Biological Activity of Fractions from Embryo Extracts of *Durum* Wheat at Different Phases of Ripening[1]

Seed dormancy is the consequence of metabolic obstruction, which becomes established during ripening and can be removed as times go on, either spontaneously or by the activity of physical factors such as light or temperature.

The onset of seed germination is the result of a complex series of phenomena controlled by hormones having antagonistic activity[2]. The prevalence of one of them determines the block or the activation of processes which lead to the new seedling.

The seeds of *durum* wheat are characterized by partial dormancy: some of them are always capable of slow germination[3] but their germination energy varies in dependence on ripening phase of seed[4]. HUBAC[5] found that the lack of germination in the unripe caryopses of *Triticum* cv. Fylgia was due to the presence of a high content of proline. MELETTI[4] in *Triticum* cv. Cappelli found that the germination inhibitory power modified its location in the caryopsis (embryo or endosperm) as a consequence of ripening phase. Moreover, substances having depressive activity on germination were isolated from unripe endosperms of this cultivar[6], and their activity seems correlated with the activating substances capable of diffusing when the seeds are allowed to germinate in water[4].

In the present research, the problem of chemical control of seed germination was approached by considering the wheat embryos at different phases of ripening as a tool to understand the mechanism of hormonal control of the seed dormancy. The aim was to reveal the presence of substances having regulation activity on germination and growth processes, and to evaluate their possible variation during and after the seed ripening.

Materials and methods. Unripe seeds of *Triticum durum* cv. Cappelli from plants of 1973 crop at different phases of ripening (milky-ripe, waxy-ripe, fully-ripe[7] and after-ripe seeds (non-dormant seeds, 10 months after harvest) were utilized. The embryos from seeds fixed in methanol at 4 °C were homogenized and centrifuged 3 times at 8000 rpm. The supernatant was dry reduced and the residue dissolved in water (crude extract). Fractionation was carried out by Sephadex G 10 column eluted by water at pH 6.5. The fractions, detected at 256 nm, were utilized for biological assays at concentration of 100 embryos/ml. Germination assay was performed by seeds of *Lactuca sativa* cv Great Lakes allowed to germinate, in the dark at 23 °C, in plastic dishes (5 cm ⌀) on paper moistened with 1 ml of fractions. Germination frequency was evaluated after 14, 18 and 24 h. Hypocothyl and root of *Lactuca* seedlings, from seeds grown on water at 23 °C in the dark for 36 h, were used as growth bioassay. The seedlings, with 3–5 mm long roots, were allowed to grow on 3 ml of fractions to assay, under artificial light (3500 Lux by Leuci daylight fluorescent tubes) at 28 °C for 5 days. 3 repeats of 12 plants for each fraction were performed. The activity of fractions was compared with solutions of abscissic acid (ABA) at 5 ppm, of *cis* 4-cyclohexene-1,2-dicarboxymide (Cis4)[8,9] at 5 ppm and of gibberellic acid (GA_3) at 1×10^{-5} *M*.

Results. From the crude extract of milky-ripe embryos, 9 fractions were isolated. All fractions were seen to have neither depressing nor stimulating effects on germination. The fractions 2, 3, 4, 5, 8 and 9 exibited a stimulating effect on hypocothyl growth, which was significantly higher than control for the fractions 3 and 4. The fractions 1–6 and 7 moderately depressed hypocothyl (Figure 2). The effects on root growth indicated that the fractions, except the fraction 1 which depressed growth moderately, had stimulating activity, very high for fractions 3, 4 and 9 (Figure 3).

Five fractions were isolated from waxy-ripe embryos. The fractions 2–5 produced an initial depressive effect on seed germination. All the fractions stimulated hypocothyl growth which, for seedlings treated with the fractions 1, 2 and 5, overtook the control ones, but was inferior to the hypocothyl of plants treated by GA_3 solution (Figure 2). On root growth, all fractions exibited a strong stimulating effect, greater than GA_3 solution (Figure 3).

