

## Rice responses to environmental conditions

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According to a recent FAO (Food and Agriculture Organization) report, rice consumption will increase by approximately 70% by the year 2020. The area of available arable land, however, will be unable to keep up with such an increase. For this reason, it is essential to investigate ways of optimizing rice production or, in areas with the harshest climates, to make it more "reliable".

Rice growing is based on lowland farming, with water being controlled to varying degrees throughout the cycle of the plant. This type of farming is predominant in Asia and is highly polymorphic, including irrigated, floating, rainfed lowland and mangrove swamp methods of rice growing. A detailed terminology has been provided by G.S. KHUSH (IRRI, 1984). Another type of rice growing, upland farming on non-saturated soil, also contributes to world rice production. Although it only accounts for 17% of the world's cultivated areas, it is the predominant form of rice growing in Africa and South America.

Rice has adapted to these highly contrasting environments to produce two types of plant - lowland varieties and upland varieties. Within the *Oryza sativa* species, the lowland varieties are grouped under the subspecies *indica*, while the upland varieties are in the *javanica* group of the subspecies *japonica*. Classifications based on morphological (JACQUOT and ARNAUD, 1979) and enzymatic (GLASZMANN et al., 1984) criteria confirm this distinction.

### I - Comparison of Upland and Lowland Varieties of Rice

#### a - Morphological Data

The environmental conditions is known to

have a significant effect on certain morphological characteristics of plants which are already genetically controlled. Upland varieties generally differ from lowland varieties in that they have fewer tillers: 6 instead of 8-11 (JACQUOT and ARNAUD, 1979); they are larger: 80 to 175 cm tall as opposed to 80 cm (CHANG and VERGARA, 1975); they have longer and thicker leaves and a longer panicle. The root system is deeper and more developed in upland varieties: 1 m in emerged soil as opposed to 0.4 m in flooded soil with long, thick and more divided roots. Root hairs are more numerous. The root weight-to-stem weight ratio is higher in upland varieties than in lowland varieties (NICOU et al., 1970; CHANG and VERGARA, 1975). Data concerning the morphology and structure of the leaf blade of the 2 types of rice is scarce and often contradictory. TSUNODA and TAKAHASHI (1984), like RENARD and ALLURI (1981) and HENSON (1982), reports lower stomatal density values in japonica than those observed in indica, leading to a lower resistance to gaseous diffusion in the latter. GUEYE and RENARD (1982), however, reports a lower stomatal density in lowland varieties. During a comparative study involving a upland variety and an lowland variety, we observed larger stomata and a slightly higher stomatal density in the upland variety.

In many species, a surplus of water constitutes a physical environment which hampers root development and, consequently, plant growth. It therefore runs counter to optimum production. Of all food crops, rice is the only cereal able to optimize its production in an lowland environment. One of the main reasons why rice is particularly well suited to lowland farming lies in its ability to oxygenate its root environment, PUARD et al. (1986-a). The supply of oxygen to the roots depends on the internal structure of the cortex which, in turn,

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is closely related to the farming environment (PUARD et al 1986-b). MORISSET et al. (1982) and JUSTIN and ARMSTRONG (1983) all point to the existence of aciferous lacunae in the cortex of lowland plants. JOHN et al. (1974) have shown that rice is capable of adapting its morphology and metabolism under anaerobic conditions. This result supports the observations concerning the development of lacunar spaces in corn when grown in a low-oxygen environment.

However, lacunar spaces in the cortex have been observed in a cross-section of rice root grown in a non-flooded, well aerated environment (Figure 1-a). In comparison, no aerenchymata were observed in a corn root grown under the same conditions (Figure 1-b). This difference illustrates the genetic

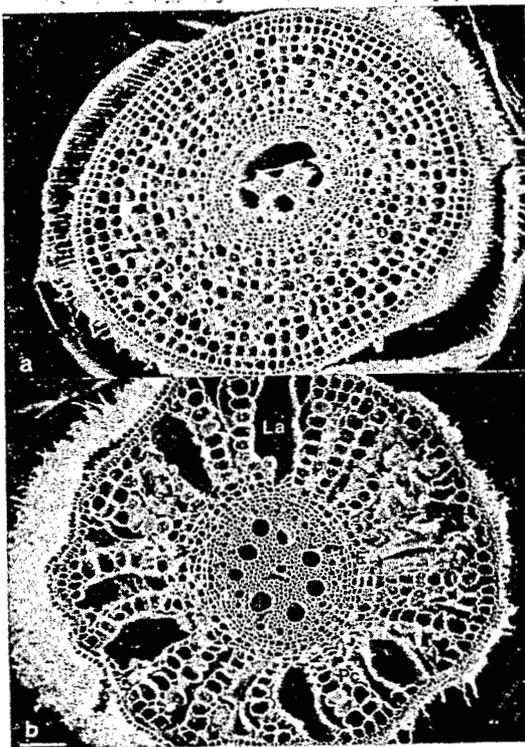


Fig. 1: Morphological comparison of root system. a) corn root (b) rice root

predisposition of rice to modify its root anatomy. The adaptability of rice is also encouraged by its environment. Several stresses, including hypoxia, cause destruction of the cortex and formation of aerenchymata.

