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EFFECT OF INDUCED MUTATION BY UV RADIATION ON COTTON GROWTH, SEEDS AND PROTEASE ACTIVITY

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ABSTRACT

The cotton has great economical value. The aim of this study to determine the variation in cotton by induced mutation. As cotton seed treated with ultraviolet rays and sodium azide, it has affected on germination of whole plant. The weight as control 1.34 g/boll, UV 1 hour 1.68 g/boll and UV 2 hours 1.82 g/boll measured from cotton plant. The protease activity also determined from aqueous extract of cotton seed as control 43.7 mg/ml, UV 1 hour 24.36 mg/ml and UV 2 hours 26.7 mg/ml respectively.

Key words: cotton seed, UV and NaN^3 treatment, protease activity.

INTRODUCTION

The cotton (*Gossypium hirsutum* L.) is the valuable crop; it is cultivated in all over the world (Song & Yamaguchi, 2003). Cotton seed used for animal, oil production and the plant of cotton produce approximately 1.6 kilogram of seed for every kilogram of cotton (Cherry and Leffler, 1984; O'Brien *et al.*, 2005). It is also known as natural purest form of cellulose and cotton fibers have great econo-mic value therefore its basic understanding of proper-ties and structure is valuable (Hu and Hsieh 1996). The first induced mutated cotton specie in the world know as MA-9 treated by X-rays (Kharkwal *et al.*, 2004). The chemical and physical mutagen produce changes in DNA (Williams *et al*., 1990; Welsh and McClelland, 1990). The agents that produces mutations are called mutagens. The physical mutagens comprise of ionizing radiation, fast eutrons and thermal neutrons and non-particulate also called as electromagnetic radiation X rays and γ rays. The non-ionizing comprises of UV radiