Physiological and molecular aspects of aspartate-derived amino acid metabolism during germination and post-germination growth in two maize genotypes differing in germination efficiency

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Abstract

The Asp-derived amino acid pathway has been studied during the early stages of development in two maize genotypes, Io and F2, differing in germination efficiency and post-germination growth. In both genotypes expression of Ask2 (monofunctional Asp-kinase-2), Akh1 and Akh2 (bifunctional Asp-kinase-homo-Ser dehydrogenase-1 and 2), increased throughout germination and post-germination growth, suggesting a developmental regulation, whereas Ask1 (monofunctional Asp-kinase-1) was expressed constitutively. The major difference between Io and F2 concerned genes encoding bifunctional enzymes, particularly Akh2, the expression of which was dramatically low in F2. ¹⁵N-Asp labelling showed differences in in vivo Asp-kinase activities between the genotypes studied. Asp flux through the Met/Thr branches was higher in Io than in F2, while the latter exhibited a higher flux of Asp through the Lys branch. Physiological results, together with the higher Akh2 expression in Io, suggest that bifunctional enzyme activity, favourable to Met/Thr, was higher in Io than in F2 and that the monofunctional pathway was boosted in F2 because of the lower competition by the bifunctional pathway, thus allowing for higher flux of Asp through the Lys branch. In conclusion, it is suggested that F2 germination and post-germination growth might have been partially inhibited due to a limitation in Met and Thr availability. A negative physiological effect related to Lys accumulation in F2 is also discussed.

Key words: AA metabolism, Akh, Ask, aspartate kinase, Asp-family pathway, germination, lysine, methionine, T50, threonine.

Introduction

Hydration of seeds induces an increase in both respiration and metabolic activity thus allowing the mobilization of C and N reserves necessary to support germination and post-germination growth until autotrophy is reached. Although the mobilization of nitrogen in germinating seeds and seedlings has received much less attention than C metabolism, several earlier studies have pointed out the crucial need for a very active amino acid (AA) metabolism including de novo synthesis and inter-conversion (Lea and Joy, 1983). In germinating seeds, the spectrum of AAs released and transported following storage protein hydrolysis is not complete and therefore does not allow cytoplasmic proteins to be synthesized (Lea and Joy, 1983). It has also been proposed that AA catabolism occurring during the early stages of germination is a source of energy as well as a source of nutrients for expanding new tissue. The supply of AAs is therefore of major importance in the control of germination (Below et al., 2000) controlled by the phytohormones abscisic acid and gibberellic acid (GarciaRubio et al., 1997). The enzymes that catalyse the key steps in amino acid catabolism in germinating seeds, such as proline dehydrogenase and arginase, are regulated either at the transcriptional or the post-transcriptional level for an optimal control of their relative activities during
specific phases of the germination process (Nakashima
et al., 1998; Goldraij and Polacco, 1999, 2000).

Both mobilization of seed storage proteins and oxidase deamination of free amino acids release ammonium
(Goldraij and Polacco, 1999, 2000) that can represent up
to 50% of N made available during seed germination
(Garcia-Rubio et al., 1997). In the absence of primary
nitrogen (NO₃⁻) assimilation, ammonium derived from the
mobilization of reserves is a major source of glutamine
(Glevarec et al., 2004). Accordingly, in a previous study
it appeared that ammonium assimilation by glutamine
synthetase was a key step in the control of germination
efficiency (Garcia-Rubio et al., 1997; Limami et al., 2002).
A quantitative genetic study showed that quantitative trait
loci (QTL) related to germination efficiency and QTLs for
GS activity during germination co-localized with two of
the GS structural genes, Gln3 and Gln4, strengthening the
idea that the corresponding enzyme activity is likely to
play a major role in the control of grain germination
(Garcia-Rubio et al., 1997; Limami et al., 2002). Besides
its metabolic role, Gln has been shown to affect either metabolism or development as a signal molecule (Palenchar
et al., 2004).

