Institut für Pflanzenernährung

der Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

Nitrate Reductase Activity in Rice as a Screening Tool for Weed Competitiveness

Wissenschaftliche Arbeit

für die

Magisterprüfung

zur

Erlangung des Grades

Magister der Agrarwissenschaften (M.Agr.)

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität-zu Bonn

Vorgelegt am 12-12-2003

von

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Institut für Pflanzenernährung

der Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

Nitrate Reductase Activity in Rice as a Screening Tool for Weed Competitiveness

Thesis submitted

In

Partial fulfilment of the requirements

For Masters of Agriculture (M.Agr.)

of the

Faculty of agriculture

University of Bonn

Submitted on 12-12-2003

by

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Bonn, den 12. Dezember 2003

Maurice Ochieng Ouko

Acknowledgements

The successful completion of this research work could not have been possible without the support of many people whose names cannot all be mentioned here. I am indebted to all of them, but in particular to my supervisor Dr. Folkard Asch. Dr. Folkard Asch has been instrumental in guiding this research, and working with him facilitated fruitful discussions, suggestions and guidance throughout the entire work. I am equally thankful and greatly indebted to Prof. Mathias Becker for providing me with the materials and the opportunity to undertake this study.

Further gratitude goes to all the members of the Institute of Plant Nutrition, university of Bonn, who in one way or another assisted me and provided a conducive working atmosphere for conducting the experiments. In particular I am thankful to Angelika Veits and Angelika Glogau for helping me with the laboratory work. Dr. Tom Eichert for helping me with the analysis of data and for being a friend and Dr. Monika Wimmer for proof reading at short notice in spite of her tight schedule.

Finally I would like to express my gratitude to my sister Margaret, her husband Alfred and daughter Seline for their support beyond measure.

Glory be to God who makes all things possible

Dedication

I dedicate this work to my parents

Mrs Mary Ouko and the late Mr. P.F. Ouko

Who sacrificed selflessly for the sake of my education.

Abstract

In West African upland and lowland direct seeded rain-fed rice ecosystems, weeds are often the most important biological constraint to rice production. Due to the aerobic soil status during the early stages of establishment, NO_3^--N is the prevalent form of soil nitrogen available. It is recognized that nitrate reductase (NR) is the rate-limiting enzyme in NO_3 assimilation and that nitrate reductase activity (NRA) can be used as a measure of the capability of a plant to assimilate NO_3^--N. The efficiency in assimilation of NO_3^--N during the early growth stages will largely determine the rice seedlings vigour and their ability to out compete weeds in the acquisition of resources such as light.

This study was conducted to investigate: (1). Do rice genotypes differ in their NRA? What does this difference depend on? (2). Is NR in rice seedlings influenced by the form and/or concentration of the substrate that triggers it's activity (3). What is the earliest possible time in seedling development to determine differences in NRA? (4). Do rice genotypes with high and early NRA compete better with weeds?

NRA of upland adapted *Oryza sativa ssp japonica* and *Oryza glaberrima* as well as lowland adapted *O. sativa ssp indica* rice genotypes were studied. The rice were grown in hydroponics with the nitrogen source varied to provide either 40 ppm NH_4^+-N, 20 ppm NO_3^--N , 40 ppm NO_3^--N or 20 ppm NH_4^+-N+ 20 ppm NO_3^--N. Leaf NRA as well as growth parameters were measured at 7, 14 and 21 days after germination (DAG). The rice genotypes were also grown in association with weeds and their weed competitiveness assessed at 17 DAG.

Rice genotypes showed wide variability in NRA. These differences were related to the nitrogen source and seedling age. Dependant on the nitrogen source, upland adapted rice genotypes showed larger variability in NRA than lowland genotypes.. Furthermore, NRA in the presence of nitrate was significantly higher than when the substrate was absent. However, higher concentrations of nitrate did not further increase NRA, implying that NR is more efficient under low than under high nitrate concentrations. NRA was present as early as 7 DAG, and a peak in NRA was observed at 14 DAG. Efficiency in NR corresponded to higher weed competitiveness. Under the experimental conditions, genotypes having high and early NRA were the most weed competitive.

In designing a screening tool for weed competitiveness based on NRA in rice, only upland adapted genotypes should be used and grown in nutrient culture containing low nitrate concentrations.

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1 Introduction

Rice is the most important food crop in the world. It is the main source of food for half of the worlds' population and is responsible for 25 – 80 % of the calories in their daily diet. It is cultivated in every continent except Antarctica.

Rice belongs to the grass family *Poaceae.* The genus *Oryza spp comprises* world wide of 25 known species. Of these only 2 are cultivated; African rice *Oryza glaberrima* Steud. and Asian rice *Oryza sativa* L.

O. sativa is agronomically more successful of the two species and as such the more widely commercially grown. However *O. glaberrima,* though low yielding is continually gaining importance in West Africa as a source for breeding material (Jones *et al.*, 1997)

Rice (*O. sativa* L.) has long been classified into two subspecies namely *indica* and *japonica* (Glaszmann, 1987; Oka, 1958, Cheng *et al.* 2002). This classification is based on their adaptation to different environmental factors such as day length, field water conditions and temperature. In addition to ecological adaptations, these subspecies differ in grain characteristics. *Indica* genotypes have long and narrow grains while the grains of *japonica* genotypes are compact and short. Generally *indica* genotypes are grown in lowlands where NH_4^+-N is the abundant form of available soil nitrogen, whereas *japonica* genotypes are grown under upland conditions where NO_3^--N is the dominant form.

Rice is cultivated in diverse ecosystems. The system of cultivation is determined by the availability of water.

Upland systems are rainfall dependent; rice is usually directly sown, and relies on natural precipitation throughout its vegetative phase. This system is mainly practiced in humid areas with a long rainy season e.g. in India and Africa. Rice grain yields in this system are low due to susceptibility to drought stress and weeds (De Datta, 1981; Gupta & Toole, 1986).

Rice is predominantly grown under wetland systems with an anaerobic soil aeration status. This system is further differentiated into rain-fed wetland/lowland and irrigated systems. In both systems the rice seeds are pre-germinated before being sown in a seedbed, and the seedlings raised therein for 10 to 50 days before being transplanted in the main field that has been ploughed, puddled and flooded.

Ideally the water level in the field is maintained at 5-10 cm to control growth and compensate for water loss via transpiration.

Due to labour shortage and/or expense, direct seeding is increasingly common in West Africa. The seeds are pre-germinated and later broadcast in the main field. Water management and weed control in such systems is usually difficult and yields are often low. Under this system weeds emerge at the same time as the rice crop and outcompete the rice for in the acquisation of resources

Most West african upland and rainfed lowland production systems involve an initial aerobic soil phase of variable duration. Under such conditions, NO_3^--N tends to be the dominat form of N_{min} while NH_4^+-N will dominate later on in saturated or flooded soils. The efficiency in assimilating NO_3^--N during the early growth stages will largely determine the rice seedlings vigour and ability to outcompete vigourosly growing upland weeds. Nitrate reductase is the rate limiting enzyme in the assimilation of nitrate, and it's activity can thus be used as a measure of the ability of rice to assimilate NO_3^--N.

1.1 Hypotheses / Objectives

The underlying hypothesis of this study is that plants having a high nitrate reductase activity (NRA) at early seedling stages more efficiently utilize NO_3^- and therefore more effectively compete with weeds.

Rice breeding for weed competitiveness in upland systems requires a simple and easy to use screening tool. This study will evaluate the possibility of developing a screening tool for weed competitiveness of rice genotypes based on early nitrate reductase activity. To do so the study will address the following questions:

a) Do genotypes differ in their NRA? What does this difference depend on?
b) What is the earliest possible time in seedling development to determine differences in NRA.
c) Is NR in rice seedlings influenced by the form and/or concentration of the substrate that triggers its activity?
d) Do upland rice genotypes with a higher early NRA compete better with upland weeds?

2 Literature Review

2.1 Nitrate Reductase

Enzymatic reduction of nitrate (NO_3^-) to nitrite (NO_2^-) was first described in 1900 In 1950's it was conclusively established that nitrate reductase (NR) is the specific enzyme, (Beevers and Hageman, 1969). While conflicting evidence exists regarding the activity of NR, the following characteristics for the enzyme are generally accepted:

a) Maximum activities of 600 to 700 µmols NO_2^- min^{-1} mg $protein^{-1}$ occurs in spinach (Paneque & Losada, 1966) and corn leaves (Schrader *et al.*, 1968).
b) NR activity can be induced by both nitrate (Beevers & Hagemann 1969 reviewed by Crawford, 1995; Wang *et al.*, 2000) and molybdenum (Gunes, 1996; Campbell, 1996).
c) There are two forms of NR: NR-NADH and NR-NADPH. This is dependent on the preferred electron donor/co-factor (Campbell, 1999)

2.2 Plant Nitrogen – Acquisition via the Uptake of Nitrate

NO_3^--N and NH_4^--N are the major sources of mineral nitrogen available to plants. Most of the absorbed NH_4^+ is incorporated into organic compounds (mainly glutamate) in roots, whereas NO_3^-, which is mobile in the xylem, is transported to the vacuoles for storage.

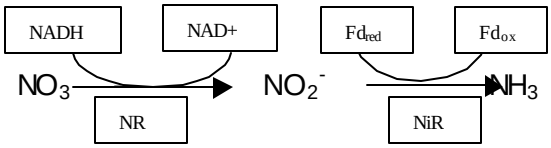
In addition, NO_3^- contributes to the maintenance of the cation-anion balance and osmoregulation in plant tissues.

Ullrich (1992) stated that the nitrate uptake system of higher plants consists of a constitutive, low affinity transport system (LATS), (a carrier system or an anion channel) and an inducible, high affinity transport system (HATS). The latter is regulated by cellular energy supply and by intracellular nitrate consumption, the activity of which depends on the proton electrochemical gradient. The HATS system is regarded as an H+/anion co-transport carrier mechanism, which produces transient plasmamembrane depolarisation upon addition of nitrate. The depolarisation is counteracted by the plasmamembrane H^+-ATP-ase, which is induced by nitrate (Santi *et al.*, 1995).

In addition to the nitrate uptake system, plants have an inducible nitrate efflux system, requiring both RNA and protein synthesis. However, the efflux system has a much slower turnover rate than the uptake system (Aslam *et al.*, 1996).

Nitrate taken up from external media by the roots, once in the cells, must be reduced to ammonium before being incorporated into amino acids and proteins. Reduction of nitrate is an energy-intensive process that is carefully regulated by the plant.

The process of uptake and assimilation of NO_3^--N by higher plants proceeds as follows:



Once taken up nitrate, is either stored in the vacuole or reduced to nitrite by nitrate reductase (NR). NO_2^- enters the chloroplast (or plastid in the root) and is reduced to ammonia (NH_3) by nitrite reductase (NiR). NH_3 can be incorporated into glutamate by glutamine synthetase (Crawford 1995, Lam *et al.*, 1995). Reducing energy is provided in the form of NAD (P) H for NR and reduced ferredoxin for NiR. Glutamate provides the immediate carbon skeletons. Because NO_3^- assimilation produces several hydroxide ions, organic acids are required for pH homeostasis. Coordinating these steps is a regulatory network that is responsive to both internal and external signals e.g. pH, CO_2 etc.

The first step in the above mentioned process is the reduction of nitrate to nitrite by the enzyme nitrate reductase (NR). This step has been shown to be the rate-limiting step in the nitrate reduction pathway (Beevers & Hageman, 1969; Campbell, 1989; Solomonson and Barber, 1990). Therefore, the activity of NR can be used as an indicator of a plant's ability to use nitrate as a nitrogen source (Vogel and Dawson, 1991). In addition, nitrate reductase activity has been proposed as a selection criterion in crop improvement programs by Chirkova & Belonogova (1994) and Eichelberger *et al* (1989a, 1989b). Attempts have been made to relate grain yield and agronomic characters in varieties of maize, alfafa and wheat with nitrate reductase activity (Deckard *et al.*, 1973; Sherrard et al., 1986; Feil *et al.*, 1993; Below, 1995).

2.2.1 Synthesis and Distribution of Nitrate Reductase

The reduction of NO_3^- to NO_2^- via NR competes for energy and carbon skeletons with both the efflux of nitrate from the cell and the transport of nitrate into the vacuole.

NR is located primarily in the cytosol of root epidermal and cortical cells and shoot mesophyll cells (Fedora *et al.*, 1994; Bercz i & Moller, 2000; Siddiqi & Glass, 2002). NR transfers two electrons from NAD (P) H to nitrate via three redox centres composed of two prosthetic groups (flavin adenine dinucleotide (FAD) and heme) and a molybdenum cofactor (MoCo), which is a complex of molybdenate and pterin.

Most of the studies on NR in higher plants have focused on the extraction of the enzyme in leaf tissue, primarily because of its abundance in such tissue and the availability of material (Beevers & Hageman, 1969). However, there is evidence that nitrate assimilation is not solely associated to leaves.

NR has been extracted from different tissues such as petioles, cotyledons, cell cultures, stems, roots, corn scutella and cornhusks. However, the activity of enzyme that is extractable from these tissues varies, from traces to 60 µmols of NO_2^- g^{-1} h^{-1} as measured by crude homogenate activities (Hageman & Hucklesby, 1971). Chlorophyll containing tissues generally have higher NRA than non-chlorophyll containing tissue (Hageman & Hucklesby, 1971, Crawford & Arst, 1993).

The location of nitrate reductase in a plant appears to be related to ecological adaptation. Fast growing pioneer species tend to assimilate nitrogen in the leaves, whereas slow growing late-succession species assimilate nitrogen in the roots (Brent *et al.*, 2002). In some plants, NR is primarily active in leaves (Smirnoff *et al.* 1984, Gebauer & Stadler, 1990 Brent, 2002) near the source of energy, and in others it occurs in the roots (Minotti & Jackson, 1969; Gojon *et al.,* 1991; Downs *et al.*, 1993; Stöhr *et al*., 2000, 2001), near the nitrate source, and in some NRA has been observed in both shoots and roots (Yoneyama & Kumazawa, 1975; Jiang, 1999; Pattinson, 2000).

In comparing the activity of leaf and root NR in different plant species, Sanderson and Cocking (1964) observed that the activity in roots was consistently lower than that in leaves. However the properties of NR from roots were identical to those of leaf extracts and it was concluded by the authors that the two NR molecules were identical, thus excluding the possibility that microbial or non-specific enzymes were being measured.

Miflin (1967) reported that the specific activity of NR is slightly higher in roots than in leaves. However, if activity is calculated on the basis of fresh weight of the plant part, the shoots have a much greater amount of the enzyme. This was illustrated by Wallace and Pate (1965), who studied NR in field peas, and showed that shoots contain about the same amount of enzyme as roots, but only when nitrate levels in the nutrient medium were maintained at 10 ppm. When the plants were grown under low levels of nitrate, the shoots had 3 to 12 times more enzyme than the roots. Roots from young maize seedlings demonstrated only 20% of the enzyme activity observed in shoots. Wallace and Pate (1967) used the site of the activity of NR to categorize plants into those that reduce nitrate in roots e.g. *Pisum ssp, Vicia spp, Lupinus spp* and *Raphanus spp*, and those that did not e.g. *Xanthium spp*.

It is probable that the synthesis of NR is stimulated at high nitrate levels (Remmler & Campbell, 1986; Campbell, 1988).

It has been shown that the distribution of nitrate assimilation between leaves and roots in some plants, e.g. *Pisum spp*, can be altered by changing the nitrate concentration in the growing medium. Pate (1968) reports that the entire nitrate is metabolized in the roots of *Pisum spp* and only reduced nitrogen is found in the sap when the plants are cultured on low levels of nitrate. If the plants are supplied with nitrate levels higher than 10 ppm, nitrate is transported to the shoots and nitrate reductase is induced there.

A similar response to the distribution of NRA in field peas grown on low nitrate is reported in etiolated barley seedlings grown in the absence of nitrate (Boutard, 1966) In contrast, *Xanthium spp* transports 95% of the supplied nitrate to the shoots and only negligible amounts are metabolized in the roots.

A shift in the extent of NRA from the roots to the leaves was also reported by Boutard (1966) and Coupe et al (1967) in studies carried out on illuminated barley seedlings grown under increasing nitrate concentrations.

2.2.2 Nitrate Reductase Regulation

The uptake and use of NO_3^- is a process consuming high amounts of energy. The uptake and transport of NO_3^- and its conversion from a +5 oxidation state of NH_4^+ consumes a substantial portion of the electrons produced by photosynthesis (Raven, *et al.*, 1992). Moreover, the intermediate products of the assimilation: NO_2^- and NH_3

are potentially toxic to plant tissue at low concentrations. Therefore higher plants have developed complex regulatory mechanisms controlling NO_3^- assimilation.

NR activity is regulated by NO_3^-, carbon and nitrogen metabolites (sucrose and glutamine), light, phytohormones, and external CO_2 levels (Kaiser & Brendle-Behnisch, 1991; Huber *et al.*, 1992a,b; Shiraishi *et al.*, 1992; Kaiser & Huber, 1994; Li *et al.*, 1995; Sivasankar & Oaks, 1996)

There are two possible regulatory mechanisms for NRA: (1) alteration of the activity level of the existing enzyme in the cytoplasm and (2) by synthesis of new enzyme or degradation of existing enzyme. In the nitrate reductase pathway the step involving nitrate reductase is considered the logical point at which regulation of nitrate assimilation by the plant occurs because:

a) Nitrate reductase is the first enzyme involved in the nitrate reductase pathway.
b) NR is the rate-limiting step in the nitrate reduction pathway (Crawford & Arst, 1993).
c) The activity of nitrate reductase is substrate inducible (Aslam & Huffacker, 1989, Campbell, 1996).
d) Toxic effects of excess levels of nitrite and ammonium ions necessitate regulation in their production (Zsoldos, 1997).

2.2.3 Nitrate Reductase Induction

NR is considered an adaptive enzyme because its activity is inducible by nitrate in intact plants and plant tissue and by molybdenum in tissue from molybdenum deficient plants.

Molybdenum is involved in the NR induction process and does not merely activate a pre-existing enzyme (Solomonson *et al*., 1984 ; Yu *et al.*, 1999). Furthermore, the induction of NR by molybdenum shows the same sensitivity to inhibitors of protein synthesis, as it does in reaction to nitrate. Optimum induction is often obtained in tissues when the induction medium is at pH 4-5, and contains 10-100 mM nitrate. (Afridi & Hewitt, 1965).