#### b - Transfer of Atmospheric Oxygen to Roots

SOLDATENKOV and CHERKOVA (1963) have pointed out, that given the low diffusion of oxygen between the liquid phase and the soil, the plant must be able to convey atmospheric oxygen to the rhizosphere. BARBER et al. (1962) used oxygen isotope  $^{15}\text{O}$ , a short-lived radioactive nuclear indicator, to demonstrate how rice conveyed oxygen taken from the atmosphere to the roots. Prior to this experiment, EVANS and ERBERT (1960), also using  $^{15}\text{O}$ , demonstrated the transfer of oxygen from the atmosphere to the roots. Rice has a higher oxygen molecule transfer ratio than other crops (JENSEN et al. 1967). GREENWOOD (1967) and VARTAPETIAN (1973) revealed a process in which oxygen was released from the roots to the rhizosphere. This mechanism is well known in hydrophytic plants (JUSTIN and ARMSTRONG, 1983) such as the marsh rush (HALDEMANN and BRANDLE, 1983). This mechanism has also been demonstrated in many cultivated crops, notably the sunflower (WAMPLE and REID, 1975), barley (TSUNODA and TAKAHASHI, 1984), corn (PRIOUL and GUYOT, 1985). Some species are able to survive in a flooded environment if the stress period does not exceed a few days. The ability to adapt to flooding, however, expressed in terms of yield, seems to be particularly related to the plant's ability to oxygenate its roots. This is a passive mechanism, linked to the difference in partial oxygen pressures between the atmosphere and rhizosphere (PUARD et al. 1986-a). Having first confirmed, using four species (corn, barley, sunflower, rice), that a plant's ability to transport oxygen was related to its resistance to flooding, we revealed an intra-specific variability of this type in a sample of thirteen varieties of rice grown in the Camargue region in the south of France. The methods developed are currently used to obtain varietal selection criteria.

According to laboratory measurements made in a controlled environment on forty-day-old plants, the percentage of oxygen supplied for root respiration by transfer from the aerial parts of the plant, differs according to the species. This percentage also depends on

the farming environment. Results show that rice is capable of satisfying 70% of its oxygen requirements by the transfer of atmospheric oxygen. A classification system composed of four plant categories has been drawn up for this parameter (Figure 2).

The transfer of atmospheric oxygen to the roots implies leaf activity. Two varieties were used to illustrate this point; one was an upland variety known as IAC 55-40. These plants were roughly the same age with a similar leaf area. Under experimental conditions and with an energy of 400 mM, photosynthesis was not significantly different and was in the area of 16 mg of CO<sub>2</sub> dm<sup>-2</sup> h<sup>-1</sup>. Transpiration ranged from 1.6 g to 1.9 g of water per dm<sup>-2</sup> h<sup>-1</sup>. Rojofotsy maintained the oxidizing power of its rhizosphere and the oxygen concentration balanced out at 5.5 mg l<sup>-1</sup>, i.e. 67% of the saturation. IAC 55-40, however, exhausted the oxygen supply of the environment. This plant therefore consumed more oxygen than it was able to transfer. These results confirm the hypothesis that, on average, upland varieties transfer less oxygen than lowland varieties (PUARD, 1987).

Oxygen consumption figures expressed

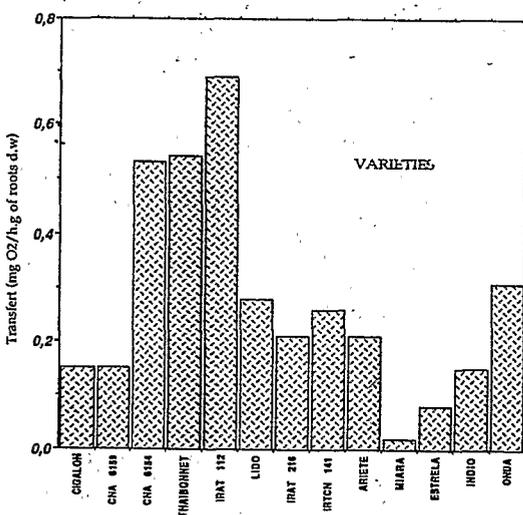


Fig. 2: Comparison of some cultivated varieties for their oxygen transport capacities

gram of root fresh weight showed that IAC 55-40 consumed 0.50 mg. g<sup>-1</sup> h<sup>-1</sup>, whereas the lowland variety Rojofotsy only consumed 0.08 mg. g<sup>-1</sup> h<sup>-1</sup>. Microscopic examination, taken 5 cm from the apex, revealed a volume of lacunar spaces (16.5%) in Rojofotsy. The proportion of these spaces indicates that Rojofotsy would be suitable for cultivation in rainfed lowland. Given the difference in morphological structure between lowland and upland varieties, leaf area would seem to affect the plant's capacity to transfer oxygen. Results show that this capacity depends on leaf area in a non-proportional manner. When leaf area was reduced by 80% by ablation, the quantity of oxygen transferred only decreased by 35 to 60% of its initial value for the different plants tested

#### c - Maintaining the Oxidizing Power of the Rhizosphere

In a natural environment, root respiration is not the only factor involved in oxygen consumption in the rhizosphere. The biomass of the soil also plays a part in the use of oxygen from the soil; consequently the flooded environment quickly becomes hypoxic and stressful. One of the consequences of the transfer of atmospheric oxygen to the roots is that the soil maintains its oxidizing power, VIZIER and PUARD (1989). In rice plantations in Camargue in southern France, we demonstrated that a plot of cultivated rice could maintain the oxidizing power of the rhizosphere ( $P_e + pH > 12$ ) with a pH in the region of 7 at the end of growth. This value remained higher than that obtained under the same flooding conditions on bare soil. The varying ability of plants to transfer oxygen can thus be seen in a slower development of the soil's reducing potential. Six Italian varieties belonging to the lowland group japonica were tested in six 20 m<sup>2</sup> plots. The pH of the soil, an important factor in redox potential, was not significantly different for the six plots and remained close to 7 throughout the experiment. Redox potential was measured 20 times a week at a depth ranging from -5 to -10 cm. The values obtained between the 9th and 12th week, i.e. 60 measurements, were then compared. The impact of environmental parameters (nutrition, soil working, etc.) is of prime importance and partly conceals the role played by the plant. Three significantly different categories of plant were observed. Of the six varieties studied, four showed that the oxygen transfer measured in the laboratory was related to the maintenance of oxidizing

