World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:5, No:11, 2011

Simulation of Increased Ambient Ozone to Estimate Nutrient Content and Genetic Change in Two Thai Soybean Cultivars

Orose Rugchati and Kanita Thanacharoenchanaphas

Abstract**—**This research studied the simulation of increased ambient ozone to estimate nutrient content and genetic changes in two Thai soybean cultivars (Chiang Mai 60 and Srisumrong 1). Ozone stress conditions affected proteins and lipids. It was found that proteins decreased, but lipids increased. Srisumrong 1 cultivars were more sensitive to ozone stress than Chiang Mai 60 cultivars. The effect of ozone stress conditions on plant phenotype and genotype was analyzed using the AFLP technique for the 2 Thai soybean cultivars (Chiang Mai 60 and Srisumrong 1).

Keywords**—**simulation, ambient ozone estimate, nutrient content, genetic changes , Thai soybean

I. Introduction

TROPOSPHERIC ozone has progressively become the major air pollutant in many parts of the world. Ozone is regarded as one of the most harmful air pollutants that suppresses crop growth and yield [1]. Ozone, which is a powerful oxidizing agent, is responsible for more damage to vegetation than any other air pollutant. Many studies have shown that average concentrations of ozone in many regions have increased. The change may come about from climate changes and human-emitted precursor[2]. It is anticipated that many factors will contribute to an ozone increase of 20-25% between 2015 and 2050, and 40-60% by 2100 [3].

Soybeans are one of the economic crops that is known for its sensitivity to ozone[4]. It has been estimated that soybean yields are suppressed about 10% by 50 ppb ozone concentration (seasonal mean 7 h daily concentration) [5]. The ambient concentration of ozone in the lower part of the northern region of Thailand is around 20-30 ppb, and some supplemental factors can push this level to be 100-200 ppb [6], which is high enough to cause adverse effects on many components of the ecosystems including crop production and some food nutrients.

Soybeans [Glycine max (L.) Merr.] are becoming an important economic crop in Thailand as a major source of protein, energy, polyunsaturated fats, fiber, vitamins, minerals, and other nutrients, for both humans and livestock [7]-[9]. Soybean seeds contain an average of 36–38% protein and 19% oil, on a dry weight basis [10]- [12], but both genetic and environmental factors can strongly affect the seed composition [7]. Soybean proteins are a complex polymorphic mixture of polypeptides, and, like many species of the Leguminosae, soybeans contain four groups of proteins: the enzymes involved in metabolism, which do not exceed 1% of the total protein; structural proteins including both ribosomal and chromosomal; membrane proteins; and storage proteins[7], [9].

Amplified fragment length polymorphisms (AFLPs) are polymerase chain reaction (PCR)-based markers for the rapid screening of genetic diversity. AFLP methods rapidly generate hundreds of highly replicable markers from DNA of any organism; thus, they allow high-resolution genotyping of fingerprinting quality. AFLP methods primarily generate dominant rather than co-dominant markers. Because of their high replicability and ease of use, AFLP markers have emerged as a major new type of genetic marker with broad application in systematics, pathotyping, population genetics, DNA fingerprinting, and quantitative trait loci (QTL) mapping [13].

To predict the protein content and genetic characteristics of soybean cultivars, we carried out an experiment to evaluate and understand how elevated ozone concentrations affect protein content. Changes in plant phenotype and genotype were studied using the AFLP technique for the 2 Thai soybean cultivars (Chiang Mai 60 and Srisumrong 1).

II. Material And Method

A. Raw Material

Two Thai soybean cultivars (Chiang Mai 60 and Srisumrong 1) from the Thai Agricultural Research Department soy bean sample were planted in ozone stress conditions during the growing period. The two soybean cultivars were exposed in an open-top chamber to three ozone treatments: non-charcoal filtered air (NCF: control) = ambient ozone, charcoal filtered air (CF: low level ozone) = < ambient ozone, and charcoal filtered air plus ozone (CF+O3: high level ozone) = > ambient ozone.

