Bull. Environ. Contam. Toxicol. (1996) 56:690-695

Bulletin of Environmental Contamination and Toxicology

Coal Fly Ash Exposure at Agronomic Levels Does Not Induce Triploidy in Maize

C. Taets, A. L. Rayburn

320 ERML, 1201 West Gregory, Department of Crop Sciences, University of Illinois, Urbana, Illinois 61801, USA

Received: 2 July 1995/Accepted: 30 September 1995

Coal burning is a major source of energy production in the United States. A major problem with coal combustion for energy production is that around 75 million metric tons of solid residue are generated each year (Murarka et al, 1987). Approximately 50 million metric tons of this residue is fly ash (Rehage and Holcomb, 1990). A major problem for electric companies is the disposal of this fly ash. The most common methods of fly ash disposal are landfilling and ash settling ponds (Suloway et al, 1983). The U.S. government supports recycling coal fly ash due to its abundance and versatility as well as environmental and economic factors (Keefer, 1993). An attractive use of fly ash would be as a soil amendment to agricultural land.

Application of coal fly ash to agricultural land could provide a means to increase the fertility of the soil while alleviating the need for quickly disappearing landfill space. Fly ash contains several nutrients such as S and B which are beneficial for plant growth (Elseewi et al. 1980; Plank and Martens, 1974). A potential problem with this land application is that, in addition to plant nutrients, fly ash also contains potentially harmful trace elements such as As, Cd, Pb, and Sb (Thicke, 1988). Although several studies have indicated potential benefits of fly ash application to agricultural land, these studies were looking at gross phytotoxic characteristics and therefore could have missed subtle genotoxic effects (Adams et al, 1972; Fail and Wochok, 1977; Thicke, 1988).

A study in our laboratory has shown that growing maize seedlings in growing media amended with fly ash results in genomic alterations (McMurphy and Rayburn, 1993). Fly ash had a significant effect on total nuclear DNA content and cell cycle parameters. These effects were small, < 5%, and did not appear to affect normal development.

Correspondence to: A. Rayburn

The concern is that these small effects could accumulate over multiple generations, resulting in detrimental effects to future generations.

During the previous study, four percent of the maize seedlings exposed to fly ash were found to be triploids. No triploid plants were found in the control treatments. It has been reported that heavy metals such as those found in fly ash cause mitotic disruption (Radecki et al, 1989). Such events could result in polyploidization. If the percent of triploid plants was increased by fly ash, this would cause a decrease in fertility and thus decrease yield. The purpose of this study was to determine if fly ash could induce triploidy in maize.

MATERIALS AND METHODS

The fly ash, classified as bituminous fly ash, was provided by the Central Illinois Public Service (CIPS) power plant at Coffeen, Illinois. Fly ash was mixed with Terra-lite® Metro-mix 200® growing medium (Hummert Seed Co, St. Louis, MO) at rates of 0, 35, and 500 tons per hectare (t/ha). These rates represent the suggested rates of fly ash application to agricultural land (Adriano et al., 1980; Thicke, 1988). Five kernels of each of the maize lines described below were planted in the various treatments in 0.7 L pots at a depth of 1.25 cm and placed in a growth chamber. The growth chamber was set for 12 hour days at 24°C and 12 hour nights at 20°C. The seedlings were analyzed two weeks after planting.

A section of the seedling extending 2.5 cm above the surface of the growing media was excised from three of the five plants. A 0.8 cm section was chopped from each of the three plants. The chopped portions of the three plants were then combined and the nuclei isolated and stained with Propidium Iodide according to McMurphy and Rayburn (1993). The nuclei were analyzed on a Coulter EPICS 750 series flow cytometer (Coulter Electronics, Hileah, FL) tuned to an excitation wavelength of 488 nm. Five thousand nuclei were analyzed per sample. The data was collected as a histogram plotted as fluorescent channel vs number of nuclei. Each histogram was analyzed for the presence of triploid individuals.

Three separate experiments were performed. In the initial experiment, kernels of crosses between diploid and tetraploid maize were analyzed. Crosses between diploid and tetraploid individuals are one method of producing a high frequency of triploid individuals. Thus, the ability of flow cytometry to detect triploids could be tested. One set of crosses involved diploid and tetraploids in the maize background R75 while the other set of crosses were in B89 background. These plants were grown in control soil and analyzed as described above.