Among the 7 fractions isolated from fully-ripe embryos, the fraction 3 had a drastic depressing effect on germination (Figure 1): other fractions appear to be similar to the control in water. The hypocothyl growth was stimulated by all fractions, and highly so by the fractions 3 and 6. The fraction 3 is characterized by a significant depress-

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FIG. 1

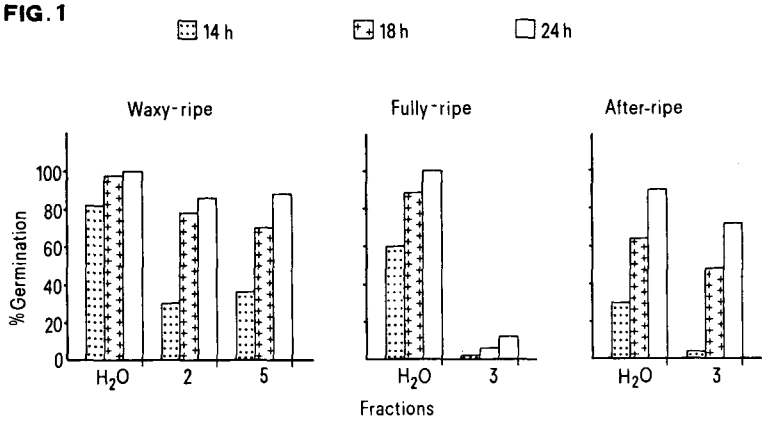

Fig. 1. Germination percentage of *Lactuca* seeds allowed to grow for 14, 18 and 24 h in the isolated fractions.

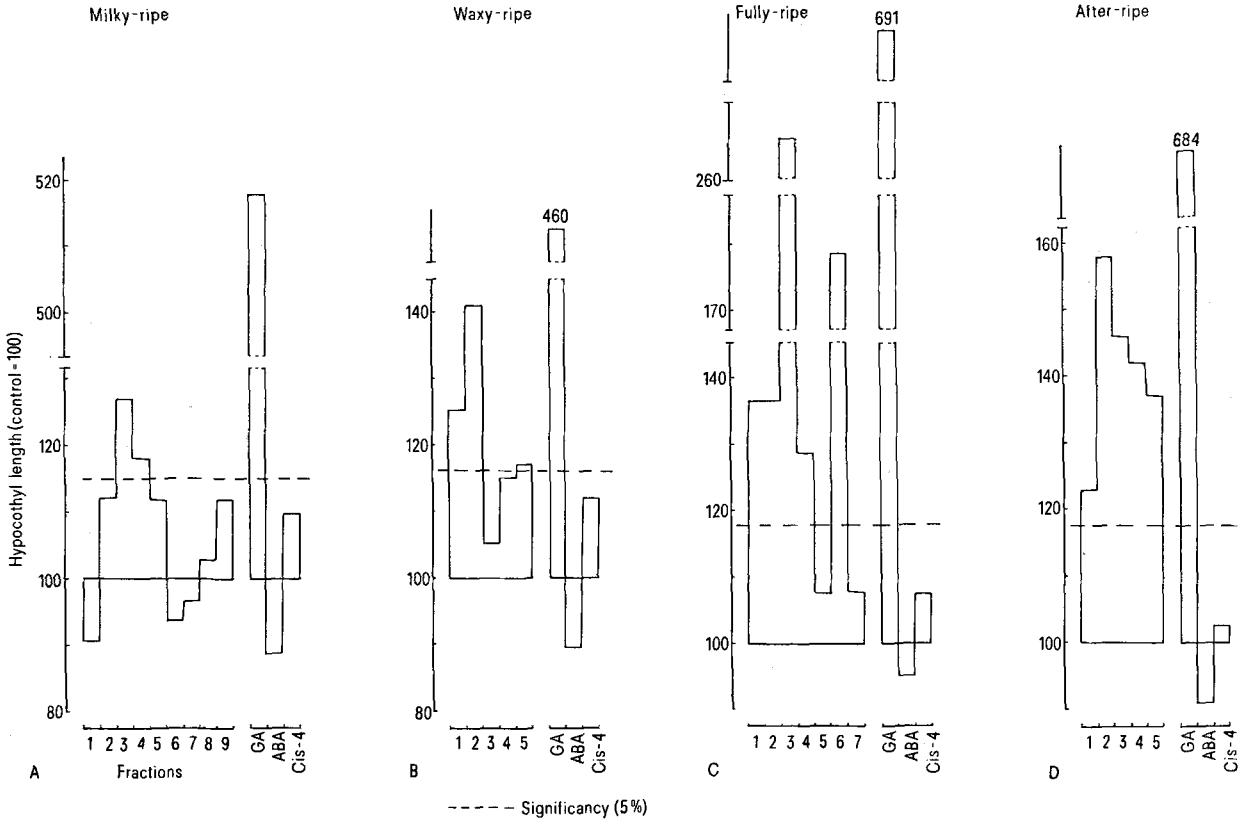

Fig. 2. Hypocothyl elongation of *Lactuca* seedlings sown in the isolated fractions.

