Title

Nitrogen deficiency-induced leaf senescence in seedlings of tropical maize cultivars as a tool to characterize grain-yielding capacity at low nitrogen supply in the field

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Introduction

Low-N stress is among the major abiotic stresses causing yield reductions in maize grown in the tropics. The access to mineral fertilizers is very unequally distributed among the world's countries and particularly limited in sub-Saharan Africa. The cultivation of N-efficient cultivars with improved grain yield under low-N conditions could help to alleviate the problem (Horst et al. 2003). The breeding process of N-efficient cultivars is more efficient when the selection is performed under the low-N target conditions (Bänziger et al. 1997). However, with decreasing soil fertility the environmental variability increases and thus heritability for grain yield declines. Therefore, secondary plant traits related to N efficiency could be used as selection traits for N efficiency, since these traits are less prone to environmental variability. The main objective of the presented study was the evaluation of N deficiency-induced leaf senescence at the seedling stage as such a trait.

N-efficient cultivars show delayed leaf senescence in field experiments

In field experiments performed in co-operation with CIMMYT, 16 contrasting maize cultivars were tested for N efficiency and the underlying mechanisms for grain yield formation (Worku 2005; Worku et al. 2007). N-efficient cultivars were found to have a high N uptake and dry matter accumulation after anthesis, while N uptake and dry matter production until anthesis were not decisive for N efficiency. Further characteristics of N-efficient cultivars were delayed leaf senescence (Schulte auf'm Erley et al. 2007), a high harvest index and high kernel numbers (Worku, 2005). N uptake and dry matter production after anthesis have frequently been found to be decisive for grain yield in cultivar comparisons both under low and high N supply (Rajcan and Tollenaar 1999; Coque and Gallais 2007). In almost all these cases, cultivars with an improved performance during reproductive growth were also characterized by delayed leaf senescence. The causal relationships between the different traits are not yet clear. Genotypic differences in delayed leaf senescence might improve dry matter production after anthesis and thus increase harvest index and yield. It may also affect N uptake, due to an enhanced C supply to the roots. This view implies a key role for leaf senescence. However, delayed leaf senescence may also be merely a symptom of increased N uptake. To unravel the relationships between leaf senescence and the other traits decisive for N efficiency, correlation coefficients between the traits were calculated (Table 1). Leaf senescence score 28 days after anthesis, with a high score representing a high ratio of senescent leaves on the plants, was negatively related to N efficiency in all investigated environments. Close relationships between leaf senescence score and dry matter accumulation and N uptake after anthesis, however, were found only in one of the experiments (Zimbabwe 2003). This finding only partially support the above described assumption that delayed leaf senescence causes improved reproductive growth and N uptake. Surprisingly, leaf senescence score correlated with kernel numbers and harvest index suggesting that leaf senescence changes the pattern of N remobilization to the kernels. Thus, although delayed leaf senescence appears to be a decisive part of N efficiency and is suited as a selection trait for N efficiency, its physiological action remains to be elucidated.

Table 1: Linear correlation coefficients between leaf senescence scores 28 days after anthesis and grain yield, dry matter accumulation after anthesis, N uptake after anthesis, harvest index and kernel number per plant of 16 maize cultivars differing in N efficiency in field experiments in Kenya (2003) and Zimbabwe (2003 + 2004) at low-N stress. Data from Worku (2005)

	Kenya	Zimbabwe	
Leaf senescence score	2003	2003	2004
Grain yield	**-0.50***	**-0.74****	**-0.44**$^{+}$
DM accumulation after anthesis	-0.22	**-0.75*****	-0.28
N uptake after anthesis	-0.28	**-0.67****	**-0.46**$^{+}$
Harvest index	**-0.63****	**-0.61***	**-0.49**$^{+}$
Kernel number	**-0.52***	**-0.76*****	**-0.55***

$^{+}$, *, ** denote level of significance at $P < 0.1$, 0.05 and 0.01, respectively (n = 16)

Leaf senescence during short-term experiments as indicator for genotypic differences in N efficiency

To test if leaf senescence might be a suitable selection trait for N efficiency also in short-term experiments, the same 16 tropical maize cultivars that were used for the field studies were grown in hydroponics (Schulte auf'm Erley et al. 2007). Leaf senescence was induced by subjecting the plants to N deficiency. The progression of leaf senescence was monitored by photosynthesis and leaf chlorophyll measurements that were estimated by SPAD values. Cultivars differed both in SPAD values and photosynthesis rates of old leaves during N deprivation. Photosynthesis rate during leaf senescence proved to be a better indicator for N efficiency in this study than leaf chlorophyll content. Significant negative correlations were found between SPAD values, photosynthesis rates in the nutrient-solution experiment and leaf senescence scores in the field experiments, and positive correlations were found between photosynthesis rates and grain yield under low-N conditions in the field. The data suggests that the assessment of the capacity of a genotype to maintain a higher photosynthetic capacity of old leaves during N deficiency-induced senescence at the seedling stage may be suited as a selection parameter for N efficiency. However, photosynthesis rate during leaf senescence could explain only up to 20 % of the cultivar differences in N efficiency, while leaf senescence in the field experiments could explain 47 % (Schulte auf'm Erley et al. 2007).

