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ORIGINAL ARTICLE

Fitness costs linked to dinitroaniline resistance mutation in *Setaria*

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A mutant Thr-239-Ileu at the α2-tubulin gene was found to confer resistance to dinitroanilines, a family of mitosis-disrupting herbicides. However, mutations affecting microtubule polymerization and cell division are expected to impact growth and reproduction, that is, the fitness of a resistant weed or the yield of a tolerant crop, although it has not been demonstrated yet. This study was designed to test this hypothesis for the growth and reproduction of near-isogenic resistant and susceptible materials that were created in F_2 and F_3 generations after a *Setaria viridis* x *S. italica* cross. Differential growth was noticeable at the very onset of seedling growth. The homozygous resistant plants, grown both in a greenhouse cabinet and in the field, were smaller and had lower 1000-grain weight and therefore a lower yield. This fitness penalty is certainly due to modified cell division kinetics. Although the presence of the mutant allele accounted for 20% yield losses, there were also measurable benefits of dinitroaniline resistance, and these benefits are discussed.
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Keywords: dinitroaniline; herbicide resistance; yield; fitness cost; cell division; tubulin

Introduction

In addition to the genetically engineered crops that now cover hundreds of millions of hectares, several commercialized herbicide-resistant varieties have been generated through 'conventional' methods (Duke, 1996). With the exception of a few cases of gene amplification (Caretto *et al*., 1994), they originated in mutant germplasms obtained through tissue culture selection, seed or microspore mutagenesis, and even from wild relatives (Tan *et al*., 2005). Nothing is known about the side effects of these genes in crops. Some insight can be gleaned from the incidence of genes captured from wild relatives; they are often at very low frequency in natural and weed populations, indicating that they suffer some fitness cost in field conditions (see Vila-Aiub *et al*., 2009 for review of fitness costs linked to herbicide resistance in weeds). When expressed in crops, there could be some yield costs to these modifications that would offset the obvious benefits, such as being able to use appropriate herbicides and to ensure weed-free crop growth. The production of novel or altered proteins could hinder overall metabolism or have pleiotropic effects. For instance, a yield penalty has clearly been observed with triazine-resistant lines of oilseed rape and foxtail millet, with a 19–25% reduction in grain yield when compared with isogenic-susceptible lines grown without the use of herbicide (Beversdorf *et al*., 1988; Darmency and Pernès, 1989). In contrast, no difference was found between Acetolactate Synthase (ALS)-inhibitor (imidazolinone)-resistant and near-isogenic susceptible lines of maize and sunflower (Boerboom and Lauer, 1997; Massinga *et al*., 2005). In *Arabidopsis thaliana*, the difference depended on nutriment availability (Purrington and Bergelson, 1997). The yield penalty question does not appear to be of great general concern, as research into this question has not been yet been reported in the academic literature for commercialized varieties of wheat, oilseed rape, rice, lentil or flax that possess the same ALS resistance mutation (Tan *et al*., 2005). Therefore, it is not clear whether herbicide resistance genes always have negative pleiotropic effects or whether herbicide-resistant mutated lines always have lower yield potential.

In this paper, we focus on an α2-tubulin mutant gene that confers resistance to dinitroaniline herbicides. It is a good candidate for creating new herbicide-resistant germplasm in cereals. Attempts to improve maize using this mutant gene have been performed through classical breeding (Landi *et al*., 1999) and through genetic engineering (Anthony and Hussey, 1999). Development of trifluralin-resistant transgenic material has also been proposed, both to improve weed control in the fields and to use as a marker gene (Anthony *et al*., 1999; Yemets and Blume, 2007). Trifluralin, a mitosis-disrupting herbicide belonging to the dinitroaniline family, has been used widely in cotton, soybeans, oilseed and wheat since the 1970s (Ashton and Crafts, 1981). A total of 10 weed species have evolved trifluralin resistance in response to repeated use of these compounds in the fields. These are primarily grass species, along with two rare dicotyledonous species (Heap, 2010). Fitness costs are expected because any mutation on tubulin genes could affect microtubule polymerization and function, and microtubules have a key role in cell replication. Such a feature has been observed for many mutations that confer dinitroaniline resistance in Protozoa (Ma *et al*., 2007).

