Article ID: 1001-0742(2005)05-0866-03 CLC number: X131.1 Document code: A

# No difference in activity of *Sod*-1 genotypes of *Poa annua* to short-term treatment of ambient gaseous organic pollution

CAI Yue-wei, CHEN Xiao-yong*

(Shanghai Key Laboratory for Ecological Processes and Restoration in Urban Areas, Department of Environmental Sciences, East China Normal University, Shanghai 200062, China. E-mail: xychen@des.ecnu.edu.cn)

**Abstract**: Our previous studies indicated that genotypes at locus *Sod*-1 of *Poa annua* changed clinally along a gradient of gaseous organic pollution. In the present study, we aimed to know whether there were differential responses of activities of different superoxide dismutase (SOD) genotypes to short-term treatment of ambient gaseous organic pollution. Significant bias from Hardy-Weinberg equibrium was observed on locus *Sod*-1, and no genotype *Sod*-1-*BB* was found. Significantly increased activities were observed in most treatments for genotype *Sod*-1-*AA* and in one treatment for genotype *Sod*-1-*AB*. However, no significant difference in SOD activities was found between the two genotypes. It was interpreted that fitness difference between the two genotypes was small and treatment duration was too short or extent of ambient organic pollution was too low to lead to differential responses. Other environmental factor effects on activities of superoxide dismutases can also explain the results.

**Keywords**: superoxide dismutase; genotype; activity; gaseous organic pollution; *Poa annua*

## Introduction

Numerous studies have indicated abundant genetic diversity in plant species, since isozymes was used as genetic markers in 1966 (Hamrick, 1989; Nybom, 2000). It is widely appreciated that genetic diversity is the base for the species to adapt the changing environments. In other words, different alleles or genotypes were thought to have differential fitness under different environments. Actually, a lot of studies have shown significant differences in frequencies of alleles or genotypes of populations from distinct habitats (Prentice, 1995; Chen, 1997; Mitton, 2004), under pollution (Prus-Glowacki, 1999; Longauer, 2001; Chen, 2003). However, new questions arise. For example, to what extent different electrophoretic phenotypes of an enzyme can affect the fitness of organisms? Do different allozyme genotypes have direct physiological effects?

The most thorough studies on allozymes have identified enzyme kinetic, physiological, and differential fitness among loci for the leucine aminopeptidase polymorphism in blue mussels, *Mytilus edulis* (Koehn, 1987), the phosphoglucose isomerase polymorphism in sulfur butterflies, *Colias eurytheme* and *C. philodice eriphyle* (Watt, 1983; 1986; 1992), the lactate dehydrogenase polymorphism in the common killfish, *Fundulus heteroclitus* (Powers, 1991; Power, 1994), and the alcohol dehydrogenase polymorphism in tiger salamanders, *Ambystoma tigrinum* (Mitton, 1986; Carter, 2000). Although there were a lot of studies have observed relationships between allozymic variation and environmental factors in plant species, there were very few studies have established phenotypic fitness among loci in plants like the above studies on animals.

*Poa annua* L. (Poaceae) is a weedy species widely distributed over the world. In the previous studies, we found that it shows genetic changes to different air pollutants (Chen, 2000; 2003). Along a gaseous organic pollution gradient, clinal changes were found in several loci. For instance, increased frequency of *Sod*-1-*AB* in *P. annua* populations was observed as the distance to the pollution source decreased and, in most heavily polluted sites, all the individual were heterozygous at locus *Sod*-1 (Chen, 2003). Because superoxide dismutases (SODs) are involved in the tolerance to stress (Alscher, 2002), we wondered whether the changes in genotype frequencies are the results of differential responses of SODs to the gaseous organic pollution.

In the present study, we treated individuals, grouped according to their genotypes, with ambient gaseous organic pollution and aimed to find whether different genotypes response differentially in activity to the pollution.

## 1 Materials and methods

### 1.1 Materials

*Poa annua* individuals were collected from clear sites (Campus of East China Normal University and nearby), and transplanted into small pots with diameter of about 5 cm. They were genotyped using PAGE as previously described. We found only two genotypes on locus *Sod*-1, as reported before. The individuals were grouped according to the genotype, heterozygote group that includes individuals with genotype *Sod*-1-*AB* and homozygote group that includes individuals with genotype *Sod*-1-*AA*.

