Environmental Pollution 159 (2011) 3714–3720

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Decrease in catalase activity of *Folsomia candida* fed a *Bt* rice diet

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ARTICLE INFO

Article history:
Received 8 April 2011
Received in revised form
13 July 2011
Accepted 16 July 2011

Keywords:
Non-target organism
Bt-rice
Collembola
Biological trait
Antioxidant enzyme

ABSTRACT

Here we report the effects of three *Bt*-rice varieties and their non-*Bt* conventional isolines on biological traits including survival, reproduction, and the activities of three antioxidant enzymes superoxide dismutase, catalase and peroxidase, in the Collembolan, *Folsomia candida*. The reproduction was significantly lower when fed Kemingdao and Huahui1 than those feeding on their non-GM near-isogenic varieties Xiushui and Minghui63 respectively, this can be explained by the differences of plant compositions depended on variety of rice. The catalase activity of *F. candida* was significantly lower when fed the *Bt*-rice variety Kemingdao compared to the near-isogenic non-*Bt*-rice variety Xiushui. This suggests that some *Bt*-rice varieties may impose environmental stress to collembolans. We emphasize that changes in activity of antioxidant enzymes of non-target organisms are important in understanding the ecological consequences for organisms inhabiting transgenic *Bt*-rice plantations.

1. Introduction

The accumulated hectarage of biotech crops from 1996 to 2010 exceeded for the first time an unprecedented 1 billion hectares. This is a record of an 87-fold increase in hectarage, making biotech crops the fastest adopted crop technology in the history of modern agriculture (James, 2010). Rice, *Oryza sativa* L., is globally one of the most important food crops, and is significant to China's goals of food and feed "self-sufficiency" and "food security" (Qiu, 2008). The Chinese Government has placed a high priority on crop biotechnology, especially biotech rice. Developing a pro-active understanding of the environmental consequences of adopting transgenic rice is a key part of ensuring the success of this new strategic approach to crop production.

Previous studies have evaluated the impact of *Bt*-crops on non-target soil organisms (NTOs). Most of this research showed no deleterious effects of *Bt*-crops on the population fitness of soil NTOs (Icoz and Stotzky, 2008; Yuan and Ge, 2010). For example, field experiments were set up to test the effect of *Bt*-rice (Kemingdao1 and Kemingdao2) on the ground-dwelling collembolan community, and a laboratory experiment was also set up to study the safety of Kemingdao1 and Kemingdao2 to *Microvelia horvathi*, a predator of collembolans. The results showed that Kemingdao1 and Kemingdao2 had no negative effects on the collembolan community and their predator in a natural environment (Bai et al., 2005, 2010). *Pirata subpiraticus*, predator of *Cnaphalocrocis medinalis* which is target insect of *Bt*-rice (KMD1 and KMD2), when preying on *Bt* rice-fed *C. medinalis* showed similar survivorship and fecundity with those preying on non-*Bt* rice-fed *C. medinalis.* Though developmental time of *P. subpiraticus* was significantly longer when it preyed on *Bt* rice-fed *C. medinalis* than on non-*Bt* rice-fed prey, a 3-year field trial indicated that *Bt*-rice did not significantly affect the density of *P. subpiraticus* (Chen et al., 2009). Xiao et al. suggested that *Bt* toxin Cry1Ac had little effects on the biomass and physiological characteristics of *Eisenia fetida*, with no acute and subchronic toxicity, and was safe for earthworms at field dose level (Xiao et al., 2005). Li et al. found no significant differences between the *Bt* and control rice plots in these arthropod community-specific parameters. The similarity of arthropod communities in the *Bt* and control rice plots was apparently high (Li et al., 2007). However, indications of adverse effects of *Bt*-crops were also shown for some soil organisms or some parameters. *Caenorhabditis elegans*, a bacteriophagous nematode, was negatively affected by both purified Cry1Ab protein and rhizosphere soil of *Bt*-maize expressing the Cry1Ab protein (Höss et al., 2008). The trophic group composition of

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doi:10.1016/j.envpol.2011.07.015

nematofauna could be affected by *Bt*-crops if they were grown in soil rich of clay (Manachini and Lozzia, 2002). Transgene products were persistent in soil and taken up by ground beetles in a *Bt*-maize agro-ecosystem even if no transgenic crop had been grown there for two years (Zwahlen and Andow, 2005). Some characteristics of the Bt toxins and the transgenic crop biotechnology may adversely affect enzymatic activity of NTOs in the soil. For example, *Bt*-crops can express Cry proteins throughout their plant tissue during their entire growth stage (Wu et al., 2002; Saxena et al., 2004) and Bt toxins can bind to soil particles and persist for long periods without losing their biological activity (Tapp and Stotzky, 1995; Palm et al., 1996; Sims and Ream, 1997) Additionally, the Bt toxin can be detected in the gut and body of some non-target species exposed to *Bt*-crops and can also be transmitted to higher trophic levels (Saxena and Stotzky, 2001a; Wandeler et al., 2002; Obrist et al., 2006). Some plant components, such as lignin, carbon, and nitrogen showed differences between transgenic and non-transgenic varieties (Saxena and Stotzky, 2001b; Flores et al., 2005; Poerschmann et al., 2005). These characteristics imply that *Bt*-crops adversely affect the fitness of some soil NTOs. However, few studies have been performed at the cellular, enzymatic or molecular levels of soil invertebrates to elucidate the mechanisms involved and inform a response strategy.

