

## GENETIC VARIATION AND SELECTION FOR NITROGEN USE EFFICIENCY IN MAIZE: A SYNTHESIS

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**ABSTRACT** - Nowadays development of varieties with a better nitrogen use efficiency (NUE, i.e. grain yield per unit of nitrogen from soil including fertilizer) is becoming a necessity both to allow the maintenance of a sufficient profit margin for the farmer and to preserve ground water from nitrate pollution. A new breeding aim could be to develop varieties adapted to low N-input. Many studies show a genetic variability for NUE at a given level of nitrogen fertilization, with a significant genotype x nitrogen fertilization (GxN) interaction. Such an interaction is relatively low, as illustrated by a highly significant phenotypic or genotypic correlation between yields at low and high N-input when the yield reduction at low N-input is not greater than about 35%. However, genetic variation is expressed differently at low and high N-input. Variation in NUE at high N-input is mainly related to variation in N-uptake, whereas at low N-input, both components of NUE could play a role, specifically nitrogen utilization efficiency, i.e. grain yield/N-uptake. GxN interaction for grain yield appears to be due to GxN interaction for kernel number. To have a good NUE at low N-input, it is necessary to reduce kernel abortion just after fertilization. Genotypes with a short anthesis-silking interval and prolific genotypes seem to have the ability to remobilize N from the stover to the grain efficiently, particularly in the early stage of embryo development, avoiding embryo or ear abortion. Leaf area duration appears to be an important factor for post-silking N-uptake and thus grain filling

and is favourable for NUE whatever the N input. Remobilization also plays an important role at low N-input. Results from QTL detection tend to confirm that genetic variation is expressed differently at low and high N-input. Selection experiments show that to have the maximum genetic advance at low N-input, it is better to select in this N condition. Finally it appears to be quite possible to develop new varieties better adapted to low N-input than present varieties.

**KEY WORDS:** Maize breeding; Nitrogen use efficiency; Nitrogen uptake; Nitrogen utilisation efficiency.

### INTRODUCTION

Combined with genetic improvement, nitrogen fertilization has been a powerful tool for increasing yield, especially for cereals. As a consequence of the law of less proportional yields, nitrogen use efficiency (NUE) nowadays is lower than in the past when a lower nitrogen fertilization was used. Another point to consider is that for maize, unlike for cereals, nitrogen fertilization is often given in only one manuring or early in the growth cycle. Consequently, under some conditions, this increases the risk of ground water pollution by nitrate leaching, which is even higher when slurry is used, as in some regions with silage maize production. Furthermore, because N fertilizers are relatively cheap in comparison to other inputs, a high level of nitrogen fertilization is like an insurance of a good harvest for the farmer. Consequently, until now, few intensive studies have been developed to reduce the use of nitrogen. However, nitrogen is costly from the point of view of energy and the risk of water pollution is too great in many countries with intensive agriculture. Nowadays the situation is changing in Europe. Because the European Community has developed a specific directive (91/676/EEC) to prevent

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**List of main abbreviations and symbols:** ASI: Anthesis-Silking Interval; df: degrees of freedom; GS: Glutamine Synthetase; G × N interaction: genotype x level of nitrogen fertilization interaction; IHP: Illinois High Protein; ILP: Illinois Low Protein; N: Nitrogen; NHI: Nitrogen Harvest Index; NNI: Nitrogen Nutrition Index; NR: Nitrate Reductase; NUE: Nitrogen Use Efficiency; QTL: Quantitative Trait Locus;  $r_p$  ( $r_G$ ) phenotypic (genotypic) coefficient of correlation; RIL: Recombinant Inbred Lines. The symbol \* or \*\* after a phenotypic coefficient of correlation means its significance at 0.05 or 0.01.

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nitrate pollution of ground water by restricting the use of nitrate fertilizers, farmers must contribute to the protection of the environment. Furthermore, due to the agricultural policy, the net income per unit of maize area is decreasing. Thus, to maintain a sufficient profit margin, while decreasing the risk of nitrate pollution and respecting the nitrate directive, farmers should try to optimize the use of nitrogen fertilizer. Both objectives for a sustainable agriculture can be met not only by using efficient farming techniques (e.g., decrease of N fertilizer supply, distribution in several applications, use of chemical forms of nitrogen) but also by using varieties which absorb and metabolize nitrogen from fertilizers well, i.e. by using varieties that have a better nitrogen use efficiency. Such objectives are valid for all countries, including those in the tropics, because in these countries most maize is grown under low N conditions, with poor soil types characterized by a low N mineralisation (BÄNZIGER *et al.*, 1997; KAMARA *et al.*, 2002). They are justified at the world level to try to satisfy the growing demand of the human population for food, while at the same time respecting the environment.

For grain maize, nitrogen use efficiency (NUE) has already been defined by MOLL *et al.* (1982), as the grain yield per unit of nitrogen from soil (including fertilizer). It is the product of nitrogen uptake efficiency, i.e. N-uptake/N from soil, and nitrogen utilisation efficiency, i.e. yield/N-uptake. For a given nitrogen fertilization, NUE is strictly related to grain yield, and nitrogen uptake efficiency is strictly related to total N-uptake. Nitrogen utilisation efficiency is an expression of the C/N ratio; for grain it can also be defined as the product of nitrogen utilisation efficiency at the whole plant level and the harvest index; it is then directly related to N partitioning, i.e. post-anthesis N-remobilization. Whole plant nitrogen utilisation efficiency is interesting to consider for silage maize production.

To develop varieties with improved NUE it is thus necessary to have genetic variability for nitrogen uptake efficiency and for nitrogen utilisation efficiency and to know the relationships of such traits to agronomic traits such as yield. Furthermore, the study of such relationships according to the nitrogen fertilization level, along with the effect of the nitrogen stress on trait means, may help us to understand how the plant functions. The present synthesis will therefore contain several parts: i) the dynamics of nitrogen within the maize plant, ii) the effect of N-stress on the agronomic trait means, iii)

genetic variability in the responsiveness to nitrogen and the genetic variability in NUE at low and high N-input, iv) the traits related to NUE and to N responsiveness, v) the detection of QTLs for NUE, vi) some results from selection experiments.

### THE DYNAMICS OF NITROGEN WITHIN THE PLANTS

When N is absorbed, after its reduction, it is allocated to the different plant parts (CLIQUET *et al.*, 1990). During vegetative growth, i.e. before anthesis, it is used for growth and invested in the leaves and the stem. Thus, early-absorbed nitrogen becomes more a part of structural walls than a part of the enzymatic pool, and the later N is absorbed during the vegetative growth, the more likely it will be invested in the enzymatic pool. A part of the absorbed nitrogen (20-30%) is also stored as NO<sub>3</sub> reserves in vacuoles of stalks and roots (CHEVALIER and SCHRADER, 1977; FRIEDRICH *et al.*, 1979). At silking, according to environmental conditions and genotypes, about 50 to 75% of the total plant nitrogen accumulated at maturity is already present.

After silking, nitrogen is accumulated within the grain in two ways: N-uptake and remobilization from the stover. Indeed, during grain filling, leaves and stalks act as source for kernels, while husks, shanks and cobs initially serve as sinks and then as sources (CLIQUET *et al.*, 1990). The use of labelled nitrogen fertilizer shows that the later N is absorbed, the greater the part allocated to the kernels (TA and WEILAND, 1992). Accumulation of nitrogen in the grain is related to the accumulation of carbon: N is required in the grain for carbohydrate accumulation through the enzymes involved in carbon metabolism (BELOW *et al.*, 1981), and according to TSAI *et al.* (1984) some grain proteins, like zein and globulin play the role of sink for nitrogen.