Photosynthesis rates and leaf-nitrogen dynamics during N deficiency-induced leaf senescence of plants cultured in hydroponics

Enzymes within the chloroplast stroma are degraded early during leaf senescence which could be responsible for the decline in photosynthesis rate (Hörtensteiner and Feller 2002). Plant and leaf-N status at the beginning of the N deficiency period might influence the onset of leaf senescence. Plant-N status is determined by N uptake during early vegetative growth and depends on N supply during that period. An efficient root-N uptake rate during the N depletion period will prolong the N supply to the leaves. Apart from improving leaf-N status, this also increases cytokinin production of the roots (Sattelmacher and Marschner 1978), which will also delay leaf senescence (Buchanan-Wollaston et al. 2003).

The leaf-senescence rate might also be influenced by the rate of N export from the leaf. The amount of N exported depends upon the breakdown of N compounds within the leaf and thus protease activity, but might also be influenced by sink strength. These findings raise the question

if cultivar differences in N deficiency-induced leaf senescence might depend on the initial leaf-N content, which may be influenced by the N supply during leaf growth, the N uptake into the leaf or the total plant, or the amount of N which is exported from the leaf. Clarification of these aspects may help simplifying and/or improving the experimental procedure for an evaluation of N deficiency-induced leaf senescence in short-term experiments as a marker for N efficiency.

Therefore, photosynthesis rates and leaf-N contents were investigated before and during N deficiency-induced leaf senescence for maize cultivars grown in hydroponics (Fig. 1). The plants were pre-cultured at two different N rates thus creating differences in N status. Photosynthesis rates decreased considerably during leaf senescence; however, this was not always related to a decrease in leaf-N content of plants pre-cultured at low-N supply. In leaves of plants pre-cultured at high N supply, photosynthesis rates and leaf-N contents declined more in parallel. The decrease in photosynthesis rate must, therefore, have been governed by other factors than leaf-N status. This suggests that N remobilization was not the initial cause for N deficiency-induced leaf senescence, but may rather reflect leaf-inherent differences in leaf senescence.

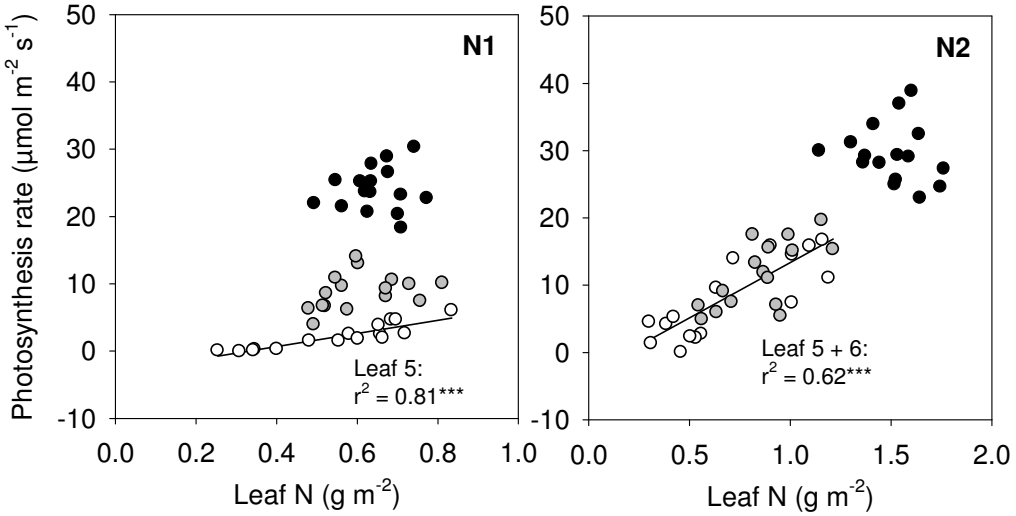

Figure 1: Relationships between photosynthesis rates and leaf-N contents of 16 tropical maize cultivars grown in nutrient solution at two N rates (N1, 0.1 mM; N2, 0.5 mM) with subsequent N depletion. Black symbols: leaf 5 counted from the base of the plants at harvest 1 (prior to induction of leaf senescence); white symbols: leaf 5 at harvest 2 (during leaf senescence); gray symbols: leaf 6 at harvest 2. *, **, *** = significant at $P < 0.05$, 0.01 and 0.001, respectively