The reduction of nitrate to nitrite in higher plants is inducible by an external nitrate supply (Rajasekhar & Oelmuller, 1987; Crawford & Arst, 1993; Cambell, 1996).

However, the level of nitrate required to induce optimal reductase activity differed widely among plant species and tissues sampled. Beevers *et al.* (1965) stated that these differences probably indicate differences in the rate of uptake of nitrate between species since the induction of NR within a given species depends on the concentration and rate of supply of nitrate to the tissue.

In intact plants, two sources of nitrate can play regulatory roles for NR: one is the nitrate stored in the leaves, the other is the nitrate translocated to the leaves from the roots.

The induction of NR in various tissues by nitrate, or molybdenum is related to the plant's development stage (Hewitt, 1975; Beevers and Hageman 1980; Hageman and Reed, 1980). Generally NR induction is related to the plant's ability to synthesize proteins. As the plant matures, the inductibility of NR decreases. It is not clear if the change in inducibility of NR with age is directly associated with a decreased capacity for protein synthesis or if it is due to an accumulation of soluble nitrogenous products which specifically repress the synthesis of NR. It may also be, that the acceleration of synthesis of NR is associated to an increased availability of soluble precursors that facilitate protein synthesis. There is a requirement for an adequate level of precursors for protein and nucleic acid synthesis before induction of NR can occur. (Lillo, 1994, De Pareira, 1998).

Schrader *et al.* (1968) determined in corn seedlings that appreciable induction of NR by nitrate is only occurs when seedlings are initially grown in medium containing NH_4 and external NO_3 introduced later. The NH_4^+ grown seedlings, though lacking NR, had higher nucleic acid, protein, soluble nucleotide, and amino acid contents than the control seedlings which were grown in water. NR induced readily when these seedlings were transferred to nitrate medium.

2.2.4 Nitrate Reductase Activity

Boutard (1966), using nitrogen deficient etiolated barley seedlings, indicated that NR was present in seedlings cultured in distilled water, and the activity was predominantly associated to roots. Addition of nitrate caused an increase in NRA in the leaves and a decrease of NRA in the roots. The increase in NRA, which was much smaller than that observed in corn, was greater in illuminated barley seedlings

than in those grown in darkness. The data of Boutard (1966) is analogous to that reported by Wallace (1975) for pea seedlings receiving low nitrate.

In studies comparing the NRA in rice (*Oryza sativa* L.) seedlings of different ages, Yang & Sung (1980) detected NRA in certain rice genotypes after two days. A peak in NRA was observed at the seventh or eleventh day after seed imbibition, a rapid decline followed thereafter. Marwaha & Juliano (1976) determined a peak in rice NRA at 10 days after germination. Yang & Sung (1980) observed considerable genotypic variation in leaf NRA. In assays conducted on 10-day-old rice seedlings, NRA values ranged from 80 to 3000 nmol NO_2^- $g^{-1}h^{-1}$. It was suggested that the variation in seedling leaf NRA was related to differences in seed weight of the various genotypes studied. Barlaan *et al.*, (1998) studied 14 irrigated lowland rice cultivars. Samples were collected from the second leaf from the top at 15, 30, 45, and 60 days after transplanting (DAT) and *in-vitro* NRA determined. Maximum NRA was reached at 15 DAT in all cultivars, and gradually declined until maturity. NRA fluctuated between 300 and 600 nmol NO_2^- $g^{-1}h^{-1}$. Shen (1969) reports a NRA of 76 nmol NO_2^- g^{-1} h^{-1} in four-day-old rice seedlings grown in distilled water. Indicating that NR may be present at low concentrations in rice seedlings as a constitutive enzyme.

Barlaan & Ichii (1996) studied 53 rice cultivars, and their results highlight the differences in NRA within cultivars and varietal groups. Plants exhibiting higher NRA were shown to be efficient nitrate assimilators. It was also observed that *indica* rice genotypes had up to 30% higher NRA than *japonica* genotypes. These results complement observations by Ta & Ohira (1982), who reported that the relative uptake of NO_3^- by *indica* rice is higher than that of *japonica.* The *indica* genotypes reduced and absorbed $^{15}NO_3^-$ more rapidly than *japonica* genotypes. These results suggest that *indica* rice have the ability to use NO_3^- as a nitrogen source more effectively than do *japonica*. Sattelmacher *et al.* (1994) pointed out that genotypes might differ in use efficiency and uptake efficiency: the two factors that are attributed to genetic variation in nutrient efficiency in crops.

Based on variation in NRA, rice genotypes may be classified into high, moderate and low NRA categories (Barlaan & Ichii, 1996). However, this classification is not standardized, since NRA is influenced by several factors such as plant part used for sampling, induction and bio assay method, plant age, growing conditions, etc.

There are several reports concerning the effect of ammonium on nitrate-induced NRA. They range from a promotion of activity (both with or without nitrate present) to

an inhibition, by repression or inactivation. Ammonium prevents the nitrate-induced appearance of nitrate reductase in fungi (Kinsky, 1961; Lewis & Fincham, 1970 Brunner *et al.*, 2000) and in eukaryotic algae (Losada *et al.*, 1970; Syrett, 1981; Berges 1997; Queseda *et al.*, 1998a; Pike *et al.*, 2002). In higher plants, the situation is less clear. Bungard *et al.* (1999) detected NRA in *Clematis vitalba* grown in hydroponics culture and supplied solely with NH_4^+ as the nitrogen source. The NRA in leaf tissue was up to three times that of the maximum level reached when the same plants were grown under NO_3^- nutrition. NH_4^+ induced activity did not occur in barley and tobacco plants grown under similar conditions. By conclusively showing that NH_4^+ can induce NRA in the complete absence of NO_3^-, Bungard *et al.* (1999) and Solomonson & Barber, (1990) proposed an alternative role for NRA, beyond the supply of reduced nitrate, such as maintaining intracellular pH. This has also been suggested by Raven & Smith (1976), Raven (1985, 1986) and Solomonson *et al.*, (1986). It has also been suggested that nitrate reduction is part of the inhibitory mechanism for nitrogenase activity in legumes (Walsh & Carroll, 1992; Kaiser *et al.*, 1997). Ammonium has been reported to inhibit the induction of nitrate reductase in barley roots (Smith & Thompson, 1971b; Kronzucker, 1999), and in roots, but not shoots of cotton plants (Radin, 1975). For *Lemna minor*, ammonium inhibition of NRA is well established (Joy, 1969; Orebamjo & Stewart, 1975a). Several reports show that ammonium has no significant effect on NRA in a number of plants (Afridi & Hewitt, 1964; Beevers *et al.*, 1965; Ingle *et al.*, 1966; Schrader & Hageman, 1967; Oaks *et al.*, 1977). Rajasekhar & Mohr (1986) showed that, in the absence of external NO3-, growth of mustard seedlings was strongly inhibited by exogenous NH_4^+, on the other hand in the absence of external NO_3, NR was induced by NH_4^+ in mustard seedlings grown in both continuous darkness and continuous far red light. Results obtained by varying the ratio of NO_3^- and NH_4^+ indicate that for inhibition of NR to occur, high concentrations of NH_4^+ are required.

Shen (1969) showed that the assimilation of nitrate by *indica* rice seedlings (IR8) is completely suppressed by NH_4^+ if the medium contained both NH_4^+ and NO_3^-. Nitrate uptake resumed only after NH_4^+ was fully depleted from the medium. NH_4^+ inhibits the first step of the nitrate reduction (nitrate to nitrite), even though the plant actively takes up nitrate (Shen, 1969). This is attributed to feedback inhibition in higher plants. Mackown *et al.* (1982a) reported inhibiting effects of NH_4^+ on NR in maize, wheat, barley, tobacco and *Aspergillus*.

If NH_4^+ -N enters the cells at high rates it causes a strong membrane depolarisation and blocks anion/H+ co-transport. This has been suggested as the primary mechanism by which NH_4^+ ions inhibit NO_3^- uptake (Ulrich, 1992). Alternatively, Andriesse *et al.* (1989) suggested that competition between the NO_3^- uptake system and the glutamine synthatase (GS) cycle for ATP may in part account for NH_4^+ inhibiting nitrate uptake, as the inhibition occurred only when the GS was active.

2.3 Rice and Weeds

Weeds are recognized as the most important biotic constraint to rice production. In water-limited situations, where flooding as a weed control measure is not a viable option, the weed problem is more severe. The weed problem in rice is highlighted by the intense labour involved in weed control and the severe impact on yield when control is not carried out at the appropriate time (Adesina & Johnson, 1995). In some extreme instances, weeds may be a reason for abandoning land after periods of cultivation (Ampong-Nyarko & De Datta, 1991).

The labour demand of transplanting rice and that of manual weeding as well as increasing labour shortage have led to an increase in the cost of weeding. This in turn has led to the preference of direct seeding in rain fed lowlands and uplands. In this case, weeds emerge at the same time as the rice, making the use of flooding as a weed control measure impractical. Intensified use of direct seeding has resulted in the emergence of new weed patterns in West Africa (Johnson, 1994).

Considering the high cost of herbicides as well as the dangers of pollution and herbicide resistance development in weeds, new strategies for weed management are required.

Crop-weed interactions are based on the competition for resources: light, water, space and/or nutrients. In most instances, weeds have a higher nutrient demand and nutrient uptake capacity, thus with limited resources weeds are more efficient competitors than the main crop.

Breeding programs that concentrate primarily on high yielding varieties, enhance the problem. These new high-yielding varieties (HYV) are increasingly replacing more weed competitive albeit lower yielding traditional varieties. The introduction and acceptance of such new varieties in upland rice ecosystems poses a challenge to the

farmers, who are faced with compromising on one factor (yield) by their preferential selection of traditional varieties (Johnson, 1994).

Commonly grown tropical *japonica* rice varieties have a high yield potential but compete poorly with weeds. In contrast, endemic *Oryza glaberrima* landraces are highly weed competitive, due to their vigour, leaf area and tillering potential during the vegetative phase (Jones, 1997). However, *O. glaberrima* yields are usually low.

Weeds are critical at the early (slow) growth stage of the rice crop, before it forms a canopy. Researchers agree that weed management should therefore focus on this stage (Zimdahl, 1980, 1988; Johnson 1996; Knezevic *et al.*, 2002; Hirohiko & Nobuyuki, 2002).

The morpho-physiology of rice cultivars affects their ability to compete with weeds in the field and influences the optimal point for weed control (Fischer *et al.*, 1995; Johnson 1996; Johnson *et al.*, 1998; Garrity *et al.*, 1992). Plant breeding can also contribute significantly to weed control. Varieties that are capable of efficiently acquiring nutrients at early stages may be able to out-compete weeds that emerge at the same time or later, resulting in better crop yields.

2.3.1 Mechanisms of Weed Competition

The definition of competition adopted by most authors is based on the concept of vying for limited resources by neighbouring plants. However, plant scientists do not agree on a standardized definition for competition though they draw largely from the widely acknowledged theories of Grime (1979) and Tilman (1982).

Grime (1979) offers a seemingly narrow definition. The theory states that competition is the tendency of neighbouring plants to utilize the same resource, and further elaborates that success in competition is a factor of resource capture. In principle, a good competitor has a high maximum relative growth rate because it captures and utilizes resources more efficiently.

Tillman's theory (1982) on the other hand, features the concentration of resources as the main principle. He states that competitive success is coupled to the ability to draw resources down to a low level and to tolerate these low levels. A highly competitive species is therefore one that has a low resource requirement.

The two theories were shown to compliment and not contradict each other depending on the system under which competition is being studied (Grace, 1990) If resources in a habitat are in abundance, competitive ability is linked to efficiency in resource

capture, while under resource limited situations, competitive ability is related to the ability of species to efficiently utililize the scarce resources. Kropff, (1993) observed that when the growth of a plant community was linked to morpho-physiological characteristics, light was the limiting resource for which the plants competed. However, in agricultural systems where in addition to light, the nutrients regime as well as soil moisture are determinants of crop production, plants compete for all resources simultaneously.

2.3.2 Competition for Nutrients

Nutrient deficiencies slow down plant growth and make the plant susceptible to diseases and pests, and less able to cope with extreme situations such as drought and soil salinity. The most important nutrients in rice production are the macronutrients nitrogen (N), phosphorous (P) and potassium (K).

Nutrient uptake has often been linked to root biomass, the assumption being that plants with a larger root biomass have a strategic advantage when acquiring resources from the medium. It would appear that this theory holds true in resource-limited situations, when supply from the immediate environment cannot meet demand. In this case, nutrient uptake by a crop plant in a mixture of plants is related to its root biomass as a factor of the total root biomass in a given volume of soil. Van van Noordwijk (1983) asserts that a species with a root system more extensive than it's relative demand is more efficient at acquiring limited resources and thereby meeting its nutrient demands.

It has been suggested that under high soil fertility, the effect of weeds is not so severe. De Datta (1970) found that under high soil fertility weeds did not reduce yields of *H-4* rice as significantly as under ordinary fertility. These results contradict the pattern of results obtained in an experiment on moist but not flooded soil in West Africa, wherby an application of 50 Kg N ha-1 to rice doubled the weed biomass in the crop (Johnson, 1996). Implying that the weeds had a higher growth rate and hence higher nutrients demand than the rice. DiTomaso (1995) and Patterson (1995) observed that applied fertilizer benefits weeds more than it does the main crop, as weeds absorb fertilizer faster and in relatively higher amounts than the crop. Radosevich *et al.* (1997) suggested that weeds acquire and accumulate nutrients in

excess of their critical concentration requirements when the mineral elements are in abundance

Nitrogen is the nutrient for which most competition exists. Heavy infestations of barnyard grass may remove 60 to 80% of the nitrogen from the soil (Holm *et al.*, 1977). Moody (1989) recorded that weeds growing in association with wet seeded rice removed upto 27.0 kg ha^{-1} of N per season.

2.3.3 Competition for Light

Sunlight supplies energy to the biosphere via the process of photosynthesis in chlorophyll bearing green plants. In rice ecosystems, light has an additional role in the regulation of growth, development and competition (Radosevich *et al.*, 1997). In most plants, a linear relationship exists between irradiance and photosynthesis, where an increase in irradiance is directly proportional to the rate of photosynthesis. Kropf (1993), stated that competition for light is an instantaneous process of resource capture and relate this efficiency to light interception of the plant species.

In mixed canopies, light interception is determined by the leaf area index of the species, the plant height and the light absorption characteristics of the leaf. Taller rice varieties are more competitive in this regard, as they are able to outgrow and intercept light more efficiently than shorter species (Jennings & Aquino, 1968).

It is known that photosynthetic characteristics are co-related to the competitive ability of C3 and C4 plants, and Pearcy *et al.* (1981) observed that competitiveness can be related to light use efficiency.

2.3.4 Competition for Water

Most authors agree that water is probably the most variable resource necessary for plant growth. Soil moisture content is highly variable and climate dependant, unless regulated by irrigation. Adequate soil moisture content generally promotes plant growth, and competition for this resource under such circumstances is not quantifiable. However, in conditions where water is limited, competition occurs.

The effect of limited soil moisture on monocultures and mixtures of weeds and crops have been studied (Patterson, 1995), while fewer studies on the mechanism involved in competition for soil moisture have been conducted.

Kropff & van Laar (1993) report that the importance of competition is determined by the length, severity and timing of the drought period. In contrast to light, water can be stored in the plant system. Competition for water starts when the plant demand cannot be met by the soil supply or by what is stored in the plant.

Two processes have been distinguished when plants compete for water: the first process is the direct competition for water. This direct competition for resource capture is an instantaneous process (Kropff & van Laar, 1993) whereby plants with a more developed root system achieve instantaneous benefit (Davis *et al.*, 1965). The second process can be characterized as an indirect effect that is influenced by the amount of absorbed radiation, by temperature, by vapour pressure deficit and by species characteristics (Radosevich *et al.*, 1997).

Plants with high water use efficiency (defined by gCO_2 fixed/g water used) are expected to be more productive during times of water scarcity than those with high water requirements (Radosevich & Holt, 1984).

2.3.5 Weed Flora

Weeds associated with rice culture may be categorized into two groups: broad leaf (dicotyledonous) and narrow leaf (monocotyledonous) weeds. Under direct seeded culture, the predominant broad leafed upland weeds are *Euphorbia heterophylla, Crotolaria spp.,* while lowland broad leafed weeds include, *Ludwigia octovalvis, Monochloria vaginalis, Eclipta alba, Marsilea quadrifolia* and *Sphenoclea zeylanica*. The common narrow leaf weeds are either grasses or sedges and include the upland weeds*, Eleusine indica* and *Digitaria spp* while narrow leafed upland weeds include *Echinochloa spp., Oryza sativa (red rice), Leptochloa chinensis* L*., Fimbristylis miliacea* and *Cyperus spp*. (Smith, 1983; Ampong-Nyarko & De Datta, 1991; Moody, 1994; Johnson, 1996; Ko *et al*., 2000). Other authors separate grassy weeds from sedges. However, weed flora is not static, the diversity of weeds depends on the rice ecosystem.

2.3.6 Impact of Weeds

Various authors attribute the largest economic impact in rice production to the grassy weeds of the genus *Echinochloa spp* (Holm *et al*., 1977; Abeysekera & Anuruddhika, 2001; Labrada, 2002). Echinochloa weed species are a major constraint to rice

production worldwide. These weeds are widely associated with rice and the damage they cause to the crop is permanent. *Echinochloa crus-galli* is found in many countries, except Central America; *E. colona* is present almost everywhere in hot climate countries; other species of Echinochloa are confined to particular ecosystems. *Echinochloa* spp. have the capacity to adopt mimetic forms to rice, with the result that their control by manual weeding is very difficult (Labrada, 2002). The barnyard grass (*Echinochloa crus-galli* (L.) Beauv is considered to be the most troublesome weed of rice in the world (Holm *et al*., 1977)

Singh *et al.* (1998) observed that rice grain and straw yields were significantly reduced due to weed infestation between 15 and 60 days after sowing. If the weeds were maintained throughout the season, grain yield reduction of up to 57% was recorded. Weed free conditions established at the early stages of seedling development were more beneficial than when weed control was carried out at later development stages.