Essential amino acids, Lys, Met, and Thr, are derived from Asp through a branched pathway with complex
regulation that allows for adaptation of the flux through
the branches to the needs of the plant at each stage of
development. Post-translational regulation of enzymes
catalysing branch points in the major routes of Asp-derived
amino acid metabolism has been well studied (Paris et al.,
2003; Rognes et al., 2003): in fact, aspartate kinase,
threonine synthase, homoserine dehydrogenase (HSDH),
dihydropicolinate synthase (DHPs), and cystathionine-γ-
synthase are allosterically regulated. Aspartate kinase, the
committing enzyme for the overall pathway, belongs to
two classes, a monofunctional enzyme (Ask) allosterically
inhibited by Lys and a bifunctional enzyme (Akh) alloste-
rically inhibited by Thr. The bifunctional Akh contains an
HSDH coding region in its C terminus. In maize, mono-
functional and bifunctional isogenes are encoded by four
isogenes, Ask1 locus on chromosome 7, Akh1 locus on chromosome 4, and Ask2 and Akh2 loci on chromosome
2 (http://www.maizegdb.org/). Manipulation of fluxes
through the branches of Asp-derived amino acid metab-
olism in mutants or transgenic plants resulted in changes
in the respective amounts of essential amino acids and,
in some cases, it dramatically affected plant develop-
ment (Heremans and Jacobs, 1997; Gaziola et al., 1999;
Galili et al., 2000; Zhu and Galili, 2004). Mutants expressing altered lysine-sensitive Ask over-accumulated
Thr, and mutants expressing lysine-insensitive DHPS
over-accumulated Lys, while double mutants that simul-
taneously expressed lysine-insensitive Ask and DHPS
over-accumulated Lys. Transgenic Arabidopsis thaliana
overexpressing DHPS in combination with defective Lys
catabolism, due to inhibition of the Lys-degrading enzyme
(LKR) by RNAi strategy, over-accumulated Lys (Zhu and
Galili, 2004). These plants were, however, affected in
seedling growth due to a putative negative physiological
effect of Lys.

After having shown the importance of Gln metabolism in
maize germination efficiency (Garcia-Rubio et al., 1997;
Limami et al., 2002), the present work aimed to extend the
investigation by evaluating the involvement of essential
amino acids metabolism in the early stages of maize
development. Molecular and physiological experiments
have been designed to compare Asp-derived amino acid
metabolism in two maize genotypes, Io and F2, differing in
germination efficiency. More generally, these two geno-
types are genetically distinct, they have been characterized
and showed differences related to agronomic and physio-
logical traits, i.e. TKW (thousand kernel weight), NR
(nitrate reductase), and GS (glutamine synthetase) activities
and nitrogen mobilization during grain filling (Hirel et al.,
2001). At the molecular level, expression of genes encod-
ing the four isogenes of aspartate kinase has been measured
by quantitative, reverse transcription, polymerized chain
reaction (q-RT-PCR). At the physiological level, changes in
amino acids have been measured by HPLC and in vivo
activity of aspartate kinase and flux through aspartate-
derived amino acid branches have been determined by
15N labelling (15N-Asp) in both genotypes throughout
ermination and post-germination growth.

Materials and methods

Plant material for germination studies

Two maize genotypes, a French flint and early line of maize (Zea
mays; F2) and an iodent late line (Io) were germinated in Petri dishes
(diameter 9 cm) on Whatman paper soaked with deionized water
and maintained in a growth chamber in darkness at 21 °C. Three replicates
of 40 grains per Petri dish were used for the germination test (T50).
Germination time was determined as the time of radicle emergence.

At various stages of germination of both lines (0, 24, 52, 74, 96 h),
30 seeds of each replicate were collected, embryos and endosperms
were separated, pooled, frozen in liquid N₂, and stored at −80 °C for
further biochemical and molecular analysis.

