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Natural variation reveals relationships between pre-stress carbohydrate nutritional status and subsequent responses to xenobiotic and oxidative stress in *Arabidopsis thaliana*

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• *Background* Soluble sugars are involved in responses to stress, and act as signalling molecules that activate specific or hormone cross-talk transduction pathways. Thus, exogenous sucrose treatment efficiently induces tolerance to the herbicide atrazine in *Arabidopsis thaliana* plantlets, at least partially through large-scale modifications of expression of stress-related genes.

• *Methods* Availability of sugars *in planta* for stress responses is likely to depend on complex dynamics of soluble sugar accumulation, sucrose–starch partition and organ allocation. The question of potential relationships between endogenous sugar levels and stress responses to atrazine treatment was investigated through analysis of natural genetic accessions of *A. thaliana*. Parallel quantitative and statistical analysis of biochemical parameters and of stress-sensitive physiological traits was carried out on a set of 11 accessions.

• *Key Results* Important natural variation was found between accessions of *A. thaliana* in pre-stress shoot endogenous sugar levels and responses of plantlets to subsequent atrazine stress. Moreover, consistent trends and statistically significant correlations were detected between specific endogenous sugar parameters, such as the pre-stress end of day sucrose level in shoots, and physiological markers of atrazine tolerance.

• *Conclusions* These significant relationships between endogenous carbohydrate metabolism and stress response therefore point to an important integration of carbon nutritional status and induction of stress tolerance in plants. The specific correlation between pre-stress sucrose level and greater atrazine tolerance may reflect adaptive mechanisms that link sucrose accumulation, photosynthesis-related stress and sucrose induction of stress defences.

Key words: *Arabidopsis thaliana*, natural variation, atrazine sensitivity, carbon nutrition, endogenous soluble sugars, oxidative stress, sucrose, xenobiotic stress.

INTRODUCTION

Glucose (Glc) and fructose (Fru) are ubiquitous sources of carbon, reducing power and energy for eukaryotic cells, and in oxygenic photosynthetic organisms, especially in higher plants, sucrose (Suc) is particularly important for transport, allocation and source–sink utilization of photosynthesis-assimilated carbon (Salerno and Curatti, 2003). These soluble sugars are also involved in signalling processes that result in altered expression of genes related to carbohydrate and nitrogen metabolism, metabolite transport and stress responses (Price *et al.*, 2004; Thum *et al.*, 2004; Loreti *et al.*, 2005; Ramel *et al.*, 2007).

Many environmental stresses lead to major changes in carbohydrate metabolism. Under conditions such as drought, desiccation, salt stress or low temperature, soluble sugars are accumulated at high concentrations, and are thought to act as protective compatible solutes (Korn *et al.*, 2008). Soluble sugars have also been involved in other stress conditions, where they do not accumulate at the same levels as those resulting from drought or freezing stress, and thus do not act as compatible solutes. Thus, pre-treatment with Suc enhances resistance of rice plants to *Magnaporthe oryzae* infection (Gomez-Ariza *et al.*, 2007) and tolerance of *Arabidopsis*

thaliana plantlets to anoxic stress (Loreti *et al.*, 2005). Exogenous supply of soluble sugars has also been shown to confer to Arabidopsis plantlets tolerance to atrazine-mediated xenobiotic and oxidative stress (Sulmon *et al.*, 2004, 2006, 2007). As in the case of induction of tolerance to anoxic stress (Loreti *et al.*, 2005), the protective effects of sugar treatment could not be ascribed to mere carbon feeding of pre-existing pathways. Induction of tolerance was associated with important modifications of gene expression related to reactive oxygen species (ROS) defence and repair mechanisms and to metabolic re-orientation, and seemed to result from complex interactions involving sugar and xenobiotic signalling (Ramel *et al.*, 2007).

Characterization of the involvement of soluble sugars in stress responses is often based on exogenous treatments (Loreti *et al.*, 2005; Ramel *et al.*, 2007). However, exogenous treatments may differ from endogenous variations, both in quantitative terms and in terms of tissue partitioning and metabolic targeting. Endogenous variations of soluble sugars have thus been studied in Arabidopsis mutants affected in genes involved in sucrose/starch partitioning, such as the starchless *pgm* mutant (Gibon *et al.*, 2004). However, mutations in key genes of starch synthesis may result in an increase in soluble sugar in compartments and metabolic pathways that differ from those that may be modified by natural selection.

In contrast, genetic and trait variation among *A. thaliana* wild-type lines (Koornneef *et al.*, 2004) provides an excellent tool to examine natural endogenous fluctuations and tolerance variations, and to gain insight into physiological, metabolic and regulatory networks in an ecological and evolutionary context (Cross *et al.*, 2006). Previous studies of Arabidopsis accessions have revealed contrasted metabolic phenotypes, including important variations in carbohydrate levels and night–day fluctuations (Cross *et al.*, 2006; Davey *et al.*, 2008). Moreover, Korn *et al.* (2008) have shown significant correlations between leaf freezing tolerance and endogenous overaccumulation of Glc, Fruc, Suc and raffinose, which are part of the response to freezing stress.

Since our previous work (Ramel *et al.*, 2007, 2009) had shown that interactions between Suc and atrazine were essential for induction of stress defences, we aimed to investigate the impact of endogenous carbon nutritional status on the efficiency of stress response induction. We thus used a natural variation approach in order to analyse relationships between pre-stress endogenous sugar variations and differential responses to xenobiotic stress involving photosynthesis inhibition and oxidative stress. Quantitative analysis of biochemical traits and physiological responses revealed that pre-stress absolute sugar levels were correlated with subsequent xenobiotic stress tolerance. Among the different sugar traits, the absolute level of Suc at the end of the day was found to be particularly important for responses of stress tolerance. This specific importance of Suc is discussed in relation to the nature of atrazine-mediated stress, which is light and photosynthesis dependent.

MATERIALS AND METHODS

Arabidopsis *accessions*

Arabidopsis thaliana accessions used in this study, Col-0, Bch-1, Bur-0, Eil-0, Lip-0, Mt-0, Pla-0, Ta-0, Te-0, Rsch-0 and Ws (Table 1), were obtained from the Nottingham Arabidopsis Stock Centre (NASC). Accessions were homogenized by single-seed propagation and were bulk amplified prior to the analysis.

TABLE 1. *Arabidopsis* thaliana *accessions used in the experiments*

Accessions	NASC no.	Geographical origin	Altitude (m)
Bch-1	N957	Bachen (Germany)	1–100
Bur-0	N1029	Burren (Ireland)	Not given
Col-0	–	Landsberg (Poland)	100
Eil-0	N1133	Eilenburg (Germany)	100–200
Lip-0	N1337	Lipowiec (Poland)	500
Mt-0	N1381	Martuba (Libya)	100–200
Pla-0	N1459	Playa de Aro (Spain)	0–100
Ta-0	N1549	Tabor (Czechoslovakia)	400–500
Te-0	N1551	Tenela (Finland)	1–100
Rsch-0	N1491	Rschew (Russia)	100–200
Ws	–	Wassilewskija (Russia)	100–200

Accessions were obtained from the Nottingham Arabidopsis Stock Centre (NASC).

Plant material and growth conditions

Seeds of *A. thaliana* were surfaced-sterilized in bayrochlore/ethanol (1 : 1, v/v), rinsed in absolute ethanol and dried overnight. Germination and growth were carried out under axenic conditions in square Petri dishes. After seeds were sown, Petri dishes were placed at 4 °C for 48 h in order to break dormancy and homogenize germination, and transferred to a control growth chamber at 22 °C under a 16 h light period regime at 85 μmol m^{-2} s^{-1}. Growth medium consisted of 0·8 % (w/v) agar in 1× Murashige and Skoog (MS) basal salt mix (M5519, Sigma-Aldrich) adjusted to pH 5·7. Direct exposure to atrazine during germination and early growth was carried out for 1 week in the absence or presence of 0·25, 0·5 or 1 μM atrazine. Transfer experiments were also carried out in order to compare the effects of atrazine on plant-lets at the same stage of development. In these experiments, plantlets were grown for 2 weeks on control MS medium until stage 1·04 of development (as described by Boyes *et al.*, 2001), and then tranferred for 5 d to fresh medium in the absence or presence of 10 μM atrazine. In the presence of this concentration of atrazine, the development of lethal injuries in Arabidopsis plantlets followed a time-dependent pattern over this period.

Analysis of plantlet growth and of photosynthetic parameters

The length of the primary root was measured every day on 30 plantlets grown on vertical plates, and the experiment was repeated at least twice. Results were given as the mean ($\pm$ s.e.m.) of these determinations. Pigments were extracted by pounding shoots of plantlets in 80 % acetone, and absorbance of the resulting extracts was measured at three wavelengths: 663, 646 and 470 nm. Levels of chlorophylls and total carotenoids (xanthophylls and carotenes) in these extracts were determined from the equations given by Lichtenthaler and Wellburn (1983). Measurements were done on three replicates of 5–10 pooled plantlets each per experiment. Results were given as the mean ($\pm$ s.e.m.) of these determinations from at least two independent experiments. Chlorophyll fluorescence and maximum photosystem II (PSII) efficiency (F_v/F_m) were measured with a PAM-210 chlorophyll fluorometer system (Heinz Walz, Effeltrich, Germany). After dark adaptation for at least 30 min, minimum fluorescence (F_0) was determined under weak red light. Maximum fluorescence of dark-adapted leaves (F_m) was measured under a subsequent saturating pulse of red light, and variable fluorescence ($F_v = F_m - F_0$) was determined. Measurements were carried out on at least 30 plants, and the experiment was repeated at least twice.