B. Sample Preparation

The soybean samples after harvesting were cleaned by air blowing, and whole seeds were selected based on shape and yellow coloring. Sample seeds were ground by hammer mill and poured through a100 mesh sieve. Ground soybean powder was kept in an aluminum foil bag under vacuum conditions at room temperature for the experiment.

[a]Department of Agro-Industry, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok Thailand, 65000, E-mail address: oroser@nu.ac.th
[b]Department of Natural Resources and Environment, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok Thailand, 65000,
**Corresponding author: E-mail address: kanitat@nu.ac.th

C. Chemical composition

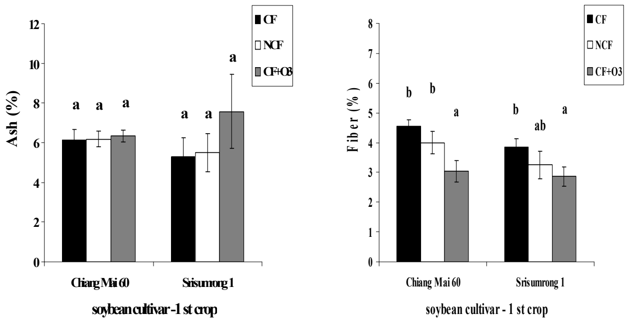
The content of moisture, protein, fiber, fat, ash, and NFE [nitrogen free extract (100 - % protein - % fat - %fiber - % ash)] were determined by the standard method of AOAC [14].

D. Phenotype Analysis

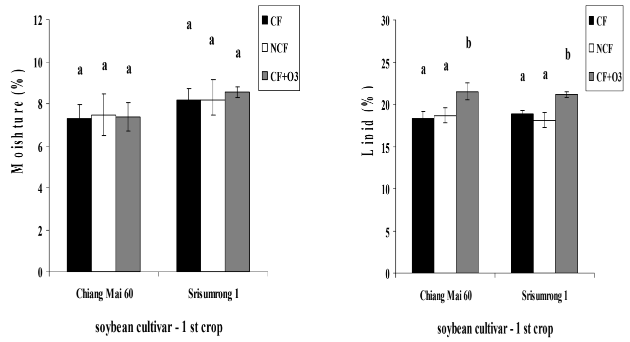
Plant phenotype and genotype were analyzed using the cDNA-AFLP procedure and performed according to the AFLP technique (DNA Technology Laboratory, Kasetsart University, 2010). The PCR program (GeneAmpR PCR System 9700, Applied Biosystem) included 24 cycles: 50 °C for 5 min, 94°C for 3 min, 94°C for 30 s, 56°C for 1 min, 72°C for 1 min, and the final extension at 72°C for 5 min with 6 selective primers: AAC-ACT, AGG-AAA, AGG-AAC, AAC-CAA, AAC-CTT and AGG-CAC. PCR products were identified on 4.5% polyacrylamide sequencing gel at 70 W for 1 h, and cDNA; fragments were visualized by silver staining. Interpretation of the data was done using the NTSYSpc program for Windows Version 2.01e Phylogenetic tree [15] - [17].

III. Results And Discussion

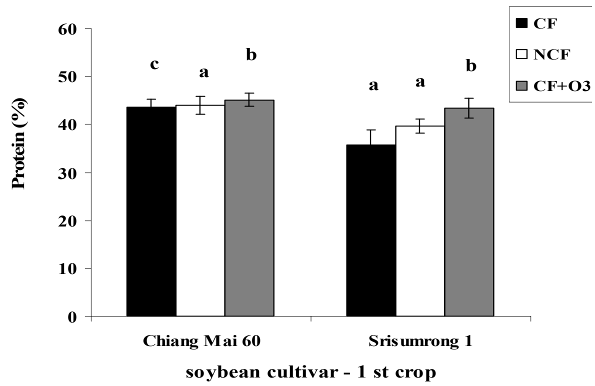
The effects of ozone stress on 2 Thai soybean cultivars' chemical composition are shown in fig 1(a) and (b) from CF, NCF and CF+O3, respectively. The data shows that ozone stress effects on both cultivars were non-significant in ash and moisture content, but did increase lipid content on both cultivars significantly for CF+O3 but not for CF or NCFt. The fiber content decreased significantly in both cultivars.