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Few studies have been carried out to investigate the fitness consequences of dinitroaniline resistance in weeds, and none have involved isogenic plant material. In goosegrass (*Eleusine indica*), there were no clear-cut differences between the two types in transplant experiments, although the resistant type often had lower inflorescence dry weight than the susceptible type (Murphy *et al*., 1986), especially under competitive conditions (Valverde *et al*., 1988; Harris *et al*., 1995). In annual bluegrass (*Poa annua*), resistant plants had lower vegetative growth but there was no consistent difference in reproduction between the two types (Lowe *et al*., 2001). Finally, in green foxtail (*Setaria viridis*), high persistence of the trifluralin-resistant type in non-treated fields over several years could support that the two types of this species have equivalent fitness (Andrews and Morrison, 1997).

We crossed resistant green foxtail plants with foxtail millet to obtain a trifluralin-resistant germplasm (Wang *et al*., 1996). Herbicide-resistant foxtail millet, *Setaria italica* (L.) Beauv., an autogamous small grain C4 cereal, was in demand because of the lack of selective herbicides and because hand weeding had become impractical for farmers. The mutation responsible for resistance was later identified as a threonine/isoleucine at residue 239 of the α2-tubulin gene (Délye *et al*., 2004). Here, we report on the impact of this α2-tubulin mutant gene on foxtail millet yield. For the first time, near-isogenic plants were used and they showed an important yield penalty. The near-isogenic material was obtained in the segregating F_2 of the initial crosses and F_3 generation of a subsequent selection and breeding scheme. In each generation, we compared resistant and susceptible plants that, on average, shared the same genetic background, with the exception of the group of linkages in which the α2-tubulin gene is located. We compared plants using field experiments and a growth cabinet experiment designed to check fine differences at the early seedling stage.

Materials and methods

Plant material

Plants from the 'Oak River' population of *Setaria viridis* (L.) Beauv. that had evolved resistance to a dinitroaniline herbicide (trifluralin) in Canada (Morrison *et al*., 1989) were crossed to the Amende-4 cultivar of *S. italica* (L.) Beauv., a spring cv. from Heilongjiang, China (Wang *et al*., 1996). Resistance was conferred by a single-gene recessive mutation in the wild parent (Jasieniuk *et al*., 1994). Some F_2 seeds collected on the F_1 were used in the garden experiment (indicated in boxes in Figure 1). Other F_2 seeds were subjected to herbicide treatment (see below) to select homozygous resistant F_2 plants. They were subsequently self-pollinated, and their progeny were selected for growth habit and ear morphology corresponding to that of the crop parent (Amende-4) over two generations. Subsequent F_4 plants showed little variability, and were used as pollen donors in a cross with one of the most popular cv. in China, Jigu 11, a summer cv. from Hebei. F_2 grains from this last cross were germinated on a trifluralin medium (see below) to select the resistant homozygous rr seedlings. At the same time, other F_2 grains were germinated on water, and therefore contained homozygous SS and rr as well as heterozygous Sr genotypes. Some 200 seedlings of the two treatments were transferred to Jiffy 7 peat pellets, planted in the garden at Dijon, bagged and individually harvested. The herbicide resistance test was carried out on the F_3 grains to identify the genotype of every maternal F_2 plant and to select the homozygous rr and SS F_3 genotypes. Then, the F_3 grains were bulked according to their genotype (indicated in boxes in Figure 1).

