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BRIEF COMMUNICATION

Comparison between Wheat Embryos Isolated Mechanically and by Floating off from Organic Solvents

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Abstract. Wheat embryos floated off from organic solvents have been compared with mechanically isolated embryos. The results of comparative investigations, including germination, *in vivo* formation of polysomes and efficiency of cell free preparations for *in vitro* protein synthesis, favour the use of floated embryos for studies of biochemical processes during germination.

Isolated embryos from wheat prove to be useful for studying germination processes. For this purpose there is a need of sufficient material. The best, but most tedious way is to dissect embryos from the caryopses by hand. Therefore many investigators prefer a method, which had been developed by JOHNSTON and STERN (1957). In principle, this method starts from ground seeds which, after screening by a set of sieves and removal of bran, are floated off from 2 M sucrose solution or from a mixture of organic solvents (cyclohexane-carbon tetrachloride). The embryos float on the surface of the solution, whereas endosperm settles at the bottom. JOHNSTON and STERN (1957) using this method obtained viable embryos with more than 90% germination capability. No negative influence due to organic solvent was detectable. The advantage of this method, yielding gram amounts of embryos, is obvious and it became a standard procedure for many investigations.

In our studies, dealing with biochemical events during germination, we made some comparative work for several reasons. First, we wanted to know whether germination of embryos isolated mechanically or by floating off from organic solvents differs. Secondly, we tested to which extent cell free preparations from embryos for the *in vitro* protein synthesis are influenced by the way the isolation is performed.

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Abbreviations used: TMV — tobacco mosaic virus; RNA — ribonucleic acid; TCA — trichloracetic acid.

Following the procedure of JOHNSTON and STERN (1957), we crushed the caryopses of winter wheat (*Triticum aestivum*, cv. 'LP 3950', F. v. Lochow-Petkus GmbH, Stöckheim BRD) in a single-ear-threshing machine at high speed but without addition of solid carbon dioxide, screened the ground material by sieving and isolated the embryos from this either by hand selection or by floating off from cyclohexane-carbon tetrachloride (10 : 24 v/v) or from cyclohexane-chloroform(10 : 50 v/v). Germination of these embryos on 0.9% agar, containing 1% sucrose and

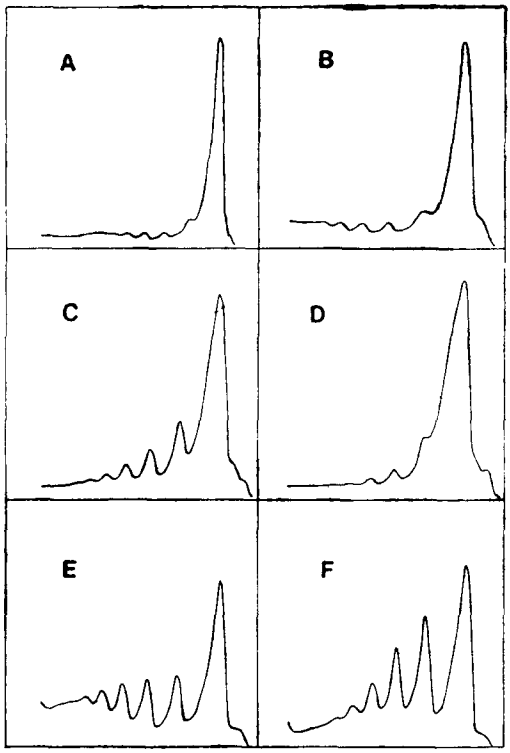

Fig. 1. Sedimentation of ribosomes from germinating wheat embryos on sucrose gradients. The germination of the respective embryos took place on filter paper in the presence of the GM-medium (CHEN *et al.* 1968) at 25 °C in the dark. Isolation of the ribosomes and the preparation of the exponential sucrose gradients (0.3 M—1.0 M) were performed according to the methods of STUTZ and NOLL (1967). Centrifugation of the gradients in 12 ml tubes was conducted in a Beckman SW41 rotor for 150 min at 4 °C and 35 000 rpm. — *A*: hand dissected embryos after 16 h germination; *B*: hand selected embryos from ground caryopses after 24 h germination; *C*: embryos floated off from cyclohexane-carbon tetrachloride, germination for 48 h; *D*: hand selected embryos after storage for 3 weeks over dry $CaCl_2$, germinated for 48 h; *E*: embryos, undissected from the caryopses, after 24 h germination; *F*: ditto. after 48 h. Sedimentation from right to left.

100 µg streptomycin/ml, for 36 h at 25 °C in the dark exhibited some differences. Compared with the seedlings from mechanically isolated embryos, the germination was strongly inhibited when cyclohexane-chloroform had been used. Seedlings arising from embryos floated from cyclohexane-carbon tetrachloride had more compact primary roots.

