Nitrogen fixation (C₂H₂ reduction) in soil samples from rhizosphere of rice grown under alternate flooded and nonflooded conditions

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Summary In a greenhouse study the influence of alternate flooded and nonflooded conditions on the N₂-ase activity of rice rhizosphere soil was investigated by C₂H₂ reduction assay. The soil fraction attached to roots represent the rhizosphere soil. Soil submergence always accelerated N₂-ase and this effect was more pronounced in planted system. Moreover, rice plant exhibited phase-dependent N₂-ase with a maximum activity at 60 days after transplanting. The alternate flooded and nonflooded regimes resulted in alterations of the N₂-ase activity. Thus, the N₂-ase activity increased following a shift from nonflooded to flooded conditions, but the activity decreased when the flooded soil was returned to nonflooded condition by draining. However, the differential influence of the water regime on N₂-ase was not marked in prolonged flooded-nonflooded cycles. Microbial analysis indicated the stimulation of different groups of free-living and associative N₂-fixing microorganisms depending on the water regime.

Introduction

Nitrogen supply to the flooded rice soils through agents other than mineral fertilizers has long been recognized. Blue green algae, photosynthetic, free-living and associative heterotrophic bacteria have been implicated in contributing the major input of biological N to rice soils. Heterotrophic N₂ fixation by bacteria in soil and in association with rice roots is of significance by virtue of high moisture content in the soil and nutrient availability in the vicinity of the roots. It has been clearly demonstrated that soil submergence has accelerated N₂ fixation which was further enhanced by organic matter application.

In rainfed tropics rice fields are subjected to intermittent dry and wet conditions. Alternate dry and wetland cropping systems have increased the availability of soil N to the higher plant. Such alterations in the water regimes might influence soil microbial processes of importance to the soil fertility. Nayak and Rao reported that soil N₂-ase was affected by alternate flooded and nonflooded conditions. Moreover, a differential influence of (NH₄)₂SO₄ and organic matter
on soil N₂ fixation was noticed under continuous flooded and non-
flooded conditions. Under situations of alternating flooded and
nonflooded conditions the decomposition of organic matter and
mineralization of soil N would differ from that of a continuously
flooded or nonflooded system. The present paper describes a green-
house study of the N₂ fixation (C₂H₂ reduction) potential as in-
fluenced by the alternate flooded and nonflooded water regimes
in planted and unplanted conditions.

Materials and methods

Greenhouse studies

An alluvial soil (pH 6.6, organic matter 1.8%, total N 0.02%, electrical conductivity 0.2
mmhos/cm, C.E.C. 18.6 meq/100g) collected from the Institute field was filled (5 kg/pot)
in porcelain pots with a lateral drainage hole padded with glasswool. The hole was plugged
with rubber cork. Two series (planted and unplanted) of six sets of pots were arranged with
three replicates for each water regime. The treatments included (a) a continuous flooded
system, (b) a continuous nonflooded system, (c) a 15-day alternating flooded-nonflooded-
flooded cycle, (d) alternating nonflooded-flooded-nonflooded cycle, (e) a 30-day alternating
flooded-nonflooded-flooded cycle and (f) alternating nonflooded-flooded-nonflooded cycle.

Water regime

Water was added up to a column of 5 cm above the soil to provide submerged conditions.
To achieve the nonflooded (60% W.H.C.) conditions in a submerged soil during the alternating
cycles, the water was allowed to drain completely (by removing the cork) and after 4–5 days
required amount of water was added to provide 60% W.H.C. (20% of moisture on a weight
basis). It took 5 days for the drained soil to reach the moisture level (20%) of the normal
nonflooded system. The level was maintained by periodical moisture determination and water
was added when required to compensate for the evaporation loss. Water was changed at an
interval of 15 and 30 days depending upon the treatment and the C₂H₂ reduction assay of the
soil was conducted at least once during the cycle.

C₂H₂ reduction

The rhizosphere and non-rhizosphere (unplanted) soils were collected periodically from the
pots. Rhizosphere represents the soil fraction attached to the root. The N₂-ase activity (C₄H₄)
reduction was analyzed in 2 g (fresh weight) soil samples in six replicates for each treatment.
The incubation of the soil samples and the nitrogenase analysis were carried out as described
earlier⁷,15,17.

The samples were placed in B-D vacutainer (Becton-Dikinson, New Jersey) tubes (75 x 13
mm) stoppered, and the gas phase was replaced with high purity C₂H₄ (10% by volume) through
a gas tight hypodermic syringe. The tubes were then incubated at 28°C for 24 h in the dark.
At the end of the incubation a 0.5 ml sample of the gas phase from each tube was analyzed for
C₂H₄ production on a GC fitted with a hydrogen flame ionization detector and a 1500 x 3 mm
column filled with 100–120 mesh Porapak-R at a column temperature of 60°C. High purity
N₂ at a flow rate of 30 ml/min served as the carrier gas. The N₂-ase activity was expressed as n
moles of C₂H₄ formed/g dry soil/day. Tubes without C₂H₄ did not evolve endogenous C₂H₄
and the C₂H₂ reduction in the drained water was negligible. Organic C was estimated by modi-
ified Walkley and Black method.

Measurements of pH and redox potential (Eh)

The changes in pH and Eh were estimated thrice during the crop growth at 40, 65 and
85 days after transplanting. The redox potential was measured with a portable redox meter