power in the farming environment. One variety (Star) stood out from the group with a mean  $P_e$  of 6.45, followed by Onda (5.87) and Lido (5.78). The other varieties were: Delta (5.73), Cigalon and Ariète (5.53). These results show that the transfer of oxygen by the plant from the atmosphere to the rhizosphere has an impact on the maintenance of the oxidizing power of the rhizosphere. The differences according to variety revealed in the laboratory were, however, attenuated by the environment. In situ measurement of the oxidation-reduction potential may be one way of testing, but the number of measurements required to obtain a significant mean value is too high. Measurement of oxygen transfer capacity under experimental laboratory conditions would seem to be a more appropriate method of varietal screening.

#### d - Photosynthesis and Transpiration

No significant difference in leaf conductivity and photosynthesis in the two types of rice was reported, TURNER *et al.* (1986). TSUNODA and TAKAHASHI (1984), however, reported a particularly high photosynthetic ratio, especially in indica. When grown under upland conditions, no difference in photosynthesis was observed between the two types rice (FUKAI *et al.*, 1985). Under aquatic conditions, however, adapted cultivars had a higher photosynthetic ratio than the upland varieties, which were not so well adapted. Water efficiency, expressed as the ratio of photosynthesis to transpiration (P/T) appeared to be higher for the upland varieties of rice. It is difficult, however, to compare transpiration and photosynthetic ratios accurately owing to the diversity of experimental conditions. Nevertheless, when illumination exceeded  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the P/T ratio was remarkably uniform regardless of type of rice and farming conditions (in soil or nutritive solutions). Water efficiency values ranged from 11.2 to  $13.1 \text{ mg CO}_2 \text{ g}^{-1} \text{ H}_2\text{O}$  (MASTEAU, 1989; BOIS *et al.*, 1984 b; ZHANG, 1991).

Throughout the plant's cycle, i.e. 150 days under experimental conditions, the gaseous exchanges ( $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) of a rice crop (cv. IRAT 13 upland variety) were studied in a cultivation chamber under controlled soil and atmospheric conditions (MOUTONNET and BOIS, 1982). Photosynthesis and transpiration increased exponentially during the vegetative phase up to the 70th day (Figure 3). Maximum values were obtained on the 95th day for

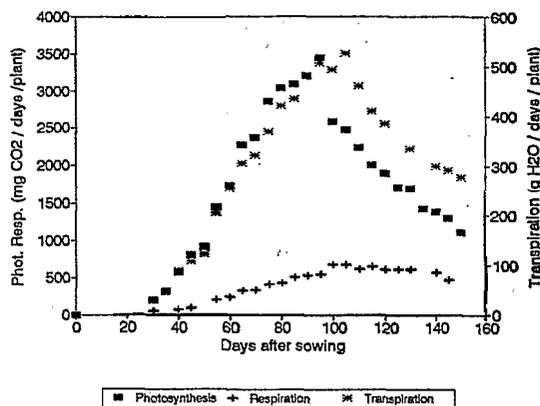


Fig. 3: Changes in the rate of Photosynthesis, respiration and transpiration of rice (cv. IRAT 13) grown in assimilation chamber.

photosynthesis and on the 110th day for transpiration. Maximum values were obtained prior to earing which occurred between the 118th and 137th day. Gaseous exchanges then decreased rapidly, this decrease being related to the senescence of the oldest leaf stages. Respiration increased up to the 100th day and remained almost stable until the 140th day. Water efficiency, evaluated by the P/T ratio, was constant throughout the vegetative phase. It decreased significantly as from the 100th day owing to the decline in photosynthesis. In terms of total transpiration for the whole crop, 265 l of water was required per kg of dry matter obtained. By comparison, for a corn crop obtained under the same conditions, 135 l of water was required per kg of dry matter. Although its photosynthesis was comparable to that of corn, a plant with C<sub>4</sub> metabolism, upland rice transpired twice as much and did not use water as effectively. Its water efficiency was not so high as that of the sunflower, a plant which, like rice, has C<sub>3</sub> metabolism.

During a nycthemeral cycle, as the light appeared at the end of the night, a stomatal opening movement was observed in rice, maximum opening being reached in 40 minutes. During the day, the stomata opened slightly to reach a plateau 3 to 5 hours after exposure to light. At night, residual transpiration was only 10 to 12% that of diurnal values. A particular characteristic was

observed in some cultivars (IRAT 13 and IRAM 499): these cultivars presented a partial reopening of the stomata, representing 1.8 to 2.5 times the minimum transpiration value (PICARD et al. 1981; BOIS et al., 1984 a). This nocturnal reopening was generally absent (cv. Morbérékan, Iguape cateto) or occurred only in the last two hours of the night (IR5, H105, Palawan). This property did not appear related to the type of crop. Photosynthesis developed in the same way as transpiration, though no peak values were observed. During the night, respiration was stable in all the cultivars (BOIS et al., 1984 b).