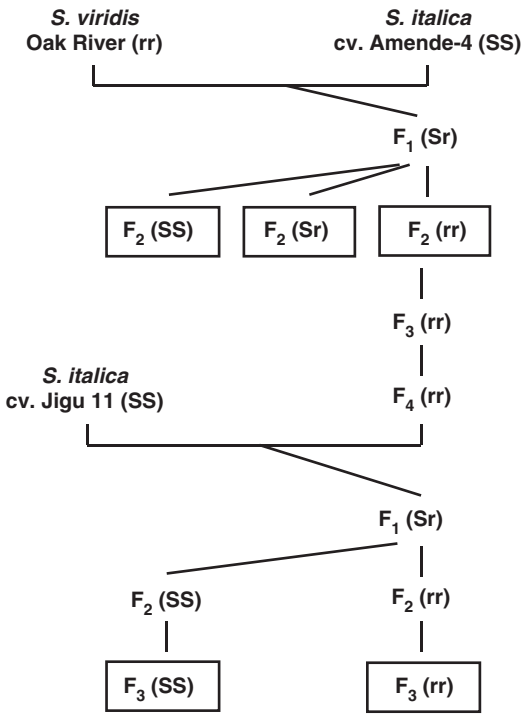

Figure 1 Breeding scheme used to produce the F_2 and F_3 materials (in the boxes). Genotypes that appeared in the progeny but were not used in the breeding plan are not indicated. S and r are the herbicide-susceptible dominant and -resistant recessive alleles, respectively.

SS, Sr and rr identification

The genotype was identified following harvest by characterization of the progeny. A total of 50 grains of each ear were germinated in a petridish on filter paper moistened with commercial trifluralin solution at 0.2 μM active ingredient (Callifort, Calliope, France: 480 g l^{-1} trifluralin). Seedlings were grown for 3 days in the dark at 25 °C, then classified as susceptible or resistant based on the shoot length ($<$ or $>$ 1.5 cm) and the look (swollen or not) of the shoot tips (Wang *et al*., 1996). Progeny of heterozygous plants produced about 17% viable seedlings instead of the 25% expected for a single-gene recessive mutation because of a distorted segregation ratio (Tian *et al*., 2006).

F_2 garden experiment

A total of 200 F_2 seeds of the initial interspecific cross were germinated on moistened paper in a petridish, grown for 3 days at 25 °C, then transferred to Jiffy 7 peat pellets and grown in a growth cabinet at 25 °C under a 16 h photoperiod. Three-leaf seedlings were planted

every 8 cm on a row in the experimental garden. The flowering date was recorded when an ear emerged from the main stem. The following measurements were taken at maturity: the height to the last leaf node, the flag leaf width, the number of ears, the length of the main ear, the seed weight of the main ear and the 1000-grain weight. The number of seeds on the main ear was estimated for each plant. Data were analyzed with the GLM procedure of SYSTAT software version 10 (SPSS Inc., Chicago, IL, USA, 1999) after log transformation of the ear length and seed weight on the main ear and seed number.

Growth cabinet experiment

Early growth differences between the genotypes could be reduced by using transplanted seedlings grown in the greenhouse, as for the previous experiment, thus mitigating any differential growth at emergence and at the very earliest stage of plant life. To determine whether this potential bias exists, we investigated the emergence and growth of young seedlings under controlled conditions. A total of 10 rr and 10 SS F_3 grains were separately sown in 8 cm^2 pots filled with 200 g of a sandy clay soil. The seedlings were grown for 7 days in a growth cabinet over a 16 h photoperiod (200 $\mu M^{-2} s^{-1}$) at 27 °C in the day and 22 °C over night. The emergence rate was scored at the end of the growth period and the emerged seedlings were randomly thinned to either one or three per pot, creating two levels of plant density and generating different levels of competition. Both low- and high-density pots, containing either rr or SS plants, were placed in three growth cabinets. The pots were given 22 ml of water and their positions were randomly changed within a density group every other day. The plant height at the last visible leaf/stem angle was measured the very day a new leaf/stem angle was visible. The plant height, the number of visible leaves and the dry aboveground biomass were measured 38 days after sowing (DAS). Regressions of plant height against leaf range were computed using block values. The data were analyzed with a fixed-effects model analysis of variance for a split-plot design, including three blocks (growth cabinets), two densities (main plots) and two genotypes.

