Pergamon

Biochemical Systematics and Ecology 30 (2002) 809–817

biochemical systematics and ecology

www.elsevier.com/locate/biochemsyseco

Genetic diversity in three perennial grasses from the Semipalatinsk nuclear testing region of Kazakhstan after long-term radiation exposure

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Received 5 July 2001; accepted 6 September 2001

Abstract

The extent and structure of the genetic diversity of plant populations from the Semipalatinsk region of Kazakhstan, adjacent to a former major nuclear test site, have been studied using RAPD (Random Amplified Polymorphic DNA) marker analyses. The DNAs from three perennial species, *Stipa capillata*, *Hordeum bogdanii*, and *Agropyron pectinatum*, each collected from heavily (HPZ), moderately (MPZ) and lightly polluted zones (LPZ) have been analyzed using RAPDs. The results show a significantly higher level of variability in plants collected from the highest radiation pollution area compared with the moderately and lightly radiation contaminated zones for *A. pectinatum* and *H. bogdanii*. Variation was five times as higher in heavily exposed *H. bogdanii*, and two times higher in *A. pectinatum* populations compared to their lightly contaminated populations. *H. bogdanii* appears to be very sensitive to radiation and as such is a good indicator species for mapping radiation pollution at nuclear test sites or nuclear accidents. © 2002 Published by Elsevier Science Ltd.

Keywords: Mutations; *Agropyron pectinatum*; *Hordeum bogdanii*; *Stipa capillata*; RAPD; Diversity; Nuclear testing radiation pollution; Semipalatinsk

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0305-1978/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0305-1978(02)00021-2

1. Introduction

The Semipalatinsk region of Kazakhstan was the site of almost all of the above- and underground nuclear tests conducted by the former USSR. Over the past 45 years, specific areas of the Semipalatinsk region periodically received very heavy dosages of radiation pollution, which led to negative effects on agricultural lands, economic development and human lives. However, due to the financial crisis in former Soviet Union very little research was performed to estimate the level of the ecological catastrophe.

The present study determined the effect of different long-term radiation dosages on the structure of genetic variation of *Agropyron pectinatum* Rish., *Hordeum bogdanii* Wilensky and *Stipa capillata* L., which are natural dominant perennial grasses in the Semipalatinsk region. The development of DNA markers, based on the polymerase chain reaction (PCR) has greatly increased the capabilities of scientists to efficiently extract more information on variability of genomes. Random Polymorphic DNA (RAPD) analysis can be very informative for populational genetics (Demeke and Adams, 1994; Lynch and Milligan, 1994). RAPDs are very sensitive to the temperature profiles and these may vary between laboratories, however, if proper laboratory procedures are followed, RAPDs can be reproducible (Adams et al., 1998).

2. Materials and methods

Several thousand spikes of different grass genera were collected in Semipalatinsk region during the summer and fall of 1994. Three perennial grass species, *A. pectinatum*, *H. bogdanii* and *S. capillata*, which are natural dominants and good representatives of the Kazakhstan steppe grasslands, were selected for DNA analysis. The predominant drifts of almost all the nuclear fallouts were towards southeast and east. Thus, the study area southeast of the epicenter was divided into three collecting zones representing high, moderate and low fallout of radioactive elements. In total, 45 populations of these three species, each consisting of at least 40 spikes from different plants, approximately equidistant from each other in a southeast direction were collected in each zone: HPZ (heavily polluted zone, within 100 km range of Semipalatinsk Test Site (STS)), MPZ (moderately polluted zone, within 300 km range) and LPZ (lightly polluted zone, 500 km from STS). The LPZ is so distant that it is used as a control in this study. Due to the limitations of time and logistics only 23 populations were chosen for DNA extraction: eight populations each of *A. pectinatum*, and *H. bogdanii,* and seven of *S. capillata* (Fig. 1). In addition, these particular populations were chosen because of their habitat similarity and because they represent the steppe region. Note that population STS in HPZ was collected from a mountainous area. For each population, DNA from 10 randomly chosen plants was extracted and used for analysis.