Le Strange, (1981) and Hill *et al*., (1989) reported that when competing with *Echinochloa spp*., the concentration of endogenous growth factors (viz auxins, gibberellins, etc.) in rice decreased, and the percentage of sterility increased. They concluded that weed competition results in a significant reduction in growth characters, yield attributes and yield. In China, 10 million tonnes (Mt) of rice are lost annually due to weed competition (Ze Pu Zhang, 2001); such a quantity of rice is sufficient to feed at least 56 million people for 1 year. In Sri Lanka, a country considered self-sufficient in rice, weeds are the major biotic stress in rice production and account for 30 to 40 percent of yield losses (Abeysekera & Anuruddhika, 2001). De Datta (1986a) and Moody (1990) reported losses of 36 to 56% in the Philippines under direct seeded conditions. In relating weed density to yield, Mishra (2000) reported yield losses of 8.5% with 75 jungle rice plants m^{-2} while 750 jungle rice plants m^{-2} resulted in yield losses of 37%.

The emerging trend by small-scale farmers of direct seeding has reduced weed pressure when rice seeds are broadcast at high seed rates. It has been experimentally demonstrated by Pantone and Baker, (1991) that by increasing crop densities it is possible to compensate for yield losses that would have been triggered by weed pressure. This however often compromises on yield and the increased cost as a result of using higher seed rates discourages its economical practice.

3 Materials and Methods

3.1 General Methods

3.1.1 Growth Conditions and Experimental Set Up

The study was conducted the at the Institute of Plant Nutrition of University of Bonn. Plants were grown in stagnant hydroponics culture. PVC trays (57 cm x 37 cm x 11.5 cm) containing 20 litres of the nutrient solution were used. Two ply Styrofoam boards were used to cover the trays. In the Styrofoam boards, 54 planting holes spaced at 6 cm x 6 cm were made. Plastic tubing, measuring 20 mm diameter and 40 mm in length was attached to the planting holes. At the bottom of the plastic tube, netting was affixed to hold the seeds and support the plant.

The Styrofoam with the planting tubings attached to it, was floated on the nutrient solution contained in the PVC trays (**Figure 1**). This ensured that at seeding all seeds were in contact with the nutrient solution at the same level.

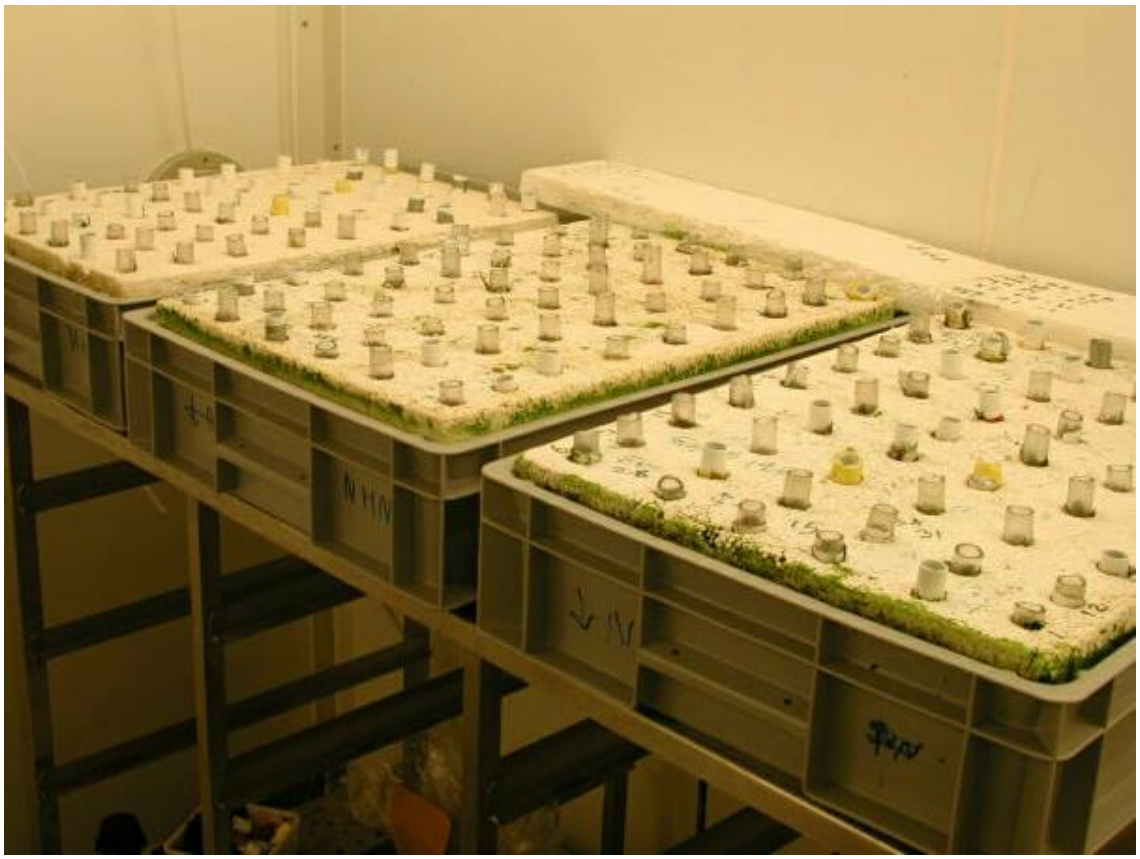


Figure 1: Experimental set up ready for sowing, showing Styrofoam plates floating on nutrient solution in 20 litre trays.

Before planting, all seeds were pre-germinated by soaking for 48 hours in petri dishes using tap water (**Figure 2**). Two seeds per planting hole were used. The pH of the nutrient solution was maintained at 5.0 ± 0.2 throughout the growing period. The pH was adjusted daily using 1 N HCl or 1M NaOH

Figure 2: Pre-germinated *Oryza sativa* and *Crotolaria spp* seeds after 48 hours

Plants were grown under controlled conditions in a climate chamber adjusted to the conditions given in **Table 1**.

Table 1: Conditions in the climate chamber.

PARAMETER	**VALUE (day / night)**
Light intensity	20 Klux
Photoperiod (h)	12 / 12
Relative humidity (%)	45 / 80
Temperature (°C)	26 / 20

Yoshida nutrient culture was used based on stock solutions given in **Table 2** (Yoshida, 1976).

Table 2: Preparation of Yoshida stock solutions and expected concentrations of individual elements in the prepared solution.

ELEMENT	REAGENT (AR GRADE)	PREPARATION (G/10 LITRES OF DISTILLED WATER)		CONC. OF ELEMENT IN NUTRIENT SOLUTION (PPM)
N	NH_4NO_3	914		40
P	$NaH_2PO_4 2H_2O$	403		10
K	K_2SO_4	714		40
Ca	$CaCl_2$	886		40
Mg	$MgSO_4 7H_2O$	3240		40
Mn	$MnCl_2 4H_2O$	15.0	Dissolved separately then combined with 500 ml of concentrated H_2SO_4. Made up to 10 litre volume with distilled water	0.5
Mo	$(NH_4)_6 .MO_7O_24 4H_2O$	0.74		0.05
B	H_3BO_3	9.34		0.2
Zn	$ZnSO_4 7H_2O$	0.35		0.01
Cu	$CuSO_4 5H_2O$	0.31		0.01
Fe	$FeCl_3 6H_2O$	77.0		2
	Citric acid (monohydrate	119		

For every 20 litres of culture solution, 25 mL of each stock solution was added (**Table 2**) to 5 litres of demineralised water. After adding each reagent the solution was stirred thoroughly to prevent coagulation and made up to final volume of 20 litres.

3.1.2 Plant Materials

The rice seeds used in the study were provided by the West African Rice Development Association (WARDA) 01 B.P. 4029, Abidjan 01, Côte d'Ivoire. The adaptations and characteristics of the cultivars used are shown in **Table 3**.

Table 3: Characteristics of rice genotypes used in the study.

CULTIVAR	SPECIES	SUB SPECIES	COUNTRY OF ORIGIN	TRADITIONAL/ IMPROVED	ECOSYSTEM
CG14	*O. glaberrima*	-	Sénégal	Traditional	Upland
Moroberekan	*O. sativa*	japonica	Cote d'Ivoire	Traditional	Upland
Avie Oufoue	*O. sativa*	japonica	Cote d'Ivoire	Traditional	Upland
Malobadjan	*O. sativa*	japonica	Cote d'Ivoire	Traditional	Upland
WAB 56-104	*O. sativa*	japonica	Cote d'Ivoire	Improved	Upland
Suakoko 8	*O. sativa*	indica	Liberia	Traditional	Lowland
Sahel 108	*O. sativa*	indica	Senegal	Improved	Lowland
CK4	*O. sativa*	indica	Guinea	Improved	Lowland
I Kong Pao	*O. sativa*	indica	Nigeria	Improved	Lowland
Sikamo	*O. sativa*	indica	Ghana		Lowland
MR123	*O. sativa*	japonica	Ghana	Improved	Upland
TOX4004	*O. sativa*	indica	Nigeria	Improved	Lowland
IS979	*O. sativa*	japonica	Cote d'Ivoire	Traditional	Upland
WAB181-18	*O. sativa*	japonica	Cote d'Ivoire	Improved	Upland
IDSA 6	*O. sativa*	japonica	Cote d'Ivoire	Improved	Upland
IG10	*O. glaberrima*		Cote d'Ivoire	Traditional	Lowland
ITA 320	*O. sativa*	indica	Nigeria	Improved	Lowland

Dr. David E. Johnson of the Natural Resources Institute, University of Greenwich, Chatham, Kent, UK provided the weed seeds used in the study. A description of the weeds is shown in **Table 4**. Maize (*Zea mays*) though not a weed was included in this study because in mixed cropping systems left over grains from the previous season may germinate in a rice field and compete with the rice plants for resources.

Table 4: Description of weeds used in the study. Maize (*Zea mays*) though not a weed was used in this study to compare resource competition

Scientific Name	Common Name	Ecosystem	Weed growth habit and weed nature
Echinochloa crus-pavonis	Barnyard grass	Lowland	Erect annual grass; Ecologically similar to rice, narrow leafed, C4
Zea mays	Maize	Upland	Erect annual grass, narrow leafed weed, C4
Crotolaria spp	Crotolaria	Upland	Broad leafed legume C3
Euphorbia heterophylla	Japanese poinsettia	Upland	Broad leafed perennial herb stems pubescent, C3

3.1.3 Influence of Nitrogen Form on Growth of Rice and Weeds

An experiment was conducted to determine the effect of the nitrogen source on seedling growth. The experiment also formed a basis for establishing a relationship between dry matter accumulation and nitrate reductase activity.

The rice cultivars described in **Table 3** were selected for the study of growth characteristics in rice as influenced by the source of nitrogen supply. The seedlings were established under the experimental conditions described in 3.1.1. and the plants sampled at 7, 14 and 21 days after sowing.

Nutrient supply was based on Yoshida culture. The nitrogen source was modified to supply nitrogen as 40 ppm NH_4, 20 ppm NO_3, 40 ppm NH_4NO_3 or 40 ppm NO_3.

The freshly harvested plants (rice/weeds) were separated into leaf, stem and roots and the fresh weights were determined. Using four replications plant material was oven dried for 24 hours at 70 °C for determination of dry weight. Leaf area of the fresh plant material was measured using a Licor 3600 meter (Licor, Lincoln, Nebraska). Other values obtained include plant height and root length.

The experiment was repeated for the weed types described in **Table 4**.

3.1.4 Determination of Nitrate Reductase Activity

Nitrate reductase activity was determined based on the method of Beevers and Hageman (1969).

The freshly sampled leaves (rice and weeds) were washed with distilled water, blotted dry, cut into 1.0 to 1.5 cm^2 segments measuring and weighed out into 0.1 g aliquots. The cut leaf tissues were then placed into 10 ml glass test tubes containing 5 ml of the infiltration medium that was composed of 100 mM KH_2PO_4 at pH 7.5, 30mM KNO_3 and 1% (v/v) propanol. The infiltration medium was vacuum infiltrated into the leaf tissues, using the following procedure:

The test tubes containing the samples were placed in an exicator and evacuated to a pressure of 30 mbar. This pressure was held for 5 minutes before being rapidly released. After releasing the vacuum, the test tubes were shaken to ensure that the tissue pieces were submerged in the medium. The evacuation procedure was then repeated. Submergence of the tissue pieces indicated successful vacuum infiltration.

Following this, the test tubes were stoppered and wrapped with aluminium foil to exclude light, which would otherwise interfere with the enzymatic processes. They were then transferred to a shaking water bath set at 30°C. After 15 minutes, 1ml of the incubation medium was extracted from each test tube and assayed for initial nitrite content (T_0). After a subsequent 30-minute incubation interval in the water bath, the test tubes containing the leaf samples were removed from the water bath and placed in a boiling water bath for 5 minutes to stop the enzymic reaction. Thereafter 1 ml samples were extracted and used to measure the final nitrite content T_{30}.

Nitrite determination was done by adding 0.5 mL of Sulphanilamide 1% (w/v) in 1.5 N HCl and an equal amount of 0.02% (w/v) N- (1-naphthyl) ethylenediamine hydrochloride to the T_0 and T_{30} samples. The samples were shaken and let stand for 15 minutes. Absorbance was read at 540 nm. using a spectrophotometer.

The difference in nitrite concentration between the two samples (T_0 and T_{30}) represents specific nitrate reductase activity (NRA) and is expressed as μmol $NO_2^-g^{-1}h^{-1}$ on a fresh weight basis. NRA for individual plants was computed by multiplying the specific NRA with the fresh weight of entire plant or plant part (leaves, stems) and expressed as μmol $NO_2^-plant^{-1}h^{-1}$

3.2 Nitrate Reductase Activity in Rice

3.2.1 Determination of Genotypic Differences in Nitrate Reductase Activity of Rice

A study was conducted to evaluate the differences in nitrate reductase activity in rice. The 17 rice cultivars listed in **Table 3** were used. All rice cultivars were established under the experimental setup and growth conditions described in 3.1.1. Each cultivars was grown in 3 rows.

NRA of the seedlings was determined at 14 days after germination as described in section 3.1.4. For each rice cultivar and treatment, four replications were carried out.

3.2.2 Influence of Nitrogen Form on Nitrate Reductase Activity in Rice

The effect that the form and concentration of nitrogen might have on the NRA of rice genotypes was determined. All rice cultivars (**Table 3**) were established under the

set up and experimental conditions described in section 1.1.1. The nitrogen source in the nutrient solution was modified, resulting in four treatments as follows:

In treatment 1 the nitrogen component of the Yoshida solution was modified by replacing the NH_4NO_3 with NH_4Cl, which provided 40 ppm NH_4. In treatment 2 the NH_4NO_3 was replaced by KNO_3 to provide 20ppm NO_3, thus establishing a low nitrogen concentration consisting solely of NO_3^-N. Treatment 3 was the standard Yoshida nutrient culture, comprising of 20 ppm NH_4 +20 ppm NO_3. In treatment 4, KNO_3 was used in place of NH_4NO_3 to supply 40 ppm NO_3.NRA was determined as described in section 3.1.4 at 7, 14 and 21 days after germination.

3.2.3 Influence of Seedling Age on Nitrate Reductase Activity of Rice

This study was carried out to establish at which seedling age nitrate reductase activity can be measured in rice, and to study NRA kinetics over different stages of seedling growth.

The 17 rice cultivars described in **Table 3** were used in this experiment. The rice genotypes were established randomly and each cultivar was grown in 3 rows under the experimental conditions described in section 3.1.1. The rice seedlings were sampled at 7,14 and 21 days after germination and analysed for NRA as described in 3.1.4.

3.2.4 Influence of Medium Conditions on the Nitrate Reductase Activity of Rice

The influence of the reduction status of the growing medium on the nitrate reductase activity of rice was assessed. An alteration in the aeration status in the root zone was achieved by growing the rice plants under different medium conditions.

*O. glaberrima (*CG14*) O. sativa ssp indica (*CK4*) and O. sativa ssp japonica (*WAB 56-104*)* rice cultivars were established in the climatic conditions described in 3.1.1 but under different medium conditions. One set of plants was grown in pots with sand as the growth medium whereas the other set was grown in hydroponics as previously described in 3.1.1.

Nutrition for both systems was based on Yoshida solution with the nitrogen form modified to supply either 40 ppm NH_4, 20 ppm NO_3, 40 ppm NH_4NO_3 or 40 ppm NO_3.

The sand cultured plants were irrigated daily so that the sand was always moist but not saturated.

Leaf nitrate reductase activity as well as growth parameters (leaf area, plant height, fresh weight and dry weight) were measured at 14 and 21 days after germination.

3.3 Nitrate Reductase Activity in Rice Weeds

3.3.1 Determination of Nitrate Reductase Activity of Weeds species

Four rice weeds adapted to different ecosystems and differing in growth habit (**Table 4**), were selected for this experiment. After pre-germination in petri dishes, the weeds were established in hydroponics similar to the rice set up as described in 3.1.1

The nitrogen treatments used to grow the weeds were identical to those used on the rice as described in section 3.2.2. At 7, 14 and 21 days after germination the weeds were sampled and analysed for NRA as described in section 3.1.4

3.3.2 Influence of Nitrogen Form on Nitrate Reductase Activity of Weeds

The effect of nitrogen source on the nitrate reductase activity of some selected rice weeds was determined. Broad leafed and narrow leafed rice types as described in **Table 4**, were established in an experimental set up similar to 3.1.1. The plants were grown in hydroponics culture using Yoshida solution, in which the N source was modified to provide 40 ppm NH_4, 20 ppm NO_3, 20 ppm 40 ppm NH_4NO_3 and 40 ppm NO_3. The weeds were sampled at 7, 14 and 21 days after germination and assayed for *in-vivo* nitrate reductase activity as described in section 3.1.4.

3.3.3 Effect of Seedling Age on the Nitrate Reductase Activity of Weeds

The relationship between NRA and seedling age of different weed species was established to provide a basis for comparing NRA in rice with that of weeds. Four weed species (**Table 4**) were selected for use in this experiment. The weeds were established randomly in rows of 3's under the experimental conditions as defined section 3.1.1. Nitrate reductase activity of the weeds was assayed at 7,14 and 21 days after germination.