F_3 field experiment

F_3 grains were sown in a randomized split-plot arrangement at two densities, 20 and 50 plants per m^2, corresponding to densities of foxtail millet production in France and China, respectively. There was 0.75 and 0.37 m interrow in the two density treatments, respectively, and five replicate blocks. Within the main plots for each density, there were pure rr and SS and mixed rr/SS subplots. Each subplot consisted of three, 5 m long rows. For the pure plantings, 300 grains were planted per row, and emerged seedlings were counted before being thinned to the desired density (that is, 75 and 92 seedlings per row for the 20 and 50 plants per m^2, respectively). For the mixed planting, pinches of five grains of the rr and SS genotype were alternately sown along each row every 7 or 5 cm according to the density. Thinning was done to ensure that only one seedling grew at each planting site.

Measurements were carried out on six plants of the central row (or six rr and six SS for the mixed planting) to avoid any border effect. Height to the last leaf node was measured, and leaf and tiller numbers were counted at two dates (54 DAS and at maturity) to detect any differential growth kinetics. The flowering date was also recorded. Flag leaf length and width, and aboveground dry biomass (excluding ears) were measured at maturity. The reproductive traits measured were the grain number and grain weight both on the main ear and for the overall plant. We also included measurements of the main ear morphology and the 1000-grain weight to describe components of reproductive allocation. The number of grains produced per unit of dry biomass was calculated for every plant. Average data for the six plants measured per treatment and replicate were analyzed using a fixed-effects analysis of variance for a split-plot design that included five blocks, densities (D) as main plots, and genotypes (G) and planting (P) factors as subplots, with $G \times D$, $G \times P$, $D \times P$ and $G \times D \times P$ interactions. We did not detect heterogeneity of variance using Levene's test, thus justifying the use of untransformed data.

Results

F_2 garden experiment

The germination rate was 75.5%, 151 seedlings were transplanted and 148 produced seeds. In all, 25% unviable seeds are not unexpected following interspecific crosses. The proportions of rr, Sr and SS genotypes were identified afterward by their progeny: 11.5, 42.6 and 46.9%, respectively. These proportions are consistent with the distorted segregation ratio previously observed (Tian *et al*., 2006). The Sr and SS genotypes did not differ in the traits measured. The rr genotype had smaller flag leaf width, lower weight of the seeds on the main ear and lower 1000-grain weight than the two other genotypes, but had more tillers (not shown) and secondary ears (Table 1). Owing to high variability, the data for the main ear length and the grain number were highly variable and only marginally significant ($P = 0.07$) but again, the rr genotype exhibited the lowest values.

Growth cabinet experiment

The grains collected on the rr F_2 plants weighed less than those collected on the SS plants, 2.67 ± 0.39 and 2.86 ± 0.22 g, respectively ($t = 4.25$, $P < 0.001$). Germination in pots was not different for rr and SS grains: 80 ± 9 and 83 ± 7%, respectively. Thirty-eight DAS results were as follows: rr plants were smaller than SS plants regardless of the density, only the rr plants in high-density pots had fewer leaves and there was no difference in plant weight among treatments (Table 2). High plant density resulted in lower plant height, number of leaves and weight than low density. However, these differences were only observed when morphological data were expressed in terms of growing days. The regression of plant height against leaf range best fit a power law $y = x^a$. The slopes were consistent across plant densities and genotypes (Table 2 and Figure 2).

F_3 field experiment

Seedling emergence was not significantly different between rr and SS, 53.9 ± 3.9 and 59.0 ± 2.2%, respectively ($t = 1.13$, $P > 0.05$). The density effect was significant for half of the traits measured (Table 1). Higher plant density

Table 1 Average values of the characters measured for the resistant and susceptible genotypes (rr, and Sr and SS, respectively) of the F_2 garden experiment and F_3 field experiment, and an F-test of the genotype effect (other significant effects are indicated in brackets)