Analysis of soluble sugars

For each accession, analysis of soluble sugars was carried out on pools of four independent samples of shoots from 2-week-old plantlets. Sampling was performed in the last hour of the day (end of day, EoD) or of the night (end of night, EoN). Experiments for sugar analysis were repeated at least twice. Samples were ground to powder in liquid nitrogen and extracted in 80 % ethanol containing 4 mM HEPES-KOH,

pH 7·5, at 80 °C for 30 min. Samples were then centrifuged for 15 min at 11 000 *g*. This initial supernatant was collected and stored on ice. The pellet was resuspended in 80 % ethanol containing 4 mM HEPES-KOH (pH 7·5), and incubated at 80 °C for 30 min. After centrifugation of the extract, for 15 min at 11 000 *g*, the supernatant was collected and stored on ice. This hot extraction of the remaining pellets was repeated further, once with 50 % ethanol in 4 mM HEPES-KOH (pH 7·5) and once with 4 mM HEPES, pH 7·5. All of the resulting supernatants were then pooled and assayed for soluble sugars (Strand *et al.*, 1999). Quantification was carried out spectrophotometrically by enzyme-based assays using the ENZYPLUS® *EZS* 864+ (RAISIO) kit.

Analysis of ROS

The nitroblue tetrazolium (NBT; N6876, Sigma-Aldrich) staining method of Rao and Davis (1999) was modified for *in situ* detection of the superoxide radical. Two-week-old plantlets were transferred for 48 and 72 h to fresh medium in the absence or presence of 10 μM atrazine. At the end of the treatment, plantlets were immersed and infiltrated under vacuum with 3·5 mg mL^{-1} NBT staining solution in potassium phosphate buffer (10 mM) containing 10 mM NaN$_3$. After infiltration, stained plantlets were bleached in acetic acid : glycerol : ethanol (1 : 1 : 3) (v/v/v) solution at 100 °C during 5 min. Plantlets were then stored in a glycerol : ethanol (1 : 4) (v/v) solution until photographs were taken. O$_2$·$^-$ was visualized as a blue colour produced by NBT reduction to formazan. Experiments were repeated four times, and each was carried out on at least 15 plantlets.

The H$_2$O$_2$ staining agent, 3,3$'$diaminobenzidine (DAB; D5637, Sigma-Aldrich), was dissolved in H$_2$O and adjusted to pH 3·8 with KOH. The DAB solution was freshly prepared in order to avoid auto-oxidation (Fryer *et al.*, 2002). Two-week-old plantlets were transferred for 48 and 72 h to fresh medium in the absence or presence of 10 μM atrazine. At the end of the treatment, plantlets were immersed and infiltrated under vacuum with 1·25 mg mL^{-1} DAB staining solution. Stained plantlets were then bleached in acetic acid : glycerol : ethanol (1 : 1 : 3, v/v/v) solution at 100 °C during 5 min, and then stored in glycerol : ethanol (1 : 4, v/v) solution until photographs were taken. H$_2$O$_2$ was visualized as a brown colour due to DAB polymerization. Experiments were repeated four times, and each was carried out on at least 15 plantlets. The specificity of DAB staining towards H$_2$O$_2$ was assessed in control infiltrations in the presence of 10 mM ascorbic acid.

Statistical analysis

Statistical analysis was carried out with the STATISTICA version 7 software and the Minitab® 15·1·1·0 software (Minitab SARL, Paris, France). Pairwise comparison of means used the non-parametric Mann–Whitney test or the parametric Student's *t*-test. Values of significance (*P*) are given in parentheses. Normality of data was analysed by the Anderson–Darling test. A one-way analysis of variance (ANOVA) associated with a Duncan test was used to compare every pair of means within and between accessions.

A two-way ANOVA was used to partition the variance of physiological parameters (F_v/F_m, shoot chlorophyll content, shoot carotenoid content) between genetic and experimental variance with the following mixed model :

$$Y_{ijkm} = (\mu + A_i + T_j + A_i) \times (T_j + \varepsilon_{jik})$$

where μ is the general mean, A_i is the random effect of the *i*th accession genotype, T_j is the fixed effect of the *j*th treatment, $A_i \times T_j$ is the interactive effect of the *i*th accession genotype with the *j*th treatment and ε_{jik} is the error of Y_{ijkm}.

A one-way ANOVA was used to partition the variance of biochemical parameters (shoot endogenous soluble sugars) into genetic variance with the following general linear model:

$$Y_{ikm} = \mu + A_i + \varepsilon_{ik}$$

where μ is the general mean, A_i is the random effect of the *i*th accession genotype and ε_{ik} is the error of Y_{ikm}.

Correlations between all of the values for the biochemical and physiological traits were measured with the Pearson coefficient calculated with Minitab® 15·1·1·0 software (Minitab SARL, Paris, France). In order to examine and visualize relationships between biochemical and physiological traits and to determine to what extent the multivariate phenotypes of accessions were distinct, a principal component analysis (PCA) based on the correlation matrix was calculated using the ADE4 package of R software.

RESULTS

Natural variation of xenobiotic sensitivity during germination and early development in the presence of atrazine

The natural variability of 11 accessions of *A. thaliana* was used to compare the effects of natural metabolic variations on the sensitivity to xenobiotic stress. Physiological and developmental parameters, such as F_v/F_m, chlorophyll content, carotenoid content and primary root growth, were measured during early development of plantlets germinated and grown on MS–agar medium in the absence or presence of varying concentrations of atrazine.