The definition of measurement endpoints is an important step in environmental risk assessment (ERA) of GM plants for NTOs (EFSA, 2010). An appropriate measurement endpoint for NTO testing is relative fitness (or some component of relative fitness), which is the relative lifetime survival and reproduction of the exposed versus unexposed non-target species as well as indicators of change also need to be defined and established at the same time (EFSA, 2010). The complexity of soil ecosystems render studies based on species diversity expensive and highly variable, and ecosystem processes (like biomass decomposition, cellulose and lignin breakdown, phosphorous and nutrient uptake) should be included in biosafety studies (Van Toan et al., 2008). In this case, it is important to make sure if *Bt*-crops impose environmental stress on NTOs. Thus, we chose the common bioindicators of various environmental stressors, i.e. antioxidant enzymes activities, as well as the traditional measurement endpoints, i.e. survival and reproduction of collembolans.

The deleterious free radicals/reactive oxygen species (ROS), including singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl ions and free hydroxyl radicals (${}^{1}O_2$, ${}^{\bullet}O_2^{-}$, H_2O_2, OH^{-} and ${}^{\bullet}OH$), which are produced during normal metabolism but increase on exposure to stressors, can cause physiological disorders and metabolic abnormalities. These radicals may react with biomolecules such as DNA, RNA, proteins and lipids causing alterations within their structures (Singh et al., 2010). Also, metabolism of plant allelochemicals, hydroxamic acids, and plant growth regulators could produce reactive oxygen species (ROS). The antioxidant enzymes are used by herbivorous insects to remove free oxygen radicals that have been derived from endogenous and exogenous sources (Lukasik, 2007). In addition, the antioxidant enzymes have been used as important bioindicators for different kinds of environmental stressors (Büyükgüzel and Kalender, 2009; Oruc, 2010; Hoffman et al., 2011). Different host plants can also impact the activity of some antioxidant enzymes (Ishikawa and Kubota, 1991; Lukasik, 2009). With reference to transgenic crops, not only is there a discrepancy between transgenic varieties and their isolines, but these differences may affect the activity of certain antioxidant enzymes of NTO's. Transgenic *Bt*-rice is one of the most important crops, so it is essential that the cellular, enzymatic and molecular levels of NTO soil invertebrates under transgenic *Bt*-rice plantations are examined in more detail.

Collembolans comprise a large group of NTO arthropods in the soil and a key biotic factor in determining the soil functions. Together with other soil-dwelling invertebrates, collembolans carried out processes in soil ecosystems, such as nutrient cycling and decomposition of organic matter, which have major ecological and agricultural significance (Moore et al., 1988). They have been used for the safety evaluation of transgenic plants for several years (Larink, 1997; Heckmann et al., 2006). *F. candida* is a commonly used sentinel species that is very sensitive to many pollutants (Fountain and Hopkin, 2005). Thus, we chose to analyze the effect of transgenic *Bt*-rice on *F. candida* fitness and at the enzymatic level. Our objectives were: 1) to assess whether the *Bt*-rice could affect the reproduction and survival of *F. candida* and 2) to determine whether *Bt*-rice could affect the somatic activity level of antioxidant enzymes that are usually affected by reverse stresses, namely superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), compared to their non-GM near-isogenic varieties.

2. Materials and methods

2.1. *Test species*

F. candida was the FCDK strain (Simonsen and Christensen, 2001; Tully et al., 2006) obtained from Department of Terrestrial Ecology, University of Aarhus and has been cultured for 10 years at Shanghai Institute of Biological Sciences for about ten years. The cultures were reared in Petri dishes (150 mm diam., with a plaster of Paris and activated charcoal (9:1 weight:weight) covering the bottom) kept at 20 °C in total darkness. The relative humidity was ~100%; adjusted weekly by addition of distilled water.