Amino acids extraction

About 100 mg of embryos were extracted in 1 ml of 80% ethanol for
1 h. During extraction, the samples were continuously agitated and
then spun for 5 min at 28 000 g. The supernatant was decanted and
the pellet subjected to further extractions in 60% ethanol and, finally,
in water. All supernatants were combined to form the hydro-alcoholic
extract. After evaporation of the extract under vacuum, organic
residues were dissolved in distilled water and extracted with the same
volume of chloroform. After centrifugation, an aqueous phase
containing the amino acids was vacuum-dried. Amino acids were
then redissolved in distilled water and passed through a 2 ml column
of Dowex 50WX8 (200–400 mesh, H-form resin; Supelco,
Bellefonte, PA, USA). Columns were washed three times with 1 ml
of water, and amino acids were eluted with 5 ml of 6 M NH₄OH and
vacuum-dried.
Gas chromatography coupled to mass spectrometry (GC/MS) for \(^{15}N\) labelled amino acids analyses

Dry samples were dissolved in 100 µl acetonitrile and derivatized using 100 µl of N-methyl-N-(tert-butyldimethylsilyl)-trifluoroaceticamide (MTBSTFA) for 30 min at 90 °C. A sample of 1 µl was injected into the gas chromatograph. The mass spectrometer was a JMS 700 (Jeol, Japan) equipped with an Optima 1 column (30 m × 0.25 mm × 0.25 µm) using a 70 eV electronic impact as ionization mode and HR–SIM (R=500) mode for data acquisition.

High pressure liquid chromatography (HPLC) for amino acids analyses

Dry samples were dissolved in 100 µl ethanol and determined as \(\alpha\)-phthalaldehyde derivatives on a C-18 column using 32-Karat software (Beckman-Coulter, Fullerton, CA, USA). The internal standard was \(\alpha\)-amino butyric acid and the gradient was produced using two eluants: 50 mM sodium acetate buffer (pH 5.9) with 200 ml l\(^{-1}\) methanol, and 100% methanol.

RNA extraction and q-RT-PCR

Total RNA was extracted as described by Verwoerd et al. (1989) from the frozen material.

Purity of RNA was assessed by determination of absorbance at both 260 nm and 280 nm. RNA was only used when the ratio Abs\(_{260\text{nm}}\)/Abs\(_{280\text{nm}}\) was higher than 1.7. After DNase treatment both 260 nm and 280 nm RNA was only used when the ratio from the frozen material.

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**Results**

**Imbibition and germination of two maize homozygous genotypes Io and F2**

The time-course of germination corresponding to radicle protrusion and the water uptake by the seeds of Io and F2 were determined at 21 °C. Germination curves (Fig. 1) showed that Io germinates faster than F2, the \(T_{50}\) (time at which 50% of seeds germinated) of Io and F2 were, respectively, 52 h and 74 h.

In order to check whether differences in germination of Io and F2 were related to imbibition properties, water uptake by both lines was monitored. Hydration of the seeds corresponded to three distinct phases, the first phase corresponded to a rapid water uptake followed by a second phase during which water uptake slowed down considerably and, finally, a third phase during which water uptake increased significantly. Water uptake by both lines was similar during the first and second phases indicating that the observed differences in germination were not linked to imbibition properties. Differences in water uptake appeared thereafter in the phase corresponding to post-germination growth and early seedling establishment, the rate of water uptake by F2 was lower than that by Io and that agreed with the slower growth rate of F2 compared with the growth of Io (Fig. 1).

**Changes in expression of genes encoding monofunctional and bifunctional isogenes of Asp kinase in embryos of Io and F2 throughout germination and post-germination growth**

The expression pattern of aspartate kinase (Asp kinase) genes was examined by real-time quantitative RT-PCR in embryos of Io and F2 throughout germination. The 18S rRNA was used as a constitutive control (Fig. 2b).