Large variations of all the photosynthetic parameters under study were observed among accessions, with 0·5 μM atrazine treatment giving the most contrasted differences (Fig. 1). Several accessions, such as Bch-1, Bur-0, Mt-0 and Pla-0, maintained significantly higher levels of chlorophyll fluorescence (Fig. 1A) or photosynthetic pigments (Fig. 1B, C), which revealed a lower sensitivity of these genotypes to atrazine treatment. In contrast, the accession Ta-0 presented the lowest values for all the physiological and developmental parameters measured (Fig. 1A–C). The other accessions including Col-0 presented intermediate values. Analysis of primary root growth in the presence of 0·25, 0·5 or 1 μM atrazine (Fig. 2) indicated that natural variation of the response to atrazine also involved differences in growth and development. Treatment with 0·5 μM atrazine gave the most contrasted responses between accessions. The Te-0 accession and the accessions previously showing lower sensitivity to atrazine in terms of photosynthetic parameters (Bch-1, Bur, Mt-0 and
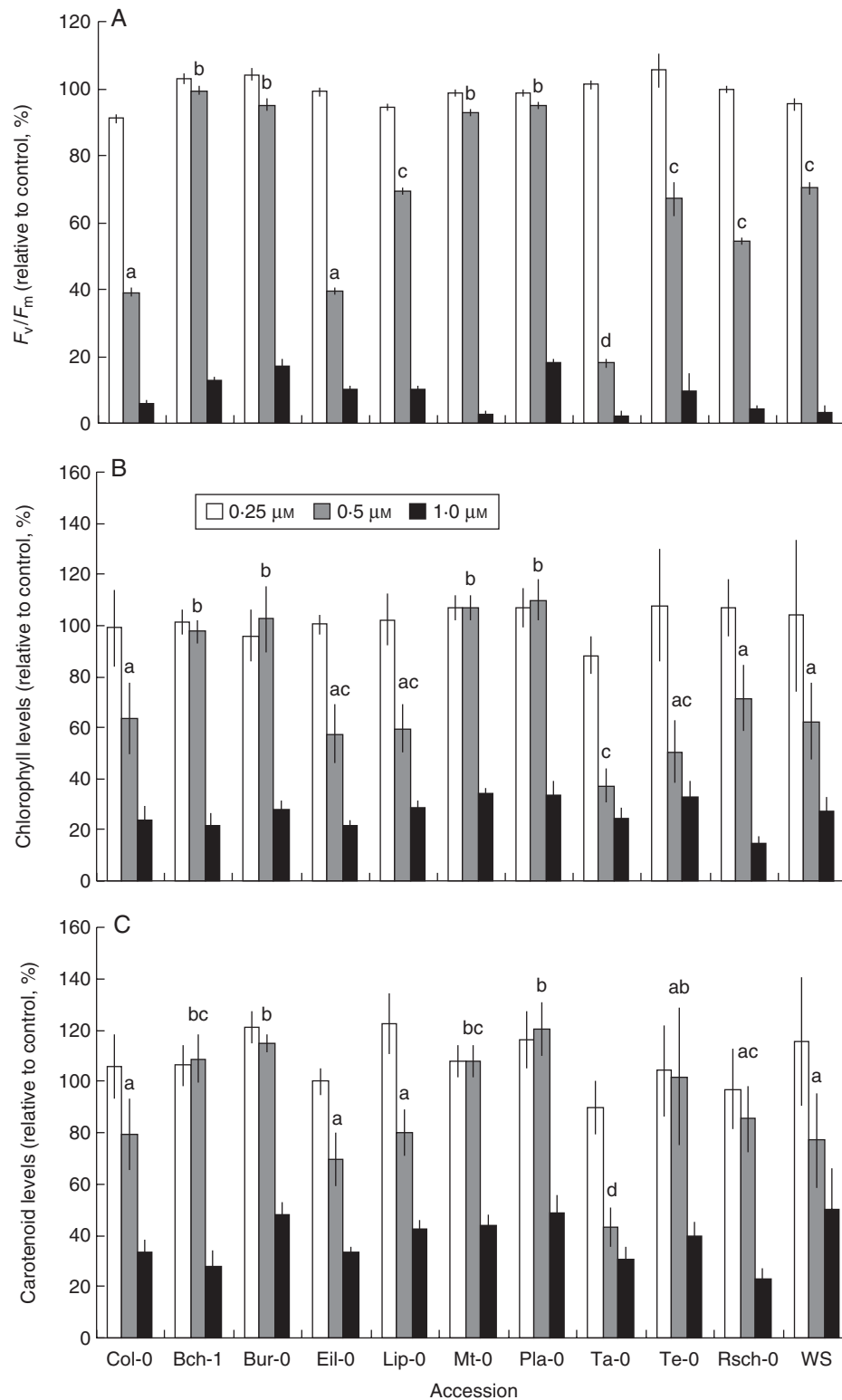
FIG. 1 Effects of atrazine on the photosynthetic apparatus of 1-week-old plantlets of the different accessions germinated and grown in the presence of atrazine. F_v/F_m was determined on whole plantlets (A); chlorophyll (B) and carotenoid (C) levels were measured on shoots. Results (mean ± s.e.m.) are expressed as a percentage relative to growth in the absence of atrazine; treatment concentrations of atrazine are as indicated. Statistical comparison between the means was carried out using one-way ANOVA followed by Duncan's test. Statistical significance of differences ($P \leq 0.05$) between accessions subjected to 0·5 µM atrazine is indicated by different letters above bars.
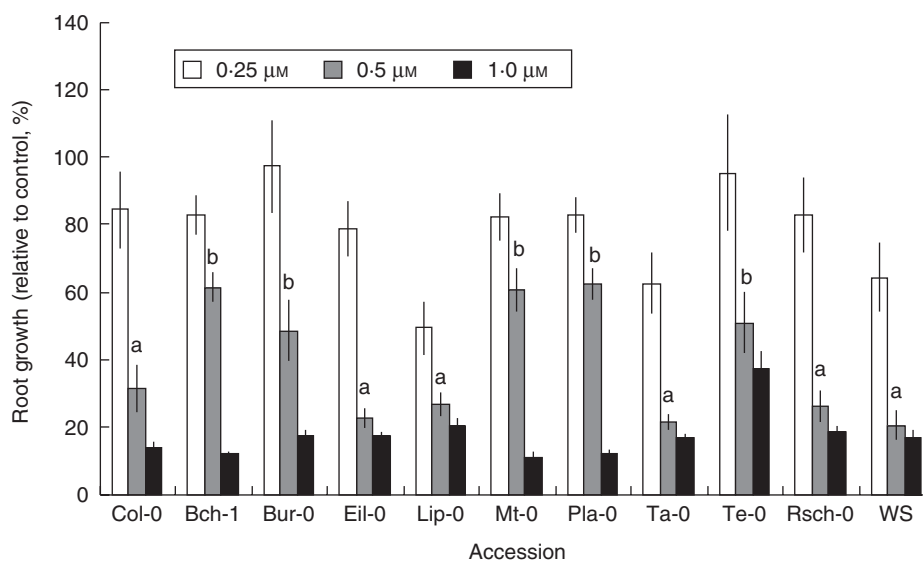
FIG. 2 Effects of atrazine on primary root growth of 1-week-old plantlets of the different accessions germinated and grown in the presence of atrazine. Results (mean ± s.e.m.) are expressed as a percentage of growth relative to control plantlets grown in the absence of atrazine; treatment concentrations of atrazine are as indicated. Statistical comparison between means was carried out using one-way ANOVA followed by Duncan's test. Statistical significance of differences ($P \leq 0.05$) between accessions subjected to 0·5 μM atrazine is indicated by different letters above bars.

Pla-0) presented the highest rates of primary root growth in the presence of 0·5 μM atrazine. All of the other accessions presented greater sensitivity to 0·5 μM atrazine.

Natural variation of xenobiotic sensitivity in Arabidopsis *plantlets after transfer to 10 μM atrazine at the same stage of development*

Transfer of 2-week-old plantlets grown on MS control medium onto 10 μM atrazine-containing medium for 5 d was carried out in order to assess the effects of atrazine on plantlets at the same stage of development. Analysis of chlorophyll fluorescence (Fig. 3A) showed two distinct groups of responses among the different accessions. Accessions that showed low or intermediate sensitivity to atrazine during early development (Bch-1, Bur-0, Mt-0, Pla-0, Te-0 and Rsch-0) were also found to be the least sensitive to transfer onto 10 μM atrazine (Fig. 3A). Analysis of chlorophyll and carotenoid levels (Fig. 3B, C) revealed a wider range of responses to atrazine and contrasted results between the transfer experiment and the direct exposure experiment during germination and post-germination (Fig. 1). The Ta-0 accession showed high sensitivity to atrazine, both during transfer onto 10 μM atrazine and during direct exposure to 0·5 μM atrazine. However, in contrast, the Mt-0 and Pla-0 accessions showed high sensitivity to transfer onto 10 μM atrazine, whereas they showed greater tolerance to 0·5 μM atrazine during germination and post-germination (Fig. 1).

All of this demonstrated differences of responses between experimental modalities and therefore suggested differences of responses in relation to developmental processes. In this transfer experiment, there was no significant difference of primary root growth between the 11 different accessions after 5 d of transfer in the presence of 10 μM atrazine (data not shown).

Natural variation of endogenous soluble sugar levels in Arabidopsis *plantlets*

The quantification of endogenous soluble sugar contents (Glc, Fru and Suc) at the end of day and at the end of night was carried out on shoots of 2-week-old plantlets for the 11 accessions grown on MS–agar medium. It thus corresponded to the carbohydrate status of plantlets prior to transfer in the absence or presence of 10 μM atrazine.

At the end of day, values of shoot total sugars ranged from 175 ng mg^{-1} fresh weight (f. wt) for Lip-0 to 754·6 ng mg^{-1} f. wt for Bur-0 (Table 2). The Lip-0 accession presented the lowest values for Glc, Suc and Fru contents, while the Bur-0 accession showed the highest values for all three soluble sugars. At the end of night, values of shoot total sugars ranged from 198·3 ng mg^{-1} f. wt for Eil-0 to 438 ng mg^{-1} f. wt for Bur-0 (Table 3). For all accessions, the pool of Suc was not entirely depleted at the end of night. Thus, at the end of night and at the end of day, the Bur-0 accession presented the highest level of total sugars in the shoots.

Important natural variation between accessions was therefore found for all soluble sugars in shoots of plantlets at the end of night and at the end of day (Tables 2 and 3), in general agreement with previous studies on rosettes of mature plants (Cross *et al.*, 2006).