*a – c : Mean differences are significant at P $\leq$ 0.05
Fig. 1 (a) Ash and fiber content of the 2 soy bean cultivars in 3 treatments of ozone stress conditions



a – c : Mean differences are significant at P $\leq$ 0.05
Fig. 1 (b) Moisture and crude fat content of the 2 soy bean cultivars in 3 treatments of ozone stress conditions



a – c : Mean differences are significant at P $\leq$ 0.05
Fig. 2 Protein content (%) of 2 the soy bean cultivars in 3 treatments of ozone stress conditions

Protein content was affected by ozone stress in all treatments with a significant amount of protein content: 43.52 + 1.66 , 44.06 + 1.9, and 45.15 + 1.37 % for CF, NCF, and CF+O3 treatments respectively in Chiang Mai 60 cultivars . The protein content varied for ozone concentration in Chiang Mai 60 cultivars more than Srisumrong1 cultivars.

A. Phenotype Analysis

The effect of ozone stress on Thai soybean cultivars by AFLP technique analysis using the similarity index is shown in fig. 3, divided into 8 groups:

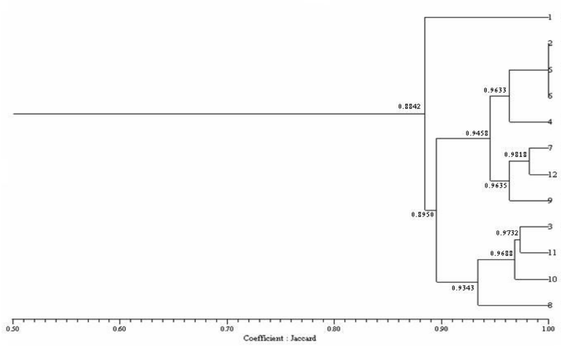


Fig. 3 Similarity Index of 2 Thai soy bean cultivars with ozone stress conditions, C1-C3 are replications

CM: Chiang Mai 60 cultivars Sr: Srisumrong
NCF : ambient ozone
CF : < ambient ozone
CF+O3 : > ambient ozone
Group 1 C1 CM CF
Group 2 C2 CM CF, C1 CM NCF, C2 CM NCF
Group 3 C2 Sr1 CF
Group 4 C1 Sr1 CNF, C2 Sr1 CF+O3
Group 5 C1 CM CF+O3
Group 6 C1 Sr1 CF, C1 Sr1 CF+O3
Group 7 C2 CM CF+O3
Group 8 C2 Sr1 CNF

This figure shows that ozone stress did not affect Chiang Mai 60 cultivar , and Srisumrong1 was more sensitive to ozone stress than Chiang Mai 60 cultivars. Ozone stress did, however, affect phenotype of both cultivars of the Thai soybean.

IV. Conclusion

This research has shown the simulation conditions of ozone increases and changes the genetics of two Thai soybean cultivars: Chiang Mai 60 cultivars and Srisumrong1cultivars. The two cultivars are the most popular for planting and distribution in Thailand due to high production yields and resistance to the environment. Proteins and lipids both are the main nutrients of soybeans that were affected by ozone stress. The proteins decreased, but lipids increased. Phenotype analysis by AFLP technique indicated the effects of ozone stress. This should be replicated with more complicated primers.

References

[1] L. Leitao, P. Goulas, and J. Biolley, “Time-course of Rubisco oxidation in baen (Phaseolus vulgaris L.),” Plant SCI, vol. 30, 2003, pp. 1-8..