As shown in Fig. 2a expression of *Ask1* was constant during germination in both lines, it was, however, at least two times higher in Io than in F2. *Ask2* and *Akh2* exhibited the same pattern of expression in both genotypes, a low level of expression during the first 24 h of imbibition, followed by a marked increase (three to four times) and stabilization of the expression between 52 h and 96 h of imbibition (Fig. 2a). *Akh1* showed an important increase during the first h of imbibition followed by a stabilization of the expression between 24 h and 96 h of imbibition in the genotype Io, whereas in the genotype F2, the

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**Table 1. Sequences of oligonucleotides used for quantitative RT-PCR**

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Oligonucleotide sequences (5’ to 3’)</th>
<th>(E)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA</td>
<td>Fwd CGTCCCTGCCCCCTTTGTACAC</td>
<td>1.18</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Rev ACACCTCCACCCGGAGCATTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ask1</em></td>
<td>Fwd CAGTGGGCGAAAACAGTGTGTTG</td>
<td>1.24</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Rev CAGCCTCAGCAACAGGAAACCATTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ask2</em></td>
<td>Fwd TGGGTCCAGGCGGTGTTATGT</td>
<td>0.99</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Rev TGCCCATGCGGTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Akh1</em></td>
<td>Fwd TGGTGCCCCCTCGTAGTGT</td>
<td>1.04</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Rev AACCAGTATGAGAGCGAAGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Akh2</em></td>
<td>Fwd GGAATTAATGCTCTAGGTATG</td>
<td>0.82</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Rev GGTACGATAATCATTTGATG</td>
<td></td>
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</tbody>
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expression increased gradually between 0 h and 74 h and then slightly decreased (Fig. 2).

Comparison of the expression of Asp kinase isogenes in both genotypes showed that (i) expression of the four isogenes was higher in Io than in F2, with the major difference for Akh2 that was expressed up to five times more in Io than in F2 at 52 h of imbibition, and (ii) in both genotypes the level of expression of Ask2 was the highest compared with the other members of Akh family (Fig. 2).

Changes in amino acid contents in embryos of Io and F2 throughout germination and post-germination growth

Free amino acids (FAA) have been determined by HPLC in embryos (Fig. 3) and endosperm (data not shown) of both genotypes, Io and F2, throughout germination. The amount of FAA was higher in Io (1.23 mmol per dry embryo) than in F2 (0.65 mmol per dry embryo).

Three amino acids, Asn, Asp, and Glu, accounted for the majority of FAA (70 mol%) in embryos and endosperm of both genotypes at the stage of dry seed (Fig. 3). This profile changed throughout germination, probably as a result of reserve protein mobilization and the subsequent reactions of amino acids transamination, oxidative deamination, and ammonium reassimilation (Limami et al., 2002). At 96 h, the major amino acids, which accounted for 70 mol% in Io, were Asn, Gln, Glu, Ala, Ser, and Val in embryos and Leu, Gln, Asn, Phe, Ala, and Val in endosperm. In F2, the major amino acids (70 mol%) were Ala, Gln, Asn, Glu, Ser, and Val in embryos and Gln, Leu, Asn, Ala, Val, Phe, and Ser in endosperm (Fig. 3).

The proportions of Asn and Asp in embryos were the only ones that decreased steadily throughout germination in both genotypes. Asn proportion decreased from 55 mol% and 42 mol% in the dry seed (0 h) to 30 mol% and 12 mol% in Io and F2, respectively, at 96 h of imbibition. The Asp proportion decreased from 10 mol% and 13 mol% to 3 mol% and 4 mol% in Io and F2, respectively, between 0 h and 96 h of imbibition. Glu, the third major amino acid in the dry seed showed a relatively stable proportion with minute changes around a mean value of 15 mol% and 12 mol% in Io and F2, respectively. Met proportion also remained fairly unchanged throughout germination in both genotypes, representing 1 mol% and 2.15 mol% in Io and F2, respectively. The proportions of all the other amino acids increased, particularly Gln, Ala, Leu, Thr, and Lys (Fig. 3).