Remobilization of nitrogen from the stover is related to protein turnover. Indeed, a compartmental analysis of experiments with labelled <sup>15</sup>N absorbed before or after silking shows that an important part of newly synthesized amino acids from N absorbed after anthesis is always allocated to the stover before remobilization to the ear, most likely in replacement of old proteins which are remobilized (GALLAIS *et al.*, 2005). An illustration of this is given by the high percentage of <sup>15</sup>N (between 20-30%) which is allocated to the stover by a post-silking <sup>15</sup>N labelling. During grain filling, when N-uptake

is limited by low C-assimilate supply to the roots or soil N depletion, it could be assumed that, due to the ear sink strength, an increasing part of newly synthesized amino acids is allocated to the kernels. Consequently, the old proteins are not completely replaced, which decreases the photosynthetic source and induces senescence. Indeed, remobilized N comes from the proteolytic degradation of leaf and stalk proteins and mainly from the photosynthetic enzyme, Rubisco. Therefore, when remobilization is active, during the ageing process, photosynthesis decreases, and thus also N-uptake (TRIBOI and TRIBOI-BLONDEL, 2002). This is another aspect pointing out the close interdependence between C and N metabolism during grain filling. We will see later that one aim of breeding could be to maintain as long as possible an efficient photosynthesis during grain filling.

The percentage of nitrogen which can be exported from the stover to the grain can vary between about 30% to more than 75% (60 to 85% in TA and WEILAND, 1992). Similarly the proportion of grain nitrogen from the stover can vary from 30 to 70%. The stover therefore plays an important role in providing N for kernel development, with an approximately equal contribution from leaves and from stalk. Roots would contribute for only 10% to the remobilized N (TA and WEILAND, 1992). What is remarkable is that such nitrogen transfer from stover to grain is nearly without dry-matter transfer. However, not all N stover is remobilized: at maturity there is residual stover N. Conceptually, two parts can be distinguished: one corresponding to nitrogen analogous to remobilized nitrogen (metabolic nitrogen) but perhaps also a part of the structural nitrogen, and the other corresponding to nitrogen which cannot be remobilized. The aim of breeding would be to have only this last part, and as reduced as possible. However, the frontier between remobilizable and non-remobilizable N is not clear.

#### **EFFECT OF NITROGEN STRESS ON TRAIT MEANS**

Different experiments have shown that grain yield is strongly affected by N stress. In the experiment of BERTIN and GALLAIS (2000), with a set of 99 recombinant inbred lines crossed to a tester, kernel number was the yield component the most affected by N-stress (-32%) whereas kernel weight was reduced by only 9% for a grain yield reduction of

38%. REED *et al.* (1988), UHART and ANDRADE (1995) and BELOW *et al.* (1981, 2000) have also shown the great sensitivity of kernel number to N-stress. This is expressed by abortion of embryos at the ear end in the lag period 10-15 days after fertilization. Indeed, the number of ovules is only slightly affected by nitrogen stress and more generally by environment (LEMCOFF and LOOMIS, 1986; UHART and ANDRADE, 1995). Such abortion could be due to a limitation in the source of photosynthetic products which, in our experiment (BERTIN and GALLAIS, 2000), also affected post-anthesis growth (-29%) much more than plant development at silking (-14%). A possible explanation is that just after fertilization the sink demand is too high in comparison with available resources, and this then leads to ovule abortion, more or less according to genotype. Plant development tends to be slower with a lower rate of leaf appearance and a delay in silking.

It is still unclear which processes determine kernel abortion under N stress. Apparently, the situation is quite different from the drought stress effect. With drought stress, abortion would be due to reduction in the flux of carbohydrates to the ear and young embryos (BÄNZIGER *et al.*, 2002). With N stress, the flux of carbohydrates also has an effect, and ANDRADE *et al.* (2002) have shown that kernel number is largely determined by the growth rate at the period bracketing fertilization. However, lack of carbohydrates is not sufficient to explain abortion. CZYZWICZ and BELOW (1994) and BELOW *et al.* (2000) using in-vitro cultured embryos showed that sucrose supply alone cannot overcome the effect of N deficiency. Nitrogen seems to play a direct role in kernel development perhaps through enzyme quantity or activity.

N-uptake, in our experiment, was reduced by 50% at silking and by 42% at maturity (for a 38% grain yield reduction). Remobilization generally starts earlier at low N-input than at high N-input as a consequence of unavailable soil N-nitrogen, and then leaf senescence, which is associated with active remobilization, is also accelerated (WEILAND and TA, 1992). The rate of translocation, i.e. % of N transferred from vegetative organs to grain, was about the same at low N-input as at high N-input. Despite approximately the same rate of remobilization from the stover, TA and WEILAND (1992) observed a higher part of N grain from remobilization at low N-input than at high N-input (62.6% vs 53%). However, the amount of remobilized N is generally

lower at low N-input. As expected, nitrogen utilization efficiency, i.e. grain yield/N-uptake, is higher at low N-input than at high N-input (+72% at silking but only +27% at maturity, in our experiment). This is due to the well-known fact that the efficiency of nitrogen decreases when the level of fertilization increases.

### GENETIC VARIATION IN NUE AND RELATED TRAITS

#### *Variation in NUE and G x N interaction*

Genetic variation can be studied from two points of view which are complementary. The first question is to know whether there is genetic variation for NUE for a given nitrogen fertilization and the second question is to know whether it is the same genotypes which are adapted to low and high N-input. Differences in NUE which directly express differences in grain yield are well-known with a normal fertilization level. It is thus more interesting to consider variability in NUE at low N-input, and variability in N-uptake and N-utilisation efficiency at different N levels.

Many studies have shown a genetic variability for NUE for a given level of N at the level of hybrids (POLLMER *et al.*, 1979; BALKO and RUSSELL 1980a; REED *et al.*, 1980; MURULI and PAULSEN, 1981; Di FONZO *et al.*, 1982; MOLL *et al.*, 1982, 1987; BRUN and DUDLEY, 1989; SMICIKLAS and BELOW, 1990; RIZZI *et al.*, 1993; LANDBECK, 1995; BÄNZIGER *et al.*, 1997; BERTIN and GALLAIS, 2000; PRESTERL *et al.*, 1996, 2002a, 2003). However, few studies have considered genetic variance according to N level. A reduction in genetic variance is often observed under stressed conditions. At low N-input, BERTIN and GALLAIS (2000) have shown that genetic variance in NUE was reduced one year and increased in another year, whereas PRESTERL *et al.* (2003) showed an increase in genetic variance in most of the conditions studied. However, if the genotypic coefficient of variation is considered, both of these studies show an increase at low N-input.

In most cases, when experiments have considered two or more sufficiently contrasted N conditions, a genotype x nitrogen level (G x N) interaction reflecting genotypic differences in responsiveness to nitrogen was also found. In the BERTIN and GALLAIS (2000) study, G x N interaction was also significant for kernel number but not for kernel weight, and the interaction observed for kernel

number per ear explained interaction observed for grain yield. This was illustrated by a high correlation between responsiveness for yield and responsiveness for kernel number ( $r_p = 0.85^{**}$ ). This underlines the role of nitrogen use in the determination of kernel number. It was interesting to note the absence of G x N interaction for plant traits at silking, which means that observed interaction for grain yield was mainly due to grain development and hence to factors determining kernel number.