Statistical analysis of environmentally and genetically determined variations

Analysis of potential variations of response to atrazine stress between the 11 accessions of *A. thaliana* was carried out by a two-way ANOVA on the whole set of absolute individual values of photosynthetic parameters (F_v/F_m, shoot chlorophyll
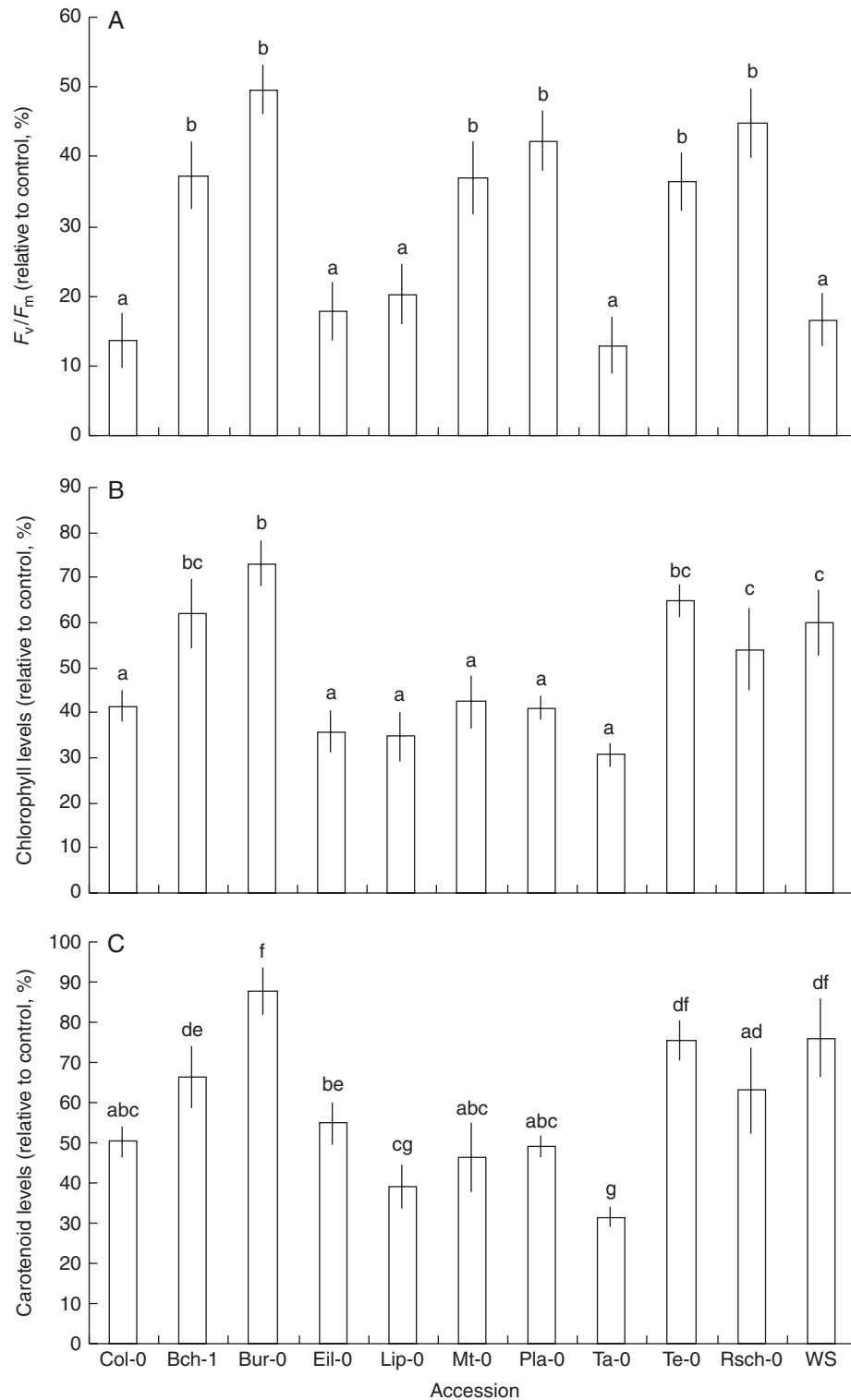
FIG. 3 Effects of atrazine on the photosynthetic apparatus of plantlets of the different accessions grown for 2 weeks in the absence of atrazine and transferred for 5 d to fresh medium in the absence or presence of 10 μM atrazine. F_v/F_m was determined on whole plantlets (A); chlorophyll (B) and carotenoid (C) levels were measured on shoots. Results (mean ± s.e.m.) are expressed as the percentage relative to the values obtained on plantlets of the same genotype transferred for 5 d to control medium in the absence of atrazine. Statistical comparison between means was carried out using one-way ANOVA followed by Duncan's test. Statistical significance of differences ($P \leq 0.05$) between accessions is indicated by different letters above bars.

TABLE 2. *Levels of endogenous soluble sugars at the end of day in plantlets of the different accessions*

Accessions	Glc $\pm$ s.e. (ng mg^{-1} f. wt)	Suc (ng mg^{-1} f. wt)	Fru (ng mg^{-1} f. wt)	Reducing sugars (ng mg^{-1} f. wt)	Total sugars (ng mg^{-1} f. wt)
Bch-1	136·7 $\pm$ 22	90 $\pm$ 10	41·3 $\pm$ 11	178	267·9
Bur-0	445·7 $\pm$ 71	236·9 $\pm$ 35	72·1 $\pm$ 16	517·8	754·6
Col-0	128·1 $\pm$ 20	70·3 $\pm$ 9	17·5 $\pm$ 1	145·5	215·9
Eil-0	262·2 $\pm$ 54	107·5 $\pm$ 17	53·9 $\pm$ 13	316·1	423·6
Lip-0	104·3 $\pm$ 8	56·4 $\pm$ 9	14·2 $\pm$ 2	118·6	175
Mt-0	115·6 $\pm$ 15	78·4 $\pm$ 7	24·3 $\pm$ 5	139·9	218·3
Pla-0	236·1 $\pm$ 39	211·5 $\pm$ 26	72·3 $\pm$ 17	308·4	519·9
Rsch-0	114·9 $\pm$ 14	107 $\pm$ 11	24·1 $\pm$ 3	139	246
Ta-0	182·7 $\pm$ 16	129·4 $\pm$ 20	44·1 $\pm$ 4	226·7	356·1
Te-0	228·1 $\pm$ 25	79·2 $\pm$ 11	42·9 $\pm$ 5	270·9	350·2
Ws	229·4 $\pm$ 55	138·4 $\pm$ 18	70·4 $\pm$ 21	299·8	438·1

Soluble sugar levels were measured as described in the Methods in shoots of plantlets grown for 2 weeks in the absence of atrazine.

TABLE 3. *Levels of endogenous soluble sugars at the end of night in plantlets of the different accessions*

Accessions	Glc $\pm$ s.e. (ng mg^{-1} f. wt)	Suc (ng mg^{-1} f. wt)	Fru (ng mg^{-1} f. wt)	Reducing sugars (ng mg^{-1} f. wt)	Total sugars (ng mg^{-1} f. wt)
Bch-1	131 $\pm$ 21	86·4 $\pm$ 11	21·7 $\pm$ 3	152·7	239·2
Bur-0	308·3 $\pm$ 22	94·6 $\pm$ 7	35·2 $\pm$ 2	343·4	438
Col-0	129·7 $\pm$ 15	101·4 $\pm$ 20	46·7 $\pm$ 7	176·4	277·8
Eil-0	103·3 $\pm$ 6	76·2 $\pm$ 28	18·8 $\pm$ 4	122·1	198·3
Lip-0	101·5 $\pm$ 6	99·6 $\pm$ 16	19 $\pm$ 2	120·5	220·1
Mt-0	134·8 $\pm$ 10	124·7 $\pm$ 15	31·8 $\pm$ 3	166·7	291·4
Pla-0	187·7 $\pm$ 7	65·9 $\pm$ 5	66·2 $\pm$ 5	253·9	319·8
Rsch-0	175·2 $\pm$ 3	82·6 $\pm$ 6	51 $\pm$ 3	226·1	308·7
Ta-0	239·7 $\pm$ 27	33·4 $\pm$ 7	65·1 $\pm$ 8	304·8	338·1
Te-0	229·3 $\pm$ 14	75 $\pm$ 13	65·3 $\pm$ 4	294·5	369·5
Ws	110 $\pm$ 5	91·4 $\pm$ 6	54·2 $\pm$ 4	164·2	255·6

Soluble sugar levels were measured as described in the Methods in shoots of plantlets grown for 2 weeks in the absence of atrazine.

TABLE 4. F *statistics of the variations of physiological parameters in plantlets of the different accessions*

Trait	Genotype effect	Treatment effect	Genotype $\times$ treatment
F_v/F_m	11·88***	2718·39***	8·65***
Chlorophyll content	7·90***	349·42***	4·46***
Carotenoid content	7·25***	207·24***	5·58***

Absolute levels of physiological parameters (F_v/F_m, shoot chlorophyll content and shoot carotenoid content) were measured as described in the Methods in plantlets grown for 2 weeks in the absence of atrazine and transferred for 5 d in the absence or presence of 10 μM atrazine. The whole set of accessions and of data was analysed using a two-way ANOVA with the genotype and treatment as main effects, and with genotype $\times$ treatment as interaction effect. *** $P < 0.001$.

content, shoot carotenoid contents) of the different arabidopsis accessions during transfer of plantlets in the absence or presence of 10 μM atrazine. The F statistics resulting from the ANOVA (Table 4) showed the main effects of genotype and treatment (absence or presence of 10 μM atrazine) and the (genotype $\times$ treatment) interaction effect. The ANOVA showed that the three physiological traits were significantly variable, thus showing significant genetic variation among the set of 11 accessions of *A. thaliana* ($F_{Fv/Fm} = 11·88$, $F_{chlorophyll} = 7·90$, $F_{carotenoid} = 7·25$). Table 4 also indicated highly significant effects of atrazine treatment on all three physiological parameters related to photosynthetic activity. In addition, significant (genotype $\times$ treatment) interactions were found for the three traits, which indicated interaccession differentiation for plasticity (Table 4).

A second analysis, using a one-way ANOVA, was carried out on the whole set of absolute individual values of endogenous soluble sugar levels (Glc, Suc and Fru) at the end of day and at the end of night in shoots of 2-week-old plantlets grown on MS–agar medium (Tables 2 and 3), in order to estimate genetically determined differences of carbohydrate status between accessions (Table 5). The results of this one-way ANOVA showed that all soluble sugar parameters measured at the end of day or at the end of night were significantly variable among the accessions, thus indicating significant genetic variation of carbohydrate status and metabolism (Table 5).