The major differences in the amino acid proportions between Io and F2 embryos, were higher levels of free Lys, Met, Thr, and Leu in F2 than in Io, whereas the latter was characterized by higher levels of free Asn and Glu.

Fate of Asp assessed in vivo by 15N-Asp labelling in Io and F2

A labelling experiment was designed to determine the fate of aspartate and in vivo activity of Asp kinase by labelling seeds with 15N-Asp (2 mM, 99% atom excess). Seeds were imbibed with sterile water for 24 h before being transferred to labelling medium. Embryo samples were collected at 24 h (48 h of imbibition), 33 h (57 h of imbibition), and 48 h (72 h of imbibition). Amino acids derived from 15N-Asp and those derived from nitrogen endogenous reserves were detected by gas chromatography coupled to mass spectrometry (GC/MS), quantified and expressed as 15N-AA mol% and 14N-AA mol% (Fig. 4).

The major labelled amino acids in both lines were Asn, Glu, Gln, Ala, and Leu (Fig. 4a). The content of 15N-Gln was two times higher in F2 (9 mol%) than in Io (4.5 mol%). Conversely, 15N-Asn was almost 4 times higher in Io (13.8 mol%) than in F2 (3.2 mol%). The contents of 15N-Glu were similar in both lines after 48 h of labelling, however, this content started to increase at least 24 h earlier in Io compared with F2. 15N-Ala was found essentially in Io in which it represented more than 1 mol% at 24 h and almost 2 mol% at 48 h of labelling. 15N-Ala in F2 constituted less than 1 mol% after 48 h of labelling. The profile of labelling of Leu was similar in both lines with, however, a quantitative difference; the proportion of 15N-Leu was 0.9 mol% in F2 versus 0.5 mol% in Io after 48 h of labelling.

Amino acids related to aspartate pathway were also labelled. Thr showed the same profile of labelling in both lines with, however, a 24 h delay in F2; as a consequence, the proportion of 15N-Thr was lower in F2 (0.5 mol%) than in Io (1.0 mol%). Labelled Met was only found in Io where it represented 0.2 mol% after 24 h of labelling before decreasing to a low level at 48 h of labelling. Conversely, labelled Lys was only found in F2 where it increased between 33 h and 48 h to reach 0.45 mol%.

The changes in the proportions of the amino acids derived from nitrogen reserves (14N-AA) in embryos
were monitored in parallel to $^{15}$N-AA neo synthesis (Fig. 4b) during the 48 h of the labelling experiment that corresponded to the period from 48 h to 72 h of imbibition. It appears that, with the exception of Ala, all $^{14}$N-AA showed the same profile of changes as FAA reported in Fig. 4, indicating that providing the embryos with 2 mM exogenous $^{15}$N-Asp did not interfere with the fate of amino acids in embryos (Fig. 4b).

**Discussion**

The germination and hydration properties of two maize genotypes, Io and F2, were determined at 21 °C. Although 100% of the seeds of both genotypes germinated, the genotype Io was characterized by a faster germination compared with F2. The speed of hydration of both lines were, however, similar throughout the germination *sensu stricto* period, i.e. up to radicle protrusion. This result indicated that the difference in germination efficiency between Io and F2 is physiologically relevant and was not due to a difference in hydration capacity or any damage or ageing side-effects on seed integrity.

Molecular characterization of Asp kinase genes during germination and post-germination growth of Io and F2

Specific sequences of cDNA of the four Asp kinase isogenes expressed in maize were cloned and sequenced,
allowing for the determination of specific primers dedicated to gene expression studies, by q-RT-PCR in embryos and endosperm. The four genes showed low expression in endosperm while they were expressed at significant levels in embryos throughout germination and post-germination growth.