A dissection of N import and N export from the senescing leaf during the N-deficiency period was performed by ^{15}N labelling. Although there were only small net changes in leaf-N content during the N-deficiency period at N1, considerable N amounts were exported from and imported into the leaf during this time span (Table 2). Leaf-N contents before the onset of leaf senescence were more than two times higher at high compared to low-N supply. The amount of N exported during N deprivation was nearly four times higher at high N compared to low N. These results suggest that N export was mainly governed by N availability in the leaf. Cultivar differences in leaf-N content prior to leaf senescence had no impact on leaf-N content during leaf senescence (data not shown). Unexpectedly, N import represented a quantitatively not negligible part of total leaf-N even during leaf senescence, and cultivar differences in N import were also important for differences in total leaf-N during leaf senescence. Since N import was not related to total plant N uptake (data not shown), it was probably governed by leaf-inherent factors.

Table 2: Leaf N (g m^{-2}) at harvest 1 (H1; prior to leaf senescence induction), N export (g m^{-2}), Leaf N of H1 still present in the leaf at harvest 2 (Leaf N old; g m^{-2}), N import (g m^{-2}) and leaf N (g m^{-2}) at harvest 2 (H2; during leaf senescence) of 16 maize cultivars grown in nutrient solution at two N rates (N1, 0.1 mM; N2, 0.5 mM) with subsequent N depletion

N rate	Leaf N H1	N export	Leaf N old	N import	Leaf N H2
N1	0.65	0.26	0.39	0.16	0.55
N2	1.51	0.99	0.53	0.17	0.70
Cultivar	**	***	***	***	***
N rate	***	***	***	ns	**
Cult. x N	ns	*	*	ns	ns

*. ** and *** denote levels of significance at $P < 0.05$, 0.01 and 0.001, respectively, ns denotes non significant

Possible relationships influencing the induction and development of leaf senescence in short-term experiments

Some observations made by quantifying leaf and plant-N flows during N deficiency-induced leaf senescence were unexpected. First, photosynthesis rate decreased earlier and stronger than leaf-N content (Fig. 1). This could be due to the degradation of N-containing enzymes within the chloroplast stroma (Hörtensteiner and Feller 2002). Alternatively, the declining photosynthesis rate induced leaf senescence and consequently N remobilisation from the leaf (Hensel et al. 1993). Our results suggest that the decrease in photosynthesis rate might have been caused by a negative feedback regulation due to an accumulation of C assimilates in the leaves, since specific leaf weight increased during N deprivation (data not shown). Leaf-area growth and thus shoot growth is strongly decreased by N deficiency (Lawlor et al. 2001; Vos et al. 2005) mediated via cytokinins produced in the roots (Walch-Liu et al. 2003). A poor leaf growth will lead to a low carbohydrate demand and a low phloem-sap flow from matured leaves to growing leaves. This will also affect N retranslocation, since it could be shown that a low phloem-sap flow also decreases amino acid translocation (Winter et al. 1992). Thus, N retranslocation from senescing leaves under N deficiency might be delayed due to a low sink-N demand.

N import might have played a decisive role for the induction of leaf senescence, since nitrate influx regulates the induction of leaf senescence (Crafts-Brandner et al. 1984). In this study, N import was probably governed by leaf-inherent factors instead of reflecting total plant-N uptake. Nitrate-N enters the leaf by the transpiration stream. Therefore, a decrease in stomatal conductance affects N import. This might be due to the decreased photosynthesis rate or mediated by abcisic acid (ABA), which is known to induce stomatal closure. Indeed, differences in ABA contents have been found in senescing leaves of an early-senescing and a stay-green phenotype of maize (He et al. 2005).

The possible carbon and nitrogen flows in the plants which might influence leaf senescence of vegetative plants are summarized in Figure 2.

