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Effect of Bacterial 1-Aminocyclopropane-1-Carboxylate Deaminase (ACD) on Resistance to Environmental Stress caused by Salinity in Winter Wheat

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ABSTRACT

Ethylene synthesis is accelerated in response to environmental stresses like salinity. Excess amount of ethylene has a negative impact on root elongation, seedlings, growth of leaves in plants. Aim of the work was study the effect of *Pseudomonas mendocina* containing plasmid carrying gene encoding 1-aminocyclopropane-1-carboxylate deaminase (ACD) on resistance of wheat plant under salt stress. Surface sterilized seeds of wheat plant were germinated in petri plates. Uniformly buds were selected, and were used in pot and greenhouse. Salinity as 172 and 207 mM NaCl were prepared for wheat plant in pot and 207 mM in greenhouse by irrigation of NaCl solution. *acdS* gene was cloned in *P. mendocina* and transformed bacterium was used in experiments . Results in pot experiment revealed that in 172mM salinity, cloned *P. mendocina* in comparison with plants treated by wild type of *P. mendocina*, exceeding the length of the stem by 11.6%, biomass by 43.7% and root length by % 47.5. In 207 mM NaCl results were as 2.65%, 20.6% and 11.9% respectively. Experiment in greenhouse showed that wheat plants treated by a suspension of cloned *P. mendocina*, in comparison with those treated by wild type of *P. mendocina* had 38.8% greater length of stem, 23.3% more root length and 28.6% more biomass. Wheat plants treated by cloned *P. mendocina* in comparison with plants without bacterial treatment had 43.8% greater length of stem, 5.5% more root length and 28.6% more biomass. It is concluded that enhancement of ACC-deaminase activity resulted in excess resistance of wheat in saline environments.

Key words: Environmental Stress, Salinity, Winter Wheat, *Pseudomonas mendocina.*

INTRODUCTION

Soil salinity is an immense problem for agriculture. Excess amount of salt in the soil affects plant growth and development. Nearly 20% of the world's cultivated area and half of the world's irrigated lands are affected by salinity[1,2].

In hot and dry regions, most crops are grown under irrigation, and inadequate irrigation management leads to secondary salinization[3,4]. An alternative approach to overcoming some of the problems associated with growing plants in saline soils involves employing ACC deaminase- producing bacteria. Those bacteria lower the ethylene level and decrease the growth inhibition from drought stress[4,5].

Ethylene is an important growth hormone produced by almost all the plants[6, 7].

Ethylene is also known as a stress hormone, because of its involvement in evoking physiological responses in plants exposed to a variety of stresses including salt stress[8-11].

It has been proposed that, ethylene, in response to environmental stresses, is produced in two peaks[12]. The first peak is small and usually occurs a few hours after the stress. It is thought to function as a signal to turn on transcription of genes that encode proteins that help to protect the plant[13-15]. The second peak is much larger, occurs one to three days after the stress and concomitant with the appearance of visible damage to the plant[16].

Plant growth promoting rhizobacteria (PGPR), have a positive effect on plant growth and development (17,18). One of the main mechanisms used by PGPR to stimulate plant growth is a reduction of plant hormone ethylene[19].

It is found that this bacteria containing ACC-deaminase enzyme can hydrolyzes ACC into ammonia and alpha ketobutyrate, resulting in reduced production of C2H4 (20). Plants growth can be enhanced by inoculating with *Pseudomonas* sp. containing ACC-deaminase that regulate endogenous ACC levels and thus decrease ethylene under salt-stressed conditions[21- 25].

However, use of plant growth-promoting microorganisms may prove useful to facilitate plant growth in saline soils[21, 26].

Aim of the work was evaluation of selected bacteria to increase wheat plant resistance under saline conditions.

MATERIAL AND METHODS

Influence of *bacterial treatments* on salt resistance of wheat plants was studied in pot and greenhouse scales.