The expression of the Asp kinase isogenes, \textit{Ask}2, \textit{Akh}2, and \textit{Akh}1, increased throughout germination and the post-germination growth period in both genotypes Io and F2. Expression of \textit{Ask}2, \textit{Akh}2, and \textit{Akh}1 seemed developmentally regulated whereas \textit{Ask}1 showed a constitutive expression at a remarkably constant level throughout germination and the post-germination growth period in both genotypes studied. The patterns of expression of Asp kinase isogenes within each genotype were similar, isogenes were expressed at different levels with \textit{Ask}2 exhibiting the highest level of expression followed by \textit{Akh}1, \textit{Ask}1, and \textit{Akh}2 showing the lowest level of expression. This result is consistent with the fact that members of multigene families exhibit differential levels of expression and organ specificities concurrent with specific roles during plant growth and development (Brugiere \textit{et al}., 1999; Limami \textit{et al}., 1999; Terce-Laforgue \textit{et al}., 1999; Carvalho \textit{et al}., 2000a, \textit{b}). A comparison of the level of expression of Asp kinase isogenes between the Io and F2 genotypes showed that expression of Asp kinase isogenes was higher in Io, the genotype exhibiting the fastest germination rate than in the genotype F2. This genetic variability concerned the four Asp kinase genes indicating that the alleles of the genotype Io conferred a higher level of expression than those of the genotype F2, particularly \textit{Akh}2 that showed the most marked difference between the genotypes. At 52 h of imbibition that also corresponded to \textit{T50} of Io, the expression of \textit{Akh}2 was five times higher in Io than in F2. This finding is in favour of \textit{Akh}2 playing a major role, compared with the three other isogenes, in the generation of metabolic and physiological differences between the genotypes during germination and post-germination growth. Similarly, it has been shown that \textit{Ask}2 was the isogene involved in determining the differences in the content of free amino acids between the endosperms of wild-type and mutant \textit{opaque-2} during the maturation phase of maize grains (Wang and Larkins, 2001; Wang \textit{et al}., 2001).

**Fig. 3.** Variation in contents of important amino acids (mol\%) in embryos of two maize lines, Io and F2, after 0 h, 24 h, 52 h, 74 h, or 96 h of imbibition on distilled water at 21 °C.
Asp family and germination and post-germination of maize
and post-germination growth. Furthermore, this study showed that expression of these isogenes was different between genotypes Io and F2. The goal of the physiological study was to characterize the Asp family pathway and to determine whether genotypes Io and F2 exhibit differences in flux through the branches of the Asp-derived amino acid pathway. For this aim, the fate of Asp was determined \textit{in vivo} by $^{15}$N-Asp labelling during late germination/early post-germination growth. $^{15}$N-Asp labelling showed that Asp flux through Met and Thr branches was higher in genotype Io than in genotype F2, while the latter exhibited a higher flux of Asp through the Lys branch. Relative accumulation of Lys and Met/Thr are generally thought to be dependent upon the ability of DHPS (dihydropicolinate synthase) and HSDH (homoserine dehydrogenase) to compete for limiting amounts of aspartate-4-semialdehyde. In maize, homoserine is mainly synthesized by the Asp kinase–homoserine dehydrogenase bifunctional enzyme (Muehlbauer \etal, 1994). Considering that the bifunctional pathway is favourable to Met/Thr synthesis while the monofunctional Asp kinase pathway would be favourable to Lys synthesis, it is assumed that Io exhibited higher Akh bifunctional activity than F2, resulting in higher flux of Asp through Met and Thr branches. It is also suggested that the AK monofunctional pathway was favoured in F2 because of lower competition from the bifunctional pathway and this allowed for a higher flux of Asp through the Lys branch in F2. These assumptions are supported by the results at the molecular level showing that the major difference in terms of gene expression concerned genes encoding bifunctional enzymes, particularly Akh2 the expression of which was dramatically low in F2 (Fig. 2a). A genetic analysis of amino acid accumulation in the maize mutant \textit{opaque-2} showed that variability in essential amino acid (Lys, Met and Thr) contents in the endosperm is at least partly controlled at the level of gene (Ask2 and Akh2 loci) expression (Wang and Larkins, 2001; Wang \etal, 2001).