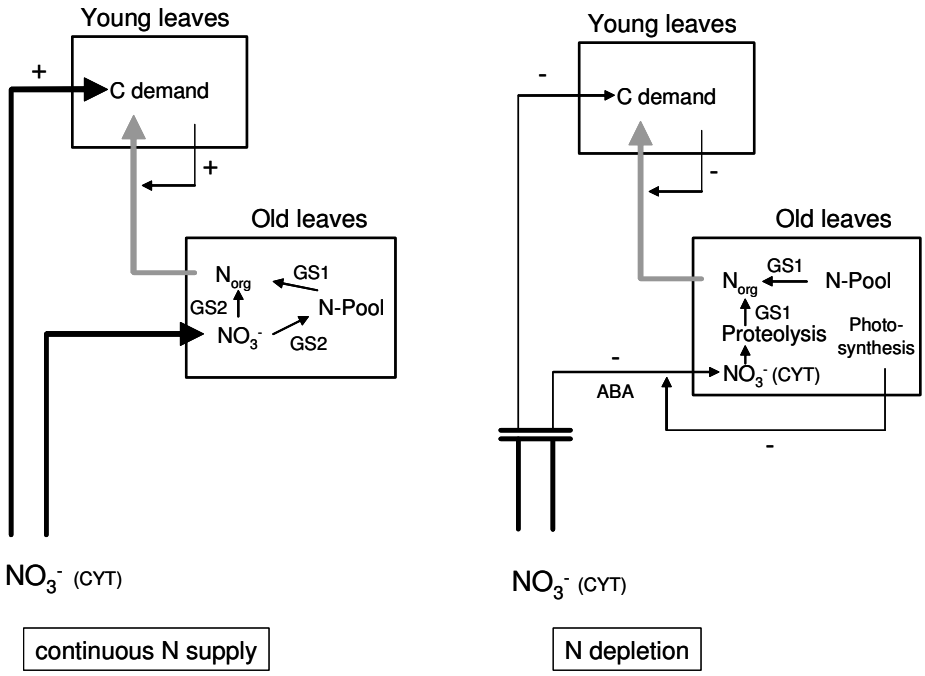

Figure 2: Schematic diagram of carbon and nitrogen flows in vegetative plants prior to the induction of leaf senescence (left) and after N deficiency-induced leaf senescence (right). During continuous N supply, nitrate and cytokinins are transported to old (fully matured) and young (growing) leaves. In old leaves cytokinin/nitrate influx prevents leaf senescence. Nitrate is assimilated by glutamine synthetase (GS2) and thus contributes to a N storage pool for later N translocation to growing tissues. Due to cytokinin influx, C demand of growing tissues is high, which promotes phloem sap inflow and thus also N retranslocation from old leaves. After N depletion, cytokinin/nitrate flux into old leaves stops, inducing leaf senescence. Nitrate influx might be decreased by a decreased photosynthesis rate or ABA. The induction of leaf senescence leads to the depletion of the N storage pool and proteolysis of N compounds. N can be retranslocated after reassimilation by glutamine synthetase (GS1). The decreasing cytokinin influx into growing leaves will decrease growth and thus also the phloem sap inflow. This might also lead to an inefficient retranslocation of N from senescing leaves.

Differences in leaf senescence between vegetative and reproductive growth

Photosynthesis rates and leaf-N contents of plants pre-cultured at low or high N supply were significantly related to leaf senescence scores at anthesis of the same cultivars grown at low-N stress in the field ($r = -0.45$ at $P<0.1$ to $r = -0.62$ at $P<0.05$). However, only photosynthesis rates during leaf senescence of plant pre-cultured at low N supply reflected leaf senescence score during reproductive growth ($r = -0.61$ at $P<0.05$) and N efficiency ($r = 0.46$ at $P<0.1$) in the field experiments. Therefore, cultivar differences in leaf senescence during reproductive growth can only partly be reproduced in a short-term nutrient-solution experiment. Several differences between vegetative and reproductive growth might influence the induction and development of leaf senescence: first, although leaf senescence might be induced by N shortage both in hydroponics and under field conditions, the timing of N shortage is dependent upon different factors. In the field, the exploration of N sources in deeper soil layers might play the most important role for N uptake during reproductive growth (Wiesler and Horst 1994). Thus in the field, root growth and morphology are the most important plant traits, which play only a minor role for N uptake in hydroponics. Secondly, source-sink relationships differ distinctly between

vegetative and reproductive growth, both for carbohydrates and as a consequence also for N. The changes in assimilate flows might influence the development of leaf senescence, or at least the parameters used to characterize leaf senescence. However, the fact that photosynthesis rate during late stages of leaf senescence was significantly correlated to leaf senescence in the field experiments and to grain yield at limiting N supply suggests that cultivar differences in specific steps of leaf senescence related to the breakdown of the photosynthetic apparatus contribute to N efficiency in the field.

Conclusions

The results presented show that the characterization of N deficiency-induced leaf senescence in short-term nutrient solution experiments is useful for the selection of N-efficient maize cultivars. However, the study also highlighted difficulties in comparing leaf senescence of seedlings grown in hydroponics to plants during reproductive growth in field experiments. Since leaf senescence is a complex process prone to many influencing factors, it will be necessary to investigate the different underlying mechanisms in more detail in order to be able to better specify the genetic base of leaf senescence. The results of this study suggest that degradation steps of the photosynthetic apparatus during late leaf senescence might be promising candidates. The identification of molecular markers specific for late senescence stages might be a promising approach.

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