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Growth of excised embryonic axes of barley on synthetic medium

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INTRODUCTION

The studies reported in the present paper were based on the results of work on decotylized embryos of *Lupinus luteus* L. carried out previously in our laboratory (Czosnowski 1962). It was found that decotylized lupin embryos cultured on Heller's mineral medium with trace elements and with sucrose utilize negligible amounts of inorganic nitrogen from the medium. Hence their growth proceeds at a low rate and so does the protein synthesis in comparison with normal (developing together with cotyledons) axes of lupin seedlings. It was further found (Czosnowski, Michejda 1964) that apart from embryos, likewise the axes of lupin seedlings exhibit this property — at least until the 12th day after germination.

Here the question should be put, how far is the inability of independent nutrition with inorganic nitrogen a specific characteristic of embryonic axes of lupin, or perhaps in general, of plants with high-protein seeds.

The present paper is a report on comparative studies carried out with excised, embryonic axes of barley (devoid of scutellum) the seeds of which are notably low-protein. The references quoted dealing with nitrogen metabolism in barley during the earliest stages of development from seed concern either intact seedlings (Folkes, Willis, Yemm 1952; Folkes, Yemm 1958; Cocking, Yemm 1961; Folkes 1959) or excised, intact embryos (including scutellum) (Brown 1906; Brown 1946).

MATERIAL AND METHODS

The test material consisted of barley grains of the spring variety 'Skrzeszowicki' originating from the Złotniki Exp. Sta. of the Department of Plant Breeding, College of Agriculture, Poznań. A grade of seeds ranging between 35 and 50 mg in weight was selected for the study. Deglumed seeds were sterilized with 0.2% mercuric chloride, rinsed several times with sterile water and left to swell for 3 hours in redistilled water.

Then, part of the grains was placed in culture test tubes, on sterile 0.7% aqueous agar, to serve as control material. From the other grains embryonic axes were isolated and planted on Heller's medium with 3% sucrose and 0.7% agar. All the operations were performed under sterile conditions.

Both the intact seedlings and isolated axes were cultured at the same time in darkness and under continuous fluorescent light (1600 lux) at 24°C in a thermostat. Samples were taken from swelled grains, seedlings and isolated axes after 1, 3, 7, 12 and 18 days of culture.

Fresh and dry weight of material dried at 75°C was determined. The determinations were made each time on 200 to 60 (in dependence on the age) seedlings or isolated axes. Total and soluble nitrogen was determined in material dried acc. to the previously described procedure (Czosnowski, Michejda 1964). Calculations were then made of protein nitrogen and the $N_{protein}/N_{soluble}$ ratio.

EXPERIMENTAL RESULTS

Initial material for experiments: deglumed barley grains left to swell for 3 hours and then tested.

The results are shown in the following Table:

	Dry matter mg/organ	Total N µg/organ	Soluble N µg/organ	Protein N µg/organ
Endosperm	37.3	679	90	589
Scutellum	0.6	91	24	66
Axis	0.7	44	12	32

Fresh and dry matter

(Figs. 1 and 2)

A. Fresh and dry matter of barley seedlings cultured on aqueous agar shows the highest rate of increase between the 1st and 7th day; later it gradually decreases, the rate being higher in darkness than under light.

B. Isolated barley axes cultured on Heller's medium with sucrose carry much lower levels of fresh and dry matter than normal seedlings.

The weight of stems after 7 days of culture is higher under light than in darkness. The root weight is unaffected by light.

Total nitrogen

(Fig. 3)

A. Total nitrogen in seedling stems shows a high rate of increase until the 12th day of culture. Later that rate of increase declines thus indicating an exhaustion of storage nitrogen in the endosperm. Light has a limited effect on the rising level of total nitrogen in stems; in respect to roots it is effectless.