Screening, enzyme assay and gene cloning

Cultured collection of rhizosphere bacteria was screened for ACD producing bacteria that were described in detail elsewhere (unpublished data). Briefly, presence of acdS-gene encoding ACD-deaminase was done by PCR (using the forward primer 5-GGGACCGGATCCTCAAGGAACAGCG CCATG- 3 and reverse primer as 5-GAACGGAAGC TTCTGGCGGCGCCAAGCTCA- 3). PGPR strains containing acdS-gene were examined for their potential to utilize ACC as a sole source of nitrogen (ACC-metabolism assay). ACC-deaminase activity measurement was accomplished by Honma and Shimomora method[27].

Amplified acdS gene was extracted and DNA was cloned into plasmid pAYS31 by restriction enzyme of BamHI . The plasmid was transformed to *P. mendocina* by thermal shock in CaCl2. Enzyme activity of produced cell was measured and compared with wild type strain. Finally, the transformed *P. mendocina* was used for evaluation of enhancement of resistance in wheat plant in high salinity condition.

Pot trial

Pot experiment was conducted at the department of genetics, Faculty of Biology, Belarusian State University, Minks, Belarus to test the comparative effectiveness of cloned *P. mendocina* in resistance of wheat under salt-stressed conditions.

Wheat seeds were sown on wet sterilized filter paper. After 4 days germinated seeds were cultured in moist soil. Sized buds with equal size were selected and transplanted into individual plastic cups of 150 ml (8 and 5 cm top and bottom diameters, respectively, and 10-cm height, with holes in the bottom) in 3 repeats of three groups. First group was treated with 10ml of bacterial suspension of cloned *P. mendocina.* The other group by 10 ml of wild type of *P. mendocina*, and the third group - 10 ml of distilled water.

The composition of soil was as (%): total N, 0.15; P_2O_5, 0.1; K_2O, 0.3; organic material content 50%, humidity 60 %.

Then plants have been irrigated by NaCl solution at a concentration of 172 and 207 mM. The results were collected within 5 weeks for wet weight, shoot and root lengths.

Greenhouse experiments

This trial was described elsewhere

(unpublished data). Briefly sized buds were transferred in plastic bag of 5 kg containing soil taken from Campus farmland. Compound soil was collected from depth of 0-30 cm, was air dried, sieved and mixed uniformly. The bags were divided into 4 groups:

First section considered as control (first control - without bacteria). The second three bags (second section) were treated by 500 ml bacterial suspension of *P. mendocina* , third section by 500 ml of cloned *P. mendocina* and last section by 500 ml of distilled water (second control without bacteria). Then, three first sections irrigated with a solution of 207 mM NaCl to maintain the level of salinity.

All treatments were irrigated by NaCl solution at a concentration of 172 and 207 mM. Results were obtained after 9 weeks.

RESULTS

Study of the ability of recombinant *P. mendocina* in expression of gene encoding 1-aminocyclopropane-1-carboxylate deaminase (ACD), revealed a proper enzyme activity in the bacterium.

Results of experiments in pot

After 5 weeks, results were obtained from pot experiments. As shown in Table 1, in the salt concentration of 172 mM the plants treated with a suspension of cloned *P. mendocina* (O) in comparison with plants treated by wild type of *P. mendocina* (K2), exceeding the length of the stem by 11.6% by weight to 43.7% in root length 47.5%.

In the other hand, wheat plants treated with a suspension of cloned *P.mendocina* (O), in comparison with plants without bacterial treatment

Table 1: Assessment of ability of cloned *P. mendocina* enhance the sustainability of wheat plants to salt stress

Parameters	Salt concentration 172 mM NaCL			Salt concentration 207 mM NaCL		
	κ1	κ2	O	κ1	κ2	O
Shoot length, Cm	34.5	31.9	35.6	34.5	34	34.9
Root length, Cm	12	8	11.8	12.25	10.1	11.3
Biomass, gr	0.26	0.19	0.273	0.36	0.34	0.41

κ1 – wheat plants without bacterial treatment κ2- wheat plants treated by wild type of *P. mendocina* (10^7 cells / ml), O- wheat plants treated with a suspension of cloned *P. mendocina* (10^7 cells / ml)

(K1), had an increase amounts as 3.2% greater length of stem, 5% more biomass and 1.7% less than the length of the root.