In the second experiment, early early synthetic kernels were planted in both control and 500 t/ha fly ash treatments. Early early synthetic was chosen due its ease in handling in laboratory situations. In the third experiment, diploid lines of W22, Va35, and H55 were selected due to their well documented status as maize inbred lines. The hybrids B73 X Mo17, Mo17 X Pa91, and B77 X H100 were chosen due to their similarity to agronomically important hybrid maize. Kernels of each of these lines were planted in both control and 35 t/ha fly ash treatments.These experiments were analyzed as described above.

RESULTS AND DISCUSSION

Several triploid plants were observed in the diploid by tetraploid crosses (Table 1). The histograms from the flow cytometric data allowed for easy discrimination of triploid individuals in the mixed

Table 1. Number and frequency of triploids recovered from diploid X tetraploid crosses

Cross	Number of Plants examined	Number of Triploids	Frequency
B89			
2n♂ X 4n♀	6	3	50%
4n♂ X 2n♀*	39	0	0%
R75			
2n♂ X 4n♀	2	2	100%
4n♂ X 2n♀*	107	1	0.9%

* one haploid plant was found in each of the crosses.

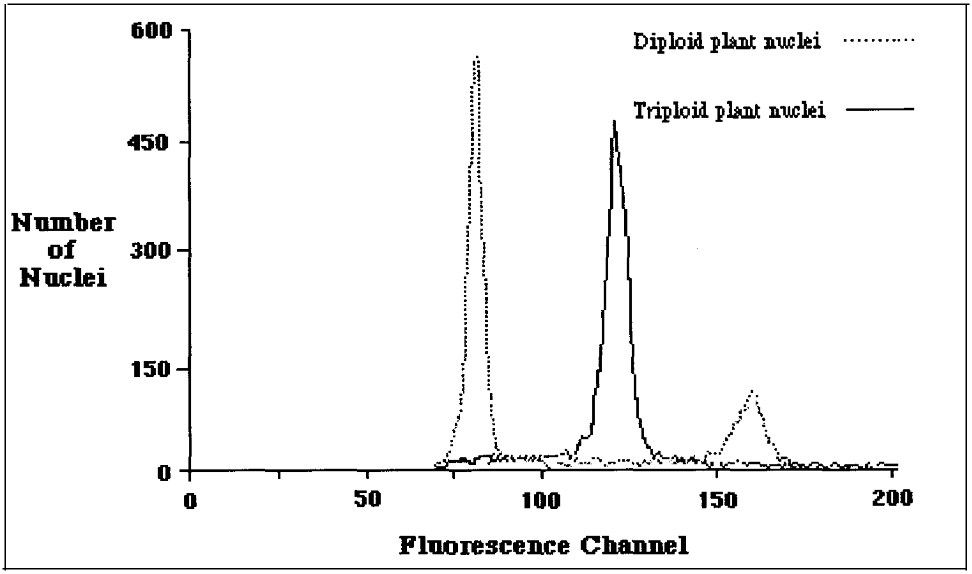

Figure 1. Overlaid histograms of nuclei from a diploid and a triploid plant. Note that the triploid peak is approximately 50 channels higher than the first diploid peak.

ploidy populations (Figure 1). These results were consistent with other reports that have documented the presence of triploid plants in various populations (DeLaat et al, 1987; Graham et al, 1994; McMurphy and Rayburn, 1991; 1993).

In the second experiment, a total of 198 early early synthetic seedlings were examined. Half of the seedlings were grown in 500 t/ha fly ash while the remaining plants were grown in control (0 fly ash) pots. No triploid individuals were found in either treatment. McMurphy and Rayburn (1993) had reported around 4% triploid individuals in seedlings grown for two weeks in fly ash. In their study one plant was found among plants grown in 500 t/ha while two plants were found among plants growing in 35 t/ha fly ash. Because no triploid plants were found in this study it is apparent that the 500 t/ha concentration does not induce triploidy.