B. The level of total nitrogen in isolated axes is much lower than in seedlings, yet the increase is notable.

After 12 days of culture under light, the total nitrogen content was found to have increased fourfold in comparison with the initial value. In roots, the increase

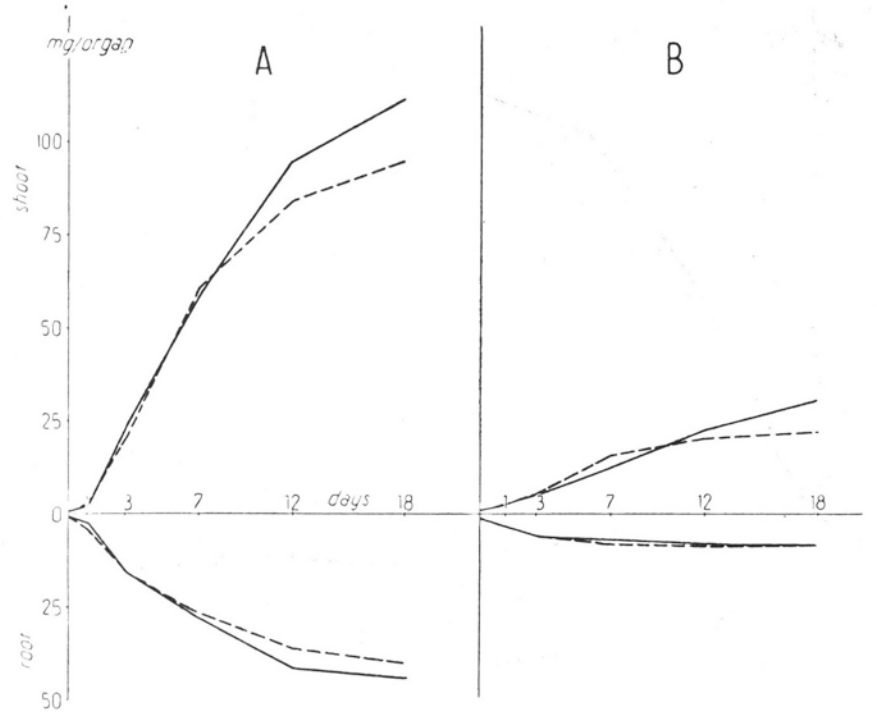

Fig. 1. Fresh weight (mg per organ) of normal seedling axes (*A*) and excised embryonic axes cultured in vitro (*B*). Above "0": shoot system, below "0": root system. Solid line — cultures under light, broken line — cultures in the dark

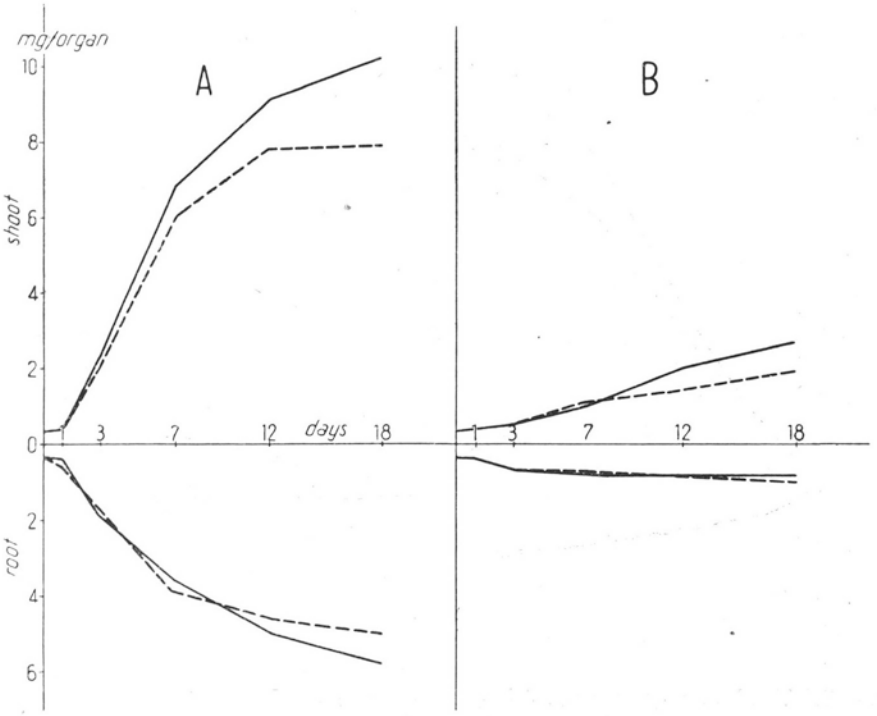

Fig. 2. Dry weight (mg per organ) of normal seedling axes (*A*) and excised embryonic axes cultured in vitro (*B*). (For further explanation see Fig. 1).