2.2. *Rice materials*

All rice varieties were planted in Dezhou, Shandong Province, China. Three *Bt*-rice varieties and their near-isogenic non-*Bt* rice varieties were used in this study. These three *Bt*-lines were Kemingdao, Huahui1 and BtShanyou63. Kemingdao, Huahui1 and BtShanyou63 lines were derived from Xiushui, Minghui63 and Shanyou63 lines, respectively. Kemingdao contain a single *Bt*-insecticidal gene *cry1Ab*, and Huahui1 and BtShanyou63 are stacked varieties expressing *cry1Ab* and *cry1Ac* dual genes (see appendix Table 1 for more detailed information on all 6 rice varieties). Leaf materials were collected in 2009, when the rice reached the jointing stage and were transported to laboratory within a few hours and frozen at −20 °C.

Table 1
The three *Bt*-rice varieties and their corresponding near-isogenic non-*Bt* rice varieties used for the experiment and their concentration of *Bt*-protein.

Diet	*Bt* protein (μg/g)[a]	*Bt* protein transformed	Remarks
BtShanyou63 (*Bt*+)	1.33 ± 0.21[A]	Cry1Ab/1Ac fused	Transformation event Shanyou63
Shanyou63 (*Bt*−)	N.D.[b]	–	Isogenic to Btshanyou63
Kemingdao (*Bt*+)	2.90 ± 0.72[A]	Cry1Ab	Transformation event Xiushui
Xiushui (*Bt*−)	N.D.	–	Isogenic to Kemingdao
Huahui1 (*Bt*+)	1.39 ± 0.21[A]	Cry1Ab/1Ac fused	Transformation event Minghui63
Minghui63 (*Bt*−)	N.D.	–	Isogenic to Huahui1
Yeast with Bt proteins	852.81 ± 365.20[B]	Cry1Ab: Cry1Ac = 1:1	–
Yeast	N.D.	–	–

[a] μg/g maize leaves (mean ± S.E). Means with the same uppercase letters are not significantly different (ANOVA, $P < 0.05$, df = 2).
[b] N.D.: Not detected.

2.3. Experimental design

The experimental set-up consisted of six diet treatments including Kemingdao, Huahui1, BtShanyou63 and Xiushui, Minghui63, Shanyou63 lines. Leaf materials of these six varieties were fed to *F. candida* respectively as a single treatment. Dry baker's yeast mixed with or without pure Bt proteins (Cry1Ab and Cry1Ac) were fed to collembolans as controls. Yeast mixed with Bt proteins was made by adding 1 mg Cry1Ab and 1 mg Cry1Ac (Case Western Reserve University) in 2 g dry baker's yeast, then finely grinding with a mortar and pestle and kept at −80 °C until used (the final concentration was 852.81 ± 365.20 mg total Bt protein/g yeast, Cry1Ab: Cry1Ac = 1:1). Five replicates were made for each treatment for survival and reproduction tests, 4 replicates for each treatment for antioxidant enzyme activity determination, 3 replicates for each treatment for organic carbon, total nitrogen and Bt toxin determination.

2.4. Observation and determination

2.4.1. Springtail survival and reproduction

Leaf material obtained from field treatments were lyophilized for 2 days at −60 °C under a vacuum of ~30 mTorr, then grinded with a plant tissue pulverizer wi50559 (Dong Xi Yi, Beijing, China) and stored at −20 °C until use. Ten specimens of 10- to 12-day old juveniles were placed in tightly closed round plastic containers (8 cm diameter, 6.5 cm height, with a plaster of Paris and activated charcoal (8:1 weight:weight) covering the bottom) for each replicate. Approximately 3 mg per replicate of the above-mentioned diets were added at the beginning of the experiment, and checked every 2 days. The diets were added or replaced if needed. The temperature was kept at 20 ± 1 °C. The relative humidity was ~100%, which was adjusted weekly by adding distilled water and the containers were aerated twice a week. The experimental duration was 28 days and the experiment was carried out in complete darkness. The surviving adults and total number of juveniles produced were counted manually when the experiment ended.

2.5. Determination of antioxidant enzymes activity

Leaf material obtained from field grown *Bt*-rice were cut into tiny pieces (about 1 mm^2), and stored at −20 °C until use. Ten mg of adults for every replicate were placed in tightly closed round plastic containers described above. Approximately 3 mg per replicate of the above-mentioned diets were added at the beginning of the experiment, and checked every 2 days. The relative humidity was set to ~100%, which was adjusted twice a week by adding distilled water, and aerated in the meantime. The experiment was carried out in total darkness for 10 days. When the experiment ended, the cultures from each replicate were transported to a 1.5-ml tube. The enzyme extract for Total SOD (T-SOD), POD and CAT was prepared by grinding the organisms with 1-ml physiological saline solution (0.9% NaCl). The extract was centrifuged for 20 min at 10,000 rpm and the supernatant was used for enzymatic assay. The T-SOD, POD and CAT activity were determined according to the reagent kit (Nanjing Jiancheng Bioengineering Institute) (Ning and Long, 2008). As indicated by kit protocol, SOD activity was assayed spectrophotometrically at 550 nm using the xanthine and xanthine oxidase system. One unit (U) of SOD activity was defined as the amount of SOD required for 50% inhibition of the xanthine in a 1-ml xanthine oxidase system reaction per milligram of total protein in the homogenate. CAT activity was based on the decomposition rate of H_2O_2 by the enzyme, which was measured as the absorbance decrease per second at 240 nm. Enzyme activity values were also expressed in CAT units, where one unit was the relative amount of enzyme needed to hydrolyze H_2O_2 per second and per gram of total proteins present in the homogenate. POD activity was determined according to the reaction principle of POD catalysis H_2O_2, and detected the changes of absorbance at 420 nm. One POD unit was defined as the amount of enzyme needed to catalyze 1 μg substrate per milligram of total protein present in the homogenate at 37 °C.