## II - Effects of Water stress in Rice

### a Impact of Water stress on Gaseous Exchange and the Role of Photorespiration

Water deficit leads to a change in the nycthemeral cycle of gaseous exchanges (BOIS et al., 1984 a). In the nocturnal phase, stomatal reopening, observed in some varieties, disappeared. In the daytime, the transpiratory cycle was completely altered: only the preliminary phase of stomatal opening was conserved. The maximum diurnal value was reached soon after exposure to light; transpiration dropped very rapidly at first, then more gradually. Photosynthesis decreased in the same way, though its decrease was proportionally less significant given the differences in resistance for CO<sub>2</sub> and water vapor transfer between the leaf and the atmosphere. Nocturnal respiration was less affected than photosynthesis; it remained between 50 and 70% of its initial value, whereas photosynthesis dropped to 10-20% of its initial value.

When the soil was dried quickly within a period of 2 to 3 days (e.g. sandy soil), the stomata began to close at the water potential thresholds of the soil, which differ from one variety to another. Lowland varieties reacted at approximately -450 mbar, while upland varieties reacted between -600 and -800 mbar.

Beyond these thresholds, the drop in transpiration was linked to the drop in soil potential. This result might appear paradoxical, for the sensitivity of stomata to water deficit is generally considered as an index of resistance to drought, N'DA KOUASSI and PUARD (1989), whereas the lowland varieties were those which presented the greatest sensitivity. This

capacity helps to preserve the plant water status during sudden changes in environment, but it handicaps photosynthesis and, therefore growth in the event of chronic water shortage. The upland varieties, which can withstand a lower potential threshold, are therefore better adapted, AHMADI and PUARD 1984). Stomatal closing affects photosynthesis less than transpiration because there is a high resistance to CO<sub>2</sub> transfer in the mesophyll which minimizes the stomatal effect. Consequently there is an increase in water efficiency (P/T ratio) at the start of the water deficit, provided that the photosynthetic mechanisms remain intact. During the rehydration phase following drying, gaseous exchanges gradually start up again. Kinetic curves showing how photosynthesis is affected by transpiration match the drying curves in IR5 and Iguape cateto, whereas they diverge in IRAT 13, H105 and Morobérékan. Photosynthesis was resumed more slowly than transpiration. This could have been due to the continuously high resistance encountered in the mesophyll or to a deterioration of the photosynthetic system (Figure 4). This hysteresis, particularly significant in IRAT 13, appeared to be a varietal characteristic (BOIS and COUCHAT, 1985 b).

By reducing CO<sub>2</sub> exchange between the leaf and the atmosphere, water stress causes a deterioration of the photosynthetic system, resulting from its inability to fix CO<sub>2</sub> in sufficient quantities. This effect has just been described for the interpretation of the drying-

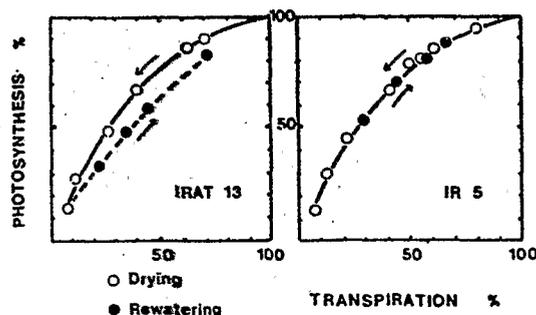


Fig. 4: Relationship between photosynthesis and transpiration during drying-rewatering sequence for two rice cultivars. (From BOIS et al. 1985).

related hysteresis curves. The fixation of oxygen via the photorespiratory system is one of several methods used by plants with C<sub>3</sub> metabolism. This method makes it possible to divert the chloroplastic reducing power generated by light, which cannot be used for photosynthetic fixation of CO<sub>2</sub>, when CO<sub>2</sub> no longer flows inside the leaf in sufficient quantities. This mechanism prevents the photosynthetic system from being destroyed. Photorespiration is highly significant in rice, which is a plant with C<sub>3</sub> metabolism. The first effect of this phenomenon is that photosynthesis is underestimated in CO<sub>2</sub> exchange measurements, since these measurements result from gross photosynthesis which consumes CO<sub>2</sub> and photorespiration which produces CO<sub>2</sub>. When placed under a 2% O<sub>2</sub> atmosphere, the rice seedlings revealed an apparent increase in photosynthesis of approximately 30%, which

was due to the inhibition of photorespiration by hypoxia.

Observations of an lowland variety and a upland variety showed that photorespiration did not differ considerably from one variety to another. Rapid water stress, followed by rehydration, tended to increase photorespiration by about 20%. This function could protect rice against water stress. Furthermore, this increase in photorespiration remained for about ten days, thus enhancing its positive effect.

#### b - Morphological Effects of Water stress

Neutron radiography was used to reveal roots in vivo through a sandy substrate (Figure 5). This method was used to study the reaction of the root system of rice to water stress (BOIS and COUCHAT, 1983 a and b). The lack of water