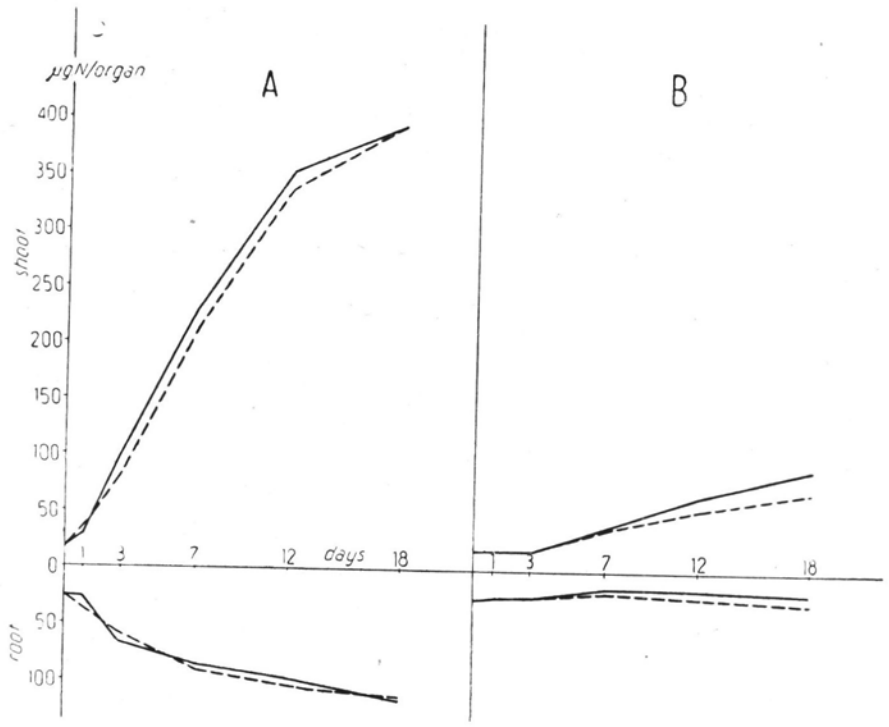

Fig. 3. Total nitrogen (µg per organ) in normal seedling axes (*A*) and excised embryonic axes cultured in vitro (*B*). (For further explanation see Fig. 1)

