Nitrogen Metabolism of Spirodela oligorrhiza
I. Utilization of Ammonium, Nitrate and Nitrite

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Received June 1, 1969

Summary. Spirodela oligorrhiza grown in axenic culture was able to use either ammonium, nitrate, or nitrite as sole source of nitrogen, although the morphology of the plants was affected. Plants utilizing ammonium contained higher levels of NH₄, arginine, asparagine and glutamine than did those utilizing NO₃, whereas concentrations of other amino acids were similar.

The utilization of NH₄ inhibited that of NO₃ by inhibiting, at least partially, NO₃ uptake, and by inhibiting almost completely the reduction of NO₃ to NO₂. NO₂ also inhibited the utilization of NO₃. NH₄ and NO₂ were taken up and assimilated simultaneously when they were supplied together in the medium.

Introduction

It is generally considered that most plants can use either ammonium or nitrate ions as sole source of nitrogen, but if the two forms are supplied together, it is frequently found that NH₄ is used first. This has been clearly shown for some lower plants, e.g. Chlorella (Pratt and Fong, 1940; Cramner and Myers, 1948; Schuler et al., 1955; Syrett and Morris, 1963) and some fungi (Rippel, 1931; Morton and MacMillan, 1954). A few organisms are briefly reported as using nitrate first, e.g. some fungi (MacMillan, reported in Morton, 1956; Szegi, 1968) and a strain of the alga Haematococcus, although this only under certain conditions (Stross, 1963). Many comparisons have been made of NH₄ and NO₃ as sole N sources for the growth of higher plants (Street and Sheat, 1958; Hewitt, 1966), but only a few workers have considered their selective utilization when the two sources are supplied together. Prianishnikov (see 1951) showed early this century that when plants such as peas or oats were grown in sand or water culture, NH₄ was taken up in greater amounts than was NO₃. Most such studies were, however, carried out under non-sterile conditions and their results are now difficult to interpret. More recently, but still using non-sterile conditions, Lycklama (1963) showed that the utilization of NO₃ by perennial rye-grass was considerably inhibited by the uptake of NH₄.
In the present work a study has been made of the utilization, that is the uptake and subsequent assimilation of NO₃, supplied singly or together with NH₄ or NO₂ as source of N for the duckweed, *Spirodea oligorrhiza*, grown under axenic, defined conditions.

**Materials and Methods**

*Culture Methods.* The strain of *Spirodea oligorrhiza* (Kurz) Hegelm. was that used by Bollard (1966). It was grown in axenic culture, essentially as described by Bollard (1966), and the only components of the medium to be deliberately varied were the compounds serving as N sources. NH₄ ions were added as (NH₄)₂SO₄, and NO₃ ions as KNO₃, and as the pH of solutions was adjusted with either KOH or H₂SO₄ this meant that the only other differences were in the concentrations of K⁺ ions (4—8 µmoles ml⁻¹) and SO₄²⁻ ions (5—7 µmoles ml⁻¹). When both NH₄ and NO₃ were present as N sources, equivalent amounts of (NH₄)₂SO₄ and KNO₃ were added to the medium. All N and carbohydrate sources were filter-sterilized. Unless stated, media were buffered against fall in pH during plant growth, by the addition of solid CaCO₃. Growth was assessed by taking fresh or dry weights, or by counting the number of fronds, and generally there was good agreement between the three methods.

*Estimation of Nitrogen Compounds in Medium.* NH₄ was estimated by titration with 0.01 N HCl using a Conway microburette (Conway, 1957) following steam-distillation (Markham, 1942). NO₃ was estimated using phenoldisulphonic acid (Humphries, 1956), NO₂ with sulphanilamide in 33% HCl and N-(1-naphthyl)-ethylendiamine hydrochloride (Snell and Snell, 1949).

*Estimation of Nitrogenous Compounds in Tissues.* Plant tissues were killed and extracted with the methanol/chloroform/water solvent (MCW) of Bieleski and Turner (1966). Soluble N estimations were carried out using this extract; other estimations were carried out using the aqueous phase of the split extract. N analyses on dried plant material or on aliquote of the MCW extract were carried out using a semi-micro-Kjeldahl method. Any NO₃ present was reduced to NH₄ with reduced iron metal (May & Baker, Dagenham, England), prior to digestion. Amino acids were estimated by the method of Bieleski and Turner (1966). NH₄ was estimated with ninhydrin (Yemm and Cocking, 1955) following microdiffusion (Conway, 1957). NO₃ was estimated with phenoldisulphonic acid (Humphries, 1956), and NO₂ was tested for by the method of Snell and Snell (1949).

**Results**

*Growth of S. oligorrhiza with either Ammonium or Nitrate as sole Nitrogen Source.* S. oligorrhiza made equally good growth with either ammonium or nitrate as sole source of nitrogen (Table 1). There was, however, some variation in the size and appearance of the fronds as shown in Table 1 and Fig. 1.

*Composition of S. oligorrhiza Growing with Different Sources of Nitrogen.* The total and soluble N contents, and levels of inorganic soluble N compounds present in plants growing with either NH₄, NO₃, or NH₄ + NO₃ as sources of N are shown in Table 2. The concentrations of the quantitatively more important constituents of the soluble amino