In the salt concentration of 207 mM the plants treated with a suspension of cloned *P. mendocina* (O) in comparison with plants treated with *P. mendocina* (K2) had an excess length of the stem at 2.65%, biomass as 20.6% and root length by 11.9%.

Furthermore, plants treated by cloned bacterium (O) in comparison with group without bacterial treatment (K1) showed 1.2% greater length of stem, 7.7% less than the length of the root and 13.9% more biomass.

Results of Greenhouse experiment

Ability of recombinant *P. mendocina* to increase the stability of wheat plants was determined after 9 weeks in salt stress condition in a greenhouse.

This experiment was conducted to determine the ability of cloned *P. mendocina* enhance the sustainability of wheat plants to salt stress. The data are presented in Fig 1-3.

As shown in Figures 1-3, in the salt concentration of 207mM NaCl, wheat plants treated

by a suspension of cloned *P. mendocina* (O) and were compared with plants treated by *P. mendocina* (K2) were 38.8% greater length of stem , 23.3% greater root length and 28.6% greater biomass. Wheat plants treated by cloned *P. mendocina* in comparison with plants without bacterial treatment (K1), had 48.3% greater length of stem, 5.5% greater root length and 28.6% more biomass.

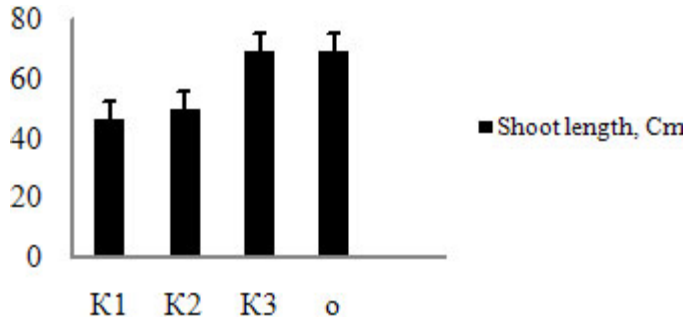

Fig. 1: Potential of wild type and cloned *P. mendocina* to promote shoot length of wheat in the salt concentration of 207mM NaCl

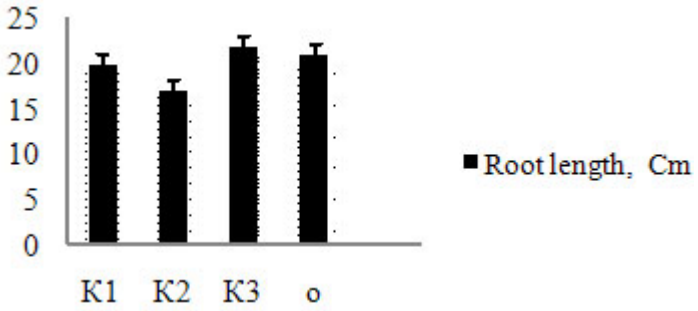

Fig. 2: Effect of P. mendocina producing ACC-deaminase on root elongation of wheat (cm) at salinity 207 mM (as under Fig 1)

