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ORIGINAL ARTICLE

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Interactions of abscisic acid and sugar signalling in the regulation of leaf senescence

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Abstract Leaf senescence can be triggered by a high availability of carbon relative to nitrogen or by external application of abscisic acid (ABA). Most *Arabidopsis* mutants with decreased sugar sensitivity during early plant development are either ABA insensitive (*abi* mutants) or ABA deficient (*aba* mutants). To analyse the interactions of carbon, nitrogen and ABA in the regulation of senescence, wild-type *Arabidopsis thaliana* (L.) Heynh. and *aba* and *abi* mutants were grown on medium with varied glucose and nitrogen supply. On medium containing glucose in combination with low, but not in combination with high nitrogen supply, senescence was accelerated and sucrose, glucose and fructose accumulated strongly. In *abi* mutants that are not affected in sugar responses during early development (*abi1-1* and *abi2-1*), we observed no difference in the sugar-dependent regulation of senescence compared to wild-type plants. Similarly, senescence was not affected in the sugar-insensitive *abi4-1* mutant. In contrast, the *abi5-1* mutant did exhibit a delay in senescence compared to its wild type. As ABA has been reported to induce senescence and ABA deficiency results in sugar insensitivity during early development, we expected senescence to be delayed in *aba* mutants. However, the *aba1-1* and *aba2-1* mutants showed accelerated senescence compared to their wild types on glucose-containing medium. Our results show that, in contrast to sugar signalling in seedlings, ABA is not required for the sugar-dependent induction of leaf senescence. Instead, increased sensitivity to osmotic stress could have triggered early senescence in the *aba* mutants.

Keywords Abscisic acid · *Arabidopsis* · Mutant · Nitrogen · Senescence · Sugar signalling

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Abbreviations ABA: Abscisic acid · aba: Abscisic acid deficient · abi: Abscisic acid insensitive · F_v/F_m: Maximum efficiency of photosystem II photochemistry

Introduction

One of the most important functions of leaf senescence is the remobilisation of nitrogen from the old leaves (Himelblau and Amasino 2001; Hörtensteiner and Feller 2002). Nitrogen deficiency can accelerate leaf senescence, especially when photosynthetic carbon supply is high (Ono et al. 1996; Stitt and Krapp 1999). High carbon/nitrogen ratios in leaves could signal a decreased requirement for investment of nitrogen in the photosynthetic apparatus, thereby increasing the amount of nitrogen available for growth and reproduction. Several lines of evidence suggest that sugars act as a signal for high carbon availability during the regulation of leaf senescence. Leaf sugar contents typically increase during senescence (Wingler et al. 1998; Masclaux et al. 2000; Quirino et al. 2001; Stessman et al. 2002) and senescence-like symptoms, such as leaf yellowing, can be triggered by sugars (Wingler et al. 1998). This correlative evidence is supported by work with mutants and transgenic plants. Tomato plants overexpressing the sugar sensor hexokinase-1 from *Arabidopsis* exhibit accelerated senescence (Dai et al. 1999), whereas senescence is delayed in the *Arabidopsis glucose-insensitive2* mutant lacking hexokinase-1 (Moore et al. 2003). Furthermore, prolonged leaf senescence has been linked to decreased source strength in Rubisco antisense plants of tobacco (Miller et al. 2000), which contain a reduced amount of sugars (Quick et al. 1991).

We have recently shown that growth on medium containing 111 mM glucose in combination with low, but not high nitrogen supply results in accelerated senescence without affecting early photosynthetic development (Wingler et al. 2004). Visible senescence

correlates with a decline in maximum photosynthetic efficiency (F_v/F_m), which can be monitored rapidly and non-destructively using chlorophyll fluorescence imaging. However, it remained unclear whether this response is caused by cross-talk of the nitrogen and sugar signalling pathways or by metabolic interactions.

In addition to sugars, treatment with abscisic acid (ABA) can induce leaf senescence (Yang et al. 2003). Moreover, the majority of sugar-insensitive *Arabidopsis* mutants are either ABA insensitive (*abi* mutants) or ABA deficient (*aba* mutants), demonstrating that ABA plays an important role in sugar responses. If ABA is required for the sugar-dependent induction of senescence, *aba* mutants would be expected to display delayed senescence in the presence of glucose. However, most sugar response mutants have been isolated in screens based on decreased sugar sensitivity during seedling development and little is known about the interactions of sugar and ABA signalling in mature or senescing plants.