### 1.2 Treatment of ambient gaseous organic pollution

Individuals used for treatment of ambient organic pollution were collected and transferred to small pots. Each pot contained one individual. These individuals were cultured in laboratory for three weeks before treatments. One week before treatment, one leaf from each individual was sampled for identifying genotype and assaying SOD activity. Individuals with similar activities were used for the treatment or as the control. The individuals were grouped according to the genotype, i.e., *AA* group and *AB* group. Twenty individuals of each group were put at the polluted site, a factory of chemical reagent. The main products of the factory are acetic acid, acetic anhydride, acetidin, triacetin, midbody of medicine and pesticide (Chen, 2003). Individuals in the laboratory were as the control. In the previous studies, we found SOD is very sensitive to air pollution (Chen, 1995). Therefore, samples were collected after 4, 8, 24, 36 and 48 h, and subject to analysis immediately.

### 1.3 Electrophoresis and assay of superoxide dismutase

Leaf samples were homogenated with Tris-HCl buffer

Foundation item: The "211 Project" Porgram and Shanghai Priority Academic Discipline Program; * Corresponding author

(pH 7.8, containing 0.04% KCl, 0.04% EDTA, 0.2% $MgCl_2$, 5% sugar, 4% PVP and 0.1% β-mercaptoethanol (V/V)). After centrifugated at 10000 r/min under 4℃ for 10 min, the supernate was subject to analysis.

Genotypes of each individual were analysed with polyacrylamide gel electrohporesis (PAGE). Zymgraphs of Cu-ZnSOD, FeSOD and MnSOD were identified using gradient polyacrylamide gels of 4% to 20%. Based on the different sensitivity to inhibitors (KCN, $H_2O_2$ or ethanol-chloroform), the three enzyme-types were identified (Luo, 1996).

SOD activities were assayed based on the inhibition of NBT photo-reaction (Chen, 1995). A unit of SOD was defined as 50% inhibition of the reaction.

## 2 Results

According to the different sensitivities to the inhibitors, zone 3 was Mn-SOD, and the other two zones were Cu-Zn-SOD(Fig.1). We termed the coded genes *Sod*-1, *Sod*-2 and *Sod*-3, respectively.

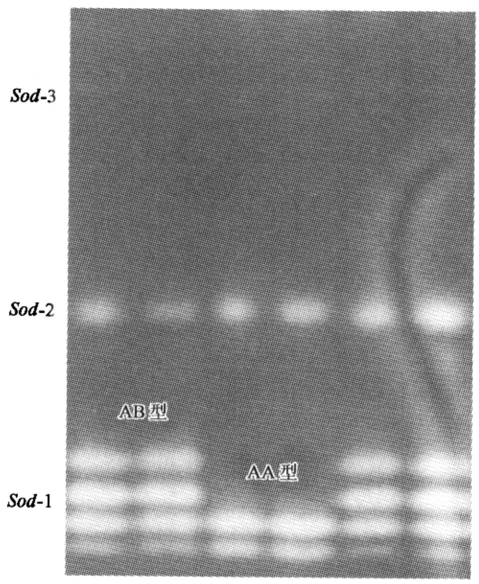


Fig.1 The zymogram of superoxide dismutase of *Poa annua*

As reported previously, only *Sod*-1 was polymorphic and we found only two genotypes on locus *Sod*-1 of *Poa annua* (Chen, 2003; Li, 2004). In polluted site, only heterozygous individuals were found, whereas heterozygous and homozygous individuals were found in clear site. Chi-square test indicated that significant bias ($P < 0.001$) from Hardy-Weinberg equilibrium was found in the two sites (Table 1).

**Table 1 Genotypic and allelic frequencies at *Sod*-1 locus of *Poa annua* in polluted and clear sites**

| Site | Sample size | Genotype frequency | | Allelic frequency | |
|---|---|---|---|---|---|
| Clear site | 261 | *AB* | 37.9% | *A* | 81.0% |
| | | *AA* | 62.1% | *B* | 19.0% |
| Polluted site | 36 | *AB* | 100% | *A* | 50.0% |
| | | *AA* | 0% | *B* | 50.0% |

Generally, activity of SOD was increased when treated with ambient organic pollution. After two days treatment, SOD activity of genotype *Sod*-1-*AA* was 164% of the control. Except for 24 h treatment, *Sod*-1 genotype *AA* showed significant increases in activity with the treatment of gaseous organic pollution(Fig.2).