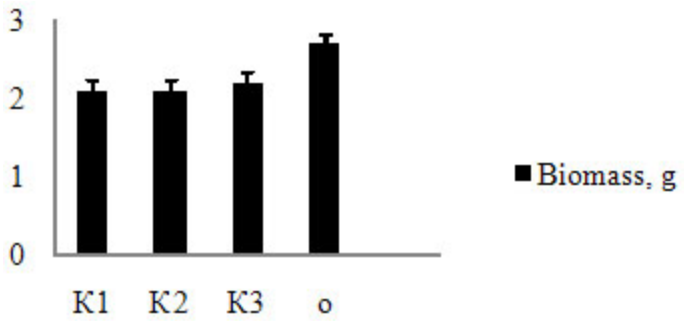

Fig. 3: Effect of bacterial treatment on biomass of wheat (cm) at 207 mM salinity (as under figure 1)

Legends

K1 – Wheat plants without bacterial treatment

K2- Wheat plants treated by wild type of *P. mendocina* (10^7 cells / ml

K3- Wheat plants in soil without adding salt and bacteria

O- Wheat plants treated with a suspension of cloned *P. mendocina*(10^7 cells / ml)

Comparison of wheat plants treated by a suspension of cloned *P.mendocina* (O), with control plants irrigated only by distilled water (K3) there was 0.29% greater length of stem, 4.1% less than the length of the root and 22.7% more biomass.

DISCUSSION

Several studies on growth promotion by bacterial ACD were conducted with dicots including canola, tomato, tobacco and mung bean. But, the bacteria are also effective with monocots (at a lesser extent) such as wheat, rye and rice. All of monocots are less ethylene sensitive than the dicots.

It has been known that PGPR containing ACC-deaminase might be useful in regulating the endogenous levels of ethylene in plants through their ACC-deaminase activity and could be promote plant growth and development[17, 28, 29]).

These bacteria are capable of reducing higher levels of C_2H_4 in plants through the activity of enzyme ACC-deaminase that hydrolyzes ACC into α-ketobutyrate and ammonia[30,31].

In this work it was proved that cloned *P. mendocina* promoted root growth because of its high ACC-deaminase activity compared to wild type of *P. mendocina* (Table 1). This study showed that bacterial ACC deaminase increased the resistance of wheat to salt stress and biomass over control (Fig. 3). It was observed that cloned *P. mendocina* containing ACC-deaminase activity promoted root, shoot and other growth contributing parameters of wheat at salinity both in pot and green house conditions.

The results are in conformity with the findings of (32) who reported that rhizobacteria with more ACC deaminase activity had more ability to decrease the intensity of ACC-induced response which confirmed the premises that ACC-deaminase activity of rhizobacteria was responsible for decreasing ACC in inoculated plant. The inoculation of rhizobacterial strains promoted root growth by lowering the endogenous inhibitory levels of ethylene because of their ACC-metabolising ability. Glick and Bashan (1997) reported the role of ACC-deaminase in reducing the production of ethylene during stressed conditions[31,33-36].

Cloned *P. mendocina* promoted root growth by lowering the endogenous inhibitory level of ethylene in roots because of their ACC deaminase activity which subsequently affected shoot, stem growth and biomass enhancement .

This opinion is supported from coefficient of correlation between in vitro ACC deaminase activity and the root elongation under stress conditions which is supported from the findings of Shaharoona *et al.* (2006, 2007) [37,38] who also reported a significant linear correlation between ACC deaminase activity of different rhizobacterial bacteria and their root growth promotion. Similar kind of findings have been reported by other scientists[39,21,23,36].

In the other study that was accomplished by the authors it was shown that cloned *P. mendocina* in pot and greenhouse enhanced resistance of wheat plant and tomato to soil pollution by heavy metals Cr, Cu or Pb and salinity. Furthermore, plants treated by cloned *P. mendocina* had enhanced growth rather than plants treated by wild type and ones without any treatment (unpublished data).

1-aminocyclopropane-1-carboxylate deaminase (ACD) reduces the production of ethylene plants, which reduces the inhibition of growth. This indicates that plants treated by cloned *P. mendocina* had not only more growth of the stem and shoot, but the best enhancement in biomass in comparison with other trials in stress conditions.