Here, we analyse the effects of sugar and nitrogen supply on senescence in *abi* and *aba* mutants of *Arabidopsis*. *abi4-1* is allelic to the sugar response mutants *gin6* (Arenas-Huertero et al. 2000), *sun6* (Huijser et al. 2000), *sis5* (Laby et al. 2000), *isi3* (Rook et al. 2001) and the salt-tolerant mutant *sañ5* (Quesada et al. 2000). In addition to being glucose-insensitive, *abi4-1* is moderately resistant to mannose (Laby et al. 2000). The *ABI4* gene encodes an APETALA2 domain transcription factor (Finkelstein et al. 1998) and is sugar inducible in young seedlings, but not in older seedlings or mature leaves (Finkelstein et al. 1998; Söderman et al. 2000; Arroyo et al. 2003). Nevertheless, altered sugar and CO_2 sensitivity has also been reported for mature *abi4* (*sun6*) mutants (Van Oosten et al. 1997; Oswald et al. 2001). In contrast to *ABI4*, *ABI5*, which encodes a basic leucine-zipper transcription factor (Finkelstein and Lynch 2000), is also glucose inducible in vegetative tissue during later stages of development (Brocard et al. 2002; Arroyo et al. 2003). The *abi5-1* mutant is moderately glucose and mannose insensitive during seedling development (Arenas-Huertero et al. 2000; Laby et al. 2000), but it has not been isolated in sugar-insensitivity screens. Two additional *abi* mutants that are not glucose insensitive, *abi2-1* and *abi1-1* (Arenas-Huertero et al. 2000), were used as controls in our experiments.

In addition, we used the ABA-deficient and glucose-insensitive *aba1-1*, *aba2-1* and *aba3-1* mutants (Arenas-Huertero et al. 2000) to analyse the role of ABA synthesis in the regulation of senescence. The ethylene-insensitive and sugar-hypersensitive *ein2-1* mutant (Cheng et al. 2002), which is allelic to mutants with delayed senescence (Oh et al. 1997), was included to analyse the interactions between ethylene and sugar signalling during senescence.

Our results show that sugar signalling during senescence is not ABA dependent and does not require ABI4. However, the ABI5-dependent sugar response pathway appears to be involved in the regulation of senescence by sugars.

Materials and methods

Plant material

Seed of *Arabidopsis thaliana* (L.) Heynh. was obtained from the Nottingham *Arabidopsis* Stock Centre (Nottingham, UK). The following mutants were used: *abi1-1*, *abi2-1* (Koornneef et al. 1984), *abi4-1*, *abi5-1* (Finkelstein 1994), *aba1-1* (Koornneef et al. 1982), *aba2-1*, *aba3-1* (Léon-Kloosterziel et al. 1996) and *ein2-1* (Bleecker et al. 1988). After sterilisation in commercial bleach, the seeds were washed in water, resuspended in 0.7% low-melting-point agarose and pipetted onto agar (1% w/v) medium (approx. 10 plants per plate). For high nitrogen (HN) treatments, half-strength Murashige and Skoog (MS) basal salt mixture (M 5524; Sigma–Aldrich, Gillingham, Dorset, UK) containing 30 mM nitrogen (10.3 mM NH_4^+ and 19.7 mM NO_3^-) was used. For low nitrogen (LN) treatments, the nitrogen concentration was lowered to 4.7 mM nitrogen (NO_3^- only) by using quarter-strength Murashige and Skoog basal salt mixture without NH_4NO_3 (M 2909: Sigma–Aldrich). The effect of this treatment was the same as for growth on non-commercial MS medium containing 4.7 mM NO_3^- and all other nutrients at half strength. After cold treatment for 2–4 days at 4°C, the plates were transferred to growth chambers and grown in vertical orientation at a photon flux density of 100 μmol m^{-2} s^{-1} for 16 h per day and a temperature of 22°C during the day and 18°C at night.

Chlorophyll fluorescence analysis

Chlorophyll *a* fluorescence was analysed using a pulse-modulated imaging fluorometer (FluorCam 700MF; Photon Systems Instruments, Brno, Czech Republic). After dark-adaptation for at least 20 min, minimum fluorescence (F_0) was measured by exposing the plants to modulated red light, before a saturating flash of white light (0.8 s duration) was applied to record maximum fluorescence (F_m) and to determine the maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes as described by Wingler et al. (2004).