Another way to show G x N interaction is to consider the correlation between high and low N levels. Genetic correlation estimated by BERTIN and GALLAIS (2000) was 0.75. An average coefficient of 0.75 was also observed by PRESTERL *et al.* (2003), with only three experiments out of 18 showing a correlation coefficient lower than 0.60. The average phenotypic correlation was 0.88. In their experiments it appears clearly that the correlation tends to decrease with the increase in the N-stress measured by the reduction in yield at low N-input. Indeed, it is expected that the G x N interaction increases when the effect of N-stress increases. BÄNZIGER *et al.* (1997) on 14 experiments with tropical maize in Mexico reported a lower genetic correlation (0.38) but for a greater N-stress (54% yield reduction at low N-input). As in the PRESTERL *et al.* (2003) experiments, variation in the genetic correlation was partly explained by the relative yield reduction at low N-input, with a lower genetic correlation at higher N-stress. However an optimum of yield reduction may exist to study genetic variability at low N-input because with a too strong N-stress, genetic variability could be suppressed and environmental variance could increase.

Some rare studies (GARDNER *et al.*, 1990) concluded that there was no G x N interaction. Clearly, results depend on the set of genotypes studied. If only hybrids from elite material are considered, specific genes for adaptation to low N-condition could have been lost by genetic drift due to selection in only favourable conditions over more than 50 years of selection, and even from domestication. Furthermore, many traits have been modified by selection for adaptation to various environments (e. g. cold and drought tolerances, disease and insect resistances, lodging resistance, stay-green, duration of the grain filling) which is expected to confer an advantage across fertility levels. As expected, modern cultivars generally outyield older ones across a range of fertility and N levels (DUVICK, 1984; DERIEUX *et al.*, 1987; COQUE and GALLAIS, 2005b) and yield

gain is associated with an increase in the C/N ratio with a decline in grain protein content, as a result of an improvement of N-uptake during the grain filling period (CASTLEBERRY *et al.*, 1984; RUSSELL, 1985; CACCO *et al.*, 1993; RAJCAN and TOLLENAAR, 1999b). Indeed, as improvement in post-anthesis N-uptake is due to improvement in photosynthesis through a greater leaf area duration, increasing carbohydrate transfer into the grain contributes to the dilution of nitrogen (TRIBOI and TRIBOI-BLONDEL, 2002).

A few studies provide an answer to the question of loss of variability for adaptation to low N-environment by modern selection. In the PRESTERL *et al.* (2003) studies, it appears that when lines are derived from landraces more genetic variability is observed. In the LAFITTE *et al.* (1997) study, with 171 landraces, different types of clusters were identified: some clusters performed well under adequate N but not under limited N, while others showed the opposite response, which indicates specific adaptation to N environments. Landraces had a large protein content in both stover and grain (probably due to their lower performances: the improved cultivars out-yielded the landraces by an average of 56%). Patterns of adaptation to adequate or limited N were not associated with any particular maize race, geographic origin, or precipitation regime at the site of collection. Clearly, landraces can hardly be used directly by the maize breeder because of their poor agronomic characteristics, but they can be used in recurrent selection to develop synthetics from which new lines can be derived in order to introduce new traits into elite material.

It must be noted, that when comparing modern varieties to unselected material or old varieties, their apparently better adaptation to high N-input than to low N-input is due to the increase in their potential. Indeed, due to the greater effect of limitation in N for high potential than for low potential genotypes, a curvilinear relationship is expected between yield at low N-input and yield potential at high N-input, and was indeed observed by BERTIN and GALLAIS (2000). With low N-input, the variation in growth and yield of high potential varieties will be restricted, and this is sufficient to generate G x N interaction.

#### **Variation in components of NUE**

Genetic variability has been observed for both NUE components (POLLMER *et al.*, 1979; DI FONZO *et al.*, 1982; MOLL *et al.*, 1982; JACKSON *et al.*, 1986; BERTIN and GALLAIS, 2000). Genetic variation in N-

uptake was also noted at silking by MOLLARETTI *et al.* (1987) and at maturity by CHEVALIER and SCHRADER (1977) and WIESLER and HORST (1993). A response to selection for N-uptake was observed in juvenile stages by TEYKER *et al.* (1989), however response to N as measured by N-use parameters was not associated with selection on seedling N-uptake. The relative importance of the variation in the two NUE components depends on the N level. At high N input, there generally appears to be greater variance for N-uptake than for nitrogen utilisation efficiency, the reverse being observed at low N-input (MURULI and PAULSEN, 1981; MOLL *et al.*, 1982, 1987; DI FONZO *et al.*, 1982; JACKSON *et al.*, 1986; LAFITTE and EDMEADES, 1994; BERTIN and GALLAIS, 2000). At high N-input, the variation is mainly related to ability to grow whereas at low N-input, due to limitation in absorbed N, variation is more related to N-partitioning (BERTIN and GALLAIS, 2000).

Variation in N-uptake depends on the correlation between dry-matter yield and N-content. At silking, at low N-input, in the BERTIN and GALLAIS (2000) study, such correlation was strongly negative ( $r_G = -0.92$ ) whereas, at high N-input, no such strong negative correlation was observed. The result was a strong reduction in genetic variance in N-uptake at silking from high N-input to low N-input. Until silking, maize functions like forage grasses for which negative correlation between dry-matter yield and protein content is well-known (LEMAIRE and GASTAL, 1997). At maturity, the reduction in the variance of N-uptake from high N-input to low N-input is not so clear and this can be explained by the lower negative correlation between grain yield and nitrogen content. The observed lower variation in N-uptake at low N-input means that N source was a limiting factor. With a high level of nitrogen, assumed to correspond to a situation with a high possible N flux from the soil to the plant, genetic differences in N-uptake can be expressed, mainly at silking. As a consequence, N-uptake was more related to plant development at silking at high N-input than at low N-input.

#### *Relationship between N-Uptake, N-Utilisation efficiency and NUE*

N-uptake and N-utilization efficiency tend to be negatively correlated. This could partly be due to the fact that they are not independent from a statistical point of view as N-utilization efficiency involves the reciprocal of N-uptake. Such a negative relationship was observed by SMICKLAS and BELOW (1990)

and also by CHEVALIER and SCHRADER (1977). In MOLL *et al.* (1987) experiments, on the average of both nitrogen levels (which renders the means more independent), the two components tend to be negatively related ( $r_p = -0.20$  between N-uptake and N-utilization for grain and  $r_p = -0.30^*$  between N-uptake and N-utilization for whole plant). NUE was related to both N-uptake ( $r_p = 0.64^{**}$ , 61 df) and to nitrogen utilisation ( $r_p = 0.52^{**}$ ). In the BERTIN and GALLAIS (2000) study, whatever the stage and N fertilization, the genetic correlation between both components was never significant and each was about equally related to NUE whatever the N fertilization. At silking, N-uptake has also been related to NUE (MURULI and PAULSEN, 1981; DI FONZO, 1982; SHERRARD *et al.*, 1986). Furthermore, it has been the main factor of genetic advance in yield (40% in old hybrid and 60% in a modern hybrid in the study of RAJCAN and TOLLENAAR, 1999b). In the PRESTERL *et al.* experiments (2002a), at high N-input, for material selected at high N-input, NUE was related only to N-uptake, whereas at low N-input, it was highly related to both components. However, for material selected at low N-input, whatever N-input, both components of NUE contribute to the explanation of its genetic variation in NUE and both appear to be relatively independent. This tends to show that for material selected at high N-input, N-uptake is the main factor of NUE, as in our study (BERTIN and GALLAIS, 2000).