CONCLUSIONS

It is concluded that cloned *P. mendocina* containing ACC-deaminase activity can effects the resistance of wheat plant in high salinity stress by lowering the ethylene, whose higher levels have inhibitory effect on root and shoot growth and biomass.

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REFERENCES

1. Barrett-Lennard, E.G., Salt of the earth: time to take it seriously, Australian centre for international agricultural research, in: the food and environment Tightrope, (H. Cadman, Canberra, Eds.), 135-145 (2000).
2. Unal M., Cakmak I , Aydin Y: Data Mining of ESTs for Genetic Improvement of Salt Tolerance in Wheat. *BIOCOMP:* 117-124 (2006).
3. Mayak S, T Tirosh, and BR Glick., Plant growth-promoting bacteria that confer resistance in tomato to salt stress. *Plant Physiol Biochem*. **42**: 565-572 (2004b).
4. Glick BR, Æ Zhenyu Cheng Æ Jennifer Czarny Æ Jin Duan; Promotion of plant growth by ACC deaminase-producing soil bacteria ; *Eur J Plant Pathol* **119**: 329-339 (2007).
5. Mayak S, T Tirosh, and BR Glick., Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci*. **166**: 525-530 (2004a).
6. Arshad M, Frankenberger WT Jr, Ethylene: agricultural sources and applications. PP. 86-99, Kluwer, New York (2002).
7. Belimov AA, Vera I Safronova, Tetsuro Mimura ; Response of spring rape (*Brassica napus* var. *oleifera* L.) to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant; Canadian *Journal of Microbiology,* **48**: 189-199 (2002), 10.1139/w02-007
8. Wang, S.Y., C.Y. Wang and A.R. Welborn, Role of ethylene under stress conditions. *In*: Alscher, R. and J. Cumming (eds.), *Stress Responses in Plant Adaptation and Acclimation Mechanisms,* Pp: 147–73 (1990). Wiley Liss, NewYork, USA
9. Abeles, F.B., Experiments with some microorganisms, which utilize ethane and hydrogen; *Ethylene in Plant Biology*. 592–601; Academic Press, New York (1993).
10. Morgan, P.G. and M.C. Drew, Ethylene and plant responses to stress. *Physiol. Pl.*, **100**: 620-30 (1997).
11. Kausar R And Sher Muhammad Shahzad; Effect Of Acc-Deaminase Containing Rhizobacteria On Growth Promotion Of Maize Under Salinity Stress ; *Journal Of Agriculture &Social Sciences.* 1813–2235, 02–4–216–218 (2006).
12. Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ. The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science*. **11**: 176-183 (2006).
13. VanLoon LC, Glick BR., Increased plant fitness by rhizobacteria. In: Molecular Ecotoxicology of Plants. H. Sandermann. eds. Springer-Verlag, Berlin: pp 177-207 (2004).
14. VanLoon LC, Bakker PAHM, Pieterse CMJ., Mechanisms of PGPR-induced resistance against pathogens. In: Plant Growth-Promoting Rhizobacteria: Present Status and Future Prospects (A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, and S. Akino, eds.): pp 50-57. OECD, Paris (1997).
15. Glick BR., Bacterial ACC Deaminase and the Alleviation of Plant Stress. *Advances in Applied Microbiology* **56**: 291-312 (2004).
16. Glick BR, Cheng ZY, Czarny J, Duan J. Promotion of plant growth by ACC deaminaseproducing soil bacteria. European Journal of Plant Pathology. *European Journal of Plant Pathology.* **119:** 329-339 (2007).
17. Glick BR, Patten CL, Holguin G, Penrose DM, Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, p 267 (1999).
18. Penrose. D.W., B.A. Moffat and B.R. Glick., Determination of ACC to assess the effect of ACC-deaminase containing bacteria on roots of canola seedlings. *Can. J. Microbiol*., **47**: 77- 80 (2001).
19. Schroth M. N., Hancock J. D. Disease-suppressive soil and root-colonizing Bacteria // Science. **216**: 1376-1381 (1982).
20. Glick, B.R., Level of ACC and related compounds in exudates and extracts of canola seeds treated with ACC-deaminase containing plant growth promoting bacteria. *J. Microbial*., **47**: 36-9 (2001).