Sugar analysis

Shoots of plants were harvested between 7 and 8 h into the photoperiod, washed thoroughly in water and extracted in 80% ethanol at 80°C. Sugars were determined enzymatically according to Stitt et al. (1989) using an ELX 808UI microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Results

Effect of glucose and nitrogen supply on sugar contents during senescence

We have shown previously that visible leaf senescence is correlated with a decline in maximum photosynthetic efficiency (F_v/F_m), a parameter that can be monitored rapidly and non-destructively using chlorophyll fluorescence imaging (Wingler et al. 2004). Senescence-dependent leaf yellowing and the decline in F_v/F_m are accelerated by glucose in combination with low, but not with high nitrogen supply (Wingler et al. 2004). In contrast to glucose, sorbitol and mannitol did not induce leaf yellowing (not shown) or an early decline if F_v/F_m in the leaf rosettes of nitrogen-deficient plants (Fig. 1), demonstrating that induction of senescence is not caused by the osmotic potential of the growth medium.

To determine the effect of nitrogen and glucose supply on internal sugar accumulation, we measured glucose, fructose and sucrose contents of Col-0 plants at four different developmental stages (Fig. 2a–c). On day 23, no signs of senescence were visible; on day 30, plants grown on glucose with a low nitrogen supply showed the first signs of senescence-dependent leaf yellowing; on day 37, senescence was clearly visible on this medium; on day 44, most plants of this treatment were dead. Glucose, fructose and sucrose accumulated strongly in plants growing on medium with glucose in combination with low nitrogen supply. Sugar contents rose during senescence (until day 37) and remained high during the final stages (day 45). In contrast, growth on 111 mM glucose in combination with high nitrogen supply only led to a moderate accumulation of sugars in the leaves. For example, fructose content was equally as high in plants grown on low nitrogen medium without glucose as in those grown on high nitrogen medium plus glucose. These results show that, especially during senescence, sugar accumulation not only depends on external glucose supply but also on the availability of nitrogen.

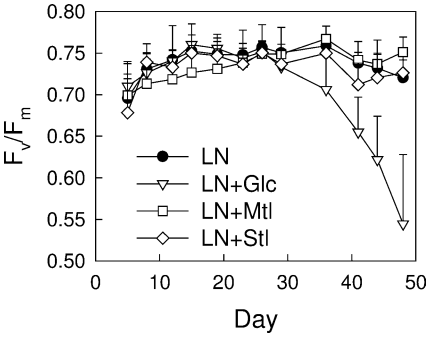

Fig. 1 Effect of glucose, sorbitol and mannitol supply on senescence. Senescence was monitored as a decline in maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes of *Arabidopsis thaliana* (Col-0) plants grown on low nitrogen (4.7 mM nitrate) medium without sugar (*LN*; *closed circles*) or with addition of 111 mM glucose (*LN + Glc*; *open triangles*), mannitol (*LN + Mtl*; *open squares*) or sorbitol (*LN + Stl*; *open diamonds*). Data are means of at least 20 plants + SD

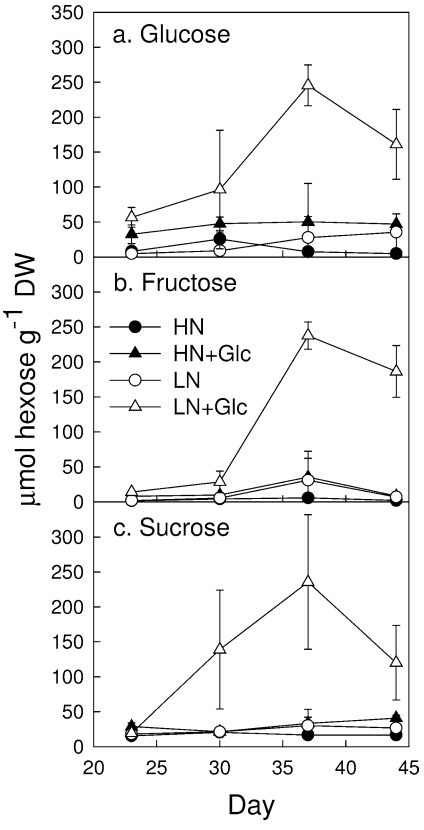

Fig. 2a–c Effect of glucose and nitrogen supply on glucose (**a**), fructose (**b**) and sucrose (**c**) contents in leaf rosettes of senescing *Arabidopsis* (Col-0) plants. The plants were grown on agar medium containing 30 mM nitrogen (*HN*; *closed symbols*) or 4.7 mM nitrogen (*LN*; *open symbols*) without sugar (*HN*, *LN*; *circles*) or with addition of 111 mM glucose (*HN + Glc*, *LN + Glc*; *triangles*). Data are means of four samples ± SD

