10*) Heydecker, W., J. Higgins and Y. J. Turner: Invigoration of seeds? *Seed Sci. & Technol.* 3: 881–888 (1975).

(*11*) Johnston, F. B. and H. Stern: Mass isolation of viable wheat embryos. *Nature* 179: 160–161 (1957).

(*12*) Lowry, O. H., N. J. Rosebrough, A. L. Farra and R. J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275 (1951).

(*13*) Marcus, A., J. Feely and T. Uddcani: Protein synthesis in imbibed seeds III. Kinetics of aminoacid incorporation, ribosomes activation, and polysome formation. *Plant Physiol.* 41: 1167–1172 (1966).

(*14*) Mory, Y. Y., D. Chen and S. Sarid: Onset of deoxyribonucleic acid synthesis in germinating wheat embryos. ibid. 49: 20–23 (1972).

(*15*) Mory, Y. Y., D. Chen and S. Sarid: De novo biosynthesis of deoxyribonucleic acid polymearse during wheat embryo germination. ibid. 55: 437–442 (1975).

(*16*) Roberts, E. H. and F. H. Abdalla: The influence of temperature, moisture and oxygen on period of seed viability in barley, broad beans and peas. *Ann. Bot.* 32: 97–117 (1968).

(*17*) Roberts, B. E. and D. J. Osborne: Protein synthesis and viability in rye grains. In *Seed Ecology.* Edited by W. Heydecker, p. 99–114. Buttersworths, London, 1973.

(*18*) Sacher, J. A.: Studies of permeability, RNA and protein turnover during ageing of fruit and leaf tissues. *Symp. Soc. Exp. Biol.* 21: 269–340 (1967).

(*19*) Savino, G., P. M. Haigh and P. De Leo: Effects of presoaking upon seed vigour and viability during storage. *Seed Science & Technology* (1977), in press.

(*20*) Savino, G., A. Dell'Aquila and P. De Leo: Effetto dell'idratazione e della disidratazione sulla sintesi proteica e respirazione in vivo in embrioni e semi di grano. *Boll. SIBS* 52: 1187–1192 (1976).

(*21*) Vidauer, W. and A. I. H. Hsiao: Persistence of phytochrome-mediated germination control in lettuce seeds for 1 year following a single monochromatic light flash. *Can. J. Bot.* 50: 687–689 (1972).

(*22*) Villiers, T. A.: Ageing and the longevity of seeds in field conditions. In *Seed Ecology*. Edited by W. Heydecker, p. 265–288, Butterworths, London, 1973.
(*23*) Villiers, T. A.: Genetic maintenance of seeds in imbibed storage. In *Crop Genetic Resources for Today and Tomorrow*. Edited by O. H. Frankel and J. G. Hawkes, p. 297–316, Univ. Press, Cambridge, 1975.