DNA was extracted from seeds using the protocol of Dellaporta et al. (1983) with the addition of 1% (w/v) polyvinilpirrolidone and Pronase E (10 mg/ml). PCR was performed in a volume of 15 μl containing 50 mM KCl, 10 mM Tris–HCl (pH 9.0),

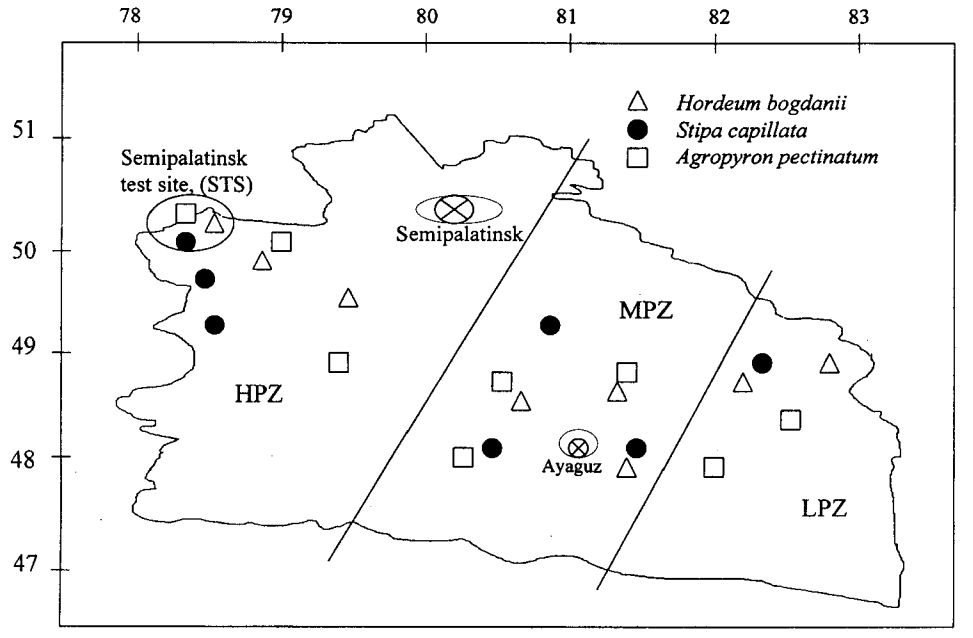

Fig. 1. Collection sites for plant populations from the Semipalatinsk region of Kazakhstan. HPZ, MPZ, LPZ=Heavily, Moderately, and Lightly Polluted Zones, respectively.

2.0 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM of each dNTP, 0.36 mM of each primer, 0.3 ng genomic DNA and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination.

Fifteen 10-mer primers (from University of British Columbia) that gave several bright bands, did not have any false bands and were found to be reproducible in replicated analyses were utilized—131: GAA ACA GCG T; 134: AAC ACA CGA G; 184: CAA ACG GAC C; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 234: TCC ACG GAC G; 239: CTG TGG CGG T; 244: CAG CCA ACC G; 249: GCA TCT ACC G; 250: CGA CAG TCC C; 268: AGG CCG CTT A; 327: ATA CGG CGT C; 338: CTG TGG CGG T; 346: TAG GCG AAC G; 399: TTG CTT GCC G.

DNA amplification was performed in a MJ Programmable Thermal Cycler (MJ Research, Inc). The thermal regimen was: 94 °C (1.5 min) for initial strand separation, then 40 cycles of 38 °C (2 min), 72 °C (2 min), 91 °C (1 min). This was followed by one cycle of 38 °C (2 min) and 72 °C (5 min) for final extension. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by UV after staining with ethidium bromide.

The genetic diversity index of Nei (1972) was calculated for comparative analysis and partitioning of total variation into variation within populations, and between populations within a zone or between zones was based on the Shannon information index (Lewontin, 1972). Similarity measures (Manhattan Metric) (SI) and principal

coordinate analysis (PCO) were computed as described in Adams (1975) and Gower (1966), respectively.

3. Results

The Shannon Information Index analysis for all three species is given in Table 1. Notice that *A. pectinatum* is more variable than *H. bogdanii* or *S. capillata* at the population, region and specific levels (Table 1).