Effect of glucose and nitrogen supply on senescence in the sugar-insensitive *abi4-1* and *abi5-1* mutants

No difference was found in the regulation of the senescence-dependent decline in F_v/F_m between the *abi4-1* mutant and its wild type Col-0 (Fig. 3a,b). In both genotypes, senescence was accelerated to the same extent by growth on medium containing glucose combined with low nitrogen supply. No decline in F_v/F_m was detected on media with high nitrogen supply or without glucose over the course of the experiment. Addition of glucose in the presence of high nitrogen supply generally resulted in higher F_v/F_m values.

On medium with glucose and low nitrogen supply, senescence was delayed in *abi5-1* compared to wild-type (Ws-2) plants (Fig. 4a,b). This effect was statistically significant (e.g. $P = 0.0015$; *t*-test for day 56). No clear differences between *abi5-1* and Ws-2 were detected during growth on the other media over the course of the experiment.

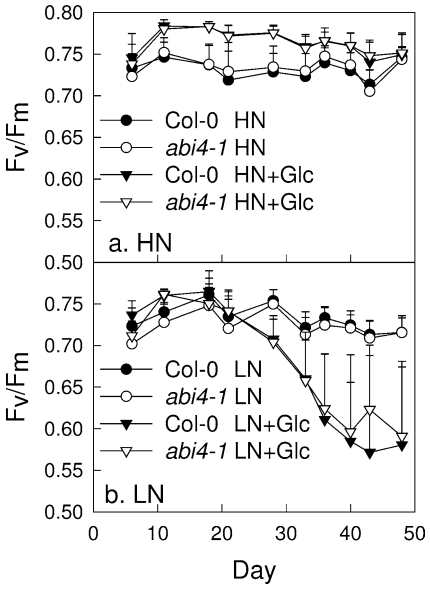

Fig. 3a, b Effect of glucose and nitrogen supply on senescence in wild-type *Arabidopsis* (Col-0; *closed symbols*) and the sugar-insensitive *abi4-1* mutant (*open symbols*). Senescence was monitored as a decline in maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes of plants grown on agar medium containing 30 mM nitrogen (*HN*; **a**) or 4.7 mM nitrogen (*LN*; **b**) without sugar (*HN*, *LN*; *circles*) or with addition of 111 mM glucose (*HN+Glc*, *LN+Glc*; *triangles*). Data are means of at least 10 plants + SD

Effect of glucose and nitrogen supply on senescence in other ABA-insensitive, ABA-deficient or ethylene-insensitive mutants

In addition to being ethylene-insensitive, *ein2* has been shown to display delayed senescence, as well as sugar hypersensitivity. In our experiments, F_v/F_m was generally reduced in *ein2-1*, especially until day 30 (Fig. 5a–d). However, there was no indication that the regulation of senescence by sugars was affected in *ein2-1*, suggesting either that EIN2 is not involved in the sugar-dependent regulation of senescence or that delayed senescence can override the effect of sugar hypersensitivity. In addition to being ABA deficient, the *aba2* and *aba3* mutants are sugar insensitive during seedling development. However, neither of these mutants showed delayed senescence in the presence of glucose. Instead, senescence was significantly accelerated in *aba2-1* ($P = 0.0006$; *t*-test for day 56), but not in *aba3-1* grown on medium with low nitrogen supply plus glucose. On other media with either high nitrogen supply or without glucose, F_v/F_m in *aba2-1* was not different from wild-type values.

Another ABA-deficient mutant, *aba1-1*, also showed a significantly accelerated decline in F_v/F_m on the medium with low nitrogen supply plus glucose ($P = 0.0024$; *t*-test for day 56) but not on the other media tested (Fig. 6 a–d). Two additional mutants, *abi1-1* and *abi2-1*, which are both ABA insensitive but not glucose insensitive, did not have any detectable senescence phenotype.