#### *Traits related to responsiveness to N-input*

Responsiveness to N-input of grain yield or kernel number was only related (negatively) to traits at low N-input: grain yield or kernel number ( $r_p = -0.70^{**}$ ), N content at silking ( $r_G = -0.74$ ), nitrogen nutrition index (NNI), N-uptake at maturity, post-anthesis N-uptake (BERTIN and GALLAIS, 2000). This means that genotypes having a poor NUE at low N-input, were those reacting more to nitrogen, but they were not the best at high N-input. Thus, G x N interaction appears to be essentially due to variation in adaptation at low N-input rather than to variation in adaptation at high N-input. It can be noted that among observed traits at silking, only NNI showed G x N interaction. However, responsiveness for yield was not related to responsiveness for NNI.

#### *Relationship between nitrogen utilization efficiency, %N and harvest index*

Nitrogen utilization efficiency which is the product of biomass yield/N-uptake and of the harvest index, can also be expressed as the ratio harvest in-

dex/N content. Our results (BERTIN and GALLAIS, 2000) show that nitrogen utilization efficiency was highly negatively related to N content ( $r_p = -0.88^{**}$  at high N-input and  $r_p = -0.90^{**}$  at low N-input) and positively related to harvest index ( $r_p = 0.75^{**}$  at high N-input and  $0.60^{**}$  at low N-input). It was also negatively related to low N-quantity in stems. To increase nitrogen utilization efficiency, it is necessary to decrease N content and to increase harvest index. These results are close to those of DI FONZO *et al.* (1982) showing that at low N-input, grain yield was related to nitrogen harvest index, i.e. N-partitioning.

#### ***Specific traits related to NUE and GxN interaction***

In general, stronger relationships between traits are observed at low N-fertility (LAFITTE and EDMEADES, 1994; BERTIN and GALLAIS, 2000; PRESTERL *et al.*, 2002a; ZAIDI *et al.*, 2003). Under favourable conditions correlations are lower because there is no competition among organs for nutrients.

#### *Anthesis-Silking Interval (ASI), prolificacy and barrenness*

When maize plants are grown in presence of some stresses such as drought or nitrogen deficiency, there results an increase in ASI (ANDERSON *et al.*, 1984; EDMEADES *et al.*, 1993; LAFITTE and EDMEADES, 1995; CAMPOS *et al.*, 2004). The consequence is that, in monogenotypic stands, there could be a deficit in ovule fertilization and also barrenness. However, in spite of the presence of pollen at silking, a negative correlation is observed between ASI and grain yield only at low N-input ( $r_G = -0.83$  in BERTIN and GALLAIS, 2000; ZAIDI *et al.*, 2003;  $r_p = -0.58^*$ ) and even in elite material (COQUE and GALLAIS, 2005b). Consistent with these correlations, TOLLENAAR (1991) observed that the interval from tassel to silk emergence tends to be shorter for the new than for old hybrids. In the experiments of BÄNZIGER and LAFITTE (1997) ASI was also negatively related to grain yield ( $r_G = -0.42$ ). Furthermore, selection for drought tolerance led to a consistently increased grain yield across N levels associated with a shorter ASI (BÄNZIGER *et al.*, 2002).

Number of ears per plant, a trait associated with ASI, was also related to NUE and its components, N-uptake and N utilisation efficiency (REED *et al.*, 1980; KAMPRATH *et al.*, 1982; ANDERSON *et al.*, 1984; MOLL *et al.*, 1987; BÄNZIGER and LAFITTE, 1997, with a genotypic correlation 0.75; ZAIDI *et al.*, 2003, with phenotypic correlations  $0.58^*$  to  $0.72^{**}$ ) mainly at

low N-input, which is consistent with the role of nitrogen metabolism in ear and kernel abortion. In MOLL *et al.* experiments, at low N-input, number of ears was correlated with NUE ( $r_p = 0.71^{**}$  with 61 df), with N-uptake ( $r_p = 0.47^{**}$ ) and with nitrogen utilisation efficiency ( $r_p = 0.30^*$ ), whereas there were no significant correlations at high N-input. Furthermore, in the selection for drought tolerance developed by BÄNZIGER *et al.* (2002) gain in yield was also associated with an increase in the number of ears per plant and kernel weight.

Genotypes for which ASI does not increase under N or drought stress could have a better nitrogen metabolism, or a physiology leading to greater yield at low N-input, as a result of a better N-partitioning avoiding ear abortion. It is well-known that a short ASI is related to prolificacy. At the extreme, true prolificacy leads to protogyny whereas normal maize shows protandry. Prolific genotypes have two favourable traits: i) first they have a high degree of translocation from the stover to the grain, a characteristic which favours yield in stress conditions; this explains why, with such material, N-utilization efficiency is more important than N-uptake, the reverse of normal maize (MOLL *et al.*, 1982, 1987; JACKSON *et al.*, 1986) ii) second, BERTIN *et al.* (1976) and BOYAT and ROBIN (1977) have shown that they have a greater nitrate reductase activity, or a nitrate reductase which will be induced in the leaf at lower light intensity.

#### *Nitrogen Nutrition Index (NNI)*

NNI can be defined according to LEMAIRE and GASTAL (1997) as the ratio of the observed N-content to a critical N content corresponding to the minimum N-content allowing the maximum dry-matter yield. In the BERTIN and GALLAIS (2000) study, NNI measured at anthesis was related to NUE only at low N-input ( $r_G = 0.84$ ). It appears that NNI has a physiological meaning, expressed early at silking and having an effect at maturity. The role of NNI appears still greater at the level of the kernel number per plant. It is related to nitrogen content and thus to the potential activity of the leaf from the point of view of photosynthesis. With active chloroplasts, N content is higher, and leaf senescence will be delayed. Indeed, in our experiment, leaf senescence three weeks after silking and NNI at silking were highly correlated ( $r_G = 0.80$ ). As a consequence, assuming that NNI is also an expression of photosynthetic activity, if photosynthetic flux is too restricted at low N-input at silking, abortion of kernels just af-

ter fertilization will occur. However the effect of senescence depends on climatic conditions which affect source capacity. Its effect can be weak or nil in favourable environments. This could explain some inconsistent results (PRESTERL *et al.*, 1996).

#### *Leaf area duration*

NUE is negatively related to leaf senescence at low N-input, with a genetic correlation  $r_G = -0.86$ , in BERTIN and GALLAIS (2000), and  $r_G = -0.48$  in BÄNZIGER and LAFITTE (1997). Furthermore, TOLLENAAR (1991) and MA and DWYER (1998) have shown that leaf area duration was the main physiological factor explaining genetic gain in yield, associated with a better NUE and more precisely with improvement in post-anthesis N-uptake (RACJAN and TOLLENAAR, 1999a,b). The greatest difference in growth and yield between old and new hybrids occurs under stress conditions (McCULLOUGH *et al.*, 1994; DWYER *et al.*, 1995) and is associated to leaf senescence. However, it must be noted that supplementary N-uptake resulting from improvement of leaf area duration was not preferentially allocated to the grain. Such improvement in maize is also at the origin of the quasi-suppression of the negative genetic correlation between protein content and grain yield. Indeed this negative correlation means that there is a deficiency in N assimilates in comparison to C assimilates. The role of leaf senescence at low N input was also evidenced by CIMMYT selection experiments (BÄNZIGER and LAFITTE, 1997; BÄNZIGER *et al.*, 1997) and the studies of PRESTERL *et al.* (1996, 2002a).