The estimates of genetic diversity for all three species based on RAPD analysis are given in Table 2. There is a general trend to be more variable from the lightly polluted to the highly polluted zone for each species. However, some anomalous populations exist such as *H. bogdanii* population 1 in the MPZ that has a variation of 0.005, that is much less than found in populations 1 and 2 of the LPZ (0.0162, 0.0109, Table 2). A total of 132, 160, and 248 RAPD bands were generated using the 15 UBC primers for *H. bogdanii*, *S. capillata*, and *A. pectinatum*, respectively. Nei's genetic diversity index in the highest polluted zone than in either the MPZ or LPZ for each of the three species (Table 2) shows that each species differs in its variability among species, irrespective of zones. However, the differences in variability between zones (within each species) indicate that the genetic diversity for each species changes from zone to zone. It is interesting that *H. bogdanii* is about 4–5 times as variable in the HPZ (0.0738) as in MPZ (0.0176) or LPZ (0.0136). *A.*

Table 1
Shannon information index analyses for within population, region and species for three grasses

Primer	*A. pectinatum* (average)			*H. bogdanii* (average)			*S. capillata* (average)		
	Population	Region	Species	Population	Region	Species	Population	Region	Species
131	0.215	0.244	0.291	0.046	0.082	0.111	0.042	0.047	0.055
134	0.116	0.141	0.213	0.017	0.018	0.024	0.147	0.155	0.199
184	0.129	0.159	0.263	0.031	0.034	0.042	0.028	0.037	0.049
212	0.187	0.247	0.308	0.045	0.065	0.088	0.103	0.138	0.185
218	0.207	0.231	0.294	0.044	0.066	0.083	0.146	0.167	0.192
234	0.209	0.231	0.341	0.008	0.009	0.011	0.061	0.069	0.094
239	0.126	0.138	0.151	0.069	0.079	0.092	0.021	0.025	0.031
244	0.122	0.146	0.176	0.022	0.025	0.029	0.0	0.0	0.0
249	0.167	0.186	0.224	0.041	0.0625	0.081	0.063	0.079	0.106
250	0.281	0.313	0.361	0.003	0.004	0.004	0.128	0.143	0.183
268	0.151	0.174	0.228	0.046	0.052	0.059	0.018	0.021	0.023
327	0.251	0.311	0.334	0.033	0.061	0.083	0.075	0.095	0.158
338	0.078	0.091	0.111	0.025	0.045	0.061	0.006	0.008	0.011
346	0.191	0.219	0.246	0.066	0.089	0.134	0.102	0.122	0.148
399	0.186	0.212	0.245	0.041	0.058	0.079	0.023	0.031	0.035
Average	0.174	0.203	0.252	0.036	0.049	0.065	0.064	0.076	0.098

Table 2
Genetic variability of wild species from Kazakhstan based on 15 RAPDs (Nei's index, HE and ANOVA). Notice the increased variation from lightly to HPZs for all three species and that *A. pectinatum* seems to be most affected by radiation

Zone	Population	*H. bogdanii*	*S. capillata*	*A. pectinatum*	Average per zone***
LPZ	1	0.0162	0.061	0.1014	
	2	0.0109		0.1056	
Average for LPZ		0.0136	0.061	0.1035	0.0594
MPZ	1	0.005	0.069	0.1319	
	2	0.0267	0.054	0.1527	
	3	0.0211	0.077	0.1655	
Average for MPZ		0.0176	0.067	0.1501	0.0783
HPZ	1	0.0492	0.121	0.2554	
	2	0.0818	0.061	0.2435	
Semipalatinsk test site	(STS)	0.0903	0.077	0.3106	
Average for HPZ		0.0738	0.086	0.2698	0.1432
Average per species*		0.035	0.074	0.1745	0.0936

*$p<0.05$, ***$p<0.001$

pectinatum was about twice as variable in the HPZ as in other zones. In contrast, *S. capillata* was not much affected (Table 2).

One way-ANOVA revealed that *H. bogdanii* and *A. pectinatum* have very different diversities among zones (Table 3). All three species show the same pattern of diversity within population > between zones > between populations within a zone. The total genetic variation for all the three species was partitioned as: 65.19% within populations; 14.09% between populations; and 20.74% between zones (Table 3).