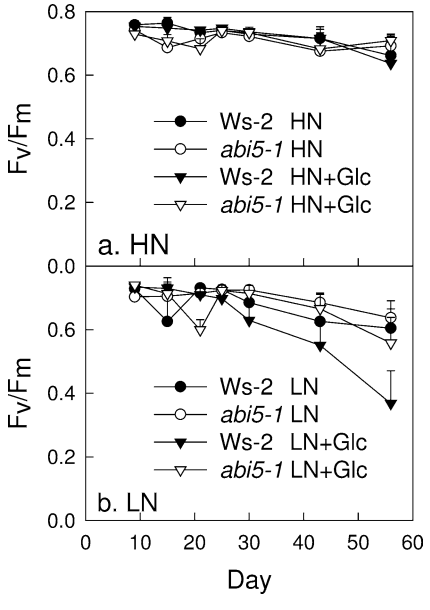

Fig. 4a, b Effect of glucose and nitrogen supply on senescence in wild-type *Arabidopsis* (Ws-2; *closed symbols*) and in the sugar-insensitive *abi5-1* mutant (*open symbols*). Senescence was monitored as a decline in maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes of plants grown on agar medium containing 30 mM nitrogen (*HN*; **a**) or 4.7 mM nitrogen (*LN*; **b**) without sugar (*HN*, *LN*; *circles*) or with addition of 111 mM glucose (*HN+Glc*, *LN+Glc*; *triangles*). Data are means of at least 10 plants + SD

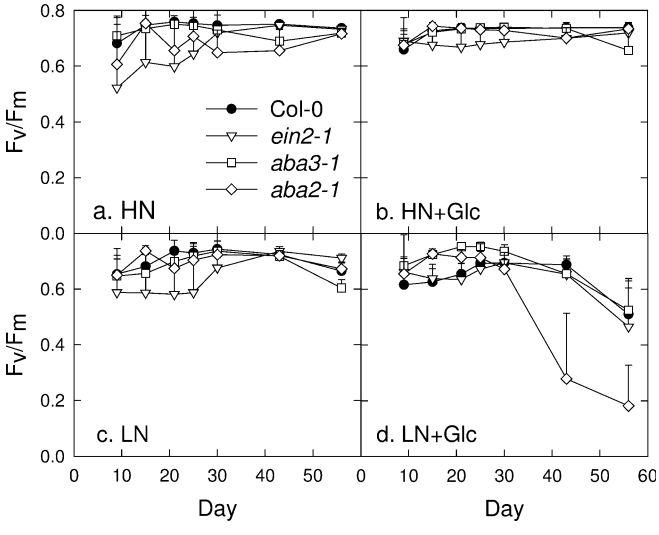

Fig. 5a–d Effect of glucose and nitrogen supply on senescence in wild-type *Arabidopsis* (Col-0; *closed circles*) and in the *ein2-1*, *aba3-1* and *aba2-1* mutants (*open symbols*). Senescence was monitored as a decline in maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes of plants grown on agar medium containing 30 mM nitrogen without sugar (*HN*; **a**) or with addition of 111 mM glucose (*HN+Glc*; **b**) and on 4.7 mM nitrogen without sugar (*LN*; **c**) or with addition of 111 mM glucose (*LN+Glc*; **d**). Data are means of at least 10 plants + SD

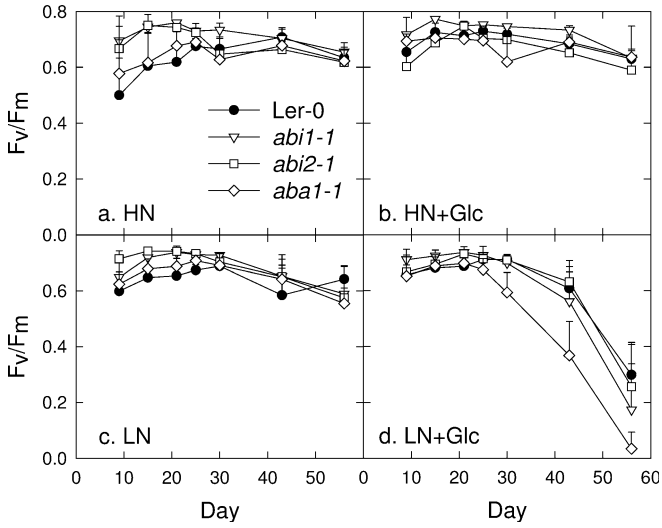

Fig. 6a–d Effect of glucose and nitrogen supply on senescence in wild-type *Arabidopsis* (Ler-0; *closed circles*) and in the *abi1-1*, *abi2-1* and *aba1-1* mutants (*open symbols*). Senescence was monitored as a decline in maximum photosynthetic efficiency (F_v/F_m) in whole leaf rosettes of plants grown on agar medium containing 30 mM nitrogen without sugar (*HN*; **a**) or with addition of 111 mM glucose (*HN+Glc*; **b**) and on 4.7 mM nitrogen without sugar (*LN*; **c**) or with addition of 111 mM glucose (*LN+Glc*; **d**). Data are means of at least 10 plants + SD