2.6. Quantification of Bt toxin and C/N ratio in rice leaf material

The content of Bt proteins in rice leaf tissue and yeast were measured by enzyme-linked immunosorbent assay (ELISA) prior to the experiment. The quantification was carried out with QualiPlate Kit for Cry1Ab/Cry1Ac (Envirologix Inc., Portland, ME, USA), and a standard curve was constructed with purified Cry1Ac protein (Case Western Reserve University) to estimate the amount of Bt proteins in the plant samples and yeast.

The organic carbon in rice leaves was measured following a modified version of the Mebius method (Nelson and Sommers, 1982). Rice leaf samples (0.5 g) were digested with 5 ml of 1 mol/L $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 (≈98%) at 150 °C for 30 min, followed by titration of the digests with standardized $FeSO_4$. Nitrogen content in leaves were assayed using Kjeltec nitrogen analysis (Foss automated Kjeltec™ instruments, Model 2100).

2.7. Statistical analyses

All statistical analyses were performed with SAS ver. 9.2 (SAS Institute Inc, Cary, NC, USA). Differences in group means of C/N ratio in rice leaf materials and of survival, reproduction and the antioxidant enzyme activity of *F. candida* were tested by two-way ANOVA on the factors including gene modification (GM), differences between 3 non-GM varieties and their interaction. Differences in group means of the same parameters between *Bt* varieties and their non-*Bt* near-isogenic varieties treatments or between yeast and yeast mixed with Bt proteins treatments were tested by *t*-test. Least significant difference (LSD) Test were applied to test for differences of the same parameters as described above and the content of Bt protein between 3 non-GM varieties and Yeast mixed with Bt proteins. A significance level of 5% was used for all tests.

3. Results

3.1. Survival and reproduction

The controls exhibited the highest level of the number of juveniles among all diet treatments. Rice diet treatments showed a 3 to 5-fold lower number of juveniles than the controls. The reproduction of non-GM Xiushui and Minghui63 varieties were significantly higher than the near-isogenic *Bt*-varieties, Kemingdao and Huahui1 ($P = 0.0214 < 0.05$; $P = 0.021 < 0.05$ respectively). However, no significant difference was observed for the reproduction of the 3 non-GM varieties. The results of two-way ANOVA showed that GM was the main factor which significantly affected the reproduction ($P = 0.03 < 0.05$) (Fig. 1).

The numbers of surviving adults were not significantly different across rice diet treatments (Fig. 2).

3.2. Activity of T-SOD, CAT and POD

The CAT activity was significantly affected by GM ($P = 0.009 < 0.01$) and interaction between GM and differences between 3 non-GM near-isogenic varieties ($P = 0.04 < 0.05$) through the results of two-way ANOVA. *F. candida* fed on the GM variety Kemingdao had extremely significantly (3.38-fold) lower CAT activity than those fed on its non-GM near-isogenic line, Xiushui ($P = 0.0026 < 0.01$) (Fig. 3).

The T-SOD activity was extremely affected by the differences across 3 non-GM varieties through the results of two-way ANOVA ($P = 0.000 < 0.01$). The T-SOD activity of *F. candida* living on Shanyou63 and Xiushui were significantly higher than those fed on Minghui63, and Shanyou63 treatment exhibited the higher T-SOD level than Xiushui treatment. Meanwhile, no significant difference was observed between all 3 *Bt* and non-*Bt* varieties pairs (Fig. 3).

The POD activity of *F. candida* in different diet treatments exhibited no differences (Fig. 3).