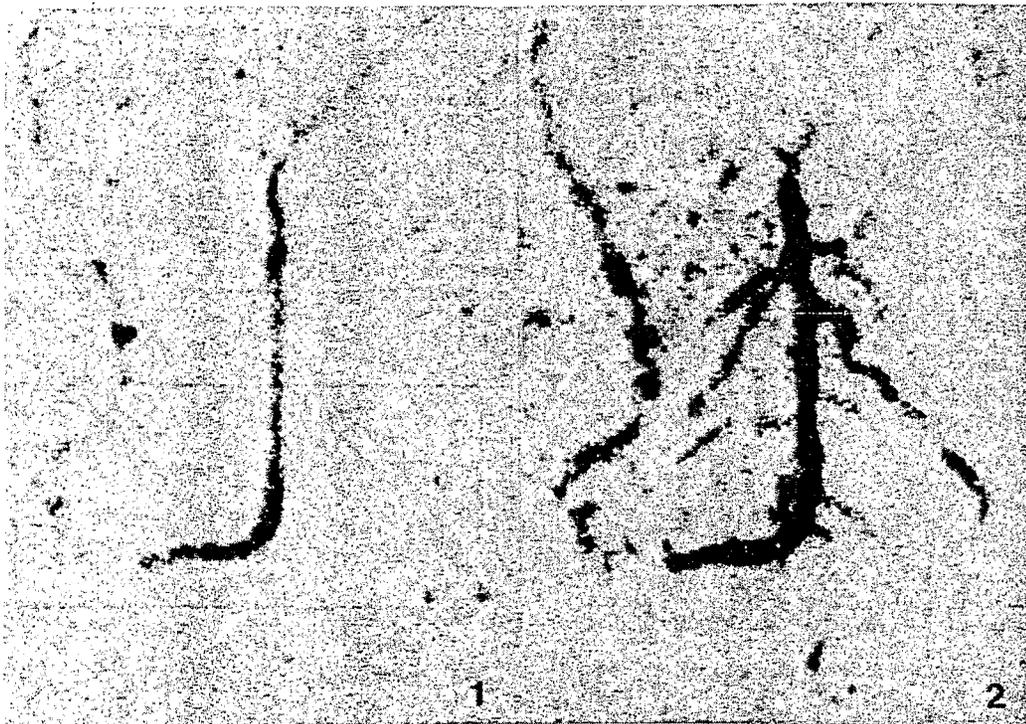


Fig. 5: Neutron radiographs of roots 1. rice roots after water stress. day 40  
2. new secondary roots after rewatering. day 44 (from BOIS and COUCHAT 1983).

inhibited growth, a phenomenon which was reflected in elongation rate and the number of growing roots. Primary root diameter was also seen to decrease by approximately 40%. After rehydration, growth resumed, characterized by an increased number of secondary roots and an increase in elongation rate.

Leaf rolling is a characteristic symptom of water deficiency in many Gramineae. It allows the plant to restrict both the quantity of luminous energy received and water loss. This mechanism is particularly well developed in rice. This is because the two sides of the leaf blade are structurally asymmetrical. Only the adaxial side possesses bulliform cells, which are large, hypertrophic, epidermal cells, located in longitudinal lines between the veins. Rolling occurs when these cells decrease in volume following a loss of turgescence. This would appear to be a hydropassive mechanism related to water potential gradients which is rapidly reversed on rehydration, though little is known of the process allowing bulliform cells to recover their turgescence. No varietal differences were reported during morphological studies. A functional difference was noted, however, between upland and lowland varieties. In the upland variety, stomatal closure and leaf rolling occurred almost simultaneously, whereas in the lowland variety, the two processes were separate, leaf movement beginning when the stomata were two-thirds closed (BOIS and COUCHAT, 1985 b). In the first case, leaf rolling, which helps to reduce water loss, was an adaptive mechanism. In the second case, it could be considered as a passive symptom of stress.

#### c -Role of Abscisic Acid and Proline in Plant Response to Water stress

Rice is known to build up large quantities of abscisic acid (ABA) in its leaves in response to water stress (KONDO et al., 1980; HENSON and QUARRIE, 1981). In corn, the drought-resistant varieties are those which build up the most ABA (PEKIC and QUARRIE, 1987). In rice, however, the reverse would seem to be true; lowland varieties build up more ABA than the upland varieties (HENSON, 1982; AUSTIN et al., 1982). This response is difficult to study owing to diurnal oscillations in ABA build-up (DINGKUNH et al., 1991). When exogenous ABA was applied to plants via the roots or excised plants, the qualitative effects on gaseous exchange were the same as those observed for water stress (NORDIN, 1976; BUNCE, 1987). QUARRIE, (1983) reported genotypic

differences in the sensitivity of rice to ABA. This point was confirmed: twenty minutes after adding  $10^{-4}$  M of ABA to the nutrient solute, rice reaction was reflected in a drop in transpiration. This reaction reflected stomatal closure. Unlike that of stoma guard cells, the turgescence of bulliform cells was not controlled by abscisic acid (BOIS et al., 1987); in this case, the drop in transpiration did not occur with a leaf rolling movement. The transpiration response time was longer (1 hour) with a less concentrated solution ( $10^{-7}$  M of ABA). Transpiration stabilized after approximately 3 hours of treatment and the level reached depended on the concentration of ABA. With a  $10^{-4}$  M solution, residual transpiration was only 40% of the initial value. It was demonstrated that residual relative transpiration was related to the ABA concentration logarithm. This relationship was different for the lowland variety and the upland variety (Figure 6). A minimum concentration was required to obtain a reaction; this threshold value was lower for the IR5 lowland variety ( $4 \times 10^{-8}$  M) than for the IRAT13 upland variety ( $3 \times 10^{-7}$  M). The rapid closure of stomata in response to the decrease in water potential of the soil of the lowland variety (result given in a previous chapter) can probably be explained by a greater sensitivity to ABA.

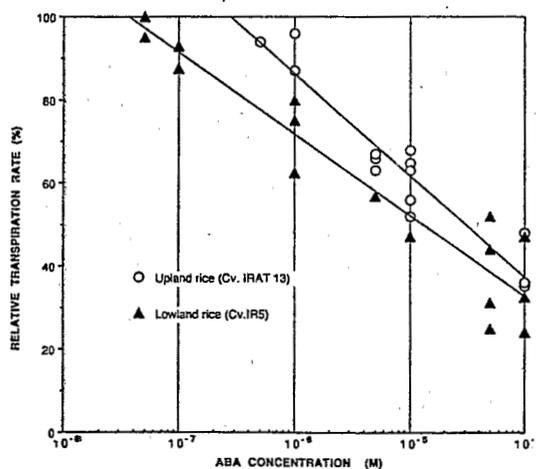


Fig. 6: Relationship between transpiration and ABA concentration in the nutrient solute for 2 rice cultivars.