Effect of glucose and nitrogen supply on visible senescence in the *abi5-1*, *aba2-1* and *aba1-1* mutants

The results of the F_v/F_m measurements were confirmed by the optical impression of the plants (Fig. 7). On medium with low nitrogen supply plus glucose, visible leaf yellowing was delayed in *abi5-1* and accelerated in *aba2-1* and *aba1-1* compared to their wild types. *abi5-1* and *aba2-1* did not show a visible senescence phenotype on any of the other media. Leaf yellowing was also visible in the old leaves of *aba1-1* plants grown on low nitrogen medium without glucose and on high nitrogen medium with glucose.

Effect of glucose and nitrogen supply on flowering in the *abi5-1*, *aba2-1* and *aba1-1* mutants

Flowering time was determined to test if altered senescence in *abi5-1*, *aba2-1* or *aba1-1* was caused by overall changes in development, as indicated by early or late flowering. In the presence of glucose, Ws-2 and *abi5-1* plants flowered earlier on low compared to high nitrogen medium (Fig. 8a). There was no difference in flowering time between *abi5-1* and its wild type Ws-2 on medium with high nitrogen plus glucose. However, flowering was slightly delayed in *abi5-1* plants on medium with low nitrogen plus glucose. Flowering was clearly accelerated in *aba2-1* plants grown on medium with low nitrogen plus glucose compared to its wild-type Col-0 (Fig. 8b). It is thus likely that early senescence on this medium was linked to accelerated development. In *aba1-1*, flowering was slightly delayed rather than accelerated on medium with low nitrogen plus glucose (Fig. 8c). Early senescence was thus not caused by accelerated plant development.

Discussion

Senescence is induced by sugar accumulation in nitrogen-deficient plants

Leaf senescence can be induced by glucose in combination with low nitrogen supply, resulting in an early decline in F_v/F_m (Wingler et al. 2004). Interactions of sugars and nitrogen in the regulation of leaf senescence could either be caused by cross-talk between the sugar and nitrogen signalling pathways or by metabolic interactions, e.g. by an effect of nitrogen supply on sugar accumulation. Our results show that the extent of sugar accumulation during growth on glucose-containing medium is nitrogen dependent (Fig. 2). Sugar accumulation was much stronger on medium with low compared to high nitrogen supply in all genotypes analysed

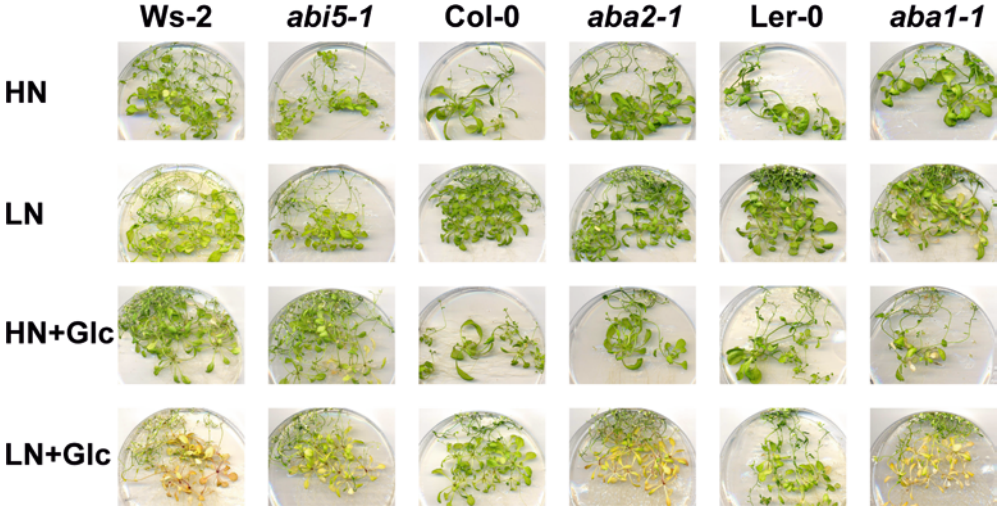

Fig. 7 Effect of glucose and nitrogen supply on visible senescence in the *abi5-1*, *aba2-1* and *aba1-1* mutants of *Arabidopsis* and their respective wild-types (Ws-2, Col-0 and Ler-0) grown for 42 days on agar medium containing 30 mM nitrogen without sugar (*HN*) or with addition of 111 mM glucose (*HN+Glc*) and on 4.7 mM nitrogen without sugar (*LN*) or with addition of 111 mM glucose (*LN+Glc*)