3.4 Weed Competition

Three weed species (*Crotolaria spp, E. heterophylla and E. crus-pavonis*) and two upland rice cultivars (WAB 56-104 and CG14) were used. CG14 is a traditional whereas WAB 56-104 is an improved cultivar. The plants were established in hydroponics under the experimental conditions described in 3.1.1, i.e. plants were supplied with either 40ppm NH_4, 20 ppm NO_3, 20 ppm NO_3, 40 ppm NH_4NO_3 or 40 ppm NO_3.

The rice and weed seeds were sown at the same dates to simulate direct seeded conditions where rice and weeds emerge at the same time and compete with each other for resources. In each of the treatments a single rice cultivar was grown in association with a single weed. In order to access inter-species competition, planting was done such that the rice and weed plants alternated.

In addition, an experimental set of monocultures of either of the weed or rice types was conducted for comparison purposes and to access intra-specific competition.

Sampling was done at 17 days after sowing. The plants were separated into leaves stems and roots and plant height, fresh weight, leaf area and dry weight were determined.

3.5 Statistical Analysis

Results for both NRA and growth parameters measured, were subjected to analysis of variance (ANOVA). Mean comparisons as wells as effect of subjects were analysed using Duncans Multiple Range Test (DMRT). When conducting the ANOVA, the data was split based on sampling date and the NRA values and dry weights considered as dependent variable, while the nitrogen treatments as well as the cultivars were independent variables. The statistical program SPSS (version 11.0) was used.

4 Results

This section presents the results from experiments conducted to assess the differential response of dry biomass and thedynamics of the nitrate reductase activity of 17 rice varieties (**Table 3**) and 4 weed species (**Table 4**). Additionally selected rice and weed species were grown in mixed culture to assess the competitiveness of rice. The results from this study are presented under the following sub-sections: Influence of the nitrogen form on growth of rice, genotypic differences in NRA of rice, influence of the nitrogen form on NRA of rice, relationship between seedling age and NRA of rice, influence of medium conditions on NRA of rice, influence of nitrogen form on the growth of weeds, differences in NRA among weed species, influence of seedling age on NRA of weeds and weed competition.

4.1 Influence of Nitrogen Form on the Growth of Rice Genotypes

Rice genotypes were grown in hydroponics using Yoshida nutrient solution in which the nitrogen form had been modified to provide either 40 ppm NH_4-N, 20 ppm NO_3-N, 40 ppm, 40 ppm NH_4NO_3-N or 40 ppm NO_3-N. The experiment was conducted to establish the most appropriate media for rice growth under the experimental conditions (3.1.1.) The total dry weights of the rice were determined at 14 and 21 DAG. For reasons of simplicity only the dry weights at 21 DAG are shown in this section, results obtained at 14 DAG are listed in the appendix.

The total plant dry weights of rice genotypes at 21 DAG are presented in **Table 5**. Differences in dry matter accumulation in response to the different nitrogen forms were observed among the rice genotypes. Generally the *indica* rice genotypes had higher total dry weights when grown in nutrient media that contained NH_4, whereas the *japonica* genotypes had highest dry weights when grown in nutrient media containing both NH_4 and NO_3 (**Table 5**).

When grown with NH_4NO_3, the *indica* genotype CK4 had the highest dry weight (194.1) however this did not differ significantly from the highest dry weight of the *japonica* genotype Avie Oufoue (178.1) (**Table 5**).

When the *japonica* genotypes were grown in medium that contained NH_4NO_3, the traditional genotypes had much higher dry weights (mean 164.1g n=3) than the traditional genotypes (mean 67.0 n=2) (**Table 5**).

Table 5: Dry weight of rice genotypes at 21 days after germination ± standard error. The plants were grown in nutrient medium that contained different forms of nitrogen. nd= not determined; trad.=traditional; impr. = improved

SPECIES	DRY WEIGHT PER PLANT (mg)					
	Trad /Impr.	Sub Species	40 ppm NH_4-N	20 ppm NO_3-N	40 ppm NH_4NO_3-N	40 ppm NO_3-N
WAB 56-104	impr.	*japonica*	110.6 ± 5.5	104.5 ± 11.3	71.3 ± 3.5	97.7 ± 8.6
WAB 181-18	impr.	*japonica*	nd	nd	62.6 ± 12.7	65.3 ± 12.1
MORROBERIKAN	trad.	*japonica*	39.5 ± 5.7	44.0	171.9 ± 8.2	53.2 ± 2.7
MALOBDJAN	trad.	*japonica*	109.7 ± 62.7	59.3 ± 36.0	144.5 ± 96.9	78.5 ± 4.9
AVIE OUFOUE	trad.	*japonica*	114.1 ± 8.2	132.9 ± 4.7	178.0 ± 12.1	nd
CG14	trad.	*nd*	140.4 ± 12.9	142.2 ± 14.4	85.7 ± 11.5	94.1 ± 5.1
MR 123	impr.	*indica*	82.4 ± 20.4	52.5 ± 8.9	118.5 ± 25.1	38.1 ± 12.0
SIKAMO	impr.	*indica*	171.2 ± 17.9	93.6 ± 8.0	125.3 ± 18.8	58.2 ± 13.3
ITA 320	impr.	*indica*	94.2 ± 49.6	31.9 ± 3.7	57.3 ± 6.3	51.1 ± 11.1
CK4	impr.	*indica*	178.3 ± 13.7	111.0 ± 8.3	137.3 ± 22.9	115.3 ± 6.6
SAHEL 108	impr.	*indica*	89.3 ± 15.4	88.6 ± 1.9	118.5 ± 2.8	70.3 ± 6.5
IKP	impr.	*indica*	nd	104.1 ± 22.2	194.1	79.7 ± 5.2
TOX4004	impr.	*indica*	105.5 ± 18.4	84.2 ± 1.7	116.3 ± 28.0	31.6 ± 5.4
SUAKOKO 8	trad.	*indica*	148.0 ± 6.0	74.6 ± 10.9	112.0 ± 4.3	90.3 ± 2.8

4.2 Genotypic Differences in NRA among Rice Types.

Selected varieties of *O. glaberrima* and *O. sativa* were grown in hydroponics. NRA was assayed at 7, 14 and 21 DAG with the aim of assessing the relationship between NRA and the ecological adaptations of rice genotypes (upland vs. lowland). At 7 DAG there were no significant differences in NRA between the rice cultivars (see appendix). Differences in NRA were more pronounced at 14 DAG than at 21 DAG. **Table 6** shows NRA of rice cultivars grown with NH_4NO_3 as the nitrogen source at 14 DAG. NRA values among the cultivars ranged from 9.7 to 19.9 µmol $NO_2^-g^{-1}h^{-1}$.

At 14 DAG, rice cultivars differed significantly. When comparing the NRA of the sub species, *indica* (15.88 µmol$NO_2^-g^{-1}h^{-1}$ (n=7)) had higher mean NRA than *japonica* (13.68 µmol $NO_2^-g^{-1}h^{-1}$ (n=5)).

Table 6: Nitrate reductase activity of rice genotypes at 14 days after germination. Letters indicate significance levels from a DMRT ($p < 0.05$). Values bearing the same letter are not significantly different from each other.

SPECIES	IMPROVED/ TRADITIONAL	SUB SPECIES	CULTIVAR	NRA (µmol $NO_2^-g^{-1}h^{-1}$)
O. sativa	traditional	*japonica*	MORROBERIKAN	10.2^{ab}
O. sativa	improved	*japonica*	WAB 56-104	9.7^{a}
O. sativa	improved	*indica*	SAHEL 108	11.7^{cd}
O. glaberima	traditional		CG14	14.4f
O. sativa	traditional	*japonica*	AVIE OUFOUE	13.8^{ef}
O. sativa	traditional	*japonica*	MALOBDJAN	13.0^{e}
O. sativa	improved	*indica*	SIKAMO	18.2^{h}
O. sativa	improved	*indica*	MR 123	18.7^{hij}
O. sativa	traditional	*indica*	SUAKOKO	14.0^{ef}
O. sativa	improved	*indica*	CK4	12.7^{de}
O. sativa	improved	*indica*	IKP	10.9^{bc}
O. sativa	improved	*indica*	ITA320	16.1^{g}
O. sativa	improved	*japonica*	WAB 181-18	15.7^{g}
O. sativa	improved	*japonica*	IS979	19.6^{ij}
O. sativa	improved	*indica*	TOX4	19.9^{j}

4.3 Influence of Nitrogen Form on the NRA of Rice

The aim of this experiment was to determine whether the nitrogen form in the nutrient medium had an influence on NRA of rice genotypes. NRA of selected upland and lowland adapted genotypes at 14 DAG is shown in **Figure 3**.

Results indicate that the specific NRA in rice genotypes varies depending on the form and concentration of nitrogen in the growing medium (**Figure 3**).

At 14 DAG, the rice cultivars adapted to upland ecosystems e.g. WAB 56-104, Morroberikan and CG14, had highest NRA when NO_3-N was the only nitrogen source used. NRA was lower in treatments that contained NH_4, i.e. NH_4-N and NH_4NO_3.

The clearest distinction in NRA with regard to nitrogen form in the nutrient medium was observed in CG14 (**Figure 3**). The NRA of plants grown in media containing either 20 or 40 ppm NO_3 was 49 and 53 % more than that of plants grown in media containing 40 ppm NH_4 (no nitrate), respectively.

The lowland rice genotypes, Sahel 108 and Suakoko 8, had identical levels of NRA when nitrate was present in the growing medium. These NRA levels were higher than that when NH_4-N was the nitrogen source (**Figure 3**).

The improved *indica* cultivar Sahel 108 had higher NRA than the traditional *indica* i.e. Suakoko 8, when both cultivars were grown in media containing NH_4 (**Figure 3**).

NRA occured in all rice genotypes when grown in medium that contained no nitrate i.e. no substrate for NR (**Figure 3**). However NRA in the absence of nitrate was low irrespective of the genotype.

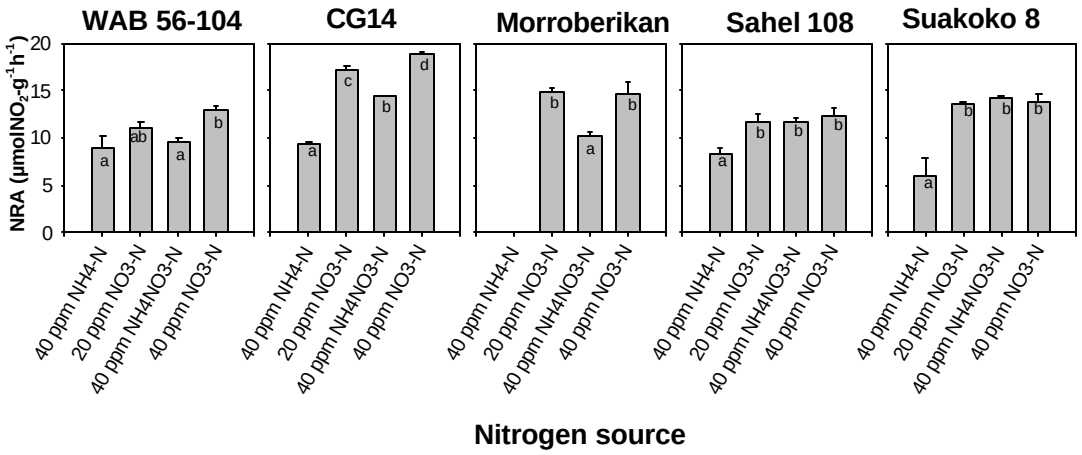


Figure 3: Nitrate reductase activity in rice genotypes at 14 days after germination (DAG). The plants were grown in nutrient media in which the nitrogen source was modified to provide either 40 ppm NH_4-N, 20 ppm NO_3-N, 40 ppm NH_4NO_3 or 40 ppm NO_3-N. NRA of Morroberikan at 14 days after germination for 40 ppm NH_4-N was not determined due to germination failure. Letters indicate significance levels from a DMRT ($p < 0.05$). Bars in each graph having the same letter are not statisticaly different

4.4 Influence of Seedling Age on the NRA of Rice

The rice cultivars shown in **Table 3** were grown in nutrient media containing different sources of nitrogen. At 7, 14 and 21 DAG the NRA of these cultivars was assayed in order to determine the relationship between seedling age and NRA. Analysis of variance showed that at 7 DAG there were no significant differences in the NRA of the rice cultivars tested. Significant differences in NRA were observed at 14 and 21 DAG. The differences in NRA at 14 DAG were more pronounced than at 21 DAG.

Variation in specific NRA with regard to seedling age was larger in the upland adapted cultivars *e.g.* WAB 56-104, Morroberikan and CG14 as compared to the lowland cultivars e*.g.* Sahel 108 and Suakoko 8 (**Figure 4**).

For a better understanding of the actual kinetics of nitrate assimilation with regard to seedling age, NRA was also computed on the basis of individual plants (**Figure 5**). In all genotypes NRA per plant increased as the plant matured, and the NRA at 21DAG was significantly higher than at 14 DAG.

An increase in NRA between 7 and 14 DAG was observed only when NO_3 was present (**Figure 4**).

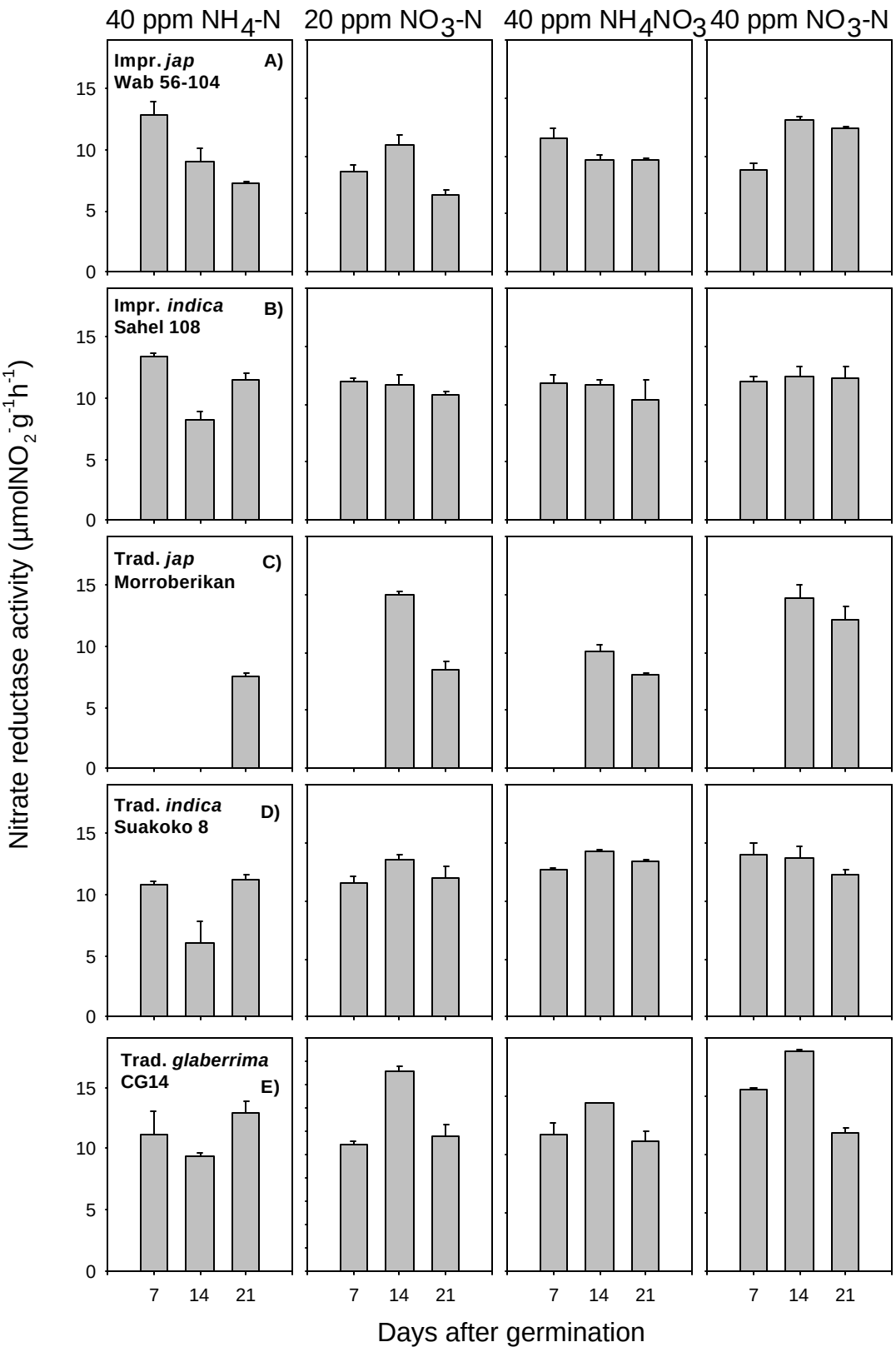


Figure 4: NRA of rice genotypes at different stages of seedling development. Note: NRA of Morroberikan at 7 days after germination for all treatments was not determined due to germination failure.