An important parameter controlling NUE is nitrogen remobilization from the stover to the grain. Due to the strength of sink ear, if N flow to the seeds is greater than N-uptake, then there is remobilization, translocation of leaf nitrogen to the grain associated with the lysis of photosynthetic proteins like Rubisco, leading to earlier senescence. There results a decrease in the photosynthetic potential and in N-uptake (TRIBOI and TRIBOI-BLONDEL, 2002; PAPONOV and ENGELS, 2003). A way to protect the photosynthetic activity is to increase the quantity of leaf proteins such that a part of them can be remobilized without negative consequences on leaf photosynthesis (TA and WEILAND, 1992). This is illustrated by a positive relationship between N-quantity at silking and post-silking dry-matter production, mainly at low N-input (DI FONZO *et al.*, 1982; BERTIN and GALLAIS, 2000). If nitrogen remobilization is not sufficient (because of insufficient N-storage), a decrease in grain protein concentration will result.

In fact it appears that the increase in leaf duration is associated with an improvement in tolerance to different types of stress: nitrogen stress, water stress and even cold stress (in autumn) (TOLLENAAR and WU, 1999). Stay-green also reduces lodging through an association with stem roots, which is an indirect proof that stay-green leaves remain photosynthetically active (TOLLENAAR *et al.*, 2000). The consequence of senescence is a decrease in photosynthesis, leading to low assimilates available for all plant functions. The ear strength then reduces translocation of carbohydrates towards roots which is essential to maintain their activity as shown by TOLLEY *et al.* (1988) and TOLLEY-HENRY and RAPER (1991) on soybean and tobacco. This also contributes to a decrease in N-uptake. Note however that leaf senescence is not always unfavourable to final grain yield, even at low N-input (TA and WEILAND, 1992). This may be due to compensation between remobilization and uptake. In the selection experiment for drought tolerance of BÄNZIGER *et al.* (2002), increase in leaf duration led to higher assimilate supply during grain filling and then to an increase in kernel weight, which was observed across all N levels. Remobilization favours protein content unlike high post-anthesis N-uptake which dilutes grain protein. This is illustrated by the comparison between Illinois High Protein (IHP) and Illinois Low Protein (ILP) material. IHP plants absorb more N during vegetative growth, synthesize protein more efficiently and have a more intense remobilization from stover to the grain (WYSS *et al.*, 1991; RIZZI *et al.*, 1996).

Breeding for chlorophyll content could delay senescence and indirectly contribute to the increase in post-anthesis N-uptake. This tends to be demonstrated by different studies. CRATS-BRANDMER and PONELEIT (1992) showed a correlation between Rubisco expression, chlorophyll content and photosynthetic activity. HAGEMAN and LAMBERT (1988) observed a correlation between photosynthetic activity and leaf N concentration. ZAIDI *et al.* (2003) showed a correlation ( $r_p = 0.54^*$  to  $0.67^{**}$ , 49 hybrids) between grain yield and chlorophyll content (SPAD) significant only at low N-input. In the PRESTERL *et al.* (2003) study, hybrids from selection at low N-input showed a higher ear leaf chlorophyll content than hybrids from selection at high N-input. Furthermore, new hybrids have a higher chlorophyll content than old (RAJCAN and TOLLENAAR, 1999b). However the correlation between chlorophyll content and stay-green state is not always significant. BÄNZIGER and

LAFITTE (1997) observed a low correlation coefficient between chlorophyll content and grain yield. Nevertheless, the value of leaf senescence as a selection criterion for adaptation to low N-input is shown by the efficiency of selection at low N-input for drought tolerance leading to a decrease in leaf senescence (LAFITTE and EDMEADES, 1994).

#### *Nitrogen Harvest Index (NHI)*

NHI is related to remobilization rate (% N at silking translocated to grain). It is the main factor determining the remobilization rate as shown by  $^{15}\text{N}$  labelling experiments (GALLAIS *et al.*, 2005). In several experiments, the remobilization rate was strongly related to NHI. As NHI is less variable than N-quantity at anthesis, it results that the quantity of remobilized N must be related to N present at anthesis. Then, at low N-input, where N-remobilization plays a greater role, the N-quantity accumulated at silking will be greater. N-quantity at anthesis is always favourable, but at high N-input, post-anthesis N-uptake can still be rather high, reducing the contribution of N anthesis to the variation in NUE. Consistent with this prediction DI FONZO *et al.* (1982) observed that grain yield was related to N leaf quantity at silking ( $r_p = 0.68^{**}$ ) and to NHI ( $r_p = 0.69^{**}$ , 15 hybrids) at low N-input. It was however not so related at high N-input, being only related to N leaf quantity at silking ( $r_p = 0.54^*$ ) and not to NHI.

#### *Root system and efficiency*

Roots may play an important role in nitrogen uptake, mainly when N is limited, that is at low N-input. It is necessary to consider the size and activity of the roots. However as they are difficult to study, few results are known about the consequences of their variation. CHEVALIER and SCHRADER (1977) observed nitrate absorption of inbred lines to be related to root dry-weight. WIESLER and HORST (1993) also found a significant correlation between N-uptake and root density; the rate of nitrate uptake, although related to the root dry-weight, was not constant, suggesting that physiological activity and root affinity for nitrate control N-uptake. KAMARA *et al.* (2002) with a set of 18 S1 lines evaluated at low N-input found that root-pulling was positively correlated to N-uptake ( $r_p = 0.68^{**}$ ) but not to N-utilisation efficiency. These results tend to confirm the expected relationship between root development and N-uptake. However, OIKEH *et al.* (1999) did not find such a correlation. Furthermore, using the data of GUINGO *et al.* (1998) showing a great

variability in the number and diameter of roots in our studied population, we have shown that there was a negative correlation between root development (root number) and grain yield, which was greater at low N-input than at high N-input. This could be interpreted as competition between two sinks (roots and ear) when N resources are limited (GALLAIS, unpublished data). It thus appears difficult to draw any conclusions about the role of roots in NUE and about what root trait to study.

#### *N-metabolism enzymatic traits*

**Nitrate reductase activity.** Nitrate reductase (NR) is the first enzyme of N metabolism. As it was thought that reduced N could be favourable, many studies have been developed on NR activity. However, NR activity was not associated with the accumulation of reduced N at maturity (REED *et al.*, 1980; SHERRARD *et al.*, 1984; MESSMER *et al.*, 1984; HIREL *et al.*, 2001), and contradictory results have been observed with positive correlation (DECKARD *et al.*, 1973), no correlation or even negative correlation (REED *et al.*, 1980; MESSMER *et al.*, 1984; HIREL *et al.*, 2001) between NR activity and grain yield (see other references in HAGEMAN *et al.*, 1976; SHERRARD *et al.*, 1986). SHERRARD *et al.* (1984, 1986) showed that selection for low NR activity led to a strain with higher leaf chlorophyll and nitrate content and a decrease in chlorophyll concentration during grain filling. Results from FEIL *et al.* (1993) displayed a positive correlation between NR activity and silage yield  $r_p = 0.73^{**}$  (12 df) or with N-uptake ( $r_p = 0.56$  to  $0.83^{**}$ ). Unlike FEIL *et al.* (1993), FAKOREDE and MOCK (1978) with selection for increased grain yield observed no change in NR activity in one population and a decrease in another. Similarly, results from EICHELBERGER *et al.* (1989) do not support the hypothesis that NR activity can be used as a selection criterion for yield, although a minimum threshold level of NR activity is required for normal productivity. Indeed, selection for high NR activity had little effect on N-traits evaluated, whereas in contrast, selection for low NR activity led to reduction in grain N-content and NUE.

