Metabolism of excised embryos of *Lupinus luteus* L.

V. Extract of yellow lupin seedlings as nitrogen source for cultured embryos

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Previous papers in this series (Czosnowski 1962, Czosnowski and Michejda 1964) have shown that the nutrient medium of Heller (1954) with 3% sucrose added is not sufficient to guarantee normal development of decotylised embryonic axes of ripe yellow lupin seeds. Particularly the nitrogen metabolism of excised embryos is deficient. Nitrogen of the medium is utilized to a very small extent and the relative levels of various nitrogen fractions in the excised axes after 12 days of culturing differ very substantially from the same parameters in normal axes of 12-day-old seedlings. One of the parameters differing very strongly is the protein-N to soluble-N ratio which is always much higher in decotylized embryos than in the axes of control plants. From these facts the conclusion was drawn that the Heller medium is an inadequate source of nitrogen and possibly its concentration is inappropriate for the plant material studied.

In the present paper data are presented on the extent to which an extract from several-days-old seedlings of yellow lupin could serve as source of nitrogen for decotylised axes.

MATERIAL AND METHODS

Culture of decotylized embryos

Seeds of *Lupinus luteus* L. var. "Express" were obtained from the Research Centre of the Institute of Soil Science, Plant Cultivation and Manuring at Przebędowo. Seeds of the weight range of 120—130 mg were sterilized by a one-minute immersion in ethanol and a 20-minute immersion in 0.2% mercuric chloride, after which they were washed several times in sterile distilled water. Cotyledonless embryos were taken from seeds after 24 hours presoaking in sterile distilled water. They were placed by twos on agar slants. The basic medium was the Heller solution with 3% sucrose added. The pH of the medium after sterilization for 20 min at 110°C was 5.6. In individual experiments the nitrate nitrogen of the medium was replaced by an extract from seedlings of yellow lupin. The culture was kept in darkness for 12 days at a temperature of 25°C.

Preparation of the extract

Seedlings of yellow lupin var. "Express" were cultivated in distilled water in darkness at a temperature of 25°C on germination dishes, and then dried at 80°C. After grinding the dried material was extracted with 80% ethanol twice at 80°C for one hour and then four times at room temperature each time for 12 hours. To the combined extracts 3 volumes of chloroform were added and after shaking the water fraction was separated. After partial evaporation at 40°C nitrogen was determined by the Kjeldahl method. The extract after bringing the nitrogen in it to a concentration of 10 mg/ml was added to the nitrogen-less Heller medium.

Composition of the experimental media

Two series of cultures were run with increasing nitrate nitrogen in the Heller medium and with increasing "extract nitrogen" in the medium. The whole experiment was replicated twice (at two different times). On each medium within each replicate 24 embryos were cultivated.

To facilitate the interpretation of the effect of nitrogen in the two forms on the response of the embryos, its concentration in the media was expressed as mg/10 ml since into the culture test tubes 10 ml of medium was supplied.

In the first series — series "H" — the Heller solution contained nitrate nitrogen in the following concentrations: 1, 3, 10 and 20 mg/10 ml. These media are referred to as H₁, H₃, H₁₀ and H₂₀. The standard Heller solution contains 1 mg N/10 ml as NaNO₃.

In the second series — series "E" — to a nitrogen-less Heller solution the extract from lupin seedlings was added so as to adjust the extract nitrogen to the following concentrations in the medium: 1, 3, 10, 15 and 20 mg/10 ml. These media are denoted as E₁, E₃, E₁₀, E₁₅ and E₂₀.

Analyses

After cultivation for 12 days the lengths of the organs were measured and the material was dried at 80°C before determining the dry weight. Analyses of the nitrogen fractions were performed as described earlier (Czosnowski and Michejda 1964).

RESULTS

The experimental results are presented in graphs 1—3.

Length increase (fig. 1)

Series H. Above 3 mgN/10 ml the length of the shoot declines. The length of the root increases up to 10 mg N/10 ml and then declines.

Fig. 1

Fig. 2

Fig. 1.: Growth in length and Fig. 2.: fresh and dry weight of decotylized embryos after 12 days of culture on media with increasing nitrate nitrogen concentration (series H) and extract nitrogen concentration (series E).

Above O level = shoot system, below O = roots. For further explanations see section "Material and methods".