PCO was performed for *S. capillata* (Fig. 2), which was least variable of the three species. The major trend (axis 1, 40%) was the separation of the heavily radiation polluted populations (STS, HPZ) from the MPZ and LPZ populations (Fig. 2). The ordination clearly differentiated all three zones, where the combination of axes 1 (40%) and 3 (15%) separated the zones (Fig. 2). Notice also that the populations are ordinated from left to right and that it corresponds with the west to east distribution of the zones.

Table 3
Analysis of genetic diversity (Shannon information index, %) for populations from the Semipalatinsk region based on 15 RAPD primers

Area	*S. capillata*	*H. bogdanii*	*A. pectinatum*	Average
Within population	65.66	60.55	69.36	65.19
Between populations within a zone	13.15	17.99	11.14	14.09
Between zones	21.19	21.54	19.5	20.74

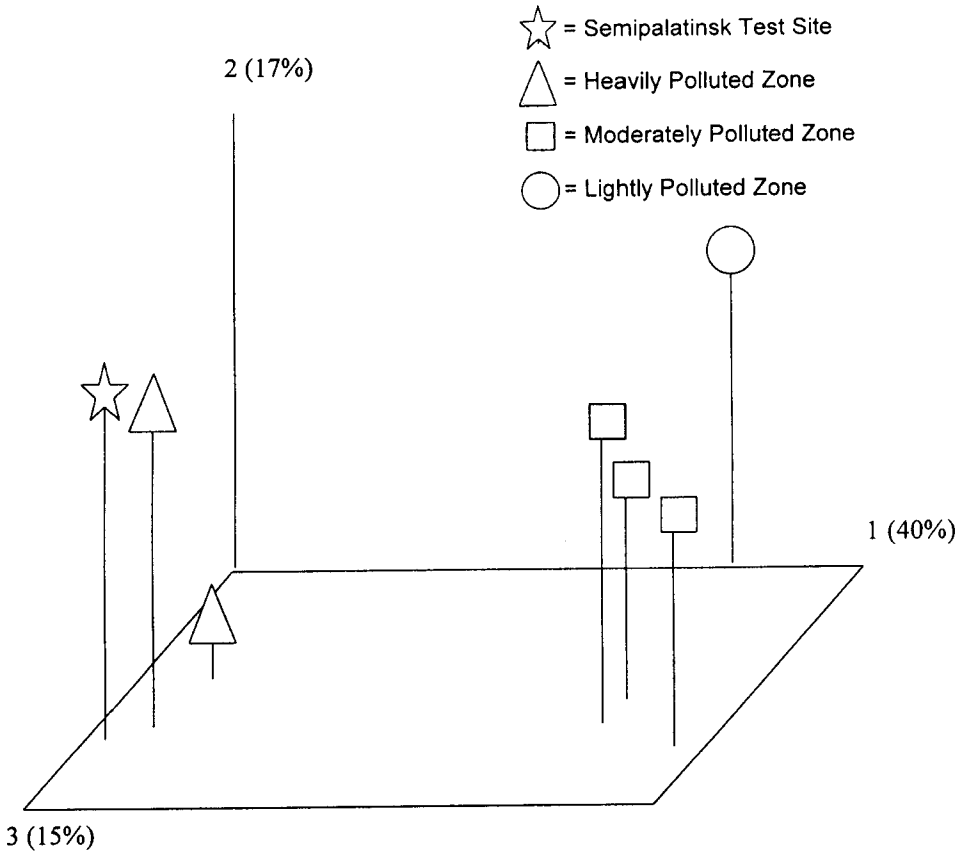

Fig. 2. Principal Coordinate Analysis (PCO) of *S. capillata* based on 160 RAPD bands. Note the separation between the test site and the HPZ and the other zones.

Table 4 lists RAPD loci which most contributed to this discrimination and describes the nature and level of variation for *S. capillata* extracted using ANOVA.