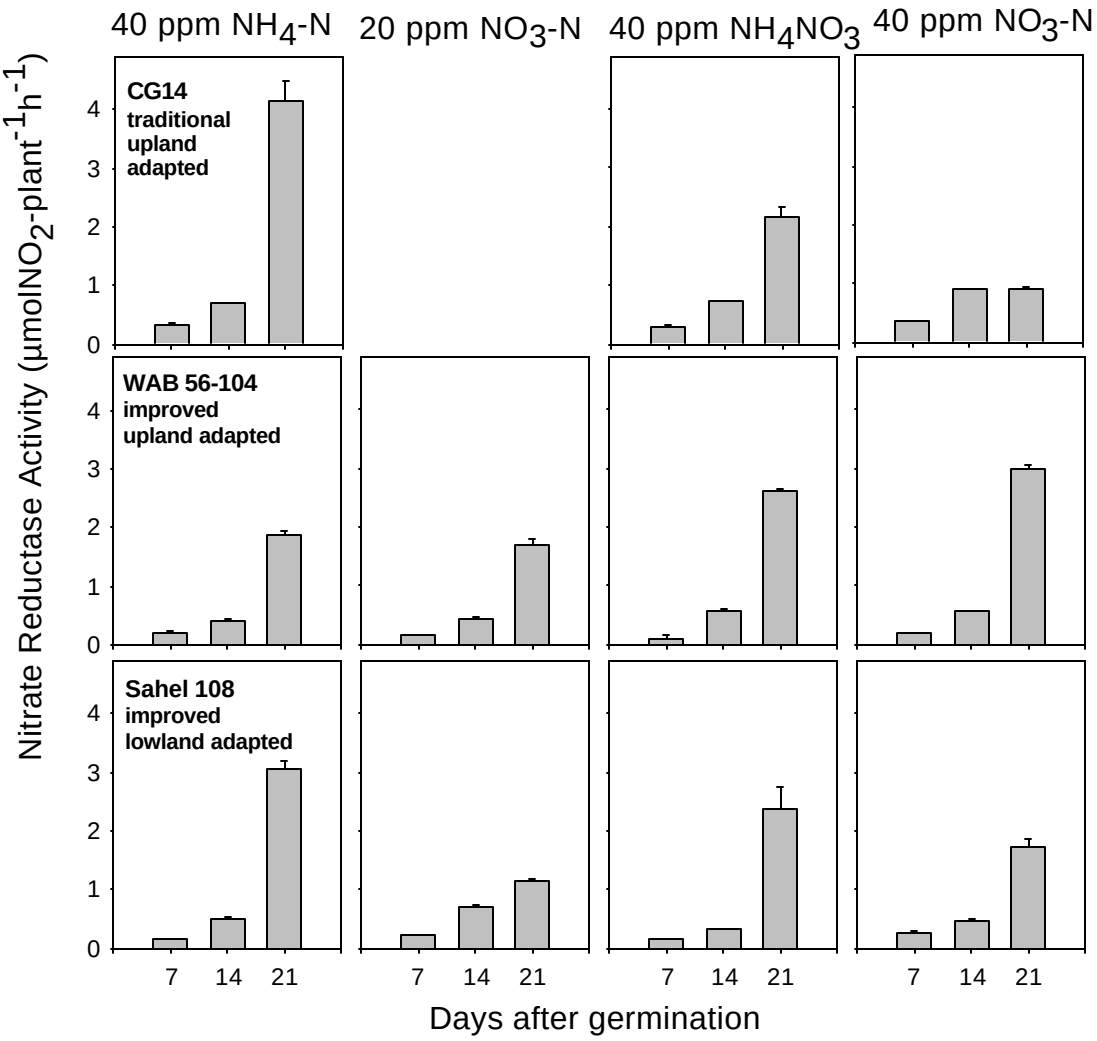


Figure 5: Nitrate reductase activity of CG14, WAB 56-104 and Sahel 108 calculated based on individual plants. NRA of CG14 at 20 ppm NO_3 was not determined due to insufficient plant material.

4.5 Influence of Growing Medium Conditions on the NRA of Rice

This experiment was conducted to assess the influence of the reductive conditions in the medium on NRA of rice genotypes.

Two upland (CG14, WAB 56-104) genotypes and one lowland (CK4) rice genotype were grown under different reductive conditions (see materials and methods). One set of these rice cultivars was grown in a sand based medium and the other set in a water based medium. NRA was determined at both 14 and 21 DAG. Results of this experiment are shown in **Figure 6**.

The NRA of the upland cultivar, WAB 56-104 at 14 and 21 DAG was higher when grown in the sand culture, as when grown in hydroponics with the nitrogen source being either 40 ppm NH_4-N, 20 ppm NO_3-N or 40 ppm NH_4NO_3. When 40 ppm NO_3-N was used the NRA of the sand grown WAB 56-104 did not differ from those grown in hydroponics (**Figure 6**).

At 14 DAG when NH_4 was present, the NRA of the sand grown CG14 was higher than those grown in hydroponics whereas at 21 DAG, when the nitrogen source was either 40 ppm NH_4NO_3 or 40 ppm NO_3-N, those in the sand culture had higher NRA than those grown in hydroponics (**Figure 6**). The lowland cultivar CK4 when grown in sand had higher NRA than those in hydroponics, in all nitrogen forms at 14 DAG. However at 21 the NRA of the sand grown CK4 was similar to that of the hydroponics grown ones (**Figure 6**).

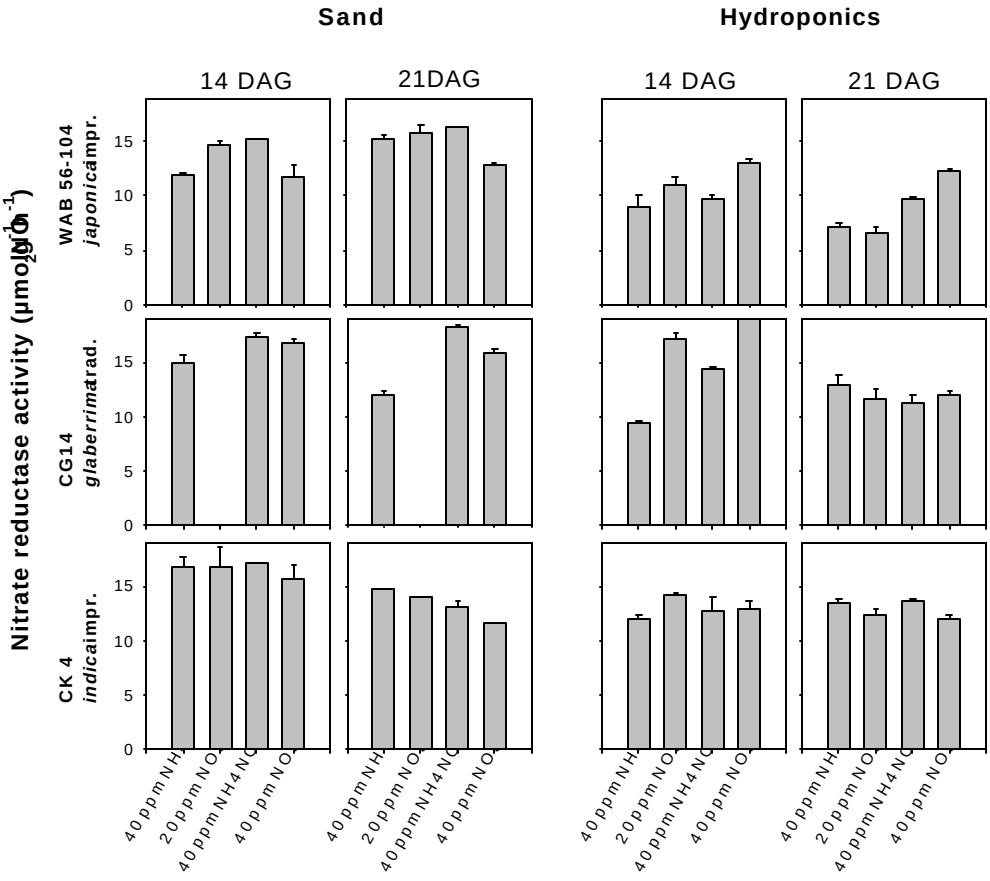


Figure 6: Nitrate reductase activity in rice genotypes at 14 and 21 days after germination (DAG). The plants were grown in either sand or hydroponics culture and supplied with different nitrogen forms. Nitrate reductase activity of CG14 grown with 20 ppm NO_3-N was not determined at either 14 or 21 DAG due to insufficient plant material.

4.6 Weed Growth under Different Nitrogen Sources

Euphorbia heterophylla, Crotolaria spp, Zea mays and *Echinochloa crus-pavonis* weeds were grown in nutrient media containing different sources of nitrogen (see materials and methods). Weeds were classified as upland adapted (*E. heterophylla, C. spp*) and lowland adapted (*Z. mays*, *E. crus-pavonis*). Weed growth was measured through determination of biomass accumulation at 14 and 21 days after germination. For reasons of simplicity since both sets of data showed parallelism only data collected at 21 DAG is presented here (**Table 7)**. Dry weights at 14 DAG are given in the appendix.

Weed growth differed depending on the form of nitrogen in the nutrient medium. Highest and lowest dry matter accumulation for lowland weeds was observed when medium containing NH_4NO_3 and 40 ppm NO_3, respectively was used. The upland weeds had higher dry matter when grown in NO_3 containing medium as compared to NH_4-N (*Crotolaria*) and NH_4NO_3 (*E. heterophylla*) (**Table 7**).

It can generally be seen that growth of the lowland weeds was favoured by the availability of both NH_4 and NO_3 whereas that of upland weeds is favoured by the presence of NO_3

Table 7: Effect of different forms of nitrogen nutrition on the total dry matter accumulation of weeds at 21 days after germination. nd = not determined.

WEED	**DRY WEIGHT PER PLANT (mg)**			
SPECIES	40 ppm NH_4	20 ppmNO_3	40 ppm NH_4NO_3	40 ppm NO_3
Crotolaria spp	nd	81.6 ± -15.3	56.0 ± -7.5	117.3 ± -10.7
Euphorbia spp	89.6 ± -9.3	165.1 -	nd	124.7 ± -35.7
Echinochloa spp	12.6 ± -4.9	16.7 ± -2.1	36.1 ± -4.5	7.8 ± -0.9
Zea mays	2728.0 ± -453.2	2090.0	4410.0 ± -77.6	1056.0 ± -542

4.7 Nitrate Reductase Activity in Rice Weeds

Upland (*Crotolaria spp and Euphorbia heterophylla*) and lowland (*Zea mays* and *Echinochloa crus-pavonis*) adapted weed species were grown in nutrient culture using different nitrogen sources (see materials and methods). The NRA of these weeds was determined at 7, 14 and 21 DAG.

As shown in **Table 8**, NRA of the weeds ranged from 2.71 to 14.79 μmol $NO^2g^{-1}h^{-1}$ at 7 DAG. At 14 DAG, the activity of NR ranged from 3.53 to 12.22 μmol $NO^2g^{-1}h^{-1}$ and at 21 DAG from 2.12 to 13.04 μmol$NO^2g^{-1}h^{-1}$.

Mean values show that regardless of the nitrogen form *Z. mays* always had the lowest NRA. Highest NRA was observed in *E. crus-pavonis* at 7 DAG grown using NH_4NO_3 as the nitrogen source.

Table 8: *in-vivo* nitrate reductase activities of selected rice weeds at 7, 14 and 21 days after germination. The plants were grown using, either 40 ppm NH_4-N, 20 ppm NO_3-N, 40 ppm NH_4NO_3 or 40 ppm NO_3-N

NITROGEN FORM	DAYS AFTER GERMINATION	NRA of WEED SPECIES (µmol $NO_2^-g^{-1}h^{-1}$)			
Crotolaria spp		40 ppm NH_4-N	20 ppm NO3-N	40 ppm NH_4NO_3	40 ppm NO_3-N
	7	5.85^{b}	4.88^{a}	8.52^{a}	5.5^{bc}
	14	4.57^{a}	5.3^{a}	10.2^{b}	7.9^{a}
	21	5.8^{b}	3.3^{a}	13.0^{d}	9.03^{b}
Euphorbia heterophylla	7	5.79^{b}	8.26^{b}	7.42^{a}	7.6^{c}
	14	4.4^{a}	8.0^{a}	7.4^{a}	8.7^{a}
	21	1.2^{a}	10.7^{c}	8.5^{c}	9.3^{ab}
Echinochloa crus-pavonis	7	6.54^{b}	6.19^{ab}	14.8^{b}	9.2^{ab}
	14	8.0^{b}	12.2^{b}	11.9^{c}	7.6^{a}
	21	5.4^{b}	7.3^{b}	5.8^{b}	11.7^{ab}
Zea mays	7	2.71^{a}	5.41^{a}	5.43^{a}	4.4^{a}
	14	3.5^{a}	7.7^{a}	6.7^{a}	8.7^{a}
	21	4.6^{b}	6.2^{ab}	2.1^{a}	6.5^{a}

4.8 Influence of Nitrogen Form on NRA of Weeds

Crotolaria spp, Euphorbia heterophylla, Zea mays and Echinochloa crus-pavonis were grown in hydroponics using nutrient solutions containing different nitrogen forms (see materials and methods). This experiment was conducted in order to assess the influence of the nitrogen form on the NRA of different weed types. The NRA of these weed species at 14 DAG is shown in **Figure 8**.

In general the lowest NRA in all weeds was found when they were grown in nutrient solution containing only NH_4 (no substrate for NR) and significantly higher NRA levels were found when nitrate was present (**Figure 8**).

The upland adapted weeds *Z. mays* and *E. heterophylla* had higher NRA when only nitrate was present as compared to when NH_4 was present. Whereas the NRA of the lowland adapted weed *E. crus-pavonis* was lowest when grown with high NO_3 concentration (**Figure 8**).

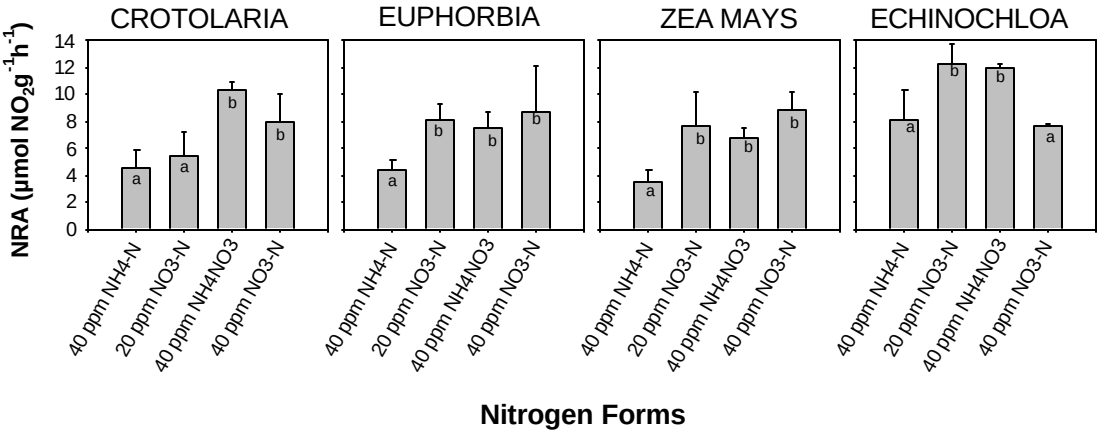


Figure 7: NRA of *Crotolaria spp, Euphorbia heterophylla, Zea mays and Echinochloa crus-pavonis* at 14 days after germination (DAG), the weeds were grown using Yoshida solution in which the nitrogen form was modified to provide either 40 ppm NH_4-N, 20 ppm NO_3-N, 40 ppm NH_4NO_3 or 40 ppm NO_3.

4.9 Influence of Seedling Age on the NRA of Weeds

In order to determine the age at which weeds are most competitive with regard to nitrate assimilation, a study was conducted in which the nitrate reductase activities of *Euphorbia heterophylla, Crotolaria spp, Echinochloa crus - pavonis* and *Zea mays* were determined at 7, 14 and 21 DAG. Results from this study for the NRA activities of *E. heterophylla* and *E. crus-pavonis* which are the most competitive rice weeds are presented in **Figure 8** and **Figure 9**. Data for *Z. mays* and *Crotolaria spp* are shown in the appendix.

Mean NRA of the weeds differed significantly at 7, 14 and 21 DAG.

The NRA of *E. heterophylla* was always highest at 21 DAG and lowest at 7 DAG when nitrate was present in the nutrient medium, the trend was reversed in the absence of nitrate (**Figure 8 A**).

In *E. crus-pavonis* NRA was highest at 14 DAG when the weeds were grown using either 40 ppm NH_4-N or 20 ppm NO_3-N, whereas with NH_4NO_3 nutrition, specific NRA was highest at 7 DAG (**Figure 8B**).

NO_3 assimilation as the seedlings grow is presented more accurately when NRA is computed on basis of individual plants (**Figure 9**). In *Euphorbia spp* an increase in NRA with increasing plant age is observed in the presence of nitrate. In *Echinochloa spp*, a similar response is only observed when the weeds are grown with 40 ppm NO_3-N (**Figure 9**).