Fig. 3. Total nitrogen, soluble nitrogen, protein nitrogen and protein-N to soluble-N ratio in decotylized embryos after 12 days of culture on media with an increasing nitrate nitrogen concentration (series H) and extract nitrogen concentration (series E).

Above O level = shoot system, below O = roots. For further explanations see "Material and methods".

Series E. At the lower nitrogen concentrations (E_1 and E_3) growth is identical. At higher concentrations it is more reduced in the shoot than in the root.

Dry weight (fig. 2)

Series H. In the shoot and root the highest value is at 10 mg N/10 ml and there is a decline in weight at both lower and higher concentrations.

Series E. In the shoot the dry weight remains at a more or less constant level. The root dry weight increases up to E_{10} and above that level declines.

Total nitrogen (fig. 3)

The initial total nitrogen content at the time of removal of the cotyledons was 260 μg per embryo.

Series H. There is a regular increase of total nitrogen content in the root and shoot from 268 μg in one axis at H_1 to 455 μg at H_{10} above which it falls up to H_{20} .

Series E. There is a regular increase from 545 μg per axis at E_1 to 1060 μg at E_{10} after which the concentration slightly falls in the roots but remains at the same level in the shoot.

Soluble nitrogen (fig. 3)

Series H. There is a similar increase in soluble nitrogen content in the root and shoot from H_1 to H_{10} (corresponding to 155 μg and 300 μg in the whole embryonic axis respectively). At H_{20} there is a drop in the content of soluble nitrogen in the shoot and a slight increase in the root. Generally in the H series the amount of soluble nitrogen in the root increases from H_1 to H_{20} .

Series E. There is an increase of soluble nitrogen in the embryonic axes from E_1 to E_{10} (from 420 to 820 μg), and then a slight drop. In the shoot there is a gradual small increase from E_1 to E_{20} . In the root there is a very rapid increment from E_1 to E_{10} followed by a slow drop at higher concentrations.

Protein nitrogen (fig. 3)

Series H. The increment of protein nitrogen in the whole embryonic axes from H_1 to H_3 (112 μg and 152 μg respectively) is followed by a slow drop at higher concentrations (to 116 μg at H_{20}).

Series E. There is an increase in protein nitrogen content of the whole embryonic axes from 125 μg at E_1 to 293 μg at E_{10} and a slight decline at higher concentrations of nitrogen. In the root protein synthesis is dynamic, while in the shoot fluctuations in the protein content are slight.

The protein-N to soluble-N ratio (fig. 3)

Series H. The $N_{\text{prot.}}/N_{\text{sol.}}$ ratio in the shoot drops from 0.54 to 0.38 with increase in the nitrogen amount supplied to the medium. In the root the corresponding drop is from 1.75 at H_1 to 0.73 at H_{20} .

Series E. The $N_{\text{prot.}}/N_{\text{sol.}}$ ratio in the shoot varies from 0.26 to 0.38 between the extremes in nitrogen supply of E_1 and E_{20} . In the root the fluctuations involve the a range from 0.19 to 0.39.

DISCUSSION

In certain conditions decotylized embryos exhibit a great independence with respect to nitrogen metabolism. For example a medium with nitrate nitrogen completely covers the nitrogen requirements of decotylized radish embryos cultured in vitro (Hoffmannowa 1967). At other times this independence is only partial as for example in barley (Michejda 1966) or maize (Oaks and Beevers 1964).

Excised embryos of yellow lupin are completely unable to utilize the nitrogen provided to the medium in nitrate form (Czosnowski 1962).

An increase in nitrate concentration of the Heller medium caused a relatively insignificant increase in total nitrogen (Kjeldahl) and the soluble nitrogen content, and this primarily in the shoots of the cultured embryonic axes (fig. 3). On the other hand the nitrogen extracted from seedlings very favourably influenced the level of the nitrogen fractions in the isolated embryos, this being most manifest in the root. A 10 mg dose of extract nitrogen per 10 ml of medium caused a 10-fold increase of the ethanol soluble nitrogen and a 2.2-fold increase of protein nitrogen content in comparison with the same dose of 10 mg of nitrogen in nitrate form per 10 ml of medium. As compared with the normal Heller medium (1mgN/10 ml) the increment was respectively 22-fold and 3.2-fold. In the shoot the increment of soluble nitrogen though distinct was very markedly weaker than in the root and the levels of protein nitrogen fluctuated around values characteristic for embryos supplied with nitrate nitrogen.