The purpose for the third experiment was two-fold: to determine if a concentration of 35 t/ha fly ash would induce triploidy as indicated from the previous study; and to investigate the possibility that different maize lines might be more sensitive to fly ash. The hybrid maize line Mo17 X B73 was included due to its agronomic importance in the corn belt, one of the major areas that has been targeted for the use of fly ash as a soil amendment. In total, 616 maize plants were analyzed. Half of the plants of each type were grown in 35 t/ha fly ash and the remaining plants grown under 0 fly ash conditions. The breakdown by maize line examined was as follows: 28 W22 plants, 108 early early synthetic plants, 120 Va35 plants, 132 H99 plants, 78 Mo17 X Pa91 plants, 66 B77 X H100 plants, and 84 B73 X Mo17 plants.

No triploid plants were detected in any of the lines and treatments. As expected, the frequency of spontaneously occurring triploids is very low (Alexander and Beckett, 1963). In addition, no triploids were found among any of the fly ash-treated plants. Indications are that 35 t/ha does not induce triploids in any maize lines. This means that maize lines that are grown on soils amended with the recommended agronomic rates may not have gross genomic alterations. This is in contrast to the results of McMurphy and Rayburn (1993). The triploids observed by McMurphy and Rayburn must have been due to an unusually high number of triploid plants in the fly ash treated samples. This was a chance event and underscores the unpredictable nature of biological organisms.

One possible explanation of these results is that the fly ash used in this study had no genotoxic properties where as the fly ash used by McMurphy and Rayburn did. We observed in this study the smaller genomic alterations that were documented by McMurphy and Rayburn. Thus, the fly ash did indeed have genotoxic properties. The conclusion of this study is that while fly ash is indeed genotoxic to maize plants, the response of the first generation plants is small with no large genomic effects such as triploidy occurring.

REFERENCES

Adams LM, Capp JP, Gilmore DW (1972) Coal mine spoil and refuse bank reclamation with power plant fly ash. Compost Sci 13:20-26

Alexander DE, Beckett JB (1963) Spontaneous triploidy and tetraploidy in maize. J Hered 54:103-106

DeLaat AMM, Gohde W, Volgelzang MJDC (1987) Determination of ploidy of single plants and plant populations by flow cytometry. Plant Breed 99:303-307

Elseewi AA, Straughan IR, Page AL (1980) Sequential cropping of fly ahs amended soils: effects on soil chemical properties and yield and elemental composition of plants. Sci Total Environ 15: 247-259

Graham MJ, Nickell CD, Rayburn AL (1994) Relationship between genome size and maturity group in soybean. Theor Appl Genet 88:429-432

Fail JL, Wochok ZS (1977) Soybean growth on fly ash amended strip mine spoils. Plant Soil 48:472-484

Keefer, RF, Bhumbla, Singh RN (1993) Accumulation of Mo in Wheat and Alfalfa Grown on Fly Ash-Amended Acid Mine Spoils. In: Keefer RF, Sajwan KS (eds) Trace Elements in Coal and Coal Combustion Residues. Lewis Publishers, Chelsea, MI, p 239

McMurphy LM, Rayburn AL (1991) Lack of relationship between relative maturity and genome size in hybrid maize. Crop Sci 31:63-67

McMurphy LM, Rayburn AL (1993) Nuclear Alterations of maize plants grown in soil contaminated with coal fly ash. Arch Environ Contam Toxicol 25:520-524

Murarka IP, Boyd RH, Harbert HP (1987) Electric utility industry solid wastes. In: Murarka IP (ed) Solid Waste Disposal and Reuse in the United States, CRC Press, Inc., Boca Raton, FL, P 95

Plank CO, Martens DC (1974) Boron availability as influenced by application of fly ash to soil. Soil Sci Soc Am Proc. 38:974-977

Radecki J, Banaszkiewicz T, Klasa A (1989) The effect of different lead compounds on mitotic activity of maize root tip cells. ACTA Physiol Plant 11: 125-130

Rehage JA, Holcombe LJ (1990) EPRI EN-6532 Project 2796-l. Electric Power Research Institute, Palo Alto, CA

Suloway JJ, Roy TM , Skelly TM, Dickersen DR, Schuller, Griffin RA (1983) Chemical and toxicological properties of fly ash. Environ. Geol. Notes 105. Illinois Department of Energy and Natural Resources, State Geological Survey Division, State Natural History Division, Champaign, IL

Thicke FE (1988) Effect of scrubber sludge and fly ash on soil properties and crop growth. PhD diss. Univ. of Illinois at Urbana-Champaign