It is worth noting the opposite patterns of $^{15}$N-Met and $^{15}$N-Thr accumulation (Fig. 4a) in genotype Io. The relative accumulation of Met and Thr has been proposed to be affected by the relative ability of cystathionine-$\gamma$-synthase and threonine synthase to compete for a common intermediate to their branches, homoserine-4-phosphate. The fact that $^{15}$N-Asp flux through the Met branch was at first higher than that through the Thr branch, followed afterwards by a decrease in $^{15}$N-Met mol\%, in parallel to an increase in $^{15}$N-Thr mol\% is consistent with the two mechanisms that have been proposed to influence partitioning of Asp flux between these two branches, namely (i) inhibition of cystathionine-$\gamma$-synthase activity by methionine, and (ii) stimulation of threonine synthase activity by S-adenosylmethionine (SAM).

**Conclusion**

The study of the Asp family pathway in genotypes Io and F2 showed differences at both the molecular and physiological levels. This conclusion poses the question of how differences between F2 and Io, in terms of speed of germination and post-germination growth, would be related to the metabolism of the Asp family pathway. In our opinion at least two reasons can be put forward, one related to metabolic roles of Met and Thr and the second related to the putative signalling role of Lys. Firstly, the results of AA contents, that represent a balance between synthesis and catabolism (Fig. 3), and $^{15}$N-AA labelling, that report on the dynamics of Asp utilization (Fig. 4a), suggest that the higher speed of germination and post-germination growth of Io was accompanied by higher rates of synthesis and catabolism of Met and Thr than in F2. Thr and, particularly, Met contents might have limited the development of F2. These AA are not only used as protein building blocks, they are also the precursors of metabolites playing key roles in plant growth as SAM. SAM is a methyl donor used in the synthesis of several plant structural components, and the carbon skeleton of the methionine moiety of SAM is used as a precursor to ethylene and to polyamine. Secondly, Lys might have participated in the partial inhibition of F2 development. Lys is, in fact, a good candidate for a role in the control of growth and development (Zhu and Galili, 2004). Lys accumulation in transgenic \textit{Arabidopsis thaliana} due to overexpression of DHPS and a defective catabolism due to inhibition of LKR by RNAi strategy exerted a negative physiological effect responsible for inhibition of seedling growth (Zhu and Galili, 2004). The Asp family pathway has received a great deal of attention from several researchers seeking to increase the contents of nutritionally important amino acids, particularly Lys, in crop grains. It appeared that the increase in Lys content by genetic manipulation of its synthesis pathway failed because its content is controlled by both synthesis and catabolism (Cattoir-Reynaerts \etal, 1981; Vauterin and Jacobs, 1994; Zhu and Galili, 2004) probably because Lys is not only a protein building block but also a signalling molecule that plays a developmental role.

**Fig. 4.** Fate of Asp determined in maize embryos by $^{15}$N labelling and the quantification of labelled ($^{15}$N-AA) and unlabelled ($^{14}$N-AA) amino acids by gas chromatography coupled to mass spectrometry (GC/MS). (a) Variation in contents of $^{15}$N labelled amino acids (mol\%) in embryos of two maize lines, Io and F2, after 24 h, 33 h, and 48 h of labelling on a 2 mM $^{15}$N-Asp solution (99\% atom excess). Seeds were imbibed with sterile water for 24 h before being transferred to labelling medium. Times on the graphs indicate the duration of labelling corresponding, respectively, to 48 h, 57 h, and 72 h of imbibition. (b) Variation in contents of amino acids derived from an endogenous nitrogen source expressed as $^{14}$N-AA (mol\%) in embryos of two maize lines, Io and F2, during the $^{15}$N-Asp labelling experiment.
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References


