Measurement of \( \text{N}_2 \) fixation in maize (Zea mays L.)—ricebean (Vigna umbellata [Thunb.] Ohwi and Ohashi) intercrops

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Abstract

The yield of \( \text{N} \) in maize (Zea mays L.) and ricebean (Vigna umbellata [Thunb.] Ohwi and Ohashi) were compared on a Tropoquall soil in North Thailand in 1984 and 1985. Both species were grown in field plots in monoculture or as intercrops at a constant planting density equivalent to 8 maize or 16 ricebean plants per m\(^2\). The contribution of symbiotic \( \text{N}_2 \) fixation to ricebean growth was estimated from measurements of the natural abundance of \( ^{15}\text{N} \) (\( \delta^{15}\text{N} \)) in shoot nitrogen and from analysis of ureides in xylem sap vacuum-extracted from detached stems.

The natural abundance of \( ^{15}\text{N} \) in the intercropped ricebean was found to be considerably less than that in monoculture in both growing seasons. Using maize and a weed (Ageratum conyzoides L.) as non-fixing \( ^{15}\text{N} \) reference plants the proportions (\( P^{15}\text{N} \)) of ricebean shoot \( \text{N} \) derived from \( \text{N}_2 \) fixation ranged from 0.27 to 0.36 in monoculture ricebean up to 0.86 when grown in a 75% maize: 25% ricebean intercrop. When glasshouse-derived calibration curves were used to calculate plant proportional \( \text{N}_2 \) fixation (\( P_{\text{R}} \)) from the relative ureide contents of field collected xylem exudates, the contribution of \( \text{N}_2 \) fixation to ricebean \( \text{N} \) yields throughout the 1985 growing season were greater in intercrop than in monocrop even at the lowest maize:legume ratio (25:75). Seasonal patterns of sap ureide abundance indicated that \( \text{N}_2 \) fixation was greatest at the time of ricebean podset. The average \( P_R \) and \( P^{15}\text{N} \) in ricebean during the first 90 days of growth showed identical rankings of monocrop and intercrop treatments in terms of \( \text{N}_2 \) fixation, although the two sets of \( P \) values were different. Nonetheless, seasonal estimates of \( \text{N}_2 \) fixation during the entire 147 days of legume growth determined from ureide analyses indicated that equivalent amounts of \( \text{N} \) could be fixed by ricebean in a 75:25 intercrop and in monoculture despite the former being planted at one-quarter the density.

Abbreviations. \( P \) = proportion of ricebean \( \text{N} \) derived from symbiotic \( \text{N}_2 \) fixation; \( P^{15}\text{N} \) as calculated from \( ^{15}\text{N} \) abundance in total \( \text{N} \) of shoots; \( P_{\text{R}} \) as calculated from the relative abundance of ureide \( \text{N} \) in xylem sap.

Introduction

The practice of cropping more than one species simultaneously (usually a legume with non-legumes) occurs widely throughout the tropics and subtropics; particularly among small farmers with limited resources. Total grain and plant \( \text{N} \) yields can often be enhanced by intercropping legumes with non-legumes (e.g. Barker and Blamey, 1985; Searle \textit{et al.}, 1981; Singh \textit{et al.}, 1986; Waghmare and Singh, 1984). It has been suggested that mixed-cropping alters rooting patterns so that a greater volume of soil is exploited than in a monoculture (Waghmare and Singh, 1984). Alternatively Singh
et al. (1986) proposed that intercropping increased the activity of soil micro-organisms and the levels of mineral N in the rhizosphere. However, improvements in the relative productivity of components of intercrops are more usually attributed to changes in the ability of the legume to fix N₂. While there is evidence to suggest that there may be transfer of fixed N from the legume to the associated crop under certain conditions (Agboola and Fayemi, 1972; Bandyopadhyay and De, 1986; Eaglesham et al., 1981; Patra et al., 1986), there is relatively little information on the contribution of N₂ fixation \textit{per se} to the total N yield of intercropping systems. Since there is some uncertainty as to the potential for N₂ fixation by legume components of intercrops (Agboola and Fayemi, 1972; Graham and Rosas, 1978; Nambiar et al., 1983; Patra et al., 1986; Suwanarit et al., 1985; Wahua and Miller, 1978), an investigation of the role played by a legume commonly used for mixed-cropping in Thailand was undertaken. Ricebean (\textit{Vigna umbellata} [Thunb.] Ohwi and Ohashi) is an ideal plant for intercropping; it has a vigorous climbing habit and is often grown in association with maize (\textit{Zea mays} L.), since Thai farmers believe this to be a more sustainable system than monoculture maize.

This paper describes the measurement, by two different methods, of N₂ fixation in ricebean grown in intercrops or in monoculture in northern Thailand. One technique uses the slight natural enrichment of $^{15}$N in soil mineral N compared with atmospheric N₂ to estimate the proportion of plant N derived from N₂ fixation (Bergersen \textit{et al.}, 1985; Shearer and Kohl, 1986). The second technique uses the proportion of ureide N in legume xylem sap as an indirect indicator of the contribution of N₂ fixation to plant growth (Herridge, 1984; McClure and Israel, 1979; Pate \textit{et al.}, 1980). Neither technique has previously been used to any great extent in tropical agricultural systems (Herridge and Peoples, 1986; Shearer and Kohl, 1986).

### Materials and methods

#### Field experimentation

Field experiments were carried out on a Tropaqualf under rainfed condition during two wet seasons, 1984 and 1985, at Chiang Mai, northern Thailand (19°N, 99°E). Maize:ricebean intercrops were compared with monocultures of both species at a constant overall density of 8 maize or 16 ricebean plants per m² using de Wit's replacement design (de Wit \textit{et al.}, 1966).

**Growing season 1984.** The maize monoculture (cv. Pacific 9 Hybrid) was planted at 0.33 × 0.375 m spacing (8 plants m⁻²) and the ricebean monoculture (local red seeded, unnamed cultivar) at 0.165 × 0.375 m (16 plants m⁻²). One maize plant was considered to be equivalent to two ricebean plants; asymptotic densities for maize and ricebean in this environment are 5.3–8 plants m⁻² and 15–20 plants m⁻² respectively (B Rerkasem and K Rerkasem, unpublished data). For the maize:ricebean intercrop at 75:25, every fourth maize plant was replaced by two ricebean plants. No fertilizer was applied, in compliance with local farm practice. There were four replicates in randomized blocks. Individual plants were harvested (Fig. 1) above ground from each plot at 56 days and 101 days after planting, except the ricebean monoculture at 101 days, in which plants from 3 m² quadrats were harvested. Samples of a weed, \textit{Ageratum conyzoides} L., growing in the experimental plots were also harvested with maize as non symbiotic-fixing reference material. All plant samples were dried at 80°C for 48 h, weighed and finely ground for total N and $^{15}$N analysis.

![Fig. 1. Sampling patterns for (a) corn in monocrop, (b) corn and ricebean in intercrop and (c) ricebean in monocrop in 1984. The arrays of plants sampled for $^{15}$N analysis are enclosed within solid lines. There were two sampling areas per plot; plot size measured 10.5 m × 7.5 m.](image-url)