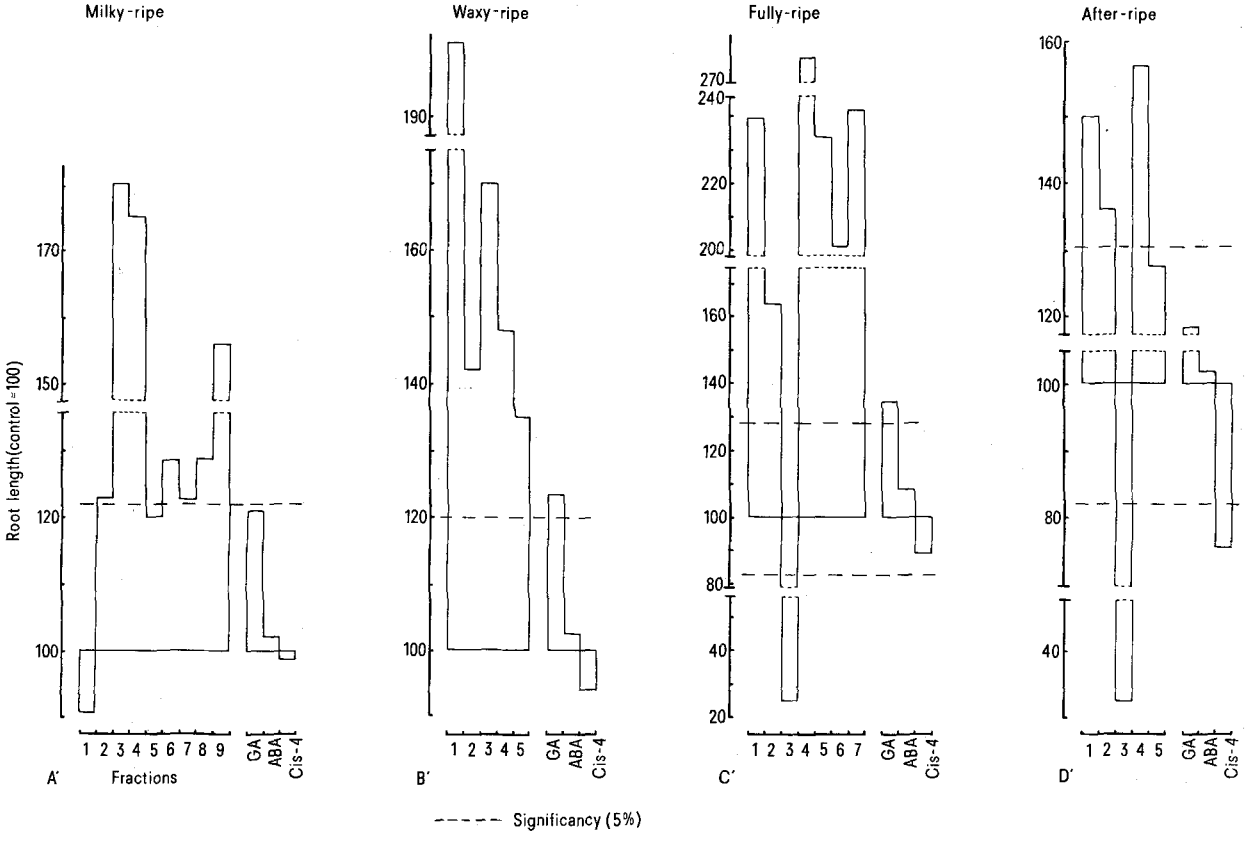

Fig. 3. Root growth of *Lactuca* seedlings sown in the isolated fractions.

ing activity on root growth: the other fractions stimulate more than controls (Figure 3).

From the after-ripe embryos, 5 fractions were isolated: only the fraction 3 exhibited a moderate depressing effect on seed germination. The fractions, when assayed on hypocothyl growth, showed a very high stimulating activity (significant values) but lower than GA_3 solution (Figure 2). The bioassay on lettuce root showed that the fraction 3 strongly depressed while other fractions stimulated growth (Figure 3).