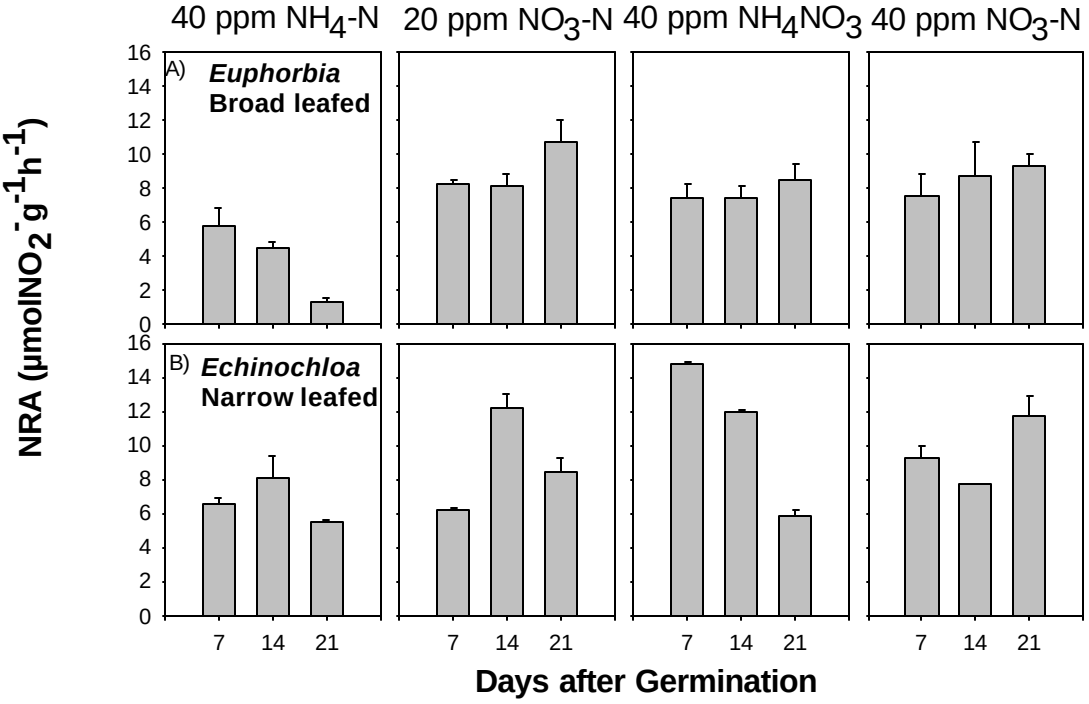


Figure 8: Nitrate reductase activities in broad-leafed (*Euphorbia heterophylla*) and narrow leafed (*Echinochloa crus-pavonis*) rice weeds at 7, 14 and 21 days after germination (DAG). Weeds were grown using different forms and concentrations of nitrogen

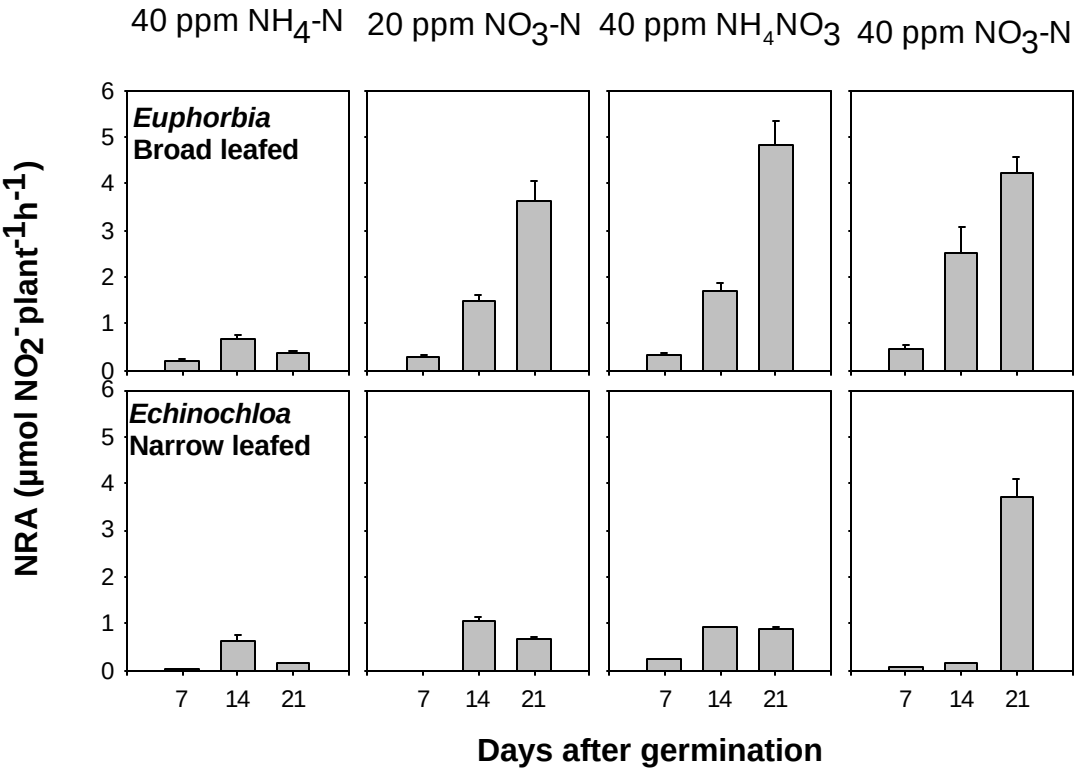


Figure 9: NRA in leaves (computed on basis of individual plants) of *Echinochloa* and *Euphorbia* at 7, 14 and 21 days after germination (DAG). The weeds were grown in nutrient media containing 40 NH_4 ppm, 20 ppm NO_3, and 40 ppm NH_4NO_3 and 40 ppm NO_3

4.10 Weed Competitiveness of Rice Genotypes

Two upland rice cultivars, CG14 and WAB 56-104 differing in NRA at 14 DAG when grown in solutions containing NO_3, were grown in competition with upland (*Euphorbia heterophylla)* and lowland *(Echinochloa spp)* weeds. The plants were grown in hydroponics using nutrient media containing either 40 ppm NH_4-N, 20 ppm NO_3-N, 40 ppm NH_4NO_3 or 40 ppm NO_3-N. Growth parameters (leaf area, total plant dry weight and leaf dry weight) of both rice cultivars and of the weed species were determined at 17 DAG.

Figure 10 shows leaf area, total plant dry weight and leaf dry weight of CG14 and WAB 56-104 grown in competition with *E. heterophylla* and *E. crus-pavonis* using nutrient medium that contained either 40 ppm of NH_4NO_3 or $N0_3$.

It was observed that the nitrogen form influenced the competitiveness of the two rice cultivars.

When NO_3-N was the only nitrogen source, CG14 was competing less effectively with E*. echinochloa* than with *E. heterophylla,* whereas WAB 56-104 was competing equally well with both weed species (**Figure 10**).

Under NH_4NO_3 nutrition, CG14 competed equally well with both weeds, whereas WAB 56-104 was less effective when grown with *E. heterophylla* than with *E. crus-pavonis* (**Figure 10**).

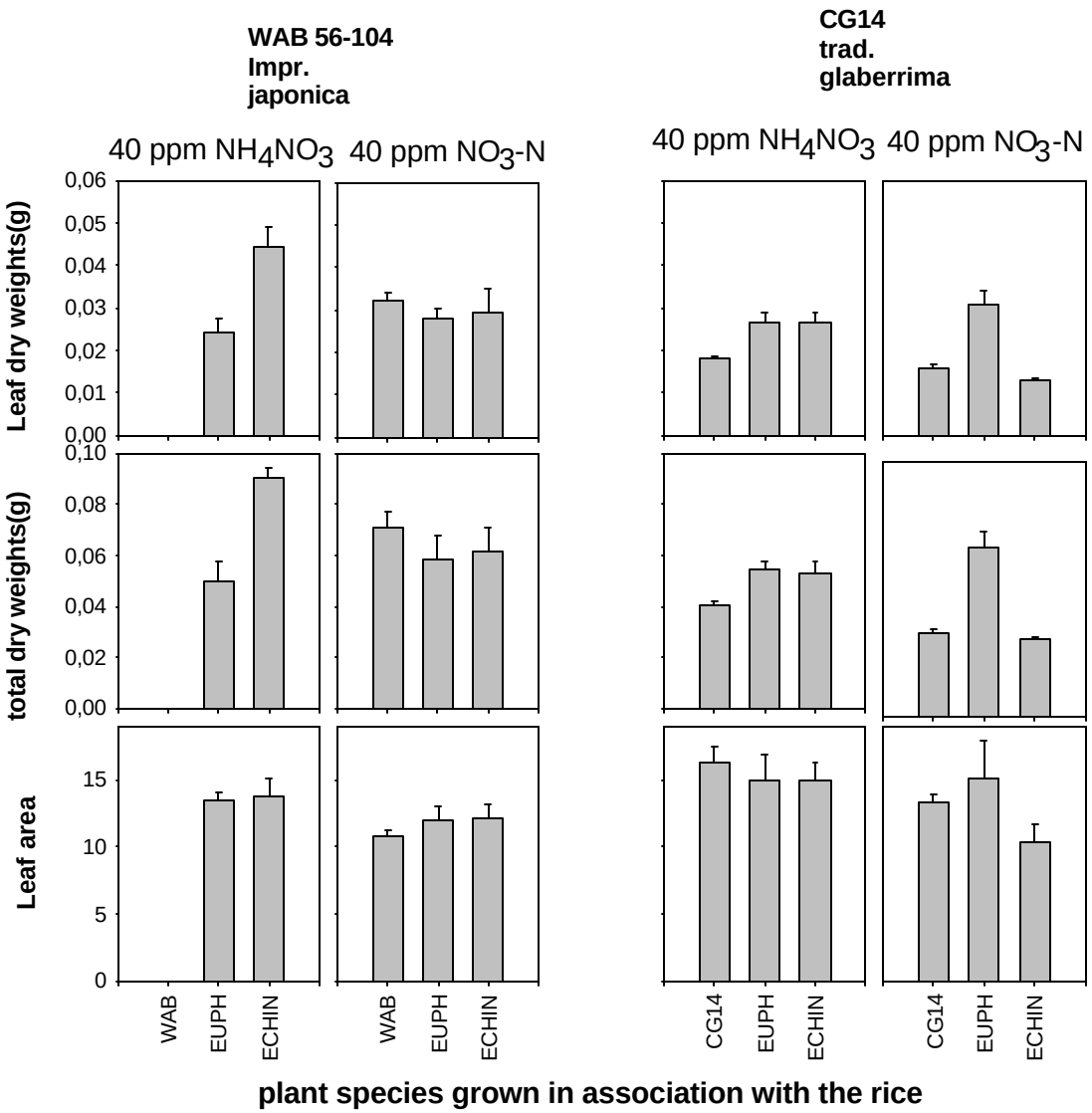


Figure 10: Growth parameters of a high NRA rice type (CG14) and a low NRA rice (WAB 56-104) at 17 days after germination when they were grown in competition with *Euphorbia heterophylla* and *Echinochloa crus-pavonis.* data for WAB 56-104 in monoculture not determined due to insufficient plant material. EUPH=*E. heterophylla*, ECH= *E. crus-pavonis*

A competitive index can be used as a measure of the competitiveness of the rice cultivars. In this study the competitive index was determined as a ratio of the dry

matter accumulation under monoculture to that accumulated under competitive conditions.

The competitive index of the rice cultivars CG14 and WAB 56-104 when grown in competition with *E. heterophylla, Crotolaria spp* and *E. crus-pavonis* are presented in **Figure 11**. Since the study focused on nitrate concentrations, the results for competition under NH_4NO_3 are not presented here (**Figure 11**). Competitive index values greater than 1 indicate strong competitive ability.

The nitrogen form influenced the competitiveness of both cultivars.

Both cultivars did not compete efficiently when grown in nutrient media containing no nitrate; they were most competitive when grown in media that contained 20 ppm NO_3-N. When the nitrate concentration in the growing medium was higher i.e. 40 ppm NO_3-N, the competitive index of both varieties declined, although this was still higher than that in the no nitrate treatment (**Figure 11**).

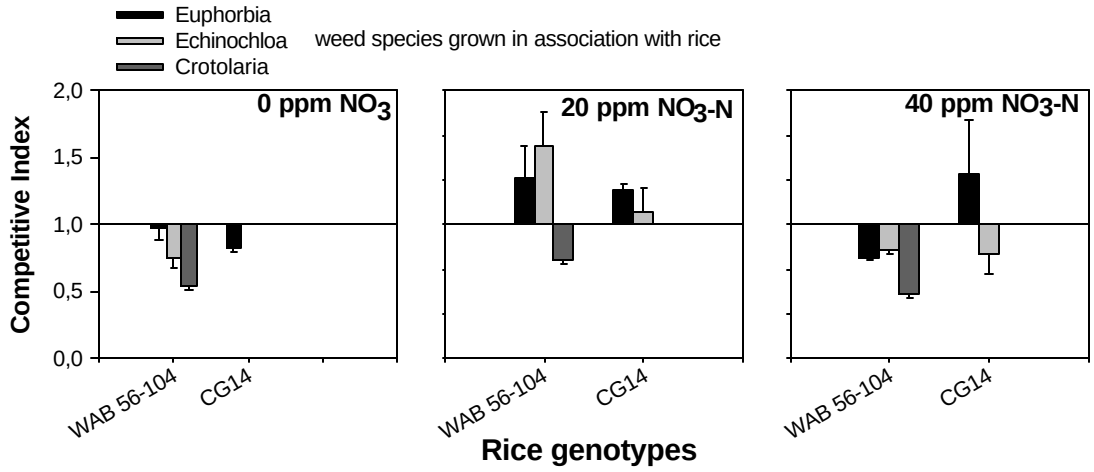


Figure 11: Weed competitiveness of rice genotypes when grown in association with *Echinochloa spp*, *Crotolaria spp* and *Euphorbia spp.* Competitive index was determined on basis of dry weights ratio i.e. rice-rice monoculture /rice-weed competitive situation

The scatter plot diagram (**Figure 12**) relates NRA of an *japonica* genotype (WAB 56-104) and of an *O. glaberrima* (CG14) to their competitive index when both were grown in association with upland weed (*E. heterophylla),* (legume*) Crotolaria spp* and (lowland weed*) E. crus-pavonis*.

At 20 ppm NO_3 inspite of having a lower NRA than at 40 ppm NO_3, the competitive index of WAB 56-104 was high when grown in association with *E. heterophylla* (**Figure 12**).

The NRA of CG14 increases with an increase in NO_3 concentration in the growing medium. When CG14 was grown in competition with *E. heterophylla* it was observed that an increase in the competitive index corresponded to an increases in NRA (**Figure 12**).

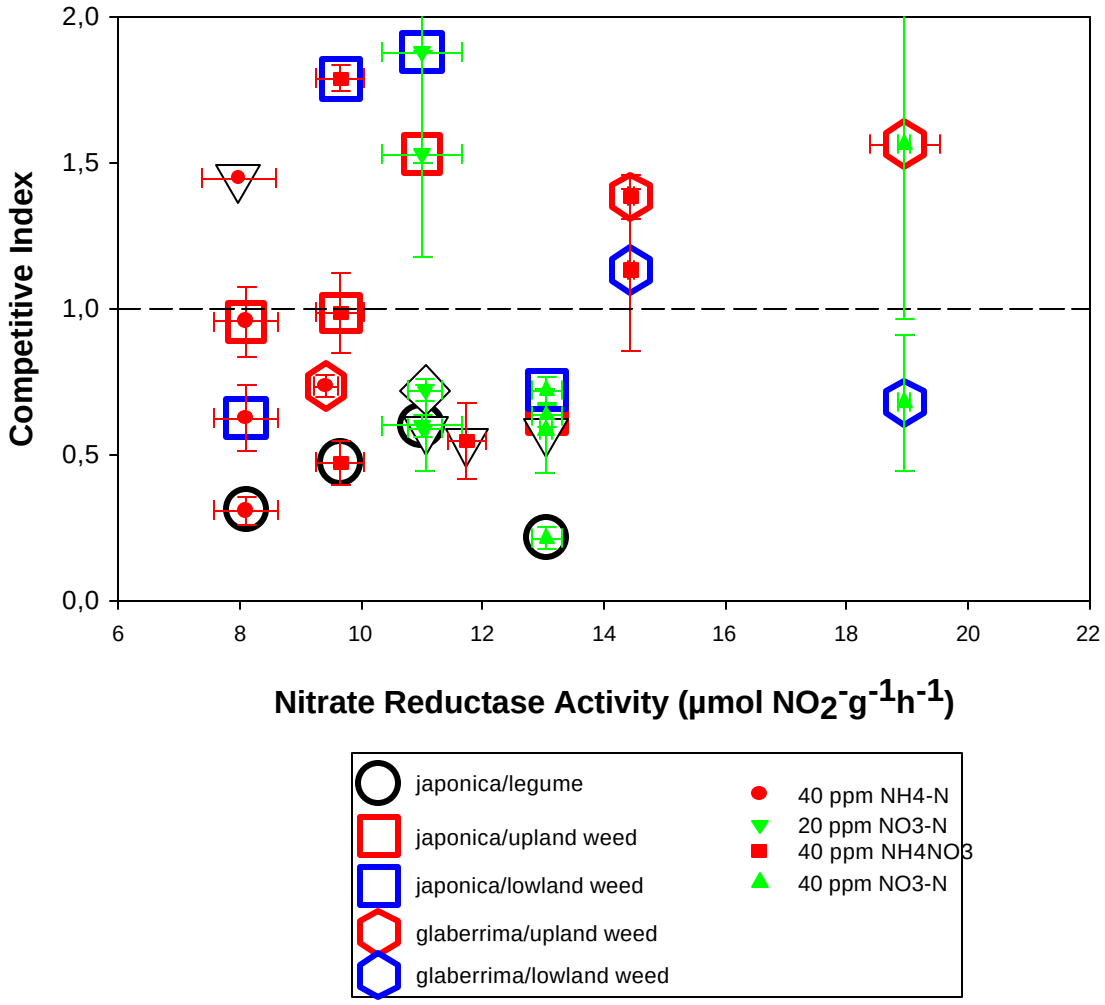


Figure 12: Scatter plot diagram showing relationship between NRA of rice types (WAB 56-104 & CG14) and their competitive index when grown in association with either *Echinochloa* spp (lowland), *Euphorbia spp* (upland), *Crotolaria spp* (legume) weeds.

5 Discussion

Weed competitiveness of rice genotypes growing under non-flooded conditions depends to a large extent on the ability of these genotypes to assimilate nitrogen early in their life cycle and to out compete associated weeds for nutrients and ultimately light.

NRA can be used as an indicator for the ability of a rice genotype to assimilate NO_3 the prevalent nitrogen form available under non-flooded conditions.

If based on early NRA, a screening tool shall be developed, a number of constraints needed to be tested:

1. Which is the most suited nitrogen form/source to be used as a substrate?
2. At which seedling age are the differences in NRA most pronounced and indicative for weed competition?
3. How do standard weeds react to the experimental conditions?
4. Is early NRA in fact related to weed competitiveness?

In this study, significant differences in leaf NRA were observed between the rice species *O. glaberrima* Steud and *O. sativa* L as well as between the subspecies *japonica* and *indica* (**Table 6**).

NRA has been used to express the ability of higher plants to use NO_3 and their capacity to reduce NO_3 to NO_2 (Chirkova & Belonogova, 1991). Results from this study indicate that some varieties are more efficient assimilators of NO_3 than others as indicated by their higher NRA values.

5.1 Growth of Rice Genotypes as Influenced by Nitrogen Form/Source

Rice, *O. sativa* as well as *O. glaberrima* can be grown in both lowland and upland systems, and has been shown to grow well in nutrient culture containing either NO_3 or NH_4 or both, even without aeration (Kronzucker *et al*., 1999).

It has however generally been accepted that rice plants are ammonophilic (Shen 1969; Wang et al., 1993; Kronzucker et al., 1998). It has however also been shown that NO3 is just as good a nitrogen source for rice as NH_4. (Malavolta, 1954; Rao, 1979; Raman *et al*., 1995).

In this study, *O. glaberrima, O. sativa ssp japonica* and *O. sativa ssp indica* rice genotypes were grown using nutrient medium that contained different forms of nitrogen. The growth parameters of the cultivars were assessed at 14 and 21 DAG. Results from the study indicate different responses among the upland and lowland adapted rice genotypes, to the form of nitrogen supplied (**Table 5**).