Characters	*F_2*				*F_3*		
	rr	*Sr*	*SS*	*F*	*rr*	*SS*	*F*
Number of tillers 54 DAS					0.98	0.61	*
Leaf number 54 DAS					9.64	9.60	NS (pd)
Height of main stem 54 DAS (cm)					23.5	24.6	NS (pd)
Flowering time (DAS)	47.4	48.6	50.4	NS	83.0	84.8	NS (i)
Leaf number at maturity					15.9	16.5	*
Main stem height at maturity (cm)	44.8	48.3	49.2	NS	81.5	93.3	***
Flag leaf length (cm)					30.0	31.2	NS
Flag leaf width (cm)	1.71b	2.01a	2.02a	*	2.48	2.65	NS (d)
Main ear length (cm)	11.5	14.9	14.6	NS	19.5	19.9	NS (d)
Main ear width (cm)					3.41	3.42	NS
Main ear weight (g)					14.8	17.6	** (d)
Grain weight on main ear (g)	2.28b	3.96a	4.29a	*	11.1	13.9	** (d)
Grain number on main ear	1071	1678	1753	NS	4469	5116	NS (d)
Number of secondary ears	25.8a	21.7a	16.3b	**	0.91	0.95	NS
Grain weight on secondary ears (g)					5.57	7.46	NS
Total grain number per plant					6705	7846	NS (d)
Total grain weight per plant (g)					16.7	21.4	* (d)
Dry biomass (except ears) (g)					17.2	20.5	NS (d)
1000-grain weight (g)	2.13b	2.29ab	2.39a	**	2.49	2.71	***
Number of grains per g of dry biomass					393	383	NS

Abbreviations: a, b, different letters within a line for F_2 indicate different values at $P<0.05$; d, significant density effect; DAS, days after sowing; i, significant effect of interaction between genotype and planting type; p, significant effect of planting type.
NS, *, ** and *** indicate F-test not significant and significant at $P<0.05$, 0.01 and 0.001, respectively.

Table 2 Average values of the measured characters for homozygous resistant (rr) and susceptible (SS) F_3 seedlings in growth cabinet, and regression parameters of the plant height in terms of the leaf range, $y=x^a$ (see Figure 2)

Density of plants per pot	*Genotype*	*Number of pots*	*Plant height (cm)*	*Number of leaves*	*Dry weight (g)*	*Regression*	
						$a \pm ASE$	*Raw R^2*
1	rr	30	17.7b	8.63a	0.432a	1.71 ± 0.04a	0.95
1	SS	26	19.4a	8.77a	0.434a	1.78 ± 0.04a	0.95
3	rr	33	14.1d	7.98c	0.271b	1.59 ± 0.04a	0.96
3	SS	31	15.5c	8.27b	0.271b	1.65 ± 0.05a	0.94

Abbreviation: ASE, asymptotic standard error.
Different letters within a column for a given temperature regime indicate different values at $P<0.05$.

resulted in higher leaf number and taller plants 54 DAS, but lower per plant biomass (21.1 ± 1.5 versus 16.6 ± 1.1 g) and grain weight (22.8 ± 2.1 versus 15.3 ± 1.1 g). No genotype interaction was detected. Interestingly, there was no density effect on secondary tiller production, 1000-grain weight or the number of grains per unit of biomass, indicating low plasticity and strict genetic control of these traits. The planting type (pure or mixed) affected the number of leaves and the plant height 54 DAS. There was a significant interaction between the planting type and the genotype for flowering time only, indicating plant competition at the early growth stage.

The genotype effect was significant for 6 of the 20 characters (Table 1). The early records at 54 DAS showed more rapid development of the tillers in the rr plants than those of the SS plants. The rr plants were smaller and had fewer leaves than the SS plants at maturity, but the final number of tillers was similar for both genotypes. There was no difference in the morphology of the flag leaf or the ear. The main ear weight and the 1000-grain weight of the rr plants were lower than the SS plants, whereas the difference in total grain number per plant and grain weight between plants was marginally

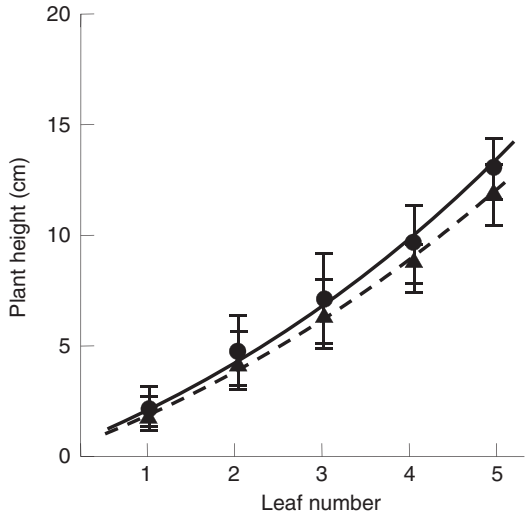

Figure 2 Regression of the plant height at the last visible leaf/stem angle on leaf range of F_3 seedlings at the three plants per pot density in the growth cabinet: slopes of rr (triangle, dotted line) and SS (circle, solid line) were not different.

significant ($P = 0.08$ and 0.09, respectively). Most trait values, except tiller number 54 DAS, were lowest in rr plants.