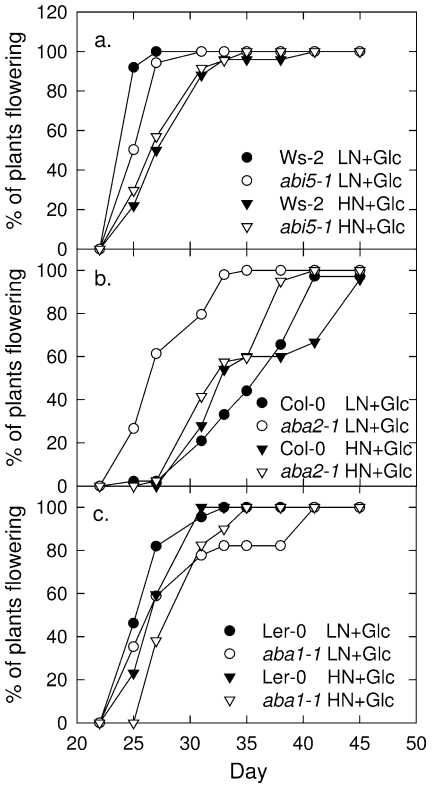

Fig. 8a–c Flowering of the *abi5-1* (**a**), *aba2-1* (**b**) and *aba1-1* (**c**) mutants (*open symbols*) and their respective wild types (Ws-2, Col-0 and Ler-0; *closed symbols*) grown on agar medium containing 30 mM nitrogen with addition of 2% glucose (*HN+Glc*; *triangles*) and 4.7 mM nitrogen with addition of 2% glucose (*LN+Glc*; *circles*). Data are means of plants grown on 4–5 separate Petri dishes

(Ws-2, *abi5-1*, Col-0, *aba2-1*, Ler-0 and *aba1-1*; data not shown). Increased sugar accumulation in nitrogen-deficient plants could be due to decreased sugar utilisation for the synthesis of amino acids and protein. Increased sugar contents have also been reported for *Arabidopsis* seedling germinated in the presence of glucose in combination with low nitrogen supply (Martin et al. 2002), *Arabidopsis* plants grown hydroponically with low nitrogen supply (Sun et al 2002), tobacco plants after nitrogen withdrawal (Paul and Driscoll 1997) and tobacco seedlings grown on low-nitrogen agar medium (Nielsen et al. 1998). However, glucose, fructose and sucrose contents were reduced in leaves of tobacco after growth at low nitrogen supply (Scheible et al. 1997, 2000) and growth in elevated CO_2 only led to sugar accumulation in nitrogen-sufficient, but not in nitrogen-deficient plants (Geiger et al. 1999). These discrepancies could result from differences between species or in the developmental stage analysed. For example, nitrogen and sugar contents were negatively correlated during early senescence, but not in non-senescing leaves of sugar maple (Schaberg et al. 2003). In our experiments, the effect of nitrogen limitation on sugar accumulation was also strongest in senescing plants.

It is likely that early senescence on medium containing glucose in combination with low nitrogen supply was caused by increased sugar contents, rather than by enhanced sugar sensitivity of nitrogen-deficient plants. Although senescence was not a response to the osmolarity of the growth medium (Fig. 1), an osmotic effect caused by the high sugar accumulation within the plants (Fig. 2) cannot be ruled out as the cause of accelerated senescence. Moreover, the accumulation of sugars could have triggered pathogen-defence responses that lead to the initiation of senescence (Yoshida et al. 2002).

Interactions of carbon and nitrogen metabolism that suggest an involvement of sugars in the regulation of leaf senescence have also been reported for other species. In sunflower, low nitrogen in combination with high light results in an accumulation of sugars and triggers an early decline in leaf nitrogen content (Ono et al. 1996). Sugar signalling may also be responsible for the shift in leaf ontogeny and accelerated senescence observed in plants growing in elevated CO_2 concentrations (Nie et al. 1995; Miller et al. 1997). However, Ludewig and Sonnewald (2000) found no correlation between sugar content and the senescence-dependent down-regulation of photosynthetic gene expression in elevated CO_2, suggesting that CO_2 effects are independent of sugar accumulation.