Indica cultivars are generally grown in lowland ecosystems where NH_4-N is generally the more prevalent form of nitrogen available. Under these reductive soil conditions nitrogen transformations result in loss of NO_3 and accumulation of NH_4. Mean dry weight values at 21 DAG show that *indica* genotypes grew better when NH_4 was present in the medium as compared to when NO_3 was the only nitrogen source present (**Table 5**). Results suggest that *indica* genotypes, which are generally grown in lowland ecosystems, are adapted to these conditions.

When NO_3 was the only source of nitrogen, growth of the *indica* genotypes was better under 20 ppm NO_3-N as compared to under 40 ppm NO_3-N, which indicates that higher NO_3 concentrations in the medium did not improve growth. These results show parallelism with conclusions drawn by Ta (1981) that indica genotypes grow better under low as compared to high nitrate conditions.

The *japonica* cultivars used in this study are generally grown in upland ecosystems. Soils in these systems are usually well aerated and NO_3-N is the prevalent form of nitrogen available. Comparisons of mean dry weight values show that these upland adapted genotypes grew better when grown in nutrient media that had both nitrogen forms as compared to when either NO_3 or NH_4 was supplied singly (**Table 5**). Results from this study confer with the observations made by Ta *et al.*, (1981), who found that growth of *japonica* rice genotypes was most favourable when the media contained NH_4NO_3. This indicates an adaptation of *japonica* genotypes to upland ecosystems.

All rice cultivars grown in nutrient medium containing NO_3 as the only nitrogen source appeared weak and were chlorotic. Growth of the rice cultivars with NO_3 as the only source of nitrogen was inferior to growth with only NH_4 or both forms as the nitrogen source (**Table 5** and appendix). This could be attributed to poor nitrate assimilation. Marwaha &Juliano (1976) reported that rice absorbs nitrate poorly during the first two weeks of growth. It has been suggested that the transformation of nitrate to asparagin in nitrate-supplied plants is responsible for poor growth during early seedling stages (cited in Youngdahl *et al.*, 1982). Willis & Carrero (1923) and the food and fertilizer technology centre,

http://www.fftc.agnet.org/library/article/bc51003.html (accessed 2002) attributed similar results to nitrate induced iron deficiency, since rapid nitrate uptake may cause physiological alkalinity, which leads to the unavailability of iron.

The differences in growth response to the nitrogen form in *japonica* and *indica* rice genotypes suggest that these subspecies differ in their ability to assimilate NO_3-N as a nitrogen source.

Results indicate that the most suitable nitrogen source for growing either *japonica* or *indica* genotypes are 20ppm NO_3 and 40 ppm NH_4NO_3. High nitrate concentrations at this early seedling stage would lead to retarded growth while using NH_4, as the only NH source does not give maximum growth.

5.2 NRA in Rice Genotypes as Influenced by the Form of Nitrogen

When the rice cultivars were grown in nutrient medium containing different sources of nitrogen, genotypes differed significantly in their NRA (See **Table 6** and appendix). These differences in NRA among the rice genotypes were related to the nitrogen source present. Variation in NRA with respect to the nitrogen source was more pronounced among the upland adapted cultivars (**Figure 3**). Yang *et al.* (1999) pointed out that preference for nitrogen forms among rice is genetically controlled and dependant upon nutrient supply levels.

In all the rice cultivars, NRA was lowest when the plants were grown using medium that contained only NH_4 as the nitrogen source. Higher NRA was observed when the nutrient medium contained NO_3 only (**Figure 3**). This is in line with the recognized substrate dependency nature of nitrate reductase (Beevers and Hageman, 1969). When both forms of nitrogen were present, NRA of the upland adapted genotypes was significantly lower than when only NO_3 was present, whereas in the lowland adapted genotypes NRA levels were similar to when only NO_3 was present.

In all genotypes, even when grown without the substrate for NR (i.e. NH_4-N treatment) NRA was observed (**Figure 3**). This NRA was not induced by trace amounts of nitrate, which might have been present in the medium as impurity, since analytical grade chemicals were used throughout the experiment. Rather it may be an indication that NR in rice is present as a constitutive enzyme, which is activated during the assay procedure by the NO_3 contained in the infiltration buffer (see

materials and methods). It might also be that the NRA measured is carried out by a non-specific enzyme that uses NO_3 in the buffer as a substrate

It has also been suggested by Bungard *et al.* (1999) that NRA in plants grown in medium that contained NH_4 is an indication that NR has an additional role in plants other than the reduction of NO_3 such as regulation of cellular pH (see literature review).

Nitrification, i.e. oxidation of NH_4 to NO_3 around the rhizosphere of plants grown in NH_4 medium has been reported by Smith *et al.* (1990) and Ta & Ohira (1982). In the present study we did not investigate oxidation at the rhizosphere which would help support theories of NRA in the absence additional NO_3 (see literature review).

At 14 days after germination the upland adapted genotypes had higher NRA than the lowland adapted ones when grown in nutrient medium that contained only NO_3. However, mean NRA values of *japonica* and *indica* genotypes were not significantly different. Only the *O. glaberrima* had significantly higher NRA than the lowland adapted *indica* under these conditions (**Figure 3**). This suggests that the upland adapted *O. glaberrima* is a more efficient assimilator of nitrate as compared to *O. sativa.* CG14 has high vigour, high tillering ability and high leaf area. Results indicate that the superior growth of CG14 in comparison with other rice cultivars is an attribute of efficiency in nitrate assimilation, which is as a result of high and early NRA. This observation is in line with the hypothesis of this study (see section 1.1).

Even though NRA in the upland adapted genotypes was higher when 40 ppm NO_3 was used as compared to when 20 ppm NO_3 was used, the differences in NRA at the two NO_3 concentrations did not differ significantly. Under field conditions where nitrogen is often the limiting factor, a plant capable of efficiently using low concentrations of nitrate is more competitive. Our results indicate that the availability of nitrate at high concentrations does not enhance nitrate assimilation under the present conditions.

In the upland adapted genotypes inhibition of NRA by NH_4 was observed when the nutrient medium used to grow the rice contained both NH_4 and NO_3 (**Figure 3**). Under these conditions the NRA was lower than when only NO_3 was used, although higher than when only NH_4 was present. In the lowland adapted genotypes NRA levels were not altered when both nitrogen forms were present. In *Lemna minor* (Orebamjo, 1975b), barley and tobacco seedlings (Bungard *et al.*, 1999) and in apple seedlings (Klepper & Hageman, 1969). NH_4 completely repressed NRA when both forms of

nitrogen were present in the growing medium. These results suggest that the extent to which NH_4 influences NR is specific to the crop species.

NRA in all rice genotypes is influenced positively by the availability of nitrate. However in upland adapted genotypes NRA is repressed by NH_4 if both nitrogen forms are present in the medium. Whereas in the lowland adapted genotypes no repression of NRA by NH_4 is observed under similar conditions. Differences in NRA between the upland and lowland adapted genotypes indicate differences in their ability to assimilate NO_3 as a nitrogen source.

Results show that NR is substrate dependant, and as such nutrient medium used in screening for the activity of the enzyme should contain nitrate.

5.3 NRA in Rice Genotypes as Influenced by Seedling Age

Studies relating specific NRA to the age of rice plants have shown that NR is detectable in 3.5-day-old seedlings (Shen 1969). Yang & Sung (1980) reported NRA in rice seedlings 4 days after imbibition. In their study a transient peak in NRA was observed at 7 to 11 days after imbibition followed by a rapid decline in activity. Marawaha (1976) reported a peak in NRA among rice genotypes at 10 DAG.

Differences in published reports with regard to the peak in NRA in rice, might be a result of inconsistencies in determining “day 1” by various authors. The reference point (day 1) used may either be sowing date, imbibition date, germination date, appearance of first leaf date, etc. In this study, germination date was used as the reference point for determining day 1.

In the present study, specific NRA was generally higher at 14 DAG, as compared to either 7 or 21 DAG (**Figure 4**). However due to the experimental set up (namely the pattern of sampling dates), the exact day after germination in which the peak occurs was not conclusively established.

Even though specific NRA was highest at 14 DAG and declined thereafter, the total amount of NRA that occurs per individual plant increases with increasing plant age (**Figure 5**). The increase in the NRA of the rice cultivars with increasing seedling age is due to an increased plant biomass.

The overall nitrate assimilation per rice plant increases, as the rice plant enters a new seedling development stage, while the specific NRA decreases seemingly because the plant has more biomass available for this process.

Based on the results, it can be said that the most appropriate time in rice seedling development, for assessing NRA is at 14 DAG. At this point maximum differences NRA among cultivars are observed. At earlier seedling stages no differences in NRA are observed while at later stages the differences are less pronounced.

5.4 Influence of Medium Conditions on NRA of Rice

In both lowland and upland adapted rice genotypes, the NRA observed when the plants were grown in sand medium as compared to stagnant hydroponics was higher. Ta, (1981) showed that the activity of NR varies dependent on medium and environmental conditions. Results show that redox potential in the growing medium influences the availability of NO_3-N and hence determines the ability of plants to assimilate nitrate.

All rice cultivars grown in nutrient medium containing NH_4 had higher NRA values in sand culture as compared to hydroponics. It is possible that since the redox potential in the sand medium was higher than that in the hydroponics, in the sand culture, nitrification (NH_4 oxidised to NO_3) lead to the relatively higher NRA under these conditions (**Figure 6**).

WAB 56-104 is an upland rice cultivar that is sensitive to flooding. When grown in the sand culture it always had higher NRA as compared to the hydroponics system. The superior nitrate assimilation of WAB 56-104 in sand culture might be due to the low redox potential under these conditions and that the relatively lower nitrate assimilation in the hydroponics system may be due to the sensitivity of this cultivar to such reductive conditions.

5.5 Influence of Nitrogen Source on the Growth of Rice Weeds

The broad leafed weeds *Euphorbia heterophylla* and *Crotolaria spp* are commonly found in upland ecosystems whereas the narrow leafed weed *Echinochloa crus-pavonis* is a lowland adapted weed. Under upland conditions NO_3 is the prevalent

form of nitrogen available whereas under lowland conditions NH_4 is the predominant form.

Among the upland adapted weeds growth was better when NO_3 was the only nitrogen source present as compared to when NH_4NO_3 *(Crotolaria*) and NH_4 (*E. heterophylla*) were the nitrogen sources.

The lowland adapted *E. crus-pavonis* had better growth when both nitrogen forms were supplied as compared to when either form was supplied singly. Results indicate that under conditions where nitrate was present, this weed was least competitive, which is in line with it's ecological adaptation.

Results indicate that nitrogen assimilation among weeds is linked to their ecological adaptations. Upland adapted weeds are expected to be more competitive under conditions where NO_3 is present. The results also show that *E. heterophylla,* which is the more economically important upland weed is more competitive under low as compared to high nitrate concentrations. The success of *E. heterophylla* as a weed might be due to its ability to efficiently use low concentrations of nitrate.

5.6 NRA of Broad Leafed and Narrow Leafed Rice Weeds

The success of a weedy plant species is linked to its ability to establish itself in an environment at the expense of the crop plant. Redinbaugh & Jones (1997) reported that high nitrate assimilation is an important factor in determining the ability of a plant species to establish itself.

NRA assays of upland and lowland adapted weeds showed that the two weed types differed in nitrate reduction, and that this was dependant on seedling age and nitrogen source (**Table 8**). The different transient trends in NRA between the two weed types suggest that they differ in their nitrate assimilation characteristics. The NRA of *E. heterophylla* increased with increasing nitrate concentration at 21 DAG whereas a similar response in *E. crus-pavonis* was observed at 14 DAG.

Yang & Sung (1980) has shown that NRA was positively co-related with seed weight. The lowland weed *E. crus-pavonis* had lowest seed weight among the weed species tested, it also had high NRA earliest. Once seed reserves are exhausted seedlings become autotrophic, it is likely that the seed reserves of *E. crus-pavonis* are exhausted earliest and hence nitrate assimilation sets in earlier than in other weeds.

This early and high NRA gives *E. crus-pavonis* a comparative advantage with regard to nitrate acquisition and is probably one of the factors that make it the most economically important rice weed in the world.

5.7 Nitrate Reductase Activity and Efficiency of Rice Cultivars

We suggest that efficiency of a plant species in nitrate assimilation is linked to high nitrate reductase activity when the nitrate concentration supplied to the plant is low. When rice cultivars were grown in media that contained 20 ppm NO_3. The NRA of CG14 as measured at 14 DAG was 33% more than that of WAB 56-104, indicating that the CG14 is a more efficient assimilator of nitrate. This view is supported by the fact that dry matter accumulation of CG14 was 27 % more than that of WAB 56-104 at the same seedling age.

5.8 Weed Competitiveness of Rice Genotypes

When the nutrient culture contained both nitrogen forms, the lowland (*Echinochloa-crus-pavonis)* and upland *(Euphorbia heterophylla)* were equally competitive when grown in association with CG14. While in association with WAB 56-104, *Euphorbia spp* was more competitive (**Figure 10**).

When the nitrogen source in the nutrient medium was 40 ppm NO_3-N the *E. crus-pavonis* was more competitive than the *E. heterophylla* when grown in association with CG14, while both weeds were equally competitive when grown in association with WAB 56-104 (**Figure 10**).

The results indicate that the competitive nature of rice cultivars is dependant on the form of nutrient supply as well as the weed species with which it is in competition When the nitrogen source was NH_4, the upland cultivars which are adapted to NO_3 assimilation, were not efficient weed competitors. Their competitiveness was enhanced when the nitrogen supply was NO_3 (**Figure 11**).

It had been previously shown that NO_3 assimilation in upland rice genotypes was most efficient when the nitrate concentration was 20 ppm (**Figure 3**). The competitiveness of these cultivars was highest at identical nitrate concentrations (**Figure 11**). This provides a direct link between efficiency in NRA and weed competitiveness of rice cultivars. Under nutrient conditions that resulted in low NRA

e.g. when NH_4 was the source of nitrogen, both rice cultivars were not strong weed competitors.

Under nutrient conditions that were sub-optimal for the weeds (20 ppm NO_3-N), rice genotypes having high NRA were able to out-compete the weeds. NO_3 acquisition under low concentrations of external NO_3 provides the comparative advantage to the rice.

High NRA efficiency in upland rice genotypes corresponded to high competitive index In designing a screening tool for weed competitiveness based on NRA of rice genotypes, results indicate that 20 ppm NO_3-N is the most suitable nitrogen source. At higher nitrate concentrations (40ppm) even though NRA is higher, there is less efficiency in nitrate use and the increase in NRA does not lead to improved weed competitiveness.

6 Conclusion

This study was conducted to determine whether NRA in rice genotypes could be related to weed competitiveness and if early and high activity conferred competitive advantage over weeds.

The NRA of upland adapted (*japonica* and *O. glaberimma*) and lowland adapted (*indica*) rice genotypes differed significantly. Generally the upland genotypes had higher NRA than the lowland types. The differences in NRA were related to the nitrogen source and indicated that activity of NR is substrate dependant and related to the ecological adaptation of the rice genotypes. Results show that specific NRA varies dependant on the stage of seedling development. The trend in NRA includes a transient peak followed by a decline in activity. In assessing the activity of NR we conclude that assays should be conducted at 14 DAG when NRA is at its highest.

In the current study the NRA of the genotypes were similar regardless of the NO_3 concentration. It was thus established that nitrate assimilation efficiency was higher at lower NO_3 concentrations. The competitiveness of upland rice genotypes was highest when NR was most efficient, i.e. at lower nitrate concentrations. CG14, which had higher earlier NRA than WAB 56-104, was also a stronger weed competitor.

Based on the results it can thus be stated that the activity of NR in upland rice genotypes can be used as a potential screening tool for weed competitiveness only if nitrogen source, weed type are taken into consideration.

Nitrate reductase activity is influenced by several factors, seedling age, substrate form, medium conditions, pH etc. In designing a screening tool, the following points should be taken into consideration:

1. The efficiency in the activity of NR and not the overall NRA should be used as a criterion.
2. Assays should be carried out when specific NRA is at its highest.
3. Only upland adapted cultivars should be used.
4. The nutrient medium must contain the substrate i.e. nitrate. However as the results show, under the experimental conditions high nitrate concentrations are not suitable. It has also been pointed out that nutrient medium containing NO_3, as the only nitrogen source is not conducive for rice growth at this early seedling stage, since favourable growth was observed when both forms were present. Future studies should use both nitrogen forms with the NH_4 at a lower

ratios to avoid NR repression. In the present study the two nitrogen forms were used in equal ratios.