The synthesis of proline under conditions of water stress is a well known phenomenon in most plants. Its physiological role, however, has not yet been fully clarified. It is known to be involved in cytoplasmic osmotic adjustment where it serves to protect enzymatic reactions and cell structures. The rate at which proline is synthesized in wheat leaves could be correlated to the degree of drought-resistance NEMMAR (1983). In rice, it was demonstrated that the proline content increased with the severity of Water deficit, DINGKUHN *et al* (1991), N'DA KOUASSI (1984) compared proline synthesis kinetics in two upland varieties of rice: Iguape cateto and LAC 29 and two lowland varieties of rice: Basmati 370 and NHTA 4, with two varieties of wheat: Bidi 17, known to be highly resistant to drought, and Mondur, which is not drought-resistant. Water stress was simulated between the 30th and 45th day by adding PEG 6000 to the nutritive medium. Under conditions of unrestricted water supply, the proline content was constant in all varieties. No difference was reported between the different types studied. However, when the "soil" water potential reached -4 bar, the proline content was seen to rise in all varieties. Differences were observed between varieties. The wheat varieties, Bidi 17 and Mondur built up considerably more proline than the rice varieties. This difference was accentuated when the "soil" water potential reached -7 bar.

The maximum proline synthesis by the plants at -9 bars (Figure 7) clearly reflected the degree of drought resistance. Two types of behavior were observed in rice: the upland varieties, Iguape cateto and Lac 29 synthesized more proline than their lowland counterparts. Wheat also showed a higher proline build-up than rice. The Bidi 17 variety from north Africa, known for its high resistance to drought, showed higher values than the Mondur variety grown in Europe which requires a lot of water. It is therefore certain that proline plays a physiological role in rice response to water stress.

### III - Effects of Hypoxia in Rice

The upland and lowland methods of rice growing each possess their own special characteristics. Of all the different methods of cultivation common in Africa, the rainfed lowland method is one of the most stressful for the plant. In addition to water stress - ranging from drought to immersion - shallow waters

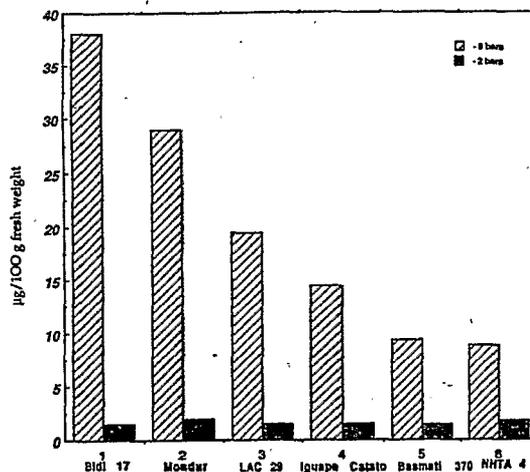


Fig. 7: Maximum proline synthesis by plants at -9 bars.

often constitute a reducing, acidic or even acidic sulfated environment, with a high risk of toxicity. Although some varieties, including lowland ones, have adapted to emerged cultivation, no variety is able to remain undamaged when exposed to alternating and repeated periods (of varying length) of drought and flooding, or even of short periods of immersion. This has serious effects on production. The development of a vegetal material adapted to this type of cultivation has been the subject of a number of research studies in the field of rice physiology.

#### a - Effects of Hypoxia on the Morphological Characteristics of Rice

A study was conducted on two varieties of rice, one upland variety called Morobérékan, the other an lowland variety known as Teksichut, grown under hydroponic conditions. In this study, root hypoxia was provoked between the 30th and 40th day. After ten days of hypoxia, root sections were observed under the electron microscope and compared with sections of a control sample grown in a well oxygenated environment. Results showed that hypoxia stimulated the development of aerenchymata in both varieties. This stimulation was more significant in the upland variety than in the lowland variety. SMIRNOFF and CRAWFORD (1983) also observed in a

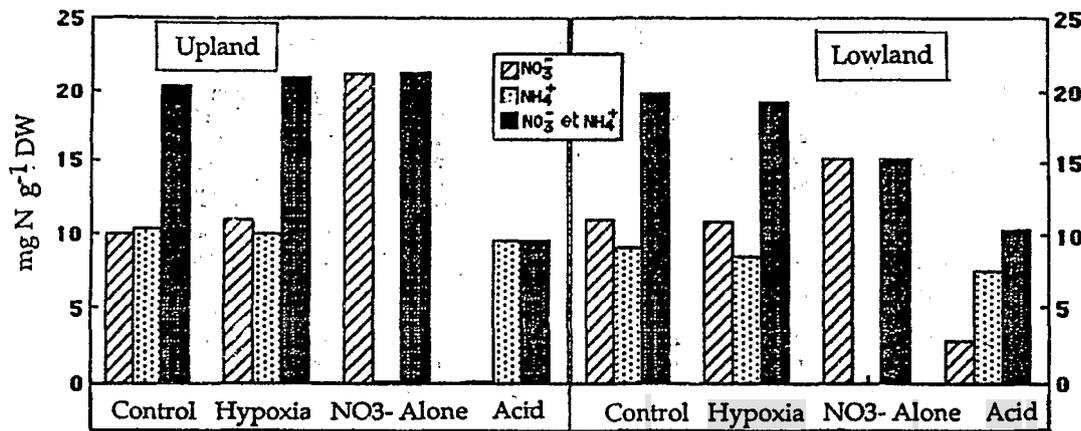


Fig. 8: Nitrogen uptake in two varieties of rice after 24-day treatment.

hydroponic environment that the aerenchymata of lowland plants were not always more developed than those of plants grown under dry conditions and that in a flooded environment, some plants, capable of withstanding flooding, could develop large lacunar spaces. The last point reflects a pathological rather than functional response and, moreover, presents the drawback of reducing the mechanical resistance of the roots, PUARD et al. (1989-a). From their analysis of root anatomy in different varieties of rice, TERASHIMA et al. (1987) observed that the relationship between the cross-section of the stele and the overall cross-section of the root varied from one ecotype to another.