Discussion

This is the first study dealing with the impact of dinitroaniline resistance on the fitness of near-isogenic resistant and susceptible plants. In previous studies, the plant material had various origins including non-crop stands. Here, we compared plant growth and reproduction of the two types of plants in F_2 and F_3 segregating progenies that, on average, shared the same genetic background with the exception of the group of linkages in which the α2-tubulin gene is located. Indeed, the large number of F_2 individuals used in the garden experiment or producing seeds for the growth cabinet and field experiments warranted similar and representative sampling of the genetic background in the two types of plants. The differences observed in the initial F_2 could reflect the differences between the two parent species, thus indicating that the r allele could be linked with genes encoding for the tiller and secondary ear number. Similarly, there could be linkage between the S allele and genes encoding for the leaf width, ear length, and ear and grain weight. Indeed, tiller and secondary ears are typically more numerous in the green foxtail than in most of the foxtail millet cultivars, and reciprocally leaf width, ear length, and ear and grain weight are higher in the foxtail millet than in its wild relative (Darmency *et al*., 1987). The association between the r allele and short main ears/numerous secondary ears was completely lost in later generations after selection and crossing to a low tillering variety, which could indicate a simple linkage. In contrast, the association between the S allele and 1000-grain weight/grain weight of the main ear persisted through to the field experiment, which could either indicate a tight linkage or a pleiotropic effect of the gene.

The rr grains used in the growth cabinet, field experiment and those harvested in the field experiment were about 7–8% lighter than SS grains. This difference in grain weight could have caused the differential juvenile growth of F_3 seedlings in the growth cabinet. However, even under high-density exacerbating competition, the rr plants were smaller and had fewer leaves than the SS plants, whereas plant weight still remained equivalent 38 DAS. This led us to believe that these differences were not a matter of resource reserves in the grains, but rather a difference in the growth kinetics. Moreover, the regression curves of plant height against leaf range revealed no difference between the two genotypes, thus indicating that grain weight had no impact on the initial seedling, at least at the earlier stage of the plant's life. These curves also show that the genetic growth program reached an equivalent stage in the two genotypes, therefore suggesting that the growth difference at 38 DAS was because of different plants' biological clock driven by different cell division rates. This hypothesis is consistent with the role of α-tubulin in cell division processes (Baskin and Cande, 1990). Tubulin alteration in resistant *Eleusine indica* resulted in aberrations to some of the microtubule functions, including wall protuberances, incomplete cell division and a decrease in the overall number of microtubules (Vaughn, 1986). In *Toxoplasma gondii*, the spontaneous resistant mutant also showed increased rates of replication defects (Ma *et al*., 2008). Similar nuclear division defects were observed due to a β-tubulin mutation in *Tetrahymena thermophila* (Smith *et al*., 2004). However, microtubules are involved in many other processes, including cell wall expansion, intracellular transport and organelle positioning; alterations to any of these could also impact plant fitness.