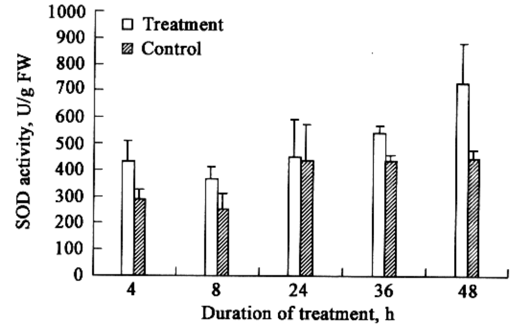


Fig.2 Changes of SOD activity of genotype *Sod*-1-*AA* of *Poa annua* treated with ambient organic pollution

Although SOD activities of genotype *Sod*-1-*AB* also increased as treatment duration increased, no significant difference was observed, except for that of 8 h treatment (Fig.3).

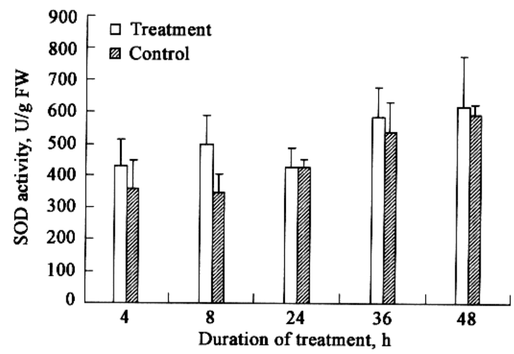


Fig.3 Changes of SOD activity of genotype *Sod*-1-*AB* of *Poa annua* treated with ambient organic pollution

Before treatment, no significant difference was observed in SOD activities between *Sod*-1 genotypes *AB* and *AA*. When treated with ambient organic pollution, significant difference in activity was found between the two genotypes after treatment for 8 h. However, when the duration lasted, no significant difference was found between the two genotypes (Fig.4).

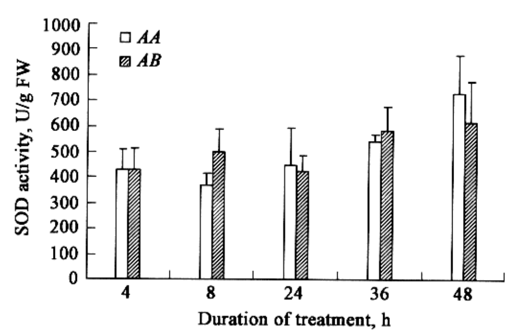


Fig.4 Comparison of SOD activities of different genotypes of *Poa annua* when treated with ambient organic pollution

## 3 Discussion

Investigation on the wild populations from polluted and clear sites indicated that genotype *AB* of locus *Sod*-1 has a fitness superior to genotype *AA*, because only genotype *AB* had been found in the most polluted sites(Chen, 2003). The present study indicated the similar situation (Table 1). Closer to pollution source, more genotype *AB* was found. In the most polluted sites, all the individuals belonged to genotype *AB*.

Under pollution stress, increased activities were found in the genotype *AA* (Table 1), indicating that SOD activity was sensitive to organic pollution, as observed in other pollution stress, such as $SO_2$ (Chen, 1995). However, no significant increases were observed in SOD activities of genotype *AA*, except one treatment. It was due to the high activities of the control, but the reason was unknown.

No significant differences were observed in SOD activities between genotype *AA* and *AB* during the treatment (Fig. 4). There were two explanations for the result. Firstly, fitness difference between the two genotypes was small and the treatment duration was too short or extent of ambient organic pollution was too low to lead to differential responses. In polluted site, the individuals suffered long-term stress of organic pollution and genotype *AA* was eliminated gradually. Fitness reduction in genotype *AA* under stress of organic pollution may be significant for long-term. To identify whether this hypothesis, it needs to treat *P. annua* for longer duration or under more serious stress.

Secondly, other environmental factors may affect the results. SODs are important scavengers of free radicals in plants. Because pests, drought, other pollutants and even changes in climate factors (such as, wind, light, temperature and rain) may lead to the increase of free radicals, changes of SOD activities are difficult to predict in the field. This is partly supported by much higher standard errors of SOD activities observed under treatment than the control in the present study (Fig. 4). Actually, biased results in the fields from theoretic predictions had been observed in a lot of studies due to the "noise" disturbance (Longauer, 2001; Li, 2004).