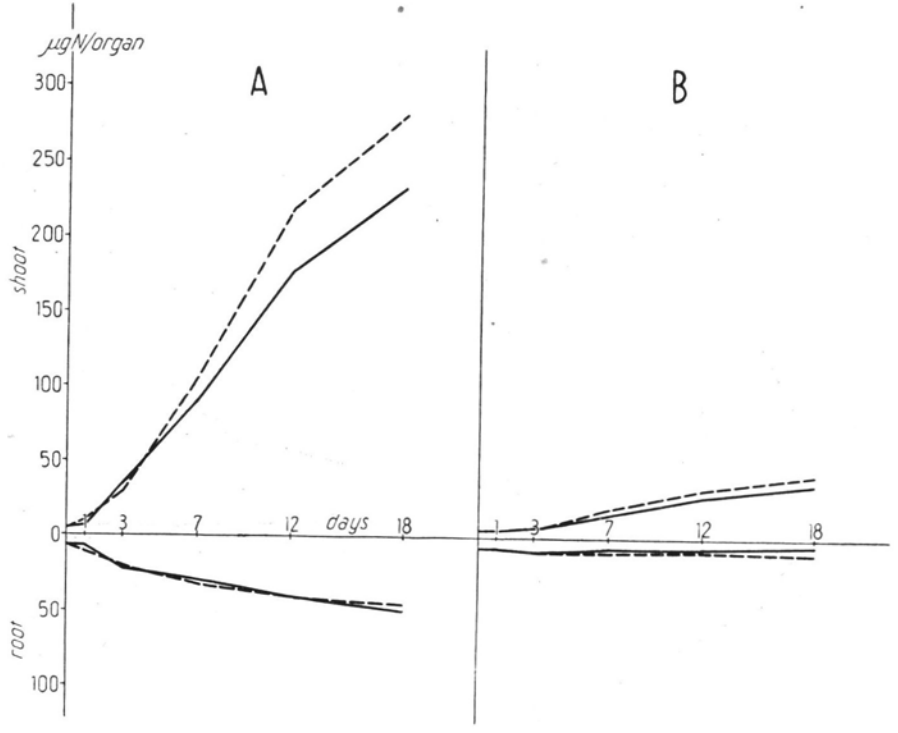

Fig. 4. Soluble nitrogen (µg per organ) in normal seedling axes (*A*) and excised embryonic axes cultured in vitro (*B*) (For further explanation see Fig. 1)

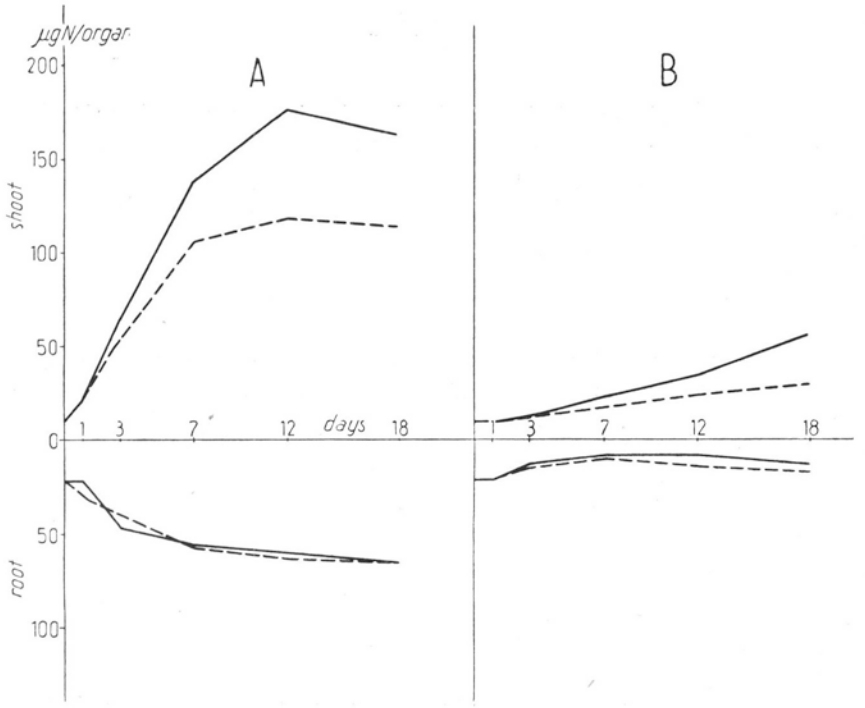

Fig. 5. Protein nitrogen (µg per organ) in normal seedling axes (*A*) and excised embryonic axes cultured in vitro (*B*). (For further explanation see Fig. 1).