The field experiment confirmed a slower leaf emission with lower stem elongation for the rr plants, both at the early stage and at maturity. The plant height difference was not observed in the initial interspecific F_2 material, but it could have been masked by the wide variability generated in the F_2 of the cross between small green foxtail and tall foxtail millet. Altered efficiency of the mutant α2-tubulin gene could cause such an effect on stem elongation. Similarly, the lower 1000-grain weight of the rr plants compared with the SS plants could indicate problems associated with cell division and grain replenishment. However, the lower 1000-grain weight alone cannot explain the lower main ear grain weight of the rr compared with the SS genotype. A reduced number of grains could also contribute to this result. Although grain number was lower in rr plants, the *P*-value was only marginally significant. Reduced main ear grain weight in rr plants could be because of a lower number of spikelets, but slower cell division kinetics in the rr genotype could hardly account for this effect. It could also arise from remains of the close linkage we previously reported between the α2-tubulin gene and a putative gametocide gene that resulted in loss of homozygous rr descendants (Tian *et al*., 2006). This possibility is consistent with a similar chromosome situation in rice, in which the corresponding *OsTubA1* gene is located near two hybrid sterility and two gametocide genes. Alternately, some tubulin genes themselves are known to control pollen tube elongation (Yu *et al*., 2009), which could directly impact pollen success.

The yield penalty extrapolated from the difference in the main ear grain weight, which is generally the main source of grain production by low tillering cultivars, would be 20%. It is a fitness cost similar to that found for atrazine resistance in the same crop (Darmency and Pernès, 1989). We recently reported that trifluralin- and atrazine-resistant lines had similar yields in multisite experiments in China (Wang *et al*., 2010b), which is consistent with a comparable reproduction cost. In all, 20% is considered to be a significant yield penalty. However, a similar reduced yield in oilseed rape was not an obstacle to the use of atrazine-resistant cultivars, which have been cultivated on more than one million hectares in Australia for many years. The benefit of using an herbicide-resistant cultivar is good weed control, especially against weeds of the same botanical family. This results in less weed competition, which balances the potential low yield (Forcella, 1987). In the case of foxtail millet, the mutation also provides cross-resistance to some herbicides of the same mode-of-action, although the level of resistance is not high enough to preserve the crop from herbicide injury at herbicide dosages currently used for good weed control in the field (Wang and Darmency, 1997). Further research on compensatory mechanisms and tolerance gain from selection might include a search of the genetic resources of foxtail millet,

which has been proposed as a possible tool to help overcome dinitroaniline-induced damages in soybean (Glover and Schapaugh, 2002). It would also be worthwhile to search the genetic resources of new mutants on both tubulin and non-tubulin genes, which actually occur spontaneously within a few generations in *Toxoplasma gondii* (Ma *et al*., 2008). Moderate yield reduction (5%) has been observed in genetically modified herbicide-resistant crops (Elmore *et al*., 2001) without hampering their widespread use. Many fitness costs linked to diverse traits in genetically modified crops have been reported, but they could be because of overexpression, silencing or breakage of native genes (Xia *et al*., 2010).

The consequence of such a high fitness cost in the absence of herbicide could be dramatic for mutant green foxtail. The difference of growth kinetics makes it less adapted to grow with the crop canopy, which results in receiving less light. The difference of seed weight makes it less suitable to develop seedlings in adverse conditions or results in smaller seedlings, thus possibly influencing the chance of reaching the adult stage and even reducing the adult reproduction (Moles and Westoby, 2004). The persistence of the trifluralin-resistant type in non-treated fields over 7 years (Andrews and Morrison, 1997) could be interpreted as the lack of S alleles in populations strongly submitted to the previous repeated treatments. As the resistance is recessive, the S alleles could not be transmitted through heterozygous plants and resulted cleared. In addition, green foxtail produces a huge quantity of seeds, and extremely dense infestations of resistant green foxtail were recorded up to 25 000 seedlings per m^2 (Andrews and Morrison, 1997), which suggests that a large reserve of resistant seeds in the soil could reinfest the field for long time, even in the absence of the selection pressure. Such high exponential infestation dynamics was documented in triazine resistance spread in the fields (Darmency and Gasquez, 1990), and the resistant type is still found at high frequency in the infested fields 20 years after, in spite of the fitness cost (Gasquez, personal communication). In contrast, the fitness cost could explain the relatively low frequency of trifluralin resistance among weeds (Heap, 2010). Higher fitness costs were found in laboratory mutants showing herbicide resistance (Roux *et al*., 2004), but the most common situation is an absence of cost or undetectable costs (Vila-Aiub *et al*., 2009), and a case of higher seed production was even observed in *Setaria* (Wang *et al*., 2010a).

Conflict of interest

The authors declare no conflict of interest.

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