Correlation analysis of biochemical, physiological and stress response parameters

In order to identify potential relationships between biochemical and physiological traits characterizing atrazine tolerance or sensitivity, Pearson correlation analyses were performed on pairs of average values for physiological traits,

which were measured in plantlets grown for 2 weeks in the absence of atrazine and transferred for 5 d in the absence or presence of 10 μM atrazine, and for quantifications of shoot endogenous soluble sugar levels, which were measured in 2-week-old plantlets prior to transfer.

The first Pearson coefficient matrix analysed correlations between physiological traits (F_v/F_m, chlorophyll and carotenoid contents) in the absence or presence of 10 μM atrazine (Table 6). No significant correlation at $P < 0.05$ was found between the level of development and functioning of the photosynthetic apparatus in the absence of atrazine and the response to atrazine stress. Correlations between physiological traits and biochemical parameters (endogenous sugars contents in shoots) in the absence of atrazine were also analysed (Table 7). No significant correlation at $P < 0.05$ was found between parameters of photosynthetic apparatus functioning (F_v/F_m, shoot chlorophyll content, shoot carotenoid content) in the absence of atrazine and parameters of soluble sugar levels in shoots at the end of day or at the end of night (Table 7). In contrast, a significant positive correlation was detected between shoot chlorophyll and carotenoid contents ($R = 0.970$) of plantlets in the absence of atrazine (Table 7). Moreover, Glc, Suc and Fru levels at the end of day in shoots were strongly and positively correlated with each other (Glc vs. Suc, $R = 0.804$; Glc vs. Fru, $R = 0.803$; and Suc vs. Fru, $R = 0.841$). The levels of reducing and total

TABLE 5. *Variation in the biochemical parameters of the different accessions*

Trait	Genotype effect
Glc_{EoD}	7.5***
Suc_{EoD}	10.19***
Fru_{EoD}	3.32**
Glc_{EoN}	19.93***
Suc_{EoN}	2.23*
Fru_{EoN}	15.12**

Absolute sugar contents were measured as described in the Methods in shoots of plantlets grown for 2 weeks in the absence of atrazine. Soluble sugar contents were measured at the end of day (EoD) and at the end of night (EoN). The whole set of accessions and of data was analysed using a one-way ANOVA. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

TABLE 6. *Correlation matrix (Pearson coefficients) of physiological traits in the absence or presence of 10 μM atrazine*

	With atrazine		
	F_v/F_m	Chlorophyll content	Carotenoid content
Without atrazine			
F_v/F_m	0.391	0.167	0.176
Chlorophyll content	0.378	0.292	0.217
Carotenoid content	0.283	0.215	0.138

Absolute levels of physiological traits (F_v/F_m, shoot chlorophyll content and shoot carotenoid content) were measured as described in the Methods in plantlets grown for 2 weeks in the absence of atrazine and transferred for 5 d in the absence or presence of 10 μM atrazine. The whole set of accessions and of data was analysed. No significant correlation at $P < 0.05$ was found.

TABLE 7. *Correlation matrix (Pearson coefficients) of endogenous sugar levels and of physiological traits after transfer in the absence of atrazine*

	F_v/F_m	Chlorophyll content	Carotenoid content	Glc_{EoD}	Suc_{EoD}	Fru_{EoD}	Reducing sugars$_{EoD}$	Total sugars$_{EoD}$	Glc_{EoN}	Suc_{EoN}	Fru_{EoN}	Reducing sugars$_{EoN}$	Total sugars$_{EoN}$
F_v/F_m	–												
Chlorophyll content	–0.298	–											
Carotenoid content	–0.327	0.970***	–										
Glc_{EoD}	0.223	–0.202	–0.185	–									
Suc_{EoD}	0.078	0.332	0.316	0.804**	–								
Fru_{EoD}	0.101	0.028	0.024	0.803***	0.841***	–							
Reducing sugars$_{EoD}$	0.207	–0.166	–0.152	0.994***	0.835***	0.864***	–						
Total sugars$_{EoD}$	0.171	–0.002	0.002	0.967***	0.924***	0.890***	0.982***	–					
Glc_{EoN}	–0.072	0.248	0.297	0.650*	0.635*	0.401	0.623*	0.651*	–				
Suc_{EoN}	–0.383	–0.288	–0.315	–0.168	–0.289	–0.353	–0.472	–0.192	–0.394	–			
Fru_{EoN}	–0.533	0.396	0.376	0.086	0.309	0.295	0.646*	0.537*	0.468	–0.545	–		
Reducing sugars$_{EoN}$	–0.191	0.310	0.348	0.582	0.623*	0.417	0.569	0.609*	0.977***	–0.472	0.646*	–	
Total sugars$_{EoN}$	–0.085	0.249	0.282	0.592	0.597	0.347	0.565	0.597	0.977***	–0.192	0.537	0.956***	–

Absolute levels of physiological traits (F_v/F_m, shoot chlorophyll content and shoot carotenoid content) were measured as described in the Methods in plantlets grown for 2 weeks in the absence of atrazine and transferred for 5 d to control medium in the absence of atrazine. Absolute levels of endogenous sugars were measured as described in the Methods in shoots of plantlets grown for 2 weeks in the absence of atrazine. Soluble sugar contents were measured at the end of day (EoD) and at the end of night (EoN). The whole set of accessions and of data was analysed. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

sugars were also highly correlated with all of the individual sugar levels. In contrast, at the end of night, no significant correlation was found between the different kinds of soluble sugars. Thus, whereas the level of total sugars at the end of day was correlated with levels of all three soluble sugars, the level of total sugars at the end of night was significantly correlated with Glc level only (Table 7). Finally, low correlations were detected between sugar levels at the end of day and sugar levels at the end of night. Glc levels at the end of day and at the end of night ($R = 0.650$), but not Fru or Suc levels, were significantly correlated. Levels of reducing and total sugars, whether at the end of day or at the end of night, also tended to be correlated, with $R = 0.569$ and $R = 0.597$, respectively

Correlations between biochemical parameters and physiological traits in the presence of 10 μM atrazine are presented in Table 8. Physiological parameters of atrazine-treated plantlets were found to show significant correlations between endogenous sugar parameters of plantlets prior to atrazine treatment. Indeed, the shoot carotenoid content was significantly and positively correlated with the level of Suc in shoots at the end of day ($R = 0.639$) and with the level of total sugars in shoots at the end of day ($R = 0.596$). Moreover, even if correlations between Suc level at the end of day and the other physiological traits were not significant, R-values were higher than those related to other sugar parameters at the end of day (Table 8). Physiological traits were not found to be correlated with the level of Suc at the end of night, but significant correlations were found with total sugar level at the end of night (total sugar $_{EoN}$ vs. chlorophyll content, $R = 0.615$; total sugar $_{EoN}$ vs. carotenoid content, $R = 0.626$). Moreover, there was a positive trend between Glc level at the end of night and the three physiological traits, with Pearson coefficients close to significance

TABLE 8. *Correlation matrix (Pearson coefficients) of endogenous sugar levels and of physiological traits after transfer in the presence of atrazine*

	F_v/F_m	Chlorophyll content	Carotenoid content
F_v/F_m	–		
Chlorophyll content	0·884***	–	
Carotenoid content	0·862***	0·970***	–
Glc$_{EoD}$	0·342	0·343	0·527
Suc$_{EoD}$	0·467	0·502	0·639*
Fru$_{EoD}$	0·247	0·353	0·518
Reducing sugars$_{EoD}$	0·335	0·355	0·542
Total sugars$_{EoD}$	0·393	0·419	0·596*
Glc$_{EoN}$	0·503	0·537	0·552
Suc$_{EoN}$	0·204	0·110	0·125
Fru$_{EoN}$	0·014	0·231	0·197
Reducing sugars$_{EoN}$	0·438	0·520	0·524
Total sugars$_{EoN}$	0·556	0·615*	0·626*

Absolute levels of physiological traits (F_v/F_m, shoot chlorophyll content and shoot carotenoid content) were measured as described in the Methods in plantlets grown for 2 weeks in the absence of atrazine and transferred for 5 d to control medium in the presence of 10 μM atrazine. Absolute levels of endogenous sugars were measured as described in the Methods in shoots of plantlets grown for 2 weeks in the absence of atrazine. Soluble sugar contents were measured at the end of day (EoD) and at the end of night (EoN). The whole set of accessions and of data was analysed. * Significant correlation at $P < 0.05$, *** $P < 0.001$.

(Table 8). Finally, in the presence of 10 μM atrazine, there were high positive correlations between all the photosynthetic parameters. Indeed, F_v/F_m was correlated with chlorophyll and carotenoid contents with, respectively, Pearson coefficients of $R = 0.884$ and $R = 0.862$, while chlorophyll and carotenoid levels were correlated with a Pearson coefficient of $R = 0.970$.