In order to differentiate between mechanical hurt resulting from grinding, and the action of the organic solvents on the embryos during floating, we carefully dissected embryos from the seeds by hand, excluding mechanical damage. One part of these embryos remained untreated as control, whereas two other parts were suspended for 15 min in the two mixtures of organic

TABLE 1
COMPARISON OF VARIOUS PARAMETERS IN EMBRYOS ISOLATED MECHANICALLY AND BY FLOATING OFF FROM ORGANIC SOLVENTS

		Germination (36 h) [%]*	Respiration [µl O_2 h^{-1}; 16 h]**
A	Hand dissected embryos	95	120 ± 14%
	ditto. treated with cyclohexane-carbon tetrachloride	85	156 ± 10%
	ditto. treated with cyclohexane-chloroform	50	69 ± 8%

Efficiency of cell free preparations for protein synthesis***

	Ribosomes	S-100	^{14}C-phenylalanine	^{14}C-leucine	poly U (100 µg)	TMV-RNA (27 µg)	Incorporation [counts min^{-1}]
B	H	H	+	—	+	—	34 132 ± 1 550
	F	F	+	—	+	—	32 400 ± 1 220
	H	F	+	—	+	—	29 300 ± 1 370
	F	H	+	—	+	—	28 650 ± 1 435
	"S-23"-system (MARCUS *et al.* 1973)						
	H		—	+	—	+	33 110 ± 2 320
	F		—	+	—	+	31 010 ± 2 120
	"S-30"-system (ROBERTS *et al.* 1973)						
	H		+	—	+	—	48 554 ± 1 320
	F		+	—	+	—	46 731 ± 1 450

Explanations: *Germination was performed on streptomycin-agar for 36 h at 25 °C in the dark. Visible primary root was taken as the criterion for germination.
**Oxygen consumption (µl O_2 h^{-1} and per mg dry embryos) was measured by Warburg technique. Dry embryos were placed on filter paper disks wetted with sucrose-streptomycin (see text) containing 10^{-4}M ammonium citrate and kept at 22 °C.
***The assays for cell free protein synthesis were conducted according to the authors cited. In the first group we used 5 A_{260} ribosomes, 100 µl freshly prepared high speed supernatant (S-100), 100 µg polyuridylic acid (poly U) and 0.16 µCi ^{14}C-phenylalanine per assay, which, at 400 µl total, was incubated for 30 min at 30 °C. In the "S-23"-system we used RNA from TMV, which was prepared as described by MARCUS *et al.* (1973). In this case the incorporation of ^{14}C-leucine (1.35 µCi/280 µl) during 30 min at 30 °C was determined.
The "S-30"-system was incubated for 60 min at 30 °C in the presence of 0.16 µCi/100 µl. All assays were carried out in triplicate.
Symbols: *H* = hand selected embryos from ground caryopses; *F* = embryos floated off from cyclohexane-carbon tetrachloride.

solvents we mentioned above. By this treatment we simulated the conditions during floating. When these three groups of embryos were germinated, they behaved in the same manner as we described above (Table 1A). Parallel to this, we also measured respiration using the Warburg technique. The results of a typical experiment are presented in Table 1.

If one considers respiration as a more precise parameter of germination than morphological aspects of the seedlings, we agree with the findings of JOHNSTON and STERN (1957), who also stated accelerated germination of embryos floated from cyclohexane-carbon tetrachloride. But it is obvious that organic solvents have some influence on the embryos. It depends on the purpose of the respective experiment if one can neglect these unknown effects. Naturally, in any case cyclohexane-carbon tetrachloride is more recommendable than others. As shown, chloroform is toxic. We also tested some other solvents like isopropanol and amylacetate but, though they were useful for floating, they caused a strong inhibition of germination.

Dry embryos contain preserved messenger RNA which, as soon as water imbibition takes place, binds to monosomic ribosomes resulting in the formation of polysomes. This process indicates the onset of protein synthesis, which does not imply synthesis of messenger RNA (CHEN *et al.* 1968). Thus the intensity of germination is reflected in the formation of polysomes and the relation between polysomes and monosomes can be thought to characterize the intensity of germination. In Fig. 1 (*A*—*F*) we show some characteristic sedimentation profiles of ribosomes from embryos at different stages of germination.

There is no fundamental difference between floated, hand selected and hand dissected embryos. Much more important than the way how the embryos had been isolated is the fact that the storage of the isolated embryos for more than two weeks results in inhibited germination. We found this in all cases, though the different batches were stored at 4 °C in a desiccator over dry calcium chloride. This phenomenon is expressed by retarded germination on agar and also in the sedimentation profile of the ribosomes in the respective embryos (Fig. 1 *D*). Of course, germination of isolated embryos is quite different from that of embryos which remain unexcised from the caryopses (Fig. 1 *E*—*F*). This difference becomes very striking soon after 24 h of germination, though fresh medium (CHEN *et al.* 1968) was added. If labelling experiments with radioactive precursors of nucleic acids, proteins *etc.* under natural conditions are intended, the handling of whole caryopses would be difficult. In our investigations we make a compromise by dissecting the seedlings after 24 or 48 h germination and incubate them for labelling in the respective medium. Another possibility would be to use the embryo-containing seed halves when natural conditions are mandatory.