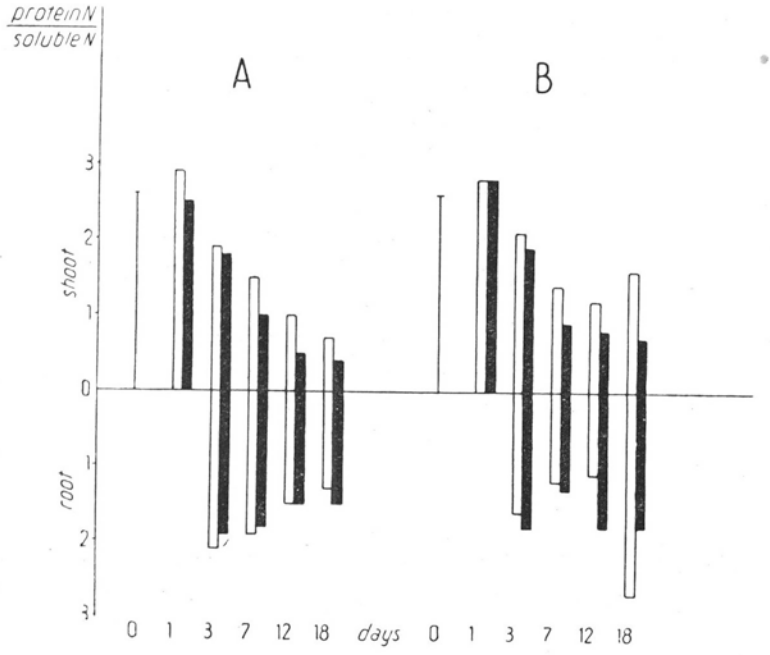

Fig. 6. $N_{protein}/N_{soluble}$ ratio in: *A* — normal seedling axes, *B* — excised embryonic axes. Above "0" level: shoot system, below "0": root system. White bars — light cultures; black bars — dark cultures.

in amount of total nitrogen is less rapid than in the stem; during the first days total nitrogen even becomes less. Czosnowski (1962) observed a similar situation in lupin and explained it by exodiffusion of nitrogen compounds into the medium.

Soluble nitrogen
(Fig. 4)

A. In seedlings, the level of soluble nitrogen in stems rapidly rises during the first 12 days of germination; its amount is lower under light than in darkness; in roots, there is no difference in this respect between light-grown and dark-grown seedlings.

B. In excised axes soluble nitrogen increases continually, though at a low rate, in stems; in roots the level of soluble nitrogen tends to become lower, especially under light.

Protein nitrogen
(Fig. 5)

A. In seedling stems of barley cultured both under light and in darkness the most rapid increase in the amount of protein takes place during the first 7 days of germination; after 12 days, breakdown processes notably prevail over protein synthesis. In roots, after 18 days of culture, the protein level continuously rises; light is effectless in this case.

B. In excised axes the amount of protein continuously increases, both in roots and in the stem. In the latter the protein increase is higher under light.

$N_{protein}/N_{soluble}$ ratio
(Fig. 6)

A. In seedlings grown with endosperm the $N_{protein}/N_{soluble}$ ratio ranges in the stem between 0.5 and 2 and in the root — between 1.5 and 2. On the whole it decreases with time and under light it is higher than in darkness.

B. In isolated axes the N_p/N_s ratio is only slightly higher than in seedlings. The downward tendency connected with the passing time, notable in seedlings, is absent in this case. After 18 days of culture under light, the N_p/N_s ratio evidently increases.

DISCUSSION

Mature, excised plant embryos are in general assumed to develop regularly on media containing mineral sources of nitrogen, commonly applied for culturing tissues and organs of plants or intact plants (Narayanaswami, Norstog 1964).

However, the growth of embryos on these media is often inferior, especially at early stages of development, to that of normal seedlings.

Czosnowski (1962) found symptoms of nitrogen starvation in isolated, embryonic axes of yellow lupin on medium containing $NaNO_3$ (Heller's solution). Other

inorganic sources of nitrogen (unpublished data) likewise proved ineffective for growth of isolated lupin axes.

Comparative studies reported in the present paper and carried out on isolated embryonic axes of barley gave different results. Barley axes are capable of assimilating nitrates (especially under light), yet utilization of nitrogen in this form, and the growth it promotes are much less abundant than in the case of normal barley seedlings. Most striking differences between axes of lupin and barley cultivated under identical conditions occur in the $N_{protein}/N_{soluble}$ ratio. In isolated axes of lupin the ratio in question was several times higher (especially in roots) than the respective values in normal axes (Czosnowski 1962); while in barley in both cases the values of this ratio are more or less alike (Fig. 6). Hence it is evident that under conditions of in vitro culture, nitrogen metabolism of barley axes is not subject to drastic shifts, in spite of its retarded course as compared with that in intact barley seedlings.