Principal component analysis

In order to classify accessions according to their response to atrazine and to their carbohydrate status, a PCA was carried out on the whole set of average values for endogenous soluble sugar levels in shoots prior to transfer and for physiological traits after transfer in the absence or presence of 10 μM atrazine.

Figure 4 shows that the first factorial plane, defined by PC1 and PC2 axes, respectively, accounted for 41·1 and 28·2 % of the observed variation, in the absence of atrazine (Fig. 4A), and for 52·2 and 22·1 % of the observed variation, in the presence of 10 μM atrazine (Fig. 4B).

As shown in Fig. 4C by the correlation circle, in the absence of atrazine, the first axis was essentially explained by shoot Suc level at the end of day, shoot Fru level at the end of day and shoot Glc level at the end of night, and the second axis was essentially explained by the three physiological traits and by shoot Glc level at the end of day. The localization of Ta-0, Rsch-0, Mt-0 and Pla-0 (Fig. 4A) indicated high levels of chlorophyll and carotenoid in shoots of these three accessions in the absence of atrazine, in contrast to Bur-0, which showed the opposite situation, and was characterized by a high level of Glc at the end of day. The other accessions had a central position characterized by intermediate values.

As shown in Fig. 4D by the correlation circle, in the presence of atrazine, the first axis was essentially explained by the physiological traits and sugar contents, except for the Fru and Suc level in shoots at the end of night. However, the variable that most contributed to this axis was the shoot Suc level at the end of day. The second axis was mainly explained by the shoot Suc level at the end of night. Bur-0, Pla-0 and Ta-0 were found to be at outlying positions (Fig. 4B). Bur-0 and Pla-0 were characterized by a high level of Suc at the end of day in shoots and low sensitivity to atrazine, while Ta-0 presented a very low level of Suc in shoots at the end of night and a high level of Fru in shoots at the end of night. The group of Rsch-0, Bch-1 and Mt-0 accessions was characterized by high levels of Suc at the end of night. Another group, including Eil-0, Col-0 and Lip-0, was characterized by low sugar levels and high sensitivity to atrazine (Fig. 4B). The central position of Te-0 and Ws accessions revealed intermediate values for all of the measured parameters.

Natural variation of atrazine-induced ROS accumulation in arabidopsis plantlets

A 5-d treatment in the presence of 10 μM atrazine reveals major injuries of the photosynthetic apparatus and of the root system under conditions of atrazine sensitivity. However, earlier modifications, such as ROS accumulation, may determine the eventual sensitivity or tolerance to atrazine. In order to determine whether endogenous variations of
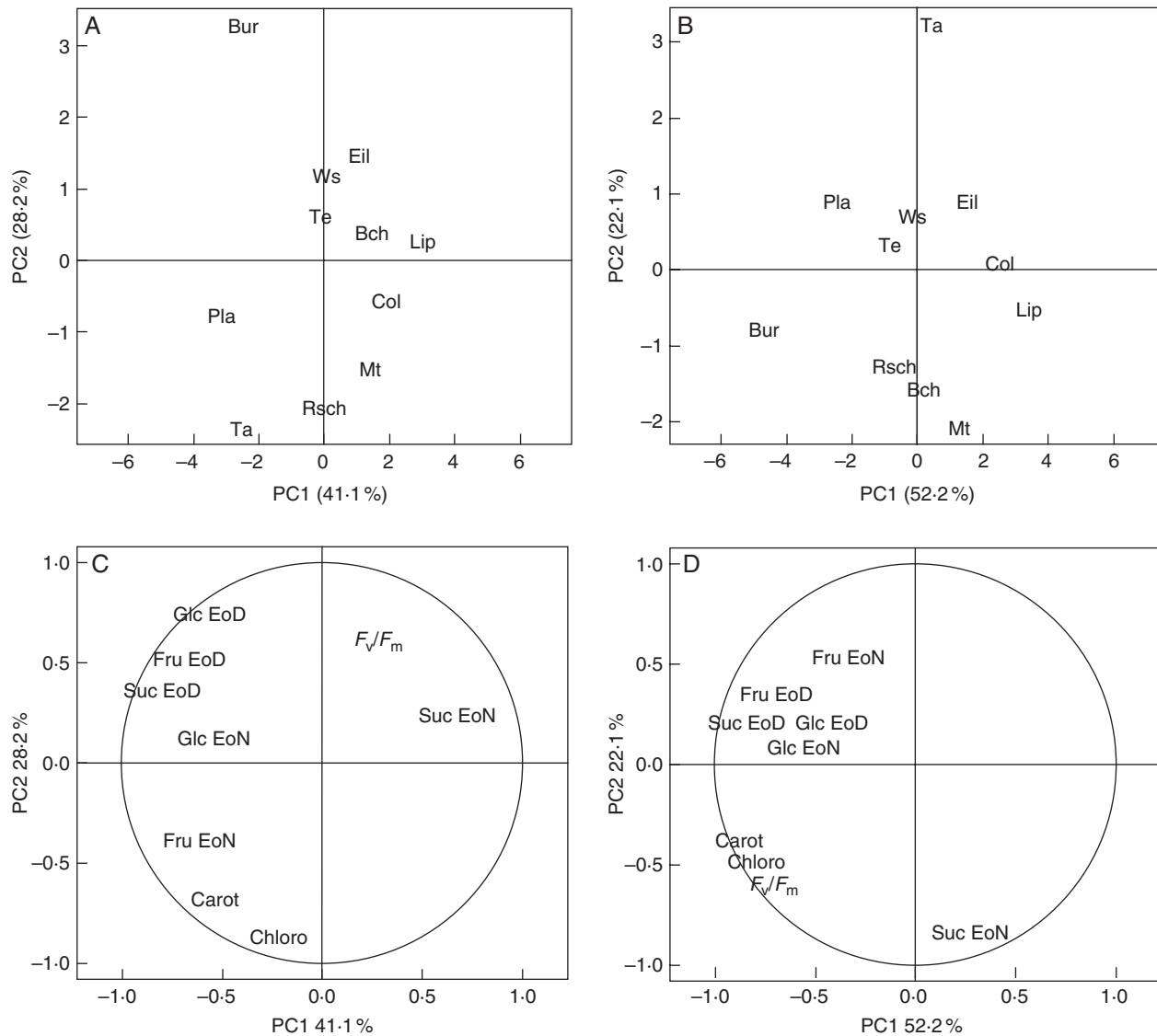
FIG. 4 Principal component analysis of endogenous soluble sugars and xenobiotic stress responses of *Arabidopsis thaliana* accessions. Principal component analysis was performed on the correlation matrix of averages of biochemical parameters [absolute levels of endogenous soluble sugars in shoots at the end of day (EoD) and at the end of night (EoN)] and of physiological traits (absolute levels of F_v/F_m, shoot chlorophylls and shoot carotenoids) measured on the 11 accessions. Soluble sugars were measured in shoots of 2-week-old plantlets prior to atrazine treatment (Tables 2 and 3) and physiological traits were measured after 5 d of transfer onto fresh medium in the absence or presence of 10 μM atrazine. (A) and (B) represent the repartition of accessions in the absence or presence of 10 μM atrazine, respectively. (C) and (D) represent the distribution of variables on the first plane (PC1 and PC2) in the absence or presence of 10 μM atrazine, respectively.

soluble sugars were related to differences in the accumulation of ROS, 2-week-old *A. thaliana* plantlets were transferred to MS medium in the absence or presence of 10 μM atrazine for 48 and 72 h. *In vivo* detection of hydrogen peroxide and superoxide anion was carried out on plantlets of the different accessions, which showed a continuum of responses (data not shown). Typical accessions, Bur-0, Pla-0, Eil-0, Col-0 and Lip-0, which showed constrasted ROS accumulation in response to atrazine, are shown in Figs 5 and 6.

Superoxide anion ($O_2 \cdot^-$) detection was performed using the NBT staining method as described in Materials and methods. In the absence of atrazine, the levels of superoxide anion were variable between the five accessions under study (Fig. 5). Although accumulation of superoxide was detectable

at 48 and 72 h for all of the accessions, the basal levels of superoxide in plantlets grown on MS medium were low in Eil-0 and Lip-0 accessions, intermediate in Col-0, and high in Bur-0 and Pla-0 accessions. The presence of 10 μM atrazine led to a strong decrease of superoxide content, which, in atrazine-sensitive plantlets (Eil-0, Col-0 and Lip-0), was more accentuated at 72 h than at 48 h. In contrast, plantlets of accessions (Bur-0 and Pla-0) showing lower sensitivity to atrazine succeeded in maintaining a significant level of superoxide content in the presence of atrazine.