The ABI5-dependent, but not the ABI4-dependent, sugar signalling pathway is involved in the regulation of senescence by sugars

Having established that sugar accumulation can trigger symptoms of leaf senescence, we were interested to find out if this response is attenuated in sugar response mutants. However, most sugar response mutants of *Arabidopsis* have been isolated based on insensitivity to very high sugar concentrations during seedling germination. In mature plants, sugar-insensitive phenotypes have only been established in a few cases, e.g. delayed senescence and decreased leaf expansion in the *gin2-1* mutant (Moore et al. 2003) and reduced feedback inhibition of photosynthesis and altered expression of the plastocyanin gene in the *sun6* (=*abi4*) mutant (Van Oosten et al. 1997; Oswald et al. 2001). However, the *ABI4* gene is mainly expressed in seeds and in young seedlings, whereas expression in vegetative tissue is very low (Finkelstein 1994; Arroyo et al. 2003). It is therefore not surprising that we could not find any effect of the *abi4-1* mutation on sugar-induced leaf senescence (Fig. 3). In addition, senescence was not affected in compost-grown *abi4-1* plants (data not shown). ABI4 therefore does not appear to be involved in the induction of leaf senescence by sugars.

In contrast to the *abi4-1* mutant, senescence was delayed in the *abi5-1* mutant (Fig. 4), demonstrating that ABI5, a basic leucine-zipper transcription factor, is involved in sugar responses during leaf senescence.

Expression of the *ABI5* gene is very low in vegetative tissue compared to seeds (Finkelstein and Lynch 2000) and is only ABA inducible in young seedlings (Lopez-Molina et al. 2001). However, more recent evidence shows that *ABI5*, in contrast to *ABI4*, can also be induced by glucose during later stages of development (Brocard et al. 2002; Arroyo et al. 2003). It would be interesting to analyse if *ABI5* is also induced during leaf senescence. There are two principal ways in which ABA and sugar signalling pathways could interact. Either sugars and ABA share components of the same signalling pathway or sugars induce ABA synthesis, e.g. due to their osmotic effect (Rook et al. 2001; Price et al. 2003). Growth of the *abi5-1* mutant at elevated CO_2 or high light would show to what extent internal sugar formation affects leaf senescence in this mutant.

Induction of senescence by sugars does not require ABA synthesis, but ABA deficiency can result in accelerated senescence

To investigate whether or not the effect of sugars during senescence is dependent on ABA synthesis, we studied leaf senescence in the ABA-deficient and sugar-insensitive *aba1-1*, *aba2-1* and *aba3-1* mutants. As senescence can be induced by ABA (Yang et al. 2003), delayed senescence could theoretically also be caused directly by ABA deficiency. However, senescence was not delayed in any of the *aba* mutants (Figs. 5, 6), showing that induction of senescence by sugars does not require ABA synthesis. While *aba3-1* showed no senescence phenotype, senescence was even accelerated in the *aba2-1* and *aba1-1* mutants. Senescence was also accelerated in the *aba1-1* mutant when it was grown in compost (data not shown). Acceleration of leaf senescence by ABA deficiency could be caused by enhanced osmotic stress due to impaired stomatal regulation, resulting in a wilty phenotype (León-Kloosterziel et al. 1996; Merlot et al. 2002). The difference between *aba1-1*/*aba2-1* and *aba3-1* can be explained by differences in the extent of ABA deficiency. *aba3-1* contains more ABA and loses less water through transpiration than the other *aba* mutants (León-Kloosterziel et al. 1996). As *aba1* mutants are impaired in epoxy-carotenoid biosynthesis and thus in xanthophyll cycle activity (Rock and Zeevaart 1991), osmotic stress may have been exacerbated by decreased photoprotection. Likewise, the *Arabidopsis aba1-3* mutant shows increased sensitivity to salinity in combination with high light (Cramer 2002). Interestingly, *aba* mutants exhibit decreased NaCl sensitivity during seedling germination (León-Kloosterziel et al. 1996; Gonzáles-Guzmán et al. 2002). Again, this shows that effects on seedling development are quite different from effects during later developmental stages. In mature plants, sugar accumulation probably increases stress in the *aba1-1* and *aba2-1* mutants, thereby inducing early senescence.

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