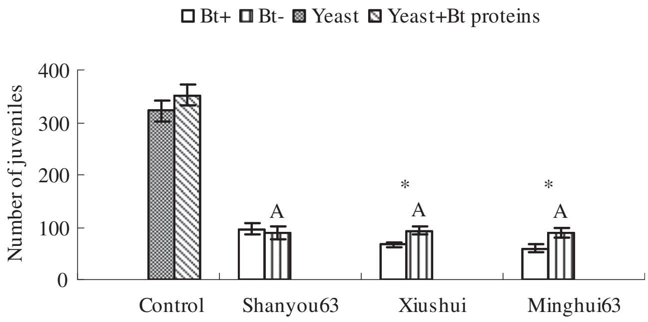

Fig. 1. The number of juveniles reared on the rice and yeast treatments. Yeast + Bt proteins: individuals fed with yeast mixed with two pure Bt proteins (final concentration of Bt proteins was 852.81 ± 365.20 mg/g, Cry1Ab: Cry1Ac = 1:1). Each value represents the average (±SE) of five replicates. Different uppercase letters indicate significant differences across non-*Bt*-rice near-isogenic variety treatments (LSD test; d.f. = 2, 12). *: Significant differences at $P < 0.05$ between *Bt*-rice varieties and their near-isogenic lines (d.f. = 1, 8).

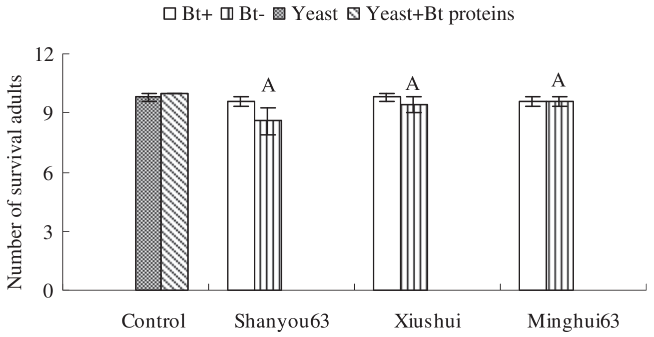

Fig. 2. The number of surviving adults reared on the rice and yeast treatments. Yeast + Bt proteins: individuals fed with yeast mixed with two pure Bt proteins (final concentration of Bt proteins was 852.81 ± 365.20 mg/g, Cry1Ab: Cry1Ac = 1:1). Each value represents the average (±SE) of five replicates. Different uppercase letters indicate significant differences across non-*Bt*-rice near-isogenic variety treatments (LSD test; d.f. = 2, 12). *: Significant difference at $P < 0.05$ between *Bt*-rice varieties treatments and their isolines treatments (d.f. = 1, 8).

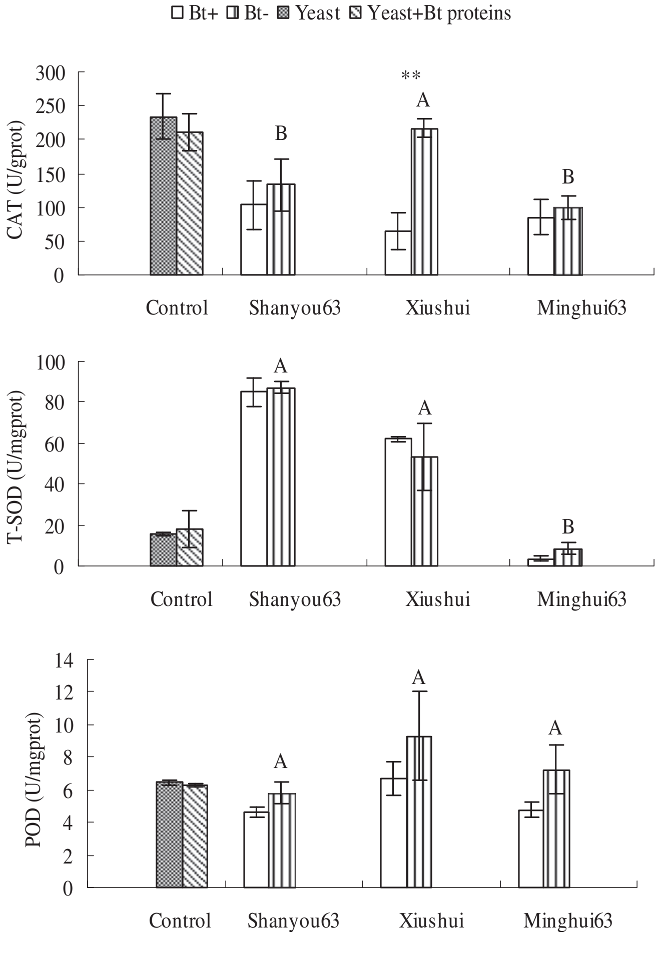

Fig. 3. The activity of 3 antioxidant enzymes of the individuals reared on rice and yeast treatments. Yeast + Bt proteins: individuals fed with yeast mixed with two pure Bt proteins (final concentration of Bt proteins was 852.81 ± 365.20 mg/g, Cry1Ab: Cry1Ac = 1:1). Each value represents the average (±SE) of four replicates. Different uppercase letters indicate significant differences across rice isoline treatments (LSD test; d.f. = 2, 9). **: Significant differences at $P < 0.01$ between *Bt*-rice varieties treatments and their isolines treatments respectively (d.f. = 1, 6).