## References:


Alscher R G, Erturk N, Heath L S, 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants [J]. Journal of Experimental Botany, 53: 1331—1341.

Carter P A, Mitton J B, Kocher T D *et al*., 2000. Maintenance of the alcohol dehydrogenase polymorphism in *Tiger Salamanders*, Ⅱ. Differences in biochemical function among allozymes [J]. Functional Ecology, 14: 70—76.

Chen X Y, Li N, Shen L *et al*., 2003. Genetic structure along a gaseous organic pollution gradient: A case study with *Poa annua* L. [J]. Environmental Pollution, 124: 449—455.

Chen X Y, Pang Y O, Qiu C H. 2000. Effects of sulfur oxidants on genetic structure of *Poa annua* populations [J]. China Environmental Science, 20: 124—127.

Chen X Y, Song Y C, 1995. Studies on the feasinility of SOD activity as bioindicators of $SO_2$ pollution [J]. Acta Phytoecologica Sinica, 19: 23—28.

Chen X Y, Song Y C, 1997. Relationships between environmental factors and allozymic variation of *Cyclobalanopsis glauca* populations in Eastern China [J]. Journal of Xiamen University (Natural Science), 36: 774—780.

Hamrick J L, Godt M J W, 1989. Allozyme diversity in plant species [M]. In: Plant population genetics, breeding, and genetic resources (Brown A. H. D., Clegg M. T., Kahler A. L. and Weir B. S. ed.). Sunderland, Massachusetts, USA: Sinauer. 43—63.

Koehn R K, Hilbish T J, 1987. The adaptive importance of genetic variation [J]. American Scientist, 75: 134—140.

Li N, Chen X Y, Shen L *et al*., 2004. Effects of traffic pollution on the genetic structure of *Poa annua* L. populations [J]. Journal of Environmental Sciences, 16: 454—457.

Longauer R, Gomory D, Paule L *et al*., 2001. Selection effects of air pollution on gene pools of Norway spruce, European silver fir and European beech [J]. Environmental Pollution, 115: 405—411.

Luo G H, Wang A G, Fu A G, 1996. The location staining method for distinguishing different types of SOD [J]. Progress in Biochemistry and Biophysics, 23: 356—359.

Mitton J B, Carey C, Kocher T D, 1986. The relation of enzyme heterozygosity to standard and active oxygen consumption and body size of tiger salamanders *Ambystoma tigrinum* [J]. Physiological Zoology, 59: 574—582.

Mitton J B, Duran K L, 2004. Genetic variation in pinon pine, *Pinus edulis*, associated with summer precipitation [J]. Molecular Ecology, 13: 1259—1264.

Nybom H, Bartish I V, 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants [J]. Perspectives in Plant Ecology, Evolution and Systematics, 3/2: 93—114.

Power D A, Smith M, Gonzalez-Villasebor I *et al*., 1994. A multidisciplinary approach to the selection/neutralist controversy using the model teleost *Fundulus heteroclitus* [J]. Oxford Surveys in Evolutionary Biology, 9: 43—107.

Powers D A, Lauerman T, Crawford D *et al*., 1991. Genetic mechanisms for adapting to a changing environment [J]. Annual Review of Genetics, 25: 629—659.

Prentice H C, Lonn M, Lefkovitch L P *et al*., 1995. Associations between allele frequencies in *Festuca ovina* and habitat variation in the alvar grasslands on the Baltic Island of Oland [J]. Journal of Ecology, 83: 391—402.

Prus-Glowacki W, Oleksyn J, Reich P B, 1999. Industrial pollutants tend to increase genetic diversity: evidence from field-grown European Scots pine populations [J]. Water Air and Soil Pollution, 116: 395—402.

Watt W B, 1992. Eggs, enzymes, and evolution-natural genetic variants change insect fecundity [C]. Proceedings of the National Academy of Sciences, USA. 89: 10608—10612.

Watt W B, Carter P A, Donohue K, 1986. Females' choice of "good genotypes" as mates is promoted by an insect mating system [J]. Science, 233: 1187—1190.

Watt W B, Cassin R C, Swan M S, 1983. Adaptation at specific loci. III. Field behaviour and survivorship differences among *Colias* Pgi genotypes are predictable from *in vitro* biochemistry [J]. Genetics, 103: 725—739.


(Received for review September 29, 2004. Accepted January 31, 2005)