Embryonic axes of barley are much more independent in their nitrogen metabolism than those of lupin. As barley belongs to plants with low-protein seeds, there might exist a correlation between the protein level in seeds and the ability of axes to utilize inorganic nitrogen during the earliest stages of development from seed.

In the present work, isolated embryonic axes of barley served as test material instead of intact embryos. It is a known fact that the scutellum plays the role of a storage organ and carries protein (Tab. 1) and fat reserves which become activated during germination (data on maize: Dure 1960; Oaks, Beevers 1964; Ingle, Beevers, Hageman 1964). Nutritional requirements, and at the same time possibilities of culturing isolated, embryonic axes of cereals have recently become the subject of research (Smirnov, Pavlov 1964). Embryos deprived of scutella are more subject to metabolic changes induced by environmental factors. This in turn may be of practical importance in genetics and plant breeding.

SUMMARY

1. Fresh and dry mater were estimated and total and soluble nitrogen determined in:

A. Axes of barley seedlings cultivated on 0.7% aqueous agar under sterile conditions;

B. Isolated embryonic axes of barley cultured under sterile conditions on Heller's mineral medium with 3% sucrose and 0.7% agar.

Culturing was carried out for 18 days, under light and in darkness.

2. Isolated axes were characterized by a slower growth (fresh and dry matter) and lower level of various forms of nitrogen, yet both the growth and the nitrogen level continuously increased. After 12 days of culture the level of total nitrogen in stems was about 4 times that in the initial material.

3. The $N_{protein}/N_{soluble}$ ratio in isolated axes was in general only slightly higher than the respective values in normal seedlings.

4. The results obtained were considered againts similar findings in studies with lupin (Czosnowski 1962). The isolated embryonic axes of barley have been found to possess a higher inde-

pendence of nitrogen metabolism than the isolated axes of lupin. Besides, nitrogen metabolism of barley axes is not affected to so great an extent by culture in vitro as the nitrogen metabolism in lupin.

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Wzrost izolowanych embrionalnych osi jęczmienia na syntetycznej pożywce

STRESZCZENIE

1. Przeprowadzono pomiary świeżej i suchej masy oraz oznaczenia azotu całkowitego i rozpuszczalnego, równolegle na:

a. osiach siewek jęczmieni hodowanych na 0,7% agarze wodnym w warunkach sterylnych,

b. izolowanych, embrionalnych osiach jęczmieni, hodowanych w warunkach sterylnych na pożywce mineralnej Hellera z 3% sacharozy i 0,7% agaru.

Hodowle prowadzono przez 18 dni równolegle — na świetle i w ciemności.

2. Izolowane osie odznaczały się znacznie powolniejszym wzrostem (mniejszą świeżą i suchą masą) i niższym poziomem badanych frakcji azotowych: azotu całkowitego (bez azotanów), rozpuszczalnego i białkowego, jednakże zarówno wzrost, jak i zawartości azotu stale się zwiększały. Po 12 dniach hodowli poziom azotu całkowitego w pędzie był ok. 4 razy wyższy niż w materiale wyjściowym.

3. Stosunek $N_{białkowego}/N_{rozpuszczalnego}$ w izolowanych osiach był na ogół nieznacznie tylko wyższy niż w przypadku normalnych siewek.

4. Przedyskutowano wyniki porównując je z analogicznymi doświadczeniami przeprowadzonymi na łubinie (Czosnowski 1962). Izolowane embrionalne osie jęczmienia odznaczają się większą samodzielnością w dziedzinie gospodarki azotowej (wykorzystują azotany zawarte w pożywce Hellera) niż izolowane osie łubinu. Ich metabolizm azotowy nie ulega w hodowli in vitro tak daleko idącym przesunięciom, jak w przypadku łubinu, o czym świadczą wartości stosunku: $N_{białkowy}/N_{rozpuszczalny}$.