In a selection experiment conducted by MESSMER *et al.* (1984) it appears that genotypes with high reduced N had greater grain yields (+10.5%) and kernel number (+6%) than the low reduced N genotypes. They also deposited dry matter in the grain at a faster rate (20%) from 35 days after anthesis to maturity. Hybrids with low NR activity and high reduced N had significantly greater yields than other

hybrids. Reduced N appears to be favourable but it is not related to NR activity. In the HIREL *et al.* (2001) experiment, leaf nitrate content at young stage (6-7 leaves) was positively related to grain yield. Their results suggest that the pool of nitrates in the vacuole can be further metabolized, leading to amino acids useful for embryo development just after fertilization (BELOW *et al.*, 2000). This is also consistent with conclusions of DI FONZO *et al.* (1982), TEYKER *et al.* (1989) and PLÉNET and LEMAIRE (1999) underlining that the plant during its vegetative growth must absorb and store an excess of mineral nitrogen which is further metabolized and translocated to the kernels.

**Glutamine synthetase (GS) activity** (HIREL *et al.*, 2000). GS catalyzes the conversion of glutamine into glutamate utilizing ammonia as substrate (resulting either from the reduction of nitrites or from organic N recycling). The translocation of stored nitrogen could be under the control of GS from which there results a significant positive correlation with grain yield and kernel number at low N-input, and with protein grain yield at high N-input. The relationship at low N-input with kernel number suggests that a high GS activity is required to avoid embryo abortion just after fertilization. It is interesting to note that RIZZI *et al.* (1996) have shown that in the material from the Illinois long-term selection experiment for protein content, material selected for a low protein content (ILP) had a low NR activity but also a low GS activity in comparison with material selected for a high protein content.

#### **Results from QTL detection**

There are only a few experiments where quantitative trait loci (QTL) for NUE and its components have been studied (AGRAMA *et al.*, 1999; BERTIN and GALLAIS, 2001).

#### *Common and specific QTLs for yield, its components*

In the BERTIN and GALLAIS (2001) study, detected QTLs for yield, its components, and plant traits at silking were not dispersed at random on the chromosomes. They were instead grouped in clusters as a consequence of relationships among traits. At high N-input, such zones seem to correspond to zones involved in plant development, including plant height, leaf area, silking date, kernel number, kernel size, ear size, grain yield. Fewer QTLs were detected at low N-input. However, their location was very often within the regions detected at high N-input. Then, for a given trait, the QTLs at low N-

input appear as a subset of QTLs at high N-input. Unlike for yield and its components, for grain composition more QTLs were detected at low N-input than at high N-input. This is quite consistent with the observed results of BERTIN and GALLAIS (2000) and DI FONZO *et al.* (1982) showing a greater role of variation in nitrogen utilisation efficiency than in nitrogen uptake efficiency at low N-input, the reverse being observed at high N-input. However, QTLs detected at low N-input for grain composition co-located in many situations with QTLs at high N-input involved in vegetative development, grain yield or its components. QTLs for ASI, leaf senescence and NNI were detected only at low N-input. Finally, QTLs specific to low N-input, i.e. those which did not co-locate with any QTLs detected at high N-input are rather few and only affect specific traits.

AGRAMA *et al.* (1999) have used a greater population size than BERTIN and GALLAIS (2001) (214 versus 99); however they have considered per se value of F3 families. Therefore the agronomic value of the results is not so clear, since the relationship between per se value and testcross value is not always high for complex traits such as grain yield (see below). On the whole, more QTLs were detected at low N-input (the opposite of the BERTIN and GALLAIS results, perhaps due to the nature of the F3 plant material used, instead of testcross families). For yield, about the same percentage of variance was explained by molecular markers (about 40%) in both N-conditions. Some common QTLs were detected but, in general, specific QTLs contributed more to the explained variation. For kernel weight, detected QTLs explained 39.8 % of the variation at high N-input and only 17.1% at low N-input, indicating that kernel weight, related to post-anthesis N-uptake, plays a greater role for variation in yield at high N-input than at low N-input, which is consistent with the BERTIN and GALLAIS (2001) results.

#### *Co-locations with some genes involved in N or C-metabolism*

Co-locations between QTLs and known genes are interesting to consider in trying to find a genetic meaning to the QTLs, although they do not necessarily imply a causal relationship (BERTIN and GALLAIS, 2001).

*Genes coding for nitrate reductase and glutamine synthetase.* NR locus on chromosome 1 and chromosome 4 coincided with a QTL affecting kernel number at high N-input and kernel weight in

both conditions. Three loci of cytosolic GS coincided with QTL for grain yield or its components (on chromosome 1, chromosome 4 and chromosome 5) and GS activity confirming the role of GS activity in yield determination. Furthermore, a two-cycle recurrent selection experiment developed from the RIL population for adaptation at low and high N-input has shown that the GS region on chromosome 9 would also be involved in adaptation at low N-input (COQUE and GALLAIS, 2005a). In addition, chloroplastic GS on chromosome 10 coincided with QTLs for ASI and leaf senescence. Finally nearly all regions of GS genes known (mapped) in maize seem to be involved in NUE (GALLAIS and HIREL, 2004). Knowing the metabolic role of GS, such coincidences cannot be due to chance. The various GS genes or combinations of these genes (GS is an octomer) code for different isoenzymes and could have different roles according to the tissue, the organ, the developmental stage and even the soil nitrogen availability (HIREL *et al.*, 2000). It may be underlined that on rice in the syntenic region a QTL for kernel weight has been detected and it coincides with a GS gene (OBARA *et al.*, 2001).

*Genes involved in C-metabolism.* C and N metabolism are inter-related. Thus, the consequence is the finding of QTLs of NUE coinciding with some C-metabolism genes coding for the following enzymes: ADPGppase, Invertase, Sucrose-Phosphate-Synthase (SPS) and Sucrose-Synthetase (SuS). Coincidences between ADPGppase genes (which control starch synthesis) and QTLs for traits related to NUE were observed on chromosome 3 (*sb2* locus) for kernel number and protein content at low N-input, and on chromosomes 5 and 8 for leaf area at high N-input and low N-input. GOLDMAN *et al.* (1993) have also found a QTL co-locating with the gene *sb2* and affecting starch content and protein content. *Sb2* is a mutant which affects ADPGppase activity (CHOUREY and NELSON, 1976), then, "normal" alleles of such a gene could be involved in quantitative variation of starch metabolism.

Invertase genes coincided with QTLs in three zones: on chromosome 2 with a QTL affecting kernel weight at high N-input as well as at low N-input, on chromosome 5 with QTLs involved in plant development at silking and grain yield, and on chromosome 10 again with QTLs of plant development at silking detected only at high N-input. The coincidences between QTLs related to NUE and invertase genes is quite consistent with the BELOW *et*

*al.* (2000) conclusion that nitrogen-induced utilization of C (leading to an increase in kernel weight) is associated with an increase in invertase activity.