21. Mayak S, Tirosh T, Glick BR, Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol Biochem* **42**: 565-572 (2004).
22. Cheng Z, Park E, Glick BR, 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can J Microbiol* **53**: 912-918 (2007).
23. Nadeem SM, Zahir ZA, Naveed M, Arshad M, Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. *Can J Microbiol* **53**: 1141–1149 (2007).
24. Saravankumar D, Samiyappan R, ACC-deaminase from *Pseudomonas Xuorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. *J Appl Microbiol* **102**: 1283–1292 (2007).
25. Ahmad Z, Usman Ghani · Muhammad Naveed · Sajid Mahmood Nadeem ; Comparative efectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions, *Arch Microbiol.,* **191**: 415–424 (2009).
26. Yildirim E. and A. G. Taylor; Effect of biological treatments on growth of bean plants under salt stress Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, New York, USA 14456. *Annual Report of the Bean Improvement Cooperative,* **48**: 176-177 (2005).
27. Honma M., Shimomura T. Metabolism of 1-aminocyclopropane-1-carboxylic acid, *Agric. Biol. Chem.,* **42**(10): 1825-1831 (1978).
28. Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR Cadmium-tolerant plant growthpromoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol Biochem* **37**: 241-250 (2005).
29. Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimove AA, Root-associated bacteria containing 1-aminocyclopropane- 1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol Fertil Soils.* **42**: 267–272 (2006).
30. Glick BR, Penrose DM, Li J., A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology.* **190**: 63-68 (1998).
31. Arshad, M., M. Saleem and S. Hussain., Perspective of bacterial ACC-deaminase in phytoremediation. *Trends Biotechnol*., **25**: 356-362 (2007).
32. Shaharoona, B., R. Bibi, M. Arshad, Z.A. Zahir and Zia-ul-Hassan., 1-Aminocyclo-propane- 1-carboxylate (ACC)-deaminase rhizobacteria extenuates ACC-induced classical triple response in etiolated pea seedlings. *Pak. J. Bot.*, **38**: 1491-1499 (2006b).
33. Glick BR, Bashan Y, Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. *Biotechnol Adv* **15**: 353-378 (1997).
34. Gómez-Jiménez, M.C., A.J. Matilla and D. Garrido. Isolation and characterization of a cDNA encoding an ACC oxidase from *Cicer arietinum* and its expression during embryogenesis and seed germination. *Aust. J. Plant Physiol.*, **25**: 765-773 (1998).
35. Matilla, A.J. Ethylene in seed formation and germination. *Seed Sci. Res.*, **10:** 111-126 (2000).
36. M. Zafar-Ul-Hye, Z.A. Zahir, S.M. Shahzad, M. Naveed, M. Arshad And M. Khalid, Preliminary screening of rhizobacteria containing acc-deaminase for promoting growth of lentil seedlings under axenic condition, Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Pakistan PP. 66-89.
37. Shaharoona B, Arshad M, Zahir ZA, EVect of plant growthpromoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett Appl Microbiol* **42**: 155-159 (2006).
38. Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007) EVectiveness of various *Pseudomonas* spp. and *Burkhaldaria*

caryophylli containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.). *J Microbiol Biotechnol*; **17:** 1300–1307

39. Dodd, I.C., A.A. Belimov, W.Y. Sobieh, B.I. Safronova, D. Grierson and W.J. Davies., Will modifying plant ethylene status improve plant productivity in water limited environments? Available at http://www.regional.org.au/au/cs/2004/poster/ 1/3/4/510 doddicref.htm (Accessed on 8th February, 2005) (2004).