Given that intercellular spaces are larger in the cortex than in other parts of the root (ARMSTRONG, 1971; ZHANG et al., 1990), significant development of the cortex increases intercellular spaces which in turn promotes the flow of oxygen in the root. Likewise, ELASHI (1979) demonstrated that the cortex-to-stele ratio increased under hypoxic conditions. The development of the cortex is therefore characteristic of the way in which rice responds to flooding. Furthermore, mesophytes such as corn and barley, have a lower cortex-to-stele ratio than rice. Root porosity, expressed by the ratio of aeriferous space volume to root volume, was higher in rice seedlings grown in flooded soil or under hypoxic conditions (KATAYAMA, 1961; DAS and JAT, 1977; PRIOUL and GUYOT, 1985). Theoretically, this would

appear to indicate that flooding stimulates the development of aerenchymata. In addition to this effect, root porosity can be affected by the relationship between the cortex and the stele, mentioned above, and by the arrangement of cortex cells. According to YOSHIDA and NISHIYAMA (1983), intercellular spaces are 2 to 3 times larger in a square arrangement than in an oblique arrangement, the square arrangement of cortical cells being a typical feature of hydrophytes.

Highly developed root porosity does not necessarily point to well developed aerenchymata. During his analysis of root anatomy, JOHN (1977), noticed that rice seedlings, grown in a nutrient solution with or without aeration, did not differ as regards development of root aerenchymata. SMIRNOFF and CRAWFORD (1983) defined aerenchymata as being "any tissue containing large, intercellular, aeriferous spaces or lacunae". According to LANN et al. (1989), aeriferous spaces are formed either by lysis of the cortical cells, or by separation (without lysis) of the cells. A distinction must be made between "normal" intercellular spaces (without formation of lacunae with or without cell lysis) and aerenchymata. As regards rice, cell lysis is the cause of the aeriferous spaces observed in the cortex (ZHANG, 1991).

In the lowland variety, the ratio of roots to aerial parts (R/PA) is reduced either by hypoxia alone or by the combined stress of hypoxia and acidity. In the upland variety, the

R/PA was only reduced by the combined stress and was not sensitive to a single separate stress. In the case of prolonged hypoxia, the primary roots died, their function being taken up by the adventive roots. These roots were often more porous than those which developed prior to the stress, the oxygen transfer capacity increased and the plant became more resistant to flooding.

#### b - Combined Effects of Hypoxia and Acidity on Gaseous Exchanges

Although rice is well-known as a plant capable of being cultivated in an acid medium, it has been shown that excessive acidity has a negative influence on development and production, DOLLING and PORTER, 1984; OSSENI, 1985; MAHLER, 1986. Similarly, many writers have stressed the inhibiting effect of anoxia on crop plants, e.g. PERIGAUD, (1967); JOHN *et al.*, (1974); PRIOUL and GUYOT, (1985). The interaction of acidity with oxygen transfer can be demonstrated: in a crop environment with a pH range of 7 to 4, it was shown that the oxygen taken by the roots diminished as acidity increased.

It was observed that the weight of dry matter (roots and aerial part) of plants grown at pH 4 was reduced compared with plants grown at pH 6. Similarly, under controlled conditions, it was shown that acidification of the farming environment caused significant slowing down of photosynthesis and transpiration; at pH 3 in 400  $\mu\text{mol. m}^{-2} \text{s}^{-1}$  light conditions, photosynthetic fixation of  $\text{CO}_2$  was only slightly greater than the compensation point. The notion of rice being acidophil should therefore be reconsidered. The deterioration phenomena reported following acidification were almost irreversible: if a rice seedlings was first left for 12 hours in a pH 3 solution in darkness, and then transferred to a pH 5.5 nutritive solution in light, it recovered its initial transpiration level more rapidly than its initial level of photosynthesis. The following day, despite a return to a more favorable pH, the plant nevertheless showed the effects of the previous high level of acidity. Equilibrium was not fully re-established until new leaves appeared.

Like acidity, hypoxia in rice causes modifications in root permeability. This has the indirect consequence, by modifying mineral and water absorption, of reducing transpiration. The resulting stomatal

regulation in turn causes a reduction in gaseous exchanges between the plant and the atmosphere and photosynthesis is affected proportionally higher than the transpiration mechanism. PRIOUL and GUYOT (1985) observed the same tendency in wheat. The results of our experiments on rice have shown that photosynthesis is not significantly different from one variety to another. Hypoxia reduces photosynthesis in both varieties and high acidity (pH4) aggravates this reduction in the lowland variety.