[2] S.J. Oltmans, A.S. Lefohn, J.M. Harris, I. Galbally, H.E. Scheel, G. Bodeker, E. Brunke, H. Claude, D. Tarasick, B.J. Johnson, P. Simmonds, D. Shadwick, K. Anlaf, K. Hayden, F. Schmidlin, T. Fujimoto, and H.E. Cuevas. “Long term changes in tropospheric ozone,” Atmos Environ, vol. 40, 2006, pp. 3156-3173.

[3] E. Singh, S. Tiwari, and M. Agrawal,.“Variability in antioxidant and metabolite levels, growth and yield of two soybean varieties: An assessment of anticipated yield losses under projected elevation of ozone,” Agr Ecosyst Environ, vol. 135, 2010, pp. 168-177.

[4] J.E. Miller, F.L. Booker, E.L. Ficus, A.S. Heagle, W.A. Pursley, S.F. Vozzo, and W.W. Heck,. “Ultraviolet-B Radiation and Ozone Effects on Growth, Yield, and Photosynthesis of Soybean,” J Environ Qual, vol. 23, 1994, pp. 83-91.

[5] A.S. Heagle. Ozone and Crop Yield. Annu Rev Phytopathol, vol. 27, 1989, pp. 397-423.

[6] http://www.pcd.or.th. Pollution Control Department of Thailand. 2004.

[7] H.B. Krishnan. “Biochemistry and molecular biology of soybean seed storage proteins,” J New Seeds, vol. 2, 2000, pp. 1–25.

[8] H. B. Krishnan. “Engineering soybean for enhanced sulfur amino acid content,” Crop Sci, vol. 45, 2005, pp. 454–461.

[9] N. C. Nielsen, R. Bassusner, and T. Beaman. The biochemistry and cell biology of embryo storage proteins. In R. A. Larkins & I. K. Vasil (Eds.), Cellular and molecular biology of plant seed development (pp. 151–220). Dordrecht: Kluwer Academic Publishers. 1997

[10] T. J. Brumm, and C. R. Jr. Hurburgh. “Quality of the 2002 soybean crop from the United States,” Louis, MO: American Soybean Association. 2002

[11] C. G. Zarkadas, Z.-R. Yu, H. D. Voldeng, and A. Minero-Amador. “Assessment of the protein quality of a new high-protein soybean cultivar by amino acid analysis,” J Agr Food Chem, vol. 41, 1993, pp. 616–623.

[12] C. G. Zarkadas, H. D. Voldeng, Z.-R. Yu, and V. Choi. “Assessment of the protein quality of nine northern adapted yellow and brown seed coated soybean cultivars by amino acid analysis,” J Agr Food Chem, vo.l 47, 1999, pp. 5009–5018.

[13] W. Waycott, B.S. Fort, E.J. Ryder and W.R. Michelmore. “Mapping morphological gene relative to molecular marker in lettuce (Lactuca sativa L.) ,” Heredity 82: 245-251.1992.

[14] Association of Official Analytical Chemists (AOAC). Official Method of Analysis Vol.2. 15th ed., The Association of Official Agricultural Chemists, Virginia.,163 p. 1995

[15] D. Corroler, N. Desmasures and M. Gueguen. “Correlation between polymerase chain reaction analysis of histidine biosyntheis operon, randomly amplified polymorphic DNA analysis and phenotypic characterization of dairy Lactococcus isolates,” Appl Microbiol Biot, vol. 51, 1999, pp. 91-99.

[16] J. Welsh, and M. McClelland. “Fingerprinting genome using PCR with arbitary primers,” Nucl Acids Res, vol. 18, 1990, pp. 7213-7218.

[17] M. Soller, and J.S. Beckman. “Genetic polymorphism in varietal identification and genetic improvement,” Theor Appl Genet, vol. 67, 1983, pp. 25-33.