H_2O_2 detection was performed using the DAB staining method as described in Materials and methods. All of the five accessions showed H_2O_2 accumulation in the absence or presence of atrazine (Fig. 6). After 48 h of transfer in the
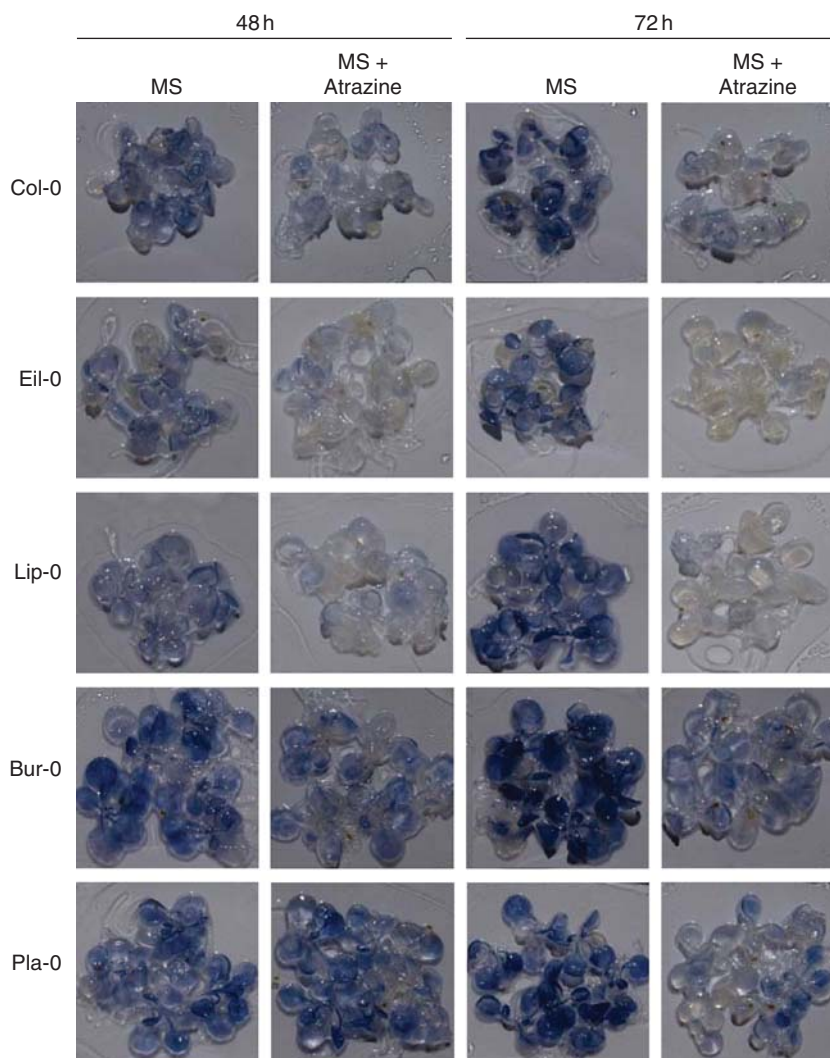
FIG. 5 Analysis of superoxide anion accumulation by NBT staining in plantlets of different accessions grown for 2 weeks in the absence of atrazine and transferred for 48 or 72 h in the absence or presence of 10 μM atrazine.

absence of atrazine, H_2O_2 contents were low, and little variation was observed between the five accessions, except for Col-0 and Eil-0 plantlets, which showed slightly higher H_2O_2 basal content. A slight discoloration was observed between 48 and 72 h of control treatment for all accessions, except for Eil-0. The presence of atrazine was found to induce accumulation of H_2O_2 at 48 and 72 h of treatment, with higher H_2O_2 contents in plantlets of atrazine-sensitive accessions (Eil-0, Lip-0 and Col-0) than in plantlets of accessions (Bur-0 and Pla-0) showing lower atrazine sensitivity.

DISCUSSION

Natural variation of absolute levels of shoot endogenous soluble sugars under non-stress conditions

Recent studies have identified and characterized natural variation of carbohydrate contents and metabolic activities in Arabidopsis under non-stress conditions (Calenge *et al.*, 2006;

Cross *et al.*, 2006; Davey et al. 2008). Thus, carbohydrate contents in rosette leaves were highly variable among recombinant inbred lines (Calenge *et al.*, 2006) or among natural accessions (Cross *et al.*, 2006). These differences of carbohydrate contents may be related to variations of photosynthetic activity, variations of carbon allocation between storage, growth or respiration, or variations of carbon utilization. The Bur-0 accession has thus been characterized, among a set of 24 accessions, by high sugar content, high starch content and low amino acid content in rosette leaves, thus indicating an imbalance between C and N metabolism (Cross *et al.*, 2006). However, Cross *et al.* (2006) pointed out that, depending on light and mineral nutrition conditions, growth and development could be correlated with absolute carbohydrate levels (Calenge *et al.*, 2006), or with enzyme activities and metabolic fluxes in central C and N metabolism (Cross *et al.*, 2006). The set of natural accessions in the present work was chosen from these previous publications on potential relationships between variations of endogenous sugar contents and growth and development.
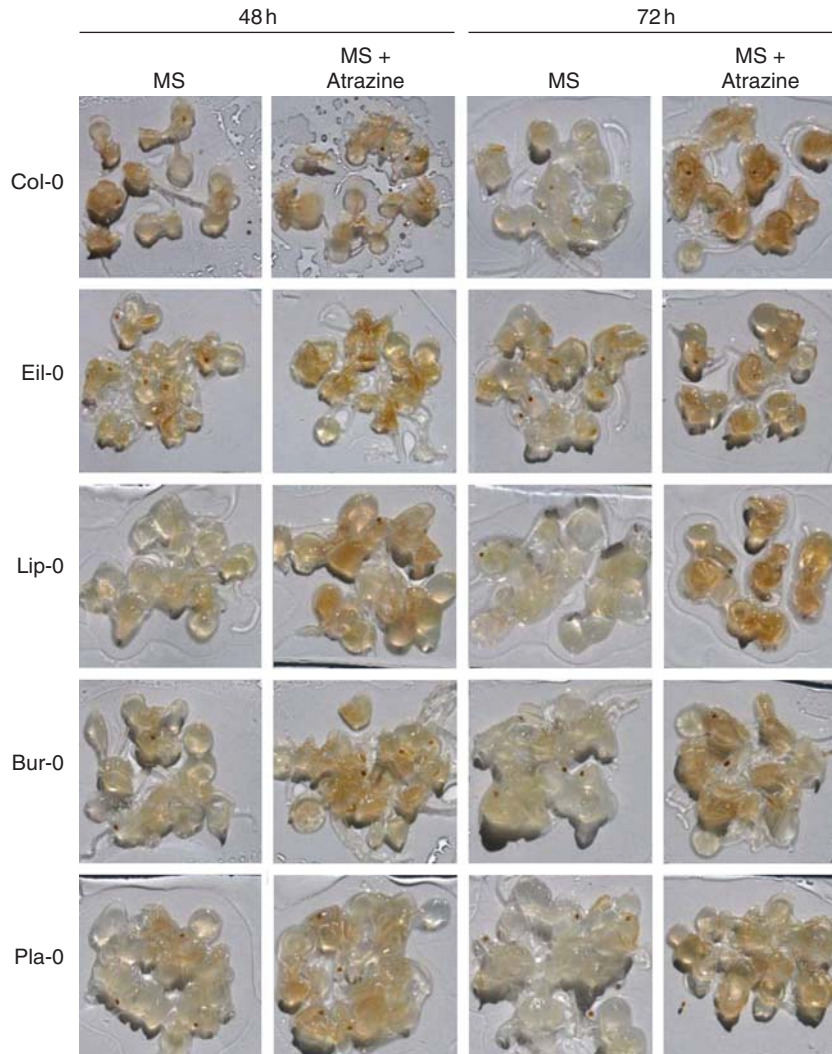
Fɪɢ. 6 Analysis of hydrogen peroxide accumulation by DAB staining in plantlets of different accessions grown for 2 weeks in the absence of atrazine and transferred for 48 or 72 h in the absence or presence of 10 μM atrazine.

Although growth conditions involved a 16 h daylength, the low level of light was likely to result in carbon limitation. These conditions (low light, long day) were identical to those of our previous studies (Ramel *et al.*, 2007, 2009), where carbon limitation was used to highlight the effects of exogenously added sucrose. Under these conditions, the different accessions showed contrasted patterns of night–day fluctuations of soluble sugar levels (Tables 2 and 3), with some accessions, such as Col-0, showing no variation between end of day and end of night soluble sugar levels. Previous studies (Matt *et al.*, 1998; Gibon *et al.*, 2004) had indicated that fluctuations of soluble sugar levels at the end of day and at the end of night depend on light growth conditions. Parallel analysis of low light–long day and higher light–long day conditions was therefore carried out, and revealed that Col-0 plantlets under higher light conditions showed normal fluctuations of low and high levels of soluble sugars, respectively, at the end of night and at the end of day (data not shown). Further work should determine

whether this unexpected lack of night–day fluctuations of soluble sugar levels under low light, carbon-limiting conditions is compounded at early stages of development of the photosynthetic apparatus, and of carbon allocation and utilization pathways.

At any rate, growth under low light–long day conditions was useful to enhance differences of endogenous sugar levels between accessions, and for the comparison of results with the effects of exogenous sugars (Ramel *et al.*, 2007, 2009). Moreover, the study of plantlets under conditions of low light is likely to be relevant to environmental situations where xenobiotic application may affect seedling establishment during springtime.