The SPS gene on chromosome 6 coincided with a QTL for senescence; on chromosome 8, another SPS locus coincided with a QTL for yield and kernel number. Such an SPS locus is also in the same zone of the QTL detected for responsiveness of yield, kernel number and senescence. Such an enzyme is involved in saccharose synthesis in the leaf, from glucose and fructose. It also plays an important role in the exportation of saccharose towards other parts of the plant, in particular the ear (PRIOUL, 1995). Furthermore, the role of carbohydrate metabolism in leaf senescence is well known. This role could be more important at low N-input than at high N-input. A *SuS* locus on chromosome 9 coincided with two QTLs detected at high N-input: one affecting yield, the other affecting senescence which is again quite consistent.

### Conclusions

QTL studies tend to confirm that variation in nitrogen utilization efficiency was greater than variation in N-uptake at low N-input and the reverse at high N-input: QTLs detected at low N-input were related to nitrogen utilization efficiency traits and QTLs detected at high N-input were more related to N-uptake traits. However, it appears difficult to conclude whether there are specific QTLs for one nitrogen condition. The same loci could be involved at low and high N input, but they could be expressed differently according to N fertilization.

## CONSEQUENCES FOR SELECTION

### **Heritabilities and $G \times E$ interaction variances**

From studies of BERTIN and GALLAIS (2000) and PRESTERL *et al.* (2003) environmental variance is often greater at low N-input than at high N-input: stressed plants are more sensitive to micro-environmental variance. However, taking into consideration of change in genetic variances, heritabilities were only slightly lower at low N-input (0.53 vs 0.69) in the BERTIN and GALLAIS study while they were equivalent in both N-conditions in the PRESTERL *et al.* (2003) experiments. BRUN and DUDLEY (1989), BÄNZIGER *et al.* (1997) found a significantly greater heritability at high N-input (with, in this last case, about the same error variances, but a significantly reduced genetic variance at low N-input, leading on

an average of 14 experiments to a heritability of 0.44 at low N-input and 0.62 at high N-input). Unlike these results, LAFITTE and EDMEADES (1994) and AGRAMA *et al.* (1999) found a tendency to a greater heritability at low N-input.

Interactions of genotypes with environment are generally higher than  $G \times N$  interaction. In the BERTIN and GALLAIS study, the variance component interactions (V) were such that for a value 100 for genetic variance,  $V(G \times Y) = 50$ ,  $V(G \times N) = 30$ ,  $V(G \times N \times Y) = 24$  (Y for year). In PRESTERL *et al.* experiments,  $V(G \times L) = 73$ ,  $V(G \times L \times N) = 73$ ,  $V(G \times N) = 50$  (L for location). This means that the interaction  $G \times N$  is affected by the site or year of test. It will thus be necessary to have several sites at least two years for the evaluation of NUE or its components. Furthermore in the PRESTERL *et al.* experiments it appears that  $V(G \times L)$  is 43% higher at low N-input than at high N-input, as a consequence of a higher sensitivity to environment of plants grown at low N-input.

### **Evaluation of lines or hybrids?**

First, it may be noted that N-stress only slightly affects yields of inbreds (BALKO and RUSSELL, 1980a). Second, in non stress conditions, correlation between per se value of lines and their testcross values for grain yield has already been shown to vary according to the germplasm. It is generally low, and rarely greater than 0.70 (HALLAUER and MIRANDA, 1981). This means that in the best situation, per se value of lines could explain about 50% of the variation among testcross progenies. Obviously, the correlation depends on the tester, and the population, but generally there are more intense correlations between values of testcross progenies of two different testers than between values of testcross progenies and their parental lines. Unlike grain yield, some traits like anthesis date, plant height and kernel weight generally show a highly significant line-hybrid correlation.

With our studied population there was no correlation between lines and hybrids at low N-uptake for grain yield and grain number per ear whereas such a correlation was significant at high N-input. The correlation for thousand kernel weight and protein content was significant at both N-conditions (GALLAIS, unpublished data). Unlike our result, PRESTERL *et al.* (2002b) studies showed that correlation between grain yield of lines and testcrosses was closer at low N-input than at high N-input; moderate to close genotypic correlations were also

observed between yield, number of ears per plant, ASI and chlorophyll content. LAFITTE and EDMEADES (1995) found no correlation between line and hybrid grain yield whatever the N conditions but there was a correlation for kernel weight. Correlation between lines and diallel GCA performances was not significant for grain number per ear. For chlorophyll content and ASI, two traits which appear important to consider for adaptation to low N-input, out of 6 types of material or conditions the correlation was significant once for ASI and twice for chlorophyll content, but with no general rules. In another experiment line-hybrid correlation was significant ( $df = 100$ ) whatever the N level for kernel weight, ASI, leaf area (but always less than 0.75). For chlorophyll content the correlation was significant at high N-input (0.51). BALKO and RUSSELL (1980b) found little relationship between lines and their hybrids for grain yield and N-uptake at low N-input. ZAIDI *et al.* (2003) observed a significant correlation between lines and hybrids for different traits (grain yield, ears per plant, chlorophyll content) with a higher correlation under low N-fertility than at normal N-input.

In conclusion, results may depend on the germplasm used, however, it appears too risky to develop costly evaluation at the level of lines, even if it appears that at low N-input, lines could be evaluated. The advantage of lines is to lead to more variation, but the environmental variances being generally greater, the broad-sense heritability is not always superior, and even if it is, it will not be sufficiently increased to compensate for a low genetic correlation between lines and hybrids. These comments also hold for QTL detection.

### ***Ideotypes for silage and for grain***

For grain, different strategies are possible, high N-uptake at silking being followed by N-uptake and N-remobilization, with contribution regulated by the availability of soil nitrogen. N-uptake is necessary to have the maximum yield at high N-input. To minimize N-residual at maturity, remobilization could also be intense, mainly at low N-input. The ideotype already proposed by SHERRARD in 1984 is still valid:

- high dry-matter and high reduced N accumulated by anthesis,
- high stalk nitrate content at anthesis,
- high rate of N-uptake and assimilation during the grain filling period,
- high rate of N movement to the developing grain
- high photosynthetic rates during grain fill and

prolonged grain-filling period without a later physiological maturity date.

Remobilization could be very interesting to consider in the last period of grain filling, just before maturity (giving interest to stay-green). For silage, as protein content in the whole plant is important, post-anthesis N-uptake will be necessary, and remobilization is also necessary to insure grain development and protein content. To summarize, post-anthesis N-uptake will always be favourable, but at low N-input intense remobilization will be necessary. For grain protein content, post-anthesis remobilization will be more favourable than N-uptake.

### ***Selection experiments for NUE***

Very few selection experiments for NUE have been developed. The important question for the breeder is to know whether it is necessary to develop specific varieties for low and high N-input or if double goal varieties can be developed. Obviously, when G x N interactions are strong it will be justified to develop specific varieties. With the selected material, such interactions appear to be relatively low, and in such a situation it seems possible to develop double goal varieties. However, the genetic advance would not be maximum at low N-input, and with the decrease in N fertilization it could appear justified to develop varieties adapted to low N-input. How to develop such selections?