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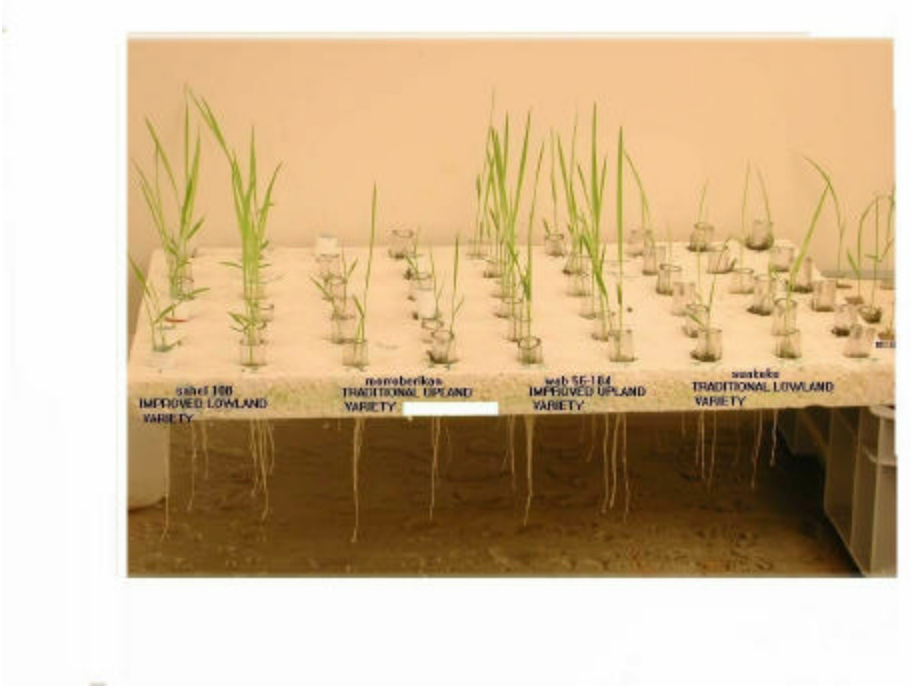
Appendix



Rice plants growing in experimental setup, note easy accessibility to roots

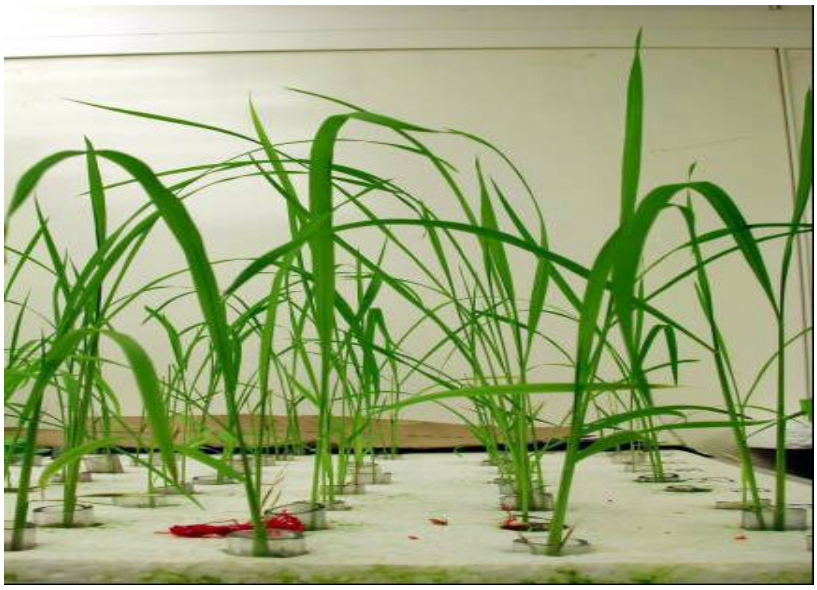


Table 9: Total dry weights of rice genotypes at 14 days after germination (DAG). The plants were grown in nutrient medium that contained different forms of nitrogen KEY: nd= not determined, Trad.=traditional, Impr. = Improved

Cultivar	**DRY WEIGHT PER PLANT (mg)**					
	Trad./Impr.	**Sub Species**	**40 ppm NH_4**	**20 ppm NO3**	**40 ppm NH_4NO_3**	**40 ppm NO_3**
WAB 56-104	Impr.	Japonica	27.8	27.9	29.8	35.4
WAB 181-18	Impr.	Japonica	14.7	7.7	27.2	22.9
MORROBERIKAN	Trad.	Japonica	15.5	24.2	29.3	20.5
MALOBDJAN	Trad.	Japonica	38.5	24.0	61.6	nd
AVIE OUFOUE	Trad.	Japonica	16.4	12.2	75.9	62
CG14	Trad.		46.9	39.7	39.8	37.0
MR 123	Impr.	Indica	23.4	27.3	43.6	29.1
SIKAMO	Impr.	Indica	42.4	43.6	28.6	30.7
ITA 320	Impr.	Indica	26.1	19.95	21.5	18.69
CK4	Impr.	Indica	41.9	19.55	46.6	53.3
SAHEL 108	Impr.	Indica	32.0	37.33	17.3	19.9
I Kong Pao	Impr.	Indica	nd	33.0	40.9	32.7
TOX	Impr.	Indica	42.7	13.4	36.3	10.2
SUAKOKO 8	Trad.	Indica	29.9	25.5	58.5	79.0

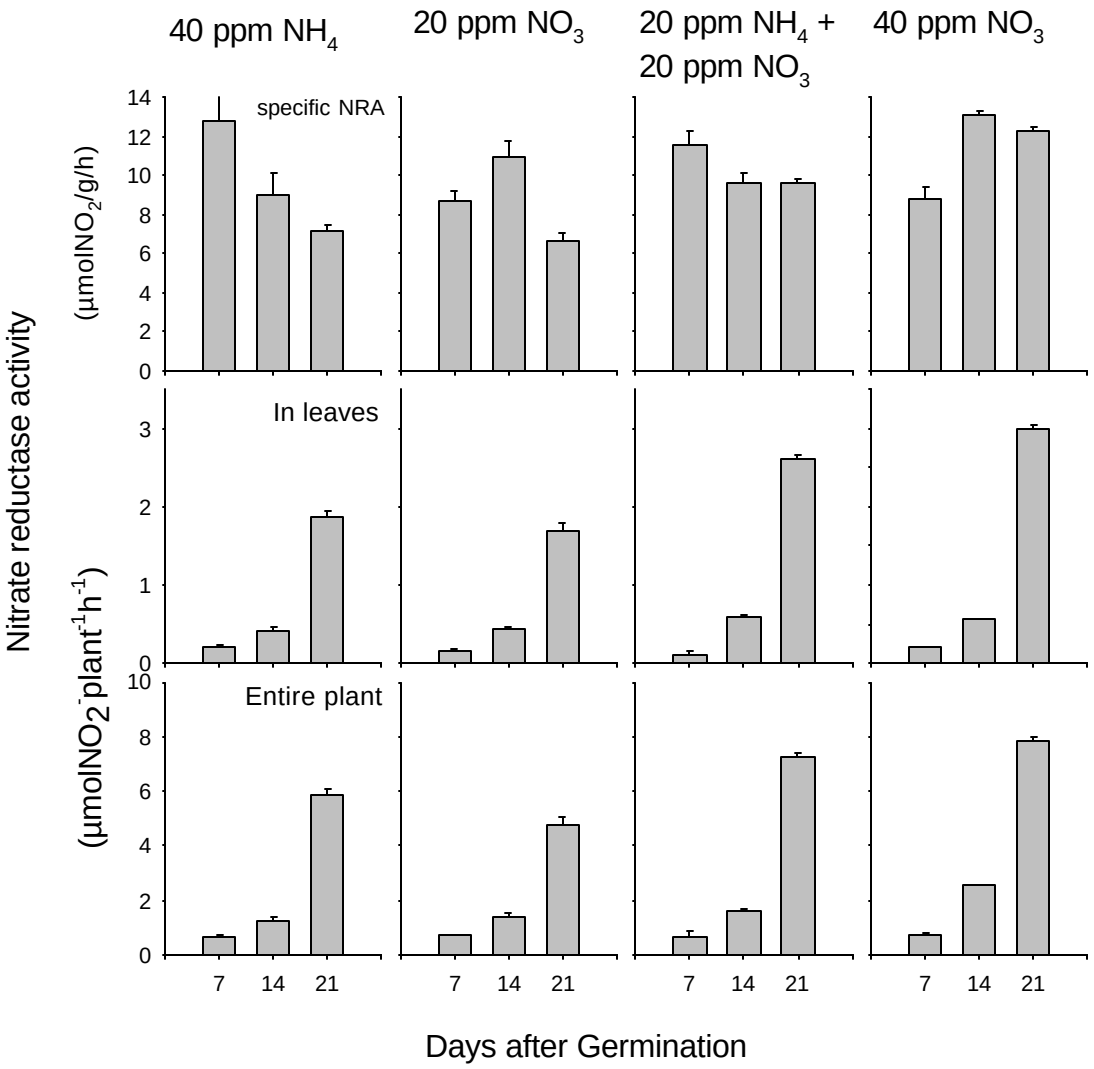


Figure 13: Nitrate reductase actovity of rice genotypes expressed on the basis of specific NRA and NRA of individual plants.

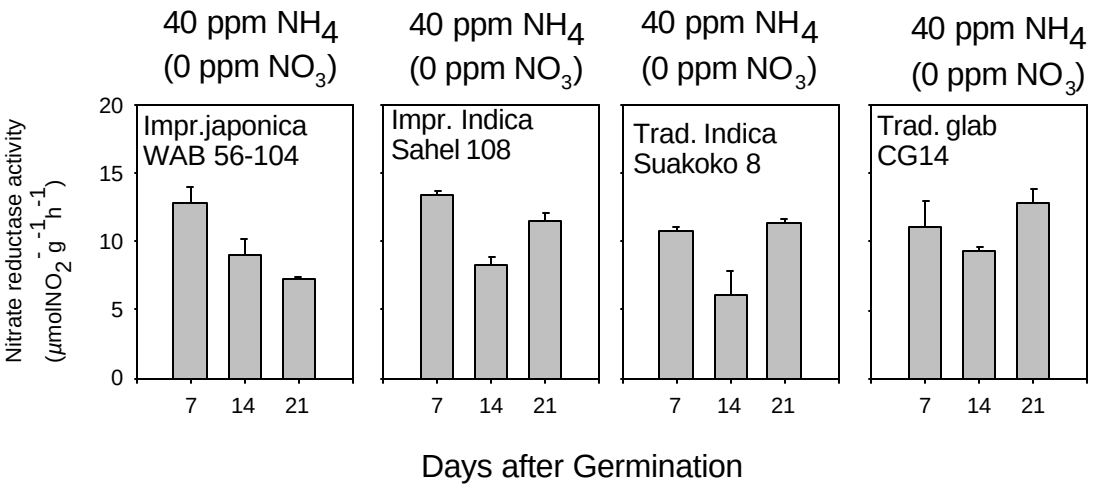


Figure 14: Nitrate reductase activity of rice genotypes in the absence of the substrate (nitrate).

Tests of Between-Subjects Effects on the NRA of rice genotypes at 7 days after germination (DAG)[b]

Dependent Variable: NRA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6096,636[a]	40	152,416	1,105	,337
Intercept	23821,230	1	23821,230	172,661	,000
GENOTYPE	2227,751	11	202,523	1,468	,154
NITROGEN	288,182	3	96,061	,696	,556
GENOTYPE * NITROGEN	3388,401	26	130,323	,945	,548
Error	14762,250	107	137,965		
Total	48075,797	148			
Corrected Total	20858,886	147			

a. R Squared = ,292 (Adjusted R Squared = ,028)

b. DAY = 7 DAG

Tests of Between-Subjects Effects on the NRA of rice genotypes at 14 days after germination (DAG)[b]

Dependent Variable: NRA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2133,983[a]	59	36,169	20,354	,000
Intercept	40226,487	1	40226,487	22636,856	,000
GENOTYPE	987,619	15	65,841	37,051	,000
NITROGEN	574,934	3	191,645	107,845	,000
GENOTYPE * NITROGEN	637,909	41	15,559	8,755	,000
Error	271,886	153	1,777		
Total	44473,132	213			
Corrected Total	2405,870	212			

a. R Squared = ,887 (Adjusted R Squared = ,843)

b. DAY = 14 DAG

Tests of Between-Subjects Effects on the NRA of rice genotypes at 21 days after germination (DAG)[b]

Dependent Variable: NRA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1636,250[a]	57	28,706	15,676	,000
Intercept	29578,361	1	29578,361	16151,974	,000
GENOTYPE	1047,983	16	65,499	35,767	,000
NITROGEN	113,096	3	37,699	20,586	,000
GENOTYPE * NITROGEN	408,178	38	10,742	5,866	,000
Error	287,507	157	1,831		
Total	35053,129	215			
Corrected Total	1923,757	214			

a. R Squared = ,851 (Adjusted R Squared = ,796)

b. DAY = 21 DAG

Tests of Between-Subjects Effects on the NRA of weed species at 7 days after germination[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	279,561[a]	15	18,637	10,326	,000
Intercept	2242,669	1	2242,669	1242,567	,000
NITROGEN	101,105	3	33,702	18,673	,000
SPECIES	148,544	3	49,515	27,434	,000
NITROGEN * SPECIES	88,110	9	9,790	5,424	,000
Error	66,780	37	1,805		
Total	2498,538	53			
Corrected Total	346,342	52			

a. R Squared = ,807 (Adjusted R Squared = ,729)

b. sampling dates = 7,00

ests of Between-Subjects Effects on the NRA of weed species at 14 days after germinatio[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	368,869[a]	15	24,591	9,433	,000
Intercept	3810,307	1	3810,307	1461,672	,000
NITROGEN	147,895	3	49,298	18,911	,000
SPECIES	111,950	3	37,317	14,315	,000
NITROGEN * SPECIES	109,024	9	12,114	4,647	,000
Error	125,127	48	2,607		
Total	4304,303	64			
Corrected Total	493,996	63			

a. R Squared = ,747 (Adjusted R Squared = ,668)

b. sampling dates = 14,00

Tests of Between-Subjects Effects on NRA of weed species at 21 days after germination[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	661,361[a]	15	44,091	17,210	,000
Intercept	3065,615	1	3065,615	1196,574	,000
NITROGEN	189,646	3	63,215	24,674	,000
SPECIES	91,033	3	30,344	11,844	,000
NITROGEN * SPECIES	380,682	9	42,298	16,510	,000
Error	122,976	48	2,562		
Total	3849,952	64			
Corrected Total	784,337	63			

a. R Squared = ,843 (Adjusted R Squared = ,794)

b. sampling dates = 21,00

'ests of Between-Subjects Effects on the NRA of weeds categories (broad and narrow leafed at 7 days after germination[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	79,218[a]	7	11,317	1,906	,091
Intercept	2156,742	1	2156,742	363,328	,000
NITROGEN	70,423	3	23,474	3,955	,014
WEEDTYPE	2,080	1	2,080	,350	,557
NITROGEN * WEEDTYPE	5,394	3	1,798	,303	,823
Error	267,123	45	5,936		
Total	2498,538	53			
Corrected Total	346,342	52			

a. R Squared = ,229 (Adjusted R Squared = ,109)

b. sampling dates = 7,00

ests of Between-Subjects Effects on the NRA of weed categories (broad and narrow leafed) a 14 days after germination[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	197,251[a]	7	28,179	5,318	,000
Intercept	3810,307	1	3810,307	719,058	,000
NITROGEN	147,895	3	49,298	9,303	,000
WEEDTYPE	24,515	1	24,515	4,626	,036
NITROGEN * WEEDTYPE	24,841	3	8,280	1,563	,209
Error	296,745	56	5,299		
Total	4304,303	64			
Corrected Total	493,996	63			

a. R Squared = ,399 (Adjusted R Squared = ,324)

b. sampling dates = 14,00

ests of Between-Subjects Effects on the NRA of weed categories (broad and narrow leafed) a 21 days after germination[b]

Dependent Variable: NRA nitrate reductase activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	384,496[a]	7	54,928	7,693	,000
Intercept	3065,615	1	3065,615	429,357	,000
NITROGEN	189,646	3	63,215	8,854	,000
WEEDTYPE	30,445	1	30,445	4,264	,044
NITROGEN * WEEDTYPE	164,405	3	54,802	7,675	,000
Error	399,841	56	7,140		
Total	3849,952	64			
Corrected Total	784,337	63			

a. R Squared = ,490 (Adjusted R Squared = ,426)

b. sampling dates = 21,00

PERSONAL DETAILS

Name	: OUKO, MAURICE OCHIENG.
Date of Birth	: 15.02.1975
Nationality	: Kenyan.
Marital Status	: Single.
Gender	: Male.
Languages	: English, Swahili and Dholuo all fluently spoken and well written.
	: German adequately spoken und writen.
Address	: Am Jesuitenhof 1/012
	: 53117 Bonn, Germany.
Email Address	: Maure_75@yahoo.com

QUALIFICATIONS : BSc Horticulture, Upper Division 2nd class (Hons), MSc Agric.

EDUCATION

2001 - 2003	: Bonn University, Institute of Plant Nutrition, MSc Agric. Thesis title : "Nitrate Reductase Activity in Rice (*Oryza sativa* L.) as a Screening Tool for Weed Competitiveness".
1994 - 1998	: Egerton University; Faculty of Agriculture, 2nd Class,upper division (Hons). Theses title: "Effect of Nitrogen and Spacing on growth of French beans (*Phaseolus vulgaris* L)."
1989 - 1992	: Aquinas High School, Nairobi, K.C.S.E mean grade B-(minus).
1981 - 1988	: Lavington Primary School; Nairobi, K.C.P.E mean grade B+(plus)

WORKING EXPERIENCE

Jun 02 - Oct 03	: Studentische Hilfskraft, University of Bonn, Institute of Plant Nutrition: Studies relating to physiological processes in rice as a result of salt stress.
Apr 99 - Feb 01	: Sigoti Complex, Nyando Tutor: Agriculture, Biology
Mar - Apr 98	: K.B.L Experimental Farm, Mau Narok: Experiment design, setup, data collection and analysis.
Feb 98	: Buds and Blooms Ltd, Nakuru: Rose flower production, irrigation and fertilizer management, disease and pest control.
Dec 97	: Catholic Justice and Peace Commission, Kisumu: Observer Kenyan general elections.

RESPONSIBILITIES

Member	: Sigoti Catholic Choir,Nyabondo Parish 2000
Supervisor	: Final year exams Sigoti Complex 1999

Representative : Students representative in the examinations council of the Faculty of Agriculture, University of Bonn 2002

PUBLICATIONS

Ouko M O, Asch F, Becker M (2002). Screening of rice genotypes for early leaf nitrate reductase activity. In Challenges to organic farming and sustainable land use in the tropics and subtropics: international research on food security, natural resource management and rural development ; book of abstract / Deutscher Tropentag 2002, Witzenhausen. (Andreas Deininger.-Kassel. ed) pp 213. Kassel Univ. Press, 2002.

Ouko M O, Asch F, Becker M (2003). Nitrate reductase activity in rice as related to weed competitiveness In Technological and Institutional Innovations for Sustainable Rural Development: international research on food security, natural resource management and rural development ; book of abstracts / Deutscher Tropentag 2003 Goettingen. (Clemens Wollny, Andreas Deininger, Netra Bhandari, Birgitte Maas, Winfred Manig, Uwe Muuss, Frank Brodbeck, Ingrid Howe.-Goettingen. Eds) pp 108. klartex GmbH, 2003.

AWARDS

Second best poster award, Deutscher Tropentag 2002.

CAREER OBJECTIVES

Conduct research in plant science with a view of gaining a better understanding into plant physiological processes.

OTHER INTERESTS

Reading : For leisure and for information.

Sports : Active participation in football,volleyball and basketball.

Photography : With specia l emphasis on landscape photography

Music : With special interest in choral music.