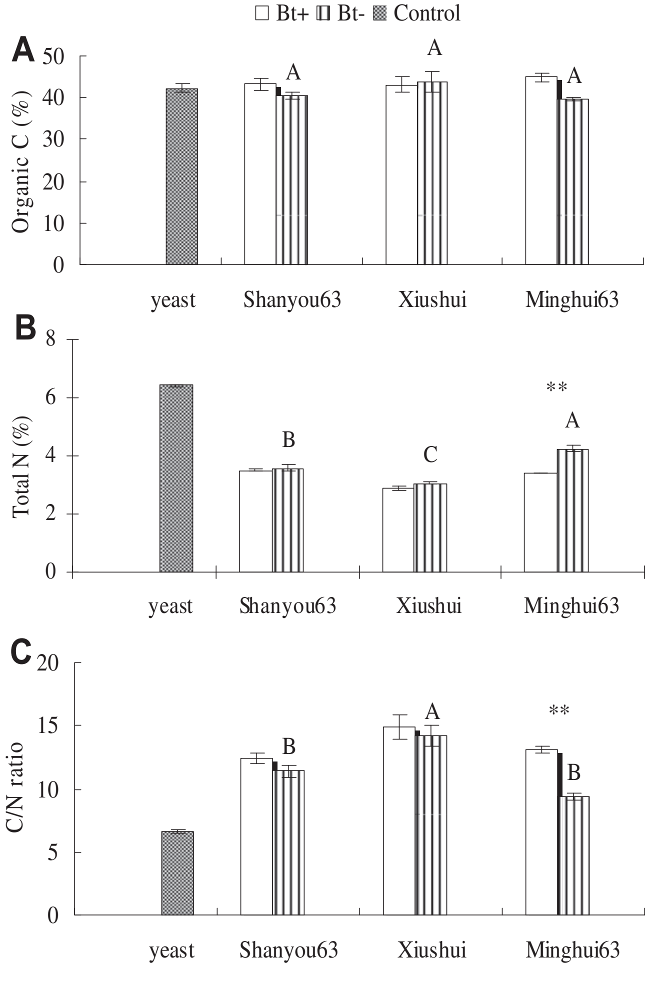

Fig. 4. The organic carbon content (A), total nitrogen content (B) and *C*/*N* ratio (C) of 6 rice leaf varieties and dry baker's yeast. Each value represents the average (±SE) of three replicates. Different uppercase letters indicate significant differences across rice non-*Bt* isoline treatments (LSD test; d.f. = 2, 6). **: Significant differences at $P < 0.01$ between *Bt*-rice varieties and their near-isogenic lines (d.f. = 1, 4).

3.3. *C*/*N* ratio

The results of two-way ANOVA showed that all GM, differences between 3 non-GM varieties and their interaction didn't affect the organic carbon contents of rice leaves (Fig. 4).

The total nitrogen content of dry baker's yeast showed 1.51- to 2.21-fold higher levels than those of the other 6 rice leaf materials. Except for the paired Minghui63 and Huahui1, the total nitrogen content of the other 2 pairs showed no significant difference between *Bt*-and non-*Bt* rice. The total nitrogen content of Huahui1 leaves was significantly lower by 1.24-fold than that of Minghui63 ($P = 0.0015 < 0.01$). Shanyou63 and Xiushui had significantly lower total nitrogen content than Minghui63, while Shanyou63 had significantly higher total nitrogen content than Xiushui. GM ($P = 0.000 < 0.01$), differences between 3 non-GM varieties ($P = 0.000 < 0.01$) and their interaction ($P = 0.001 < 0.01$) all affected the total nitrogen content of rice leaves significantly according to the two-way ANOVA (Fig. 4).

The level of *C*/*N* ratio of dry baker's yeast was lower by 1.43- to 2.26-fold than all rice varieties. The *C*/*N* ratio of Shanyou63, which was similar to Minghui63, was significantly lower than Xiushui. In all three pairs of *Bt*-rice and their isogenic varieties, the *C*/*N* ratio was similar except that Minghui63 was extremely lower than its transgenic *Bt*-line Huahui1 ($P = 0.0006 < 0.01$). Both GM ($P = 0.004 < 0.01$) and differences between 3 non-GM varieties ($P = 0.000 < 0.01$) affected the C/N ratio of rice leaves significantly according to the two-way ANOVA (Fig. 4).