#### c - Impact of Nitrogen Nutrition on the Response of Rice Plants to the Combined Effects of Hypoxia and Acidity

Hypoxia, like the combined stresses of hypoxia and high acidity, has more effect on the roots than on the aerial parts. All the factors that encourage root growth increase plant tolerance to flooding. Conversely, any factors likely to reduce root growth make the consequences of flooding more severe. We particularly concentrated our studies on hypoxia and acidity PUARD *et al.* (1989-b). The results obtained demonstrate the effects of high acidity on the formation of aerenchymata and the destruction of the cortex. This modification of the anatomic structure considerably reduces the resistance of the plant to even short periods of drought, whether natural or induced. This situation is unfortunately common in lowland cultivation where the water supply is poorly controlled, such as in shallow water.

In a treatment under normal aeration and pH conditions, the total nitrogen uptake (nitrates and ammonia) was similar in the lowland and upland varieties (figure 8). This is due to the fact that the nutritive environment contained little nitrogen in ammoniacal nitrogen.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were found in a ratio of 7 : 1. Hypoxia did not affect nitrogen uptake in either rice variety. Since rice is capable of transferring aerial oxygen to its roots, this partially compensates for root hypoxia. When  $\text{NO}_3^-$  was the only source of nitrogen, uptake in the two varieties was significantly higher than in the control.

This result is probably due to the presence of  $\text{NH}_4^+$ . It has, in fact been established (SHEN, 1969; SASAKAWA and YAMAMOTO, 1977) that assimilation of nitrates is inhibited by  $\text{NH}_4^+$ . It has been observed that suppressing  $\text{NH}_4^+$  stimulates nitrate uptake more strongly in the

upland variety than the lowland variety. In flooded or very damp soils, the nitrogen source is essentially  $\text{NH}_4^+$  due to denitrification. In well-aerated soil, on the other hand,  $\text{NO}_3^-$  predominates. It can be supposed that, due to natural selection, lowland plants are generally better adapted to ammoniacal nutrition than upland plants; this is certainly the case of rice as compared with other cereals. More specifically, within the species *Oryza sativa*, a similar difference of adaptation has occurred between the lowland and upland varieties in relation to forms of nitrogen.

Nitrogen uptake in both varieties studied was lower in an acidic medium than in the control treatment. This reduction essentially concerns uptake of  $\text{NO}_3^-$ , which is completely suppressed in the upland variety. Acidity had a greater, if not specific, depressive effect on uptake of  $\text{NO}_3^-$ . Any uptake of  $\text{NH}_4^+$ , where it occurs at all, is only slight. The difference in lowland and upland varieties concerning the equilibrium between  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake can be explained in terms of the lowland variety having better adaptation to ammoniacal nutrition. Acidity reduces total nitrogen uptake. FRIED (1965) has shown that high acidity stimulates the absorption of nitrates. This does not contradict our results since calculations of nitrate uptake also include their assimilation.

Suppressing ammonia in rice powerfully stimulates uptake of mineral elements. This effect cannot be explained as above in terms of differences between existing roots and new roots grown during treatment. The most noteworthy aspect of the varietal effect (lowland or upland) on the response of rice to the three environmental stresses studied concerns the behavior of rice in relation to ammonia and hypoxia. The lowland variety is better adapted to ammonia, the predominant source in flooded soils, and has a weaker response than the upland variety to suppression of ammonia.

### Conclusion

Wild varieties of rice have developed mechanisms for adapting to abiotic stresses such as soil and climate as well as biotic stresses. However, of the twenty or so wild and two cultivated varieties that make up the genus *Oryza*, only *Oryza sativa*, an Asiatic rice now cultivated worldwide, is beginning to be well-known. Very little work has been undertaken

on the wild varieties despite the obvious benefits to be obtained from studying these species in their varied and often hostile habitats.

We have compared certain characteristics of upland and lowland rice, both as concerns their morphology and their physiology. The simple fact that half the 146 million hectares of rice are irrigated illustrates the advantages of learning how this plant responds to water stress. This information has a bearing on photosynthesis in which the part played by photorespiration should be better analyzed. From the biochemical point of view, efforts should be concentrated on understanding the mechanisms affecting the action of ABA. The lack of effect of ABA on bulliform cells suggests that the transfer process of water potential in leaves requires investigation. There is no leaf-rolling signal. The part played by proline (clearly built up in response to stress) in osmoprotection and post-stress nitrogen storage needs investigation. It was seen using neutronographic techniques that the putting out of new roots is a key factor in plant response to water stress. The mechanisms governing this phenomenon need to be clarified.

At the time of writing, no varieties suited to cultivation in shallow water exist. Efforts should also be made to understand the combined effects of hypoxia and acidity as well as the role played by nitrogen nutrition in the response of rice plants to this dual stress. Lowland varieties adapt to the combined stresses of hypoxia and acidity; this is probably due to the highly developed cortex. In upland varieties, acidity, hypoxia and lack of nitrogen cause excessive development of aerenchymata leading to root destructuring. Therefore nitrogen does not have a positive influence and the use of nitrates during flooding is not advisable.

It is certain that the future lies with transgenic modifications of rice. Protoplasts of the variety indica haploid can be used to regenerate rice seedlings. Biotechnology programs are being developed in several places and we can look forward to seeing properties of resistance both to water stress and flooding with hypoxia and acidity being transferred to cultivated rice. From this point of view, studies of wild rice varieties with a view to transferring genes which are beneficial to cultivated varieties seem promising. Physiological studies should nevertheless be carried out, if possible under strictly controlled

conditions, to test the capabilities of the transgenic varieties thus obtained. Programs should be launched to study the maximum yield capacities of existing and transgenic varieties under strictly controlled conditions. This type of program would provide the maximum yield information required by organizations concerned with planning future rice cultivation. In this way, studies would respond to demands reflected in FAO reports concerning production requirements related to the forecast development of world rice consumption.

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