Discussion. The behaviour of wheat seed during ripening is the result of effects caused by growth substances which are acting either on its embryo or endosperm. Their relationships regulate a lot of enzymatic reactions which lead to the accumulation of photosynthetic products, the utilization of storage products and seedling growth. The results obtained confirm the existence of a variation, during the seed ripening, in the regulation capacity on seed germination and growth by the embryos.

In the milky-ripe embryos, no fraction which depressed germination was detectable. On the contrary, they contain substances which stimulate hypocothyl and root growth. Yet, since these substances appear to have stimulating efficiency lower than the assayed gibberellin, but are capable of inducing a strong stimulating effect on the root growth, they might contain cytokininlike products[10]. However, at this ripening phase, the wheat caryopsis, being capable of germination, exibits a reduced seedling growth. Since the lack of a detectable inhibitory activity in the extract from unripe embryos was detected, they may be unable to utilize storage products which are accumulating[11,12]. The waxy-embryos show some fractions (the 2nd and 5th) which moderately depress the initial phases of germination but are capable of stimulating the hypocothyl elongation.

It appears that, just before the wheat seeds attain their maximum dry weight, the embryos possess a lot of stimulating substances which are not opposed by others having depressing effects. When the seeds reduce their water content, the embryos exibit a fraction (the 3rd in the fully-ripe seed) which depresses the seed germination and root growth but is capable of highly stimulating the hypocothyl growth which depends principally on distension. As the auxins rapidly disappear when the wheat grains get ripe[11], the effects of the fraction could be due to the relative concentration of active substances present in it, giving different or opposite effects in the assayed tests[13].

The presence of germination depressing substances in the diffusates from embroys during the ripening phases has been detected[4]: similar substances do not diffuse from the endosperms. From our experiment it appears that the capacity to depress germination increases, when the seed is ripening, reachs its maximum in the ripe seeds and decreases during the after ripening phase.

The germination-depressing activity of fraction 3 from fully-ripe embryos appears to be similar to specific inhibitors like ABA or Cis4. These, on the contrary, exibit a depressing activity on seedling root growth, lower than that detected for the fraction 3.

The different and opposite effects – stimulating or depressing – due to the fraction 3 could be the consequence of the simultaneous presence of different active products in this fraction which, when isolated from ripe and after-ripe embryos, appear to be the same. A preliminary investigation to identify the active products by reagents for gibberellins and auxins did not show the presence of such promotors in the fraction 3. Different procedures are being tried to evaluate the presence of growth substances in these extracts.

Summary. Bioassay of fractions from wheat embryos revealed that, at all ripening phases, they posses natural substances which stimulate hypocothyl growth. The fully-ripe and the after-ripe embryos exibited a very effective fraction for depressing seed germination and seedling root growth; the other fractions stimulate root growth. The results are discussed in relation to current hypotheses of physiological balance between antagonist growth substances.

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Coniferyl Alcohol from Callus of *Castanea sativa* Cultured in vitro

In vitro culture is being used increasingly in studies of the metabolism and morphogenesis of forest tree species, but little has been done on the Fagaceae. Some authors[1–4] reported the successful culture of *Quercus* sp., *Fagus sylvatica* L. and *Castanea sativa* Mill. callus from cambial tissue, but none of them made studies of the metabolism of the callus.

We obtained callus tissue from cotyledons of *Castanea sativa* Mill. which have been maintained in subculture for a year. This paper describes a chemical study of exudates which appear on the surface of callus in the course of their development, one component of which has been identified as coniferyl alcohol.

Material and methods. Callus tissue of *Castanea sativa* which originated from cotyledon tissue was sub-cultured every 7–8 weeks. 7-month-old stock tissue was used for experiments. The basal medium contained the Murashige and Skoog[5] inorganic components plus benzyladenine, 0.5 ppm; indole-3-butyric acid, 1 ppm; m-inositol, 500 ppm; vitamin B_1, 1 ppm; Ca-pantothenate, 0.5 ppm; sucrose, 3% w/v and 0.7% agar. In other experiments kinetin (0.5 ppm), but not benzyladenine, was added. The pH of the medium was adjusted to 5.6 before autoclaving. The cultures were grown at 25 °C in the dark. For histological studies the explants were fixed in FAA and microtome sections were processed in the conventional manner using safranin-fast freen as the stain. For insuring localization of lignified tissues, polarized light microscopy was performed.

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