4. Discussion

4.1. Survival and reproduction

The application of *Bt*-rice, used mainly to control rice stem borers and leaf-folders, could decrease insecticide use and increase the yield and profitability of crops. Decreased insecticide use not only reduces costs of pesticides but is also beneficial to the environmental quality and the health of farmers and consumers (High et al., 2004). However, certain characteristics of *Bt*-rice and Bt toxin may make it potentially adverse to soil non-target organisms. Numerous and varied soil-dwelling invertebrates including earthworms, collembolans, mites, woodlice, and nematodes, together with the microbial communities, carry out processes in soil ecosystems, such as nutrient cycling and decomposition of organic matter, which have major ecological and agricultural significance (Moore et al., 1988). Collembolans also support secondary consumers in rice ecosystems by being preyed on by polyphagous predators, which depend on consuming collembolans in early stages of the crop season (Guo et al., 1995; Schoenly et al., 1998; Sigsgaard, 2002; Zhao et al., 2007). Hence, it is important to evaluate the impact of *Bt*-rice on collembolans.

Collembolans reared in the laboratory or studied in natural populations have been used to evaluate the effects of *Bt*-crops and/or purified Bt proteins. Overall results of these studies showed *Bt*-crops had no significant effects on springtail survival, reproduction, growth, population abundance and predation by predators (Yu et al., 1997; Al-Deeb et al., 2003; Bai et al., 2005, 2010; Bakonyi et al., 2006; Griffiths et al., 2006; Chang et al., 2011). However, different effects of *Bt*-rice on *F. candida* were observed in the present study. According to our test results, reproduction was negatively affected by feeding on Kemingdao and Huahui1 and unaffected by BtShanyou63, which had a lower but not statistically significant Bt protein content than Kemingdao and Huahui1, when compared to their respective non-GM near-isogenic lines. Nevertheless, no significant difference of reproduction was found between yeast and yeast mixed with Bt proteins treatments, even if at such significant higher Bt protein concentration than in rice materials. The springtail survival with all diet treatments was similar and almost no mortality was observed, i.e. mortality was recorded only at the normal acceptable control background level. Additionally, individuals reared on yeast and yeast mixed with Bt proteins both had significantly higher reproduction than individuals reared on rice leaf materials. These results agree with previous work reporting that the collembolans *F. candida* and *Protaphorura armata* performed better on yeast than on transgenic wheat or maize tissue and their isogenic lines as well as *P. armata* was unaffected by Bt protein (Romeis et al., 2003; Clark and Coats, 2006; Heckmann et al., 2006).

The change in reproduction of springtails with Huahui1 food was presumably caused by the nutritional quality of the diets as reflected in the C/N ratios, with lower C/N ratios representing higher food quality. Plant nutrient quality appears to be a main factor leading to the differences observed between certain *Bt*- and non-*Bt* varieties, not only for collembolans, but also for other organisms such as isopods (Clark et al., 2006). Dry baker's yeast had the lowest C/N ratio in our study, approximately 1.4- to 2.3-fold lower than rice tissue, and thus higher food quality than the leaf tissue of all the rice varieties. Yeast is generally known to be a very good food source for collembolans (Heckmann et al., 2006; Larsen et al., 2008), so this explains the observed *F. candida* reproduction when living on yeast compared to rice leaf tissue. In our study, individuals reared on Minghui63, which had a lower C/N ratio than any other rice varieties, did reproduce more than when fed the near-isogenic Huahui1 leaves. No significant differences in C/N ratio were observed for the other two transgenic/isogenic pairs. This corresponds to a report that *Oniscus asellus* and *Porcellio scaber* performed better on a low compared to a high C/N plant material diet (Zimmer and Topp, 2000). These results confirm on the one hand that the food nutritional quality may be an important factor for the reproduction of springtails for some transgenic varieties, but on the other hand other plant components not reflected in C/N may also affect the level of reproduction as observed for Xiushui (Figs. 1 and 4C). However, this probably confirms the general experience that NTOs' performance could be influenced by differences both within and among GM or non-GM varieties (Pont and Nentwig, 2005; Griffiths et al., 2007). Thus, our data showed that the reproduction of *F. candida* was more sensitive than the survival to the effects of *Bt*-rice, especially in response to the change within or among *Bt* and non-*Bt* rice varieties.

4.2. Antioxidant enzyme activities

Oxidative stress can be induced by a wide range of environmental factors, like UV stress, pathogen invasion, oxygen deprivation, metal trace elements, and pesticide action among others. Reactive oxygen species (ROS), produced under oxidative stress, have been reported to cause DNA damage, lipid peroxidation (which principally affects membrane structure and function) and protein damage. In addition, ROS can regulate cell function by controlling production or the activation of substances that have biological activities. The antioxidant enzymes, including SOD, CAT and POD, perform their function by scavenging the ROS to protect organisms from oxidative stress (Sugino, 2006). Superoxide radicals ($^{\bullet}O_2^-$) that are generated are converted to H_2O_2 by the action of SOD, CAT and POD scavenging the H_2O_2 in cells (Li et al., 2005; Ning and Long, 2008). Thus, changes in antioxidant enzyme activity can indicate if organisms are experiencing negative environmental factors. Based on this, we determined the activity levels of 3 antioxidant enzymes, T-SOD, CAT and POD, to test the effect of *Bt*-rice on *F. candida* compared to non-*Bt* rice varieties.