The very intensive accumulation of soluble nitrogen in the embryos cultured on an extract from seedlings results in a shift of the protein-N to soluble-N ratio to lower values, characteristic for a normal seedling axis, and particularly its root (Czosnowski 1962; Czosnowski and Michejda 1964).

It is obvious that an extract from seedlings cannot be considered exclusively as a source of nitrogen, primarily in the organic form, since the extract is a complicated mixture of various groups of compounds soluble in ethanol 80%. However from the general results of the experiment it appears, that the extract from seedlings was of use primarily from the point of view of nitrogen metabolism.

When culturing isolated embryos of yellow lupin (and probably not only lupin) such parameters as fresh and dry weight as well as increment in length are useless for the characterisation of the developmental normality on an artificial medium.

These characters are often very close even when, for example, the nitrogen fractions indicate very high and characteristic differences in level.

Oaks and Beevers (1964) have found that isolated embryos of maize react by an increased protein synthesis and an increase in the soluble nitrogen fraction to an addition to the medium of "leachates" obtained from detached endosperm pieces, which it was possible in turn to substitute by a synthetic mixture of amino acids.

In the case of maize and even more so in the case of lupin the positive effect of leachate from the endosperm or an extract from seedlings depends presumably on the supplementation of the nitrogen pool with a close to natural mixture of amino acids necessary for the synthesis of proteins in the fast growing portions of the isolated axes. In normal seedlings the pool is constantly supplied from reserves successively activated from the storage organs, while in vitro it is supplied with greater or lesser difficulty by an endogenous synthesis from the nitrogen compounds available in the static composition of the medium.

Only further studies can demonstrate whether the difficulties encountered by excised embryonic axes of yellow lupin are the result of inadequacy of the nitrate absorbing mechanism or whether insufficiency of the nitrate reductase — nitrite reductase — glutamic acid dehydrogenase system is responsible here.

SUMMARY

Since nitrate proved to be a completely inadequate source of nitrogen for decotylyzed embryos of yellow lupin (Czosnowski 1962), the nitrogen was supplied in the form of an ethanolic extract from 5-days old seedlings of yellow lupin.

Nitrogen from the extract acts favourably on the metabolism of excised embryos already from the lowest concentrations applied comparable to the normal nitrogen concentration used in nitrate form in the Heller medium: 1 mgN/10 ml. The extract nitrogen supplied at a concentration of 10mg/10ml results in a 22-fold increase in soluble nitrogen content in the roots as compared with that obtained after growth on the Heller medium, and a 3,2-fold increase in protein nitrogen.

The very strong accumulation of soluble nitrogen in embryos cultured on an extract from seedlings results in a shift of the $N_{\text{prot.}}/N_{\text{sol.}}$ ratio in the direction of lower values characteristic for the normal axis of a growing seedling and particular of its root.

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Metabolizm izolowanych zarodków Lupinus luteus L. V.

Streszczenie

Ponieważ azotan okazał się zupełnie nieodpowiednim źródłem azotu dla izolowanych od liścieni osi zarodków łubinu żółtego (Czosnowski 1962, Czosnowski i Michejda 1964), dostarczono im azotu w postaci ekstraktu etanolowego z pięciodniowych siewek łubinu żółtego.

Azot ekstraktowy oddziałuje na metabolizm izolowanych zarodków wybitnie korzystnie począwszy od najniższych stosowanych w pożywce stężeń, porównywalnych z normalnym stężeniem azotu azotanowego w pożywce Hellera (1 mg N/10 ml). Azot ekstraktowy w stężeniu 10mg/10ml daje 22-krotny wzrost azotu rozpuszczalnego w korzeniu w porównaniu z pożywką Hellera, oraz 3,2-krotny wzrost azotu białkowego.

Bardzo silne gromadzenie azotu rozpuszczalnego w organach izolowanych zarodków hodowanych na ekstrakcie z siewek powoduje przesunięcie stosunku $N_{\text{białkowy}}/N_{\text{rozpuszczalny}}$ w kierunku niższych wartości, charakteryzujących normalną oś siewki, szczególnie w korzeniu (Czosnowski 1962).