4. Discussion

One of the important aspects of ecological and agricultural recovery from radiation pollution is the understanding of the effect of radiation on organisms and their populations. Whereas laboratory controlled research on the effect of the radiation on individuals has been conducted extensively (El-Mancy and Salisbury, 1974; Teramura, 1983; Tano, 1986), little research had been done on at the population level until the recent nuclear accident near Chernobyl, Ukraine. Available publications from Hiroshima and Nagasaki bombings and Chernobyl region yield quite controversial results. Unlike research on human populations from Hiroshima and Nagasaki that

Table 4
RAPD loci for *S. capillata*, extracted on the basis of ANOVA calculation, that describe the difference of populations among HPZ, MPZ and LPZ

RAPDs	*F* ratio	Description of RAPDs frequency
134C	2.87	Higher frequency in populations HPZ1, HPZ2, and STS
134D	3.59	Lower frequency in populations HPZ1, HPZ2, and STS
184D	3.65	Lower frequency in populations HPZ1 and STS
218B	6.34	Absence in populations HPZ1, HPZ2, and STS
212E	8.99	Presence only in population HPZ1
227H	2.25	Presence only in population HPZ1
250C	7.32	100% present in populations HPZ1, HPZ2, and STS
250F	6.31	Lower frequency in populations MPZ3, HPZ1, and STS
250J	6.39	High frequency in populations HPZ1, HPZ2, and STS
327C	9.55	Absence in populations HPZ1, HPZ2, and STS
346H	2.17	Higher frequency in population STS
346F	27.88	Absence in population STS
346J	3.91	Presence only in populations HPZ2 and STS

claimed almost no effect on genetic changes (Neel et al., 1988), publications from the Chernobyl region point to substantial genetic variation for a number of species, including human populations (Abramov et al., 1992; Baker et al., 1996; Dubrova et al., 1996). However, Baker et al., 1996 suggested that high genetic variation under declining radiation level may have been coupled with other remaining pollutants, such as high concentrations of heavy metals, to cause some of the effects.

Overall available information on the effect of radiation stress on plant populations is very limited. Most of the studies have been descriptive rather than experimental and have provided three conclusions. First, studies have shown that different genotypes within the same species and different species respond differently to radiation exposure (Pozolotina, 1981; Elstner et al., 1989; Smolders, 1993). Secondly, different types of cytogenetic damages may occur in the same population (Grinikh and Shevcnenko, 1992). Thirdly, radiation exposure may alter the extent and structure of genetic variation and diversity in plant populations (Elstner et al., 1989; Abramov et al., 1992).

However, hypotheses, let alone answers, are lacking for important basic questions on the effect of radiation on plant populations. One key question is whether radiation stress will increase or decrease the genetic diversity within or between populations over the short and long distances from the epicenter. Abramov et al. (1992) examined the extent of genetic variation via starch gel electrophoresis of leaf proteins of several loci in populations of *Arabidopsis thaliana* growing within 30 km of Chernobyl. In addition to an increase in the mutation rate, they reported that the overall amount of genetic variation both within and between populations had decreased. They concluded that radiation stress removed many radiation-sensitive genotypes leading to fewer genotypes per population and a decrease in genetic diversity within the populations.

In this study, we performed comparative RAPD analysis for three different domi-

nant, perennial grass species growing at different distances from the nuclear test site. Our results show a difference between species in total genetic diversity (Table 2). In addition, all three species showed a tendency towards increasing genetic diversity index values from LPZ to MPZ, despite ecological similarity of the collected sites. The exception was the sharply different mountainous STS location. Though most of the variation was concentrated within populations, the variation of genetic diversity between zones for all three species exceeded the variation of diversity between populations within a zone. Thus, the genetic diversity of these wild grass populations appeared to increase with increasing exposure to radiation over the course of these exposures.

There are, of course, alternate hypotheses for these data. Despite apparent ecological similarities of the collected sites, natural geographic differences and other parameters also might explain the outcome of our results. It is possible that the more western populations (near the test site) were just naturally more variable even before nuclear testing. That could be true for one species, but seems unlikely for all three taxa analyzed in this study. Also, as noted by Prister et al. (1992), the amount of radiation exposure received by a specific site may significantly differ from site to site, even over short distance. The experiments for genetic diversity of the species over short distances from STS and within the STS are underway to address these questions.

Acknowledgements

This research was supported by Fulbright Fellowship provided by CIES, USA (to YT) and funds from Baylor University.

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