Study of indirect selection shows that, whatever the nitrogen environment, when they are too different, response to selection is expected to be lower than response to direct selection. In our experiment, selection at low N-input for response at high N-input was less efficient than direct selection at high N-input, whereas selection at high N-input for response at low N-input was close to direct selection at low N-input (COQUE and GALLAIS, 2005a). This is due to the fact that heritabilities were nearly similar at both low and high N-input. Consequently even with a high genetic correlation between low and high N-input, indirect selection cannot be more efficient than direct selection (GALLAIS, 1983). To have, for example, selection at high N-input for response at low N-input more efficient than selection at low N-input, it will be necessary to have a heritability significantly greater at high N-input. Reciprocally, to have selection at low N-input for response at high N-input more efficient than direct selection at high N-input, it would be necessary to have a heritability significantly greater at low N-input. Consistent with this theoretical conclusion, in the exper-

iments of PRESTERL *et al.* (2003) showing a greater heritability at low N-input, selection at high N-input for response at low N-input was on average 70% of expected direct response by selection at low N-input. Relative efficiency of indirect selection was greater than 1 in only two experiments where heritability at high N-input was significantly higher than that at low N-input. In another experiment (PRESTERL *et al.*, 2002a) hybrids selected at low N-input (L x L) had a better grain yield than hybrids selected at high N-input (+11.5%) when both types of hybrids were evaluated at low N-input. However, when evaluated at high N-input they were not significantly different. The L x L hybrids showed higher N-uptake (+12%) at low N-input. In contrast, no differences in nitrogen utilisation efficiency were observed between the two types of hybrids. Then improvement in NUE at low N-input was due to improvement in nitrogen uptake.

The greater efficiency of direct selection at low N-input was also observed in CIMMYT experiments by LAFITTE and EDMÉADES (1994), BÄNZIGER *et al.* (1997). It is interesting to underline that a selection for drought tolerance has increased the tolerance to nitrogen stress, showing that both criteria must be considered simultaneously (BÄNZIGER *et al.*, 1999). Improvement was associated with a significant reduction in barrenness and in the anthesis-silking interval, increase in kernel number per ear and harvest index. Leaf senescence was also delayed and there was a tendency for an increase in the nitrogen harvest index, indicating that leaf N was used more efficiently for grain production whatever the N-condition. Biomass grain yield (100 kg/ha/year under severe N stress and 75 kg/ha/year under well-fertilized conditions) and N accumulation were more improved in severe N conditions. Increase in biomass was larger under severe N stress. Such a result showing greater gains in stress conditions is the opposite of the results of CASTELBERRY *et al.* (1984) which showed that correlated response from selection under optimal conditions is expected to decrease as N stress increases. This correlated response to selection can be due to the improvement of stay-green and post-anthesis N-uptake, an important criterion for both stresses. It could also be the result of the development of the root system (not studied), with a larger root density in deeper soil layers.

A selection experiment of MURULI and PAULSEN (1981) displays about the same type of results. Under conditions of low N supply the synthetic select-

ed at low N-input outyielded the one selected at high N-input by approximately 35%, whereas at high N-input a yield advantage of 25% was in favour of the synthetic selected at high N-input. Furthermore low N selection did not respond to N, unlike high N selection. However some authors concluded that it is better to select at high N-input, even for performance at low N-input. BRUN and DUDLEY (1989) showed that selection in high N-environments resulted in higher predicted gains in N response over both N levels than selection in lower N environments. This was also the conclusion of LAFITTE and EDMÉADES (1995) and is mainly due to the reduction in heritability due to an increase in environmental error at low N-input. Nevertheless, there can obviously exist specific genes for adaptation to nitrogen stress which could be revealed only in such a condition.

It can then be concluded that to have the maximum genetic advance at low N-input, it will be better to select at this N condition. Even if the target area corresponds to moderate N deficiency, it will be better to evaluate the material with a sufficient stress (leading to a yield reduction of about 35-40%) in order to express specific genetic variability for low N-input. If the contrast between the two levels of evaluation is not sufficient, G x N interactions would not appear, and the two nitrogen levels would be like replications of each other. To select for a general adaptation to variation in N level, it will be better to select in both conditions.

#### *The use of physiological traits*

The use of physiological traits to assist selection could be efficient if easily measured key traits could be identified. Qualities of such traits are the following: high heritability, greater than the primary traits (yield), high genetic correlation with the primary trait, easy to measure on a great number of genotypes, measurable in young stage and nondestructive to reproductive tissue (GALLAIS, 1983). In classical phenotypic selection their use can be restricted to the evaluation of parental material with the aim of combining different components of NUE as already suggested by MOLL *et al.* (1982). However, some traits would be easier to use than others. In particular senescence can be easily evaluated with relative accuracy by use of a chlorophyllmeter (COQUE and GALLAIS, unpublished). Furthermore, the use of marker-assisted selection could change the situation completely by allowing a genotypic selection.

### ***The use of marker-assisted selection***

The difficulty of selection for NUE at low N-input is that there is a large environmental effect: in several experiments, the heritability appears to be lower than that at high N-input, due to a large experimental error, and variances of interactions with locations and years are generally larger than variance of G x N interaction (BERTIN and GALLAIS, 2000; PRESTERL *et al.*, 2002a, 2003). Then this implies more replications within a site and more sites than breeding at high N-level. Thus, phenotypic selection at low N-input will be more costly than phenotypic selection at high N-input. Furthermore, the associated traits, such as physiological traits (enzyme activities) are difficult to use, because they are difficult to measure on a great number of genotypes. Molecular markers bring a solution to such problems through marker-assisted selection. When favourable genomic regions (QTLs) have been detected, markers could be used for their transfer (accumulation) in one genotype. The advantage of markers is that such transfer can be developed without agronomic evaluation, even in off-season, thereby saving time. Marker-assisted selection is costly for the QTL studies, but afterwards it saves time, and allows a quick development of new material or even varieties. Marker-assisted back-cross method can be used for this (BOUCHEZ *et al.*, 2002). Some types of recurrent selection aimed at increasing the frequency of favourable alleles can also be used. The main factor for the efficiency of such a method is the accuracy in QTL detection, which implies using a sufficiently large population size (at least 300 RIL) and different environments in different years. Markers can also be used for the transfer of favourable chromosome segments from genetic resources to elite material by limiting the risk of introgression of a too high genome percentage of the donor parent.

### **CONCLUSIONS**

Many studies have shown variation in NUE, in particular at low N-input. It appears that expression of genetic variation in NUE is not the same at low N-input as at high N-input because N metabolism or limiting factors are different in both N-conditions. At low N-input, variation in N-utilization efficiency is more important than variation in N-uptake, the reverse being observed at high N-input. Genes explaining variability at low N-input are probably not the same as at high N-input. Therefore, it appears to

be quite possible to improve NUE at different nitrogen levels of fertilization but genotypes adapted to high N-input will not necessarily be adapted to low N-input. The higher response to selection for a given N-environment is generally observed by a direct selection in this environment. It is interesting to note that criteria for adaptation to low N-input appear to be close to those for adaptation to drought stress, in particular leaf area duration, ASI and number of ears per plant. Marker-assisted selection can help very efficiently for the development of the best varieties, either with specific adaptation to low N-input or with general adaptation to N level of fertilization. In particular, it could be a way to use a genetic variability which is difficult to evaluate, such as variability for physiological traits, or even NUE at low N-input.

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