No significant difference of each antioxidant enzyme activity was observed between yeast and yeast mixed with Bt proteins treatments, which means Bt proteins don't have negative effects on antioxidant enzyme activities of *F. candida*. The T-SOD activity of individuals reared on BtShanyou63, Shanyou63, Kemingdao and Xiushui was significantly higher than those reared on yeast, Huahui1 and Minghui63, while those fed on Shanyou63 and BtShanyou63 was significantly higher than those living on Kemingdao and Xiushui. When each *Bt*-rice variety was compared to its non-GM near-isogenic line, no significant differences were observed. These results showed that the elevated T-SOD activity of *F. candida* fed with BtShanyou63, Shanyou63, Kemingdao and Xiushui was caused by the difference between basic varieties and not the products of the *Bt*-gene modification. Transgenic rice lines did not exhibit any negative effect on the activity of T-SOD of the *F. candida* that ate them compared to their near-isogenic rice lines food source. The CAT activity of the Kemingdao diet treatment was significantly lower than its non-GM near-isogenic variety (Xiushui) treatment. Moreover, Kemingdao significantly affected the reproduction of *F. candida* compared to its isogenic variety. This was consistent with the result of reproduction that individuals fed Kemingdao showed significantly lower numbers of juveniles than those fed Xiushui. However, though collembolans reared on Huahui1 exhibited significantly lower reproduction than those reared on its near-isogenic non-*Bt* variety, they had similar CAT activity level. Thus it was reasonable to assume that some other kind of antioxidant enzymes of *F. candida* may be affected by Huahui1, and CAT may not be the only antioxidant enzyme that could be affected by *Bt*-rice. Saxena and Stotzky reported that altered expression of secondary plant metabolites following transformation events may influence the overall impact of a GM crop (Saxena and Stotzky, 2001b). The very low enzymatic activity of the CAT enzyme in *F. candida* when fed Kemingdao is difficult to explain, but it may be worth to focus on the possibility of pleiotropic effects: the unintentionally change in plant metabolism and/or the composition of the GM rice as a consequence of the genetic modification in a way that could affect *F. candida* (NTO) – plant relationships.

5. Conclusions

At present, not much research has been reported regarding the effect of *Bt*-crops on the enzyme activity of non-target organisms. Perhaps this is because *Bt*-crops have rarely been investigated for effects on the important biological traits of non-target organisms. Our results showed that catalase activity of *F. candida* may be decreased when fed with certain *Bt*-crop varieties, compared with those fed with non-*Bt* crop varieties (Kemingdao and Xiushui in our study). Moreover, though most studies have generally indicated few or no significant detrimental effects on soil non-target organisms, it is important also to determine if a transgenic crop could result in environmental stress. Our results indicate that the antioxidant enzymes may be important in relation to this. This is the first report on the impact of transgenic *Bt*-rice on the enzymatic activity of non-target organisms. We used *F. candida* as widely used indicator specie. However, it is not an herbivore, even though it may feed on plant materials. It is presumably not adapted to consume plant materials, so this may actually elicit stress responses that would not be detected in a true herbivore. Therefore, we recommend testing the rice material with a herbivorous collembolan, such as *Protaphorura fimata*, in a future study. Furthermore, we also recommend testing GMO rice root material, as this is also a likely route of exposure for soil organisms. Finally, we used limited number of rice varieties in this study, so we recommend to include more other rice varieties in future research to make sure if the change of antioxidant enzyme activity was caused by *Bt* gene modification or rice varieties. Transgenic *Bt*-rice is a promising technology for the management of insect pests because it has few demonstrated side effects on non-target organisms. However, antioxidant enzymes could be impacted by feeding on certain *Bt*-crops that this may have chronic effects manifested over the long term. This work is an important step toward understanding the ecological consequences of transgenic *Bt*-rice plantations.

Acknowledgements

We are grateful to Professor Marvin Harris from Texas A&M University for reviewing the manuscript draft. This project was supported by The Innovation Program of Chinese Academy of Science (KSCX2-EW-Z-6), National Nature Science Fund of China (Nos. 31030012, 30921063 and 40971157) and the Special Program for New Transgenic Variety Breeding of the Ministry of Science and Technology, China (Nos. 2009ZX08012-005B and 2012ZX08011002). Paul Henning Krogh was supported by grant no 3304-FVFP-09-B-004 from The Danish Food Industry Agency programme “Future crops – application of biotechnology with special focus on genetically engineering for the benefit of the whole society”.

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