Absolute carbohydrate levels in shoots, whether at the end of day or at the end of night, were not found to be correlated with PSII efficiency (F_v/F_m), chlorophyll levels or carotenoid levels in plantlets under non-stress conditions (Table 7). This may agree with the absence of correlation between soluble carbohydrate levels and growth (Cross *et al.*, 2006). On the

other hand, the analysis of superoxide content in plantlets of the different accessions grown on control medium showed higher levels of superoxide in the Bur-0 accession (Fig. 5). Superoxide radical is formed mainly by reduction of oxygen at the PSI site (Asada *et al.*, 1974), with oxygen serving as an electron acceptor. Higher levels of superoxide may thus reflect higher activity in PSI, and could be interpreted as a result of higher cyclic photophosphorylation activity in the Bur-0 accession.

Natural variation of pre-stress endogenous soluble sugars is related to major traits of xenobiotic and oxidative stress tolerance

The protective roles of soluble sugars under different kinds of stress conditions (Sulmon *et al.*, 2004; Loreti *et al.*, 2005; Gomez-Ariza *et al.*, 2007; Ramel *et al.*, 2007; Banti *et al.*, 2008; Morkunas and Bednarksi, 2008) have been demonstrated by exogenous sugar pre-treatment or by continuous supply of exogenous sugars. However, endogenous Suc levels in Arabidopsis leaves vary between 1 and 3 mM (Gonzali *et al.*, 2006), and are thus lower than Suc levels resulting from exogenous sugar treatments (Graham *et al.*, 1994; Martin *et al.*, 2002; Sulmon *et al.*, 2004; Loreti *et al.*, 2005; Ramel *et al.*, 2007). It thus remained unclear whether endogenous levels of soluble sugars played an important role in stress responses.

The different modalities of atrazine treatment revealed significant differences of sensitivity to various atrazine concentrations between the different accessions (Figs 1–3). Several accessions, such as the Bur-0 accession, were much less sensitive to atrazine stress. Moreover, the present set of natural accessions, chosen on the basis of their varying sugar contents, pointed to a qualitative link between pre-stress endogenous sugar levels in shoots and the response of plantlets to subsequent xenobiotic stress. Accessions showing the highest basic levels of endogenous sugars were generally less sensitive to atrazine stress. Statistical analysis (Table 8) revealed consistent trends and significant correlations between improved tolerance to atrazine treatment and various traits of carbohydrate metabolism, especially the level of total sugars at the end of the night, the level of total sugars at the end of the day and the level of sucrose at the end of the day.

A recent study (Korn *et al.*, 2008) has demonstrated a strong relationship between freezing tolerance and the overaccumulation of different sugars as compatible solutes in *A. thaliana* accessions. The present work outlines a novel kind of relationship, between pre-stress carbohydrate metabolism and subsequent xenobiotic and oxidative stress, that does not involve overaccumulation of compatible solutes.

This relationship may have been enhanced by the carbon-limiting conditions of low light that were applied to plantlets, and may be less pronounced under high light conditions. Nevertheless, situations of multiple stresses, such as combination of low light and abiotic stressors, are relevant to environmental conditions (Mittler, 2006). Thus, the demonstration of this relationship between endogenous carbohydrate metabolism and stress response points to an important integration of nutritional status and stress tolerance in plantlets.

Involvement of pre-stress endogenous soluble sugars in xenobiotic stress tolerance

Parallel transcriptomic and biochemical analysis of control, atrazine alone, Suc alone and atrazine plus Suc treatments had shown important interactions of atrazine and Suc effects, with early induction of specific transcription factors (Ramel *et al.*, 2007, 2009). In these previous studies, the effects of immediate atrazine exposure interacted with endogenous sugar levels that were determined by the exogenous treatment. It could therefore be reasoned that, in the present work, where pre-stress endogenous sugar levels were correlated with subsequent atrazine tolerance, atrazine first interacted with pre-existing endogenous sugar levels, and that the effects of this primary interaction were important for induction and further development of tolerance mechanisms.

Atrazine-related stress is light and photosynthesis dependent, and involves imbalance of ROS dynamics, with accumulation of singlet oxygen (Rutherford and Krieger-Liszkay, 2001; Ramel *et al.*, 2009), a decrease of superoxide levels (Ramel *et al.*, 2009) and accumulation of hydrogen peroxide (Ramel *et al.*, 2009). The differences between accessions in terms of pre-stress shoot endogenous sugar levels and of stress responses were shown to be linked to important effects on hydrogen peroxide and superoxide dynamics (Figs 5 and 6). Thus, H_2O_2 accumulation in the presence of atrazine (Fig. 6) was decreased in accessions that were the least sensitive to atrazine. Analysis of superoxide radical levels (Fig. 5) also showed that superoxide levels were maintained in the accessions that were the least sensitive to atrazine. Atrazine binding to D1 protein of PSII and inhibition of electron feeding to PSI block oxygen partial reduction at the PSI level and decrease superoxide radical production. The maintenance of superoxide radical levels in the Bur-0 and Pla-0 accessions could thus be ascribed to maintenance of photosynthetic activity in the presence of atrazine. All of these effects on ROS dynamics in accessions with various pre-stress endogenous sugar levels in shoots were similar to the positive effects of exogenous Suc on ROS dynamics (Ramel *et al.*, 2009), and were in agreement with the importance of pre-stress carbon nutritional status, as discussed above.

Defence systems are generally thought to be costly in terms of carbon and energy investment (Rhoades, 1979). Stress responses and response-associated costs are thus likely to increase only when the benefits outweigh the cost of tolerance response (Zangerl and Bazzaz, 1992; Karban and Baldwin, 1997). It is reasonable to assume that efficient investment in stress responses requires decision-like mechanisms, whereby the plant can sense available carbon resources and reorchestrate metabolic and stress defence processes in response to environnemental cues. The positive effects of pre-stress endogenous sugar levels on tolerance to atrazine may be related to such mechanisms.

Pre-stress Suc level was the only single soluble sugar parameter that was found to be positively correlated with greater atrazine tolerance (Table 8). Suc, as a central metabolite between photosynthesis, carbon transport and carbon utilization (Salerno and Curatti, 2003), plays an important role in the regulation of carbon allocation (Chiou and Bush, 1998). Exogenous treatment studies have also shown that, in the

tolerance to anoxic stress (Loreti *et al.*, 2005) and to atrazine stress (Sulmon *et al.*, 2004, 2006), Suc treatment was much more efficient than Glc treatment. Finally, exogenous Suc has been shown to induce in a specific way a number of stress-related genes (Solfanelli *et al.*, 2006). These Suc-specific processes may be due to Suc-specific signalling pathways, which remain to be characterized (Couée *et al.*, 2006), or to better uptake or utilization of exogenous Suc, when compared with Glc (Banti *et al.*, 2008).

Analysis of natural endogenous variations avoids such differential effects related to uptake and metabolic utilization of exogenous molecules. The specificity of Suc among the various endogenous biochemical traits of carbohydrate metabolism therefore established the importance of Suc as an endogenous player of stress defence regulations and tolerance. It further showed that the correlation with improved atrazine tolerance could not be primarily ascribed to intrinsic reducing properties of soluble sugars, since Suc is a non-reducing disaccharide. The correlation between pre-stress Suc level at the end of the day and greater atrazine tolerance may therefore reflect adaptive mechanisms that link Suc accumulation, photosynthesis-related stress and induction of stress defences. Characterization of the natural variation of these mechanisms will imply further work involving quantitative trait locus analysis.

Differential impact of xenobiotics on natural plant populations

Because of its widespread use and of its persistence, atrazine has been detected as a common contaminant in soils, streams, rivers and lakes (Solomon *et al.*, 1996; Clark *et al.*, 1999). Numerous studies have shown the effects of this herbicide on reduction of photosynthesis, chlorophyll synthesis, cell growth and nitrogen fixation in aquatic photosynthetic communities (Millie and Hersh, 1987; Hersh and Crumpton, 1989; Weiner *et al.*, 2007).

Atrazine pollution can range from 0·5 nM to 0·14 μM in surface waters of river and urban basins, and peaks ranging from 0·3 to 1·4 μM can be reached in agricultural basins (Weiner *et al.*, 2007). In the present study, sub-lethal or lethal atrazine concentrations were in the range (from 0·25–1 μM) of runoff pollutions in agricultural basins, or at the level of acute lethal exposure (10 μM).

Natural variation of sensitivity in Arabidopsis accessions (Figs 1–3) was shown to occur under all of these concentrations. The lowest atrazine concentration of 0·25 μM negatively affected primary root growth of plantlets (Fig. 2). The different accessions also showed important differences of sensitivity during exposure to a high, lethal, atrazine concentration (Fig. 3). It thus seemed that even a low level of runoff atrazine pollution could have an impact on seedling establishment of natural populations, and exert selective effects on specific genotypes. Since such levels of pollution, or even higher levels of pollution, may occur in basins of intensive agriculture, further work is required in order to determine whether herbicide runoff pollution could have large-scale effects on the population dynamics of wild plant species, and what consequences such effects could have on the functioning of terrestrial ecosystems.

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