We also checked if the efficiency of cell free systems for the *in vitro* synthesis of protein, prepared from wheat embryos, is dependent on the isolation method. Following the methods of MARCUS (1972) and MARCUS *et al.* (1973), we prepared from hand selected and floated embryos ribosomes — once washed — and high-speed supernatants "S-100" (MARCUS 1972) and assayed them crosswise with preparations from hand selected and floated embryos. We conducted the polyuridylic acid-directed incorporation of ^{14}C-labelled phenylalanine (MARCUS 1972). In the case of the "S-23"-system (MARCUS *et al.* 1973) we also carried out TMV-RNA catalyzed amino acid incorporation into TCA precipitable material. We further checked the system which has recently been described by ROBERTS *et al.* (1973). In Table 1 B the data of some typical series are given. It should be pointed out that the efficiency of the assays varied from one series to the other. The presented data were obtained from freshly isolated ribosomes and high speed supernatants (S-100) in order to exclude effects arising from storage. With respect to the efficiency, it should be mentioned that it depends to a great extent on the quality of transfer RNA, which we prepared according to the method of VANDERHOEF *et al.* (1970). The treatment of S-100 with DEAE-cellulose, by which the system becomes dependent on the supply of exogenous transfer-

RNA (MARCUS 1972), also increases the efficiency of the cell free system. We obtained a twofold increase when we used "S-100-DEAE" (not shown here). In general, we could not find great differences between preparations from embryos isolated mechanically or by floating off from cyclohexane-carbon tetrachloride (Table 1). As mentioned above the negative effect of long storage was also reflected in a strong reduction of the efficiency in the respective assays.

Summarizing, our comparative investigations favour the floating method which was introduced by JOHNSTON and STERN (1957). We reinvestigated this method, and after including some additional parameters, as the formation of polysomes *in vivo* and effectiveness of cell free systems for protein synthesis, we agree with the results and consequences of these authors.

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BOOK REVIEW

SEMIKHATOVA, O. A.: **Energetika Dykhaniya Rasteniï pri Povyshennoï Temperature.** [Energetik der Pflanzenatmung bei erhöhter Temperatur]. — Nauka, Leningradskoe Otdelenie, Leningrad 1974. 112 S. Rbl. 0,53.

Diese Monographie der Verfasserin aus dem Botanischen Institut der Akademie der Wissenschaften der UdSSR in Leningrad ist einer speziellen Thematik gewidmet — dem Verhalten der Pflanzen unter Stress-Bedingungen (hier erhöhte Temperaturen in der Wüste) und fusst auf den experimentellen Resultaten der Verfasserin. Als Pflanzenreaktion auf die ungünstigen Bedingungen wurde der Energiestoffwechsel im Laufe der Atmung untersucht. Im 1. Kapitel des Büchleins werden die geeigneten Charakteristiken des energetischen Stoffwechsels der Pflanzen bei hohen Temperaturen ausgesucht und beschrieben, im 2. Kapitel mit Hilfe von Entkopplergiften und ^{32}P-Anlagerung die qualitativen Seiten der Atmung, in Kapitel 3 die quantitativen Eigenschaften der Pflanzenatmung bei hohen Temperaturen untersucht. Der Einfluss von hohen Temperaturen auf isolierte Mitochondrien und die Sauerstoffaufnahme, den Atmungsquotient und das Phosphatenergiepotential sind der Inhalt der letzten Kapitels. Es konnte gezeigt werden, dass es bei hohen Temperaturen nicht zur Entkopplung von Oxidation und Phosphorylierung kommt, wie bisher angenommen wurde. Ungünstige Temperaturbedingungen unterbrechen nicht die Energieversorgung der Zellen, sondern steigern deren Energiebedarf. Die festgestellten Tatsachen und Schlussfolgerungen werden in einer ausführlichen Zusammenfassung in russischer und englischer Sprache summiert und diskutiert. Es folgt ein Literaturverzeichnis (286 Zitationen, leider einige Druckfehler in Zitationen) und Inhaltsverzeichnis. Das Büchlein ist durch 23 graphische Darstellungen illustriert und mit 10 Tabellen ausgestattet. Ein Sach- und Autorenregister wären zu begrüssen. Das Buch wurde sehr sorgfältig zusammengestellt und reflektiert die reichen Erfahrungen der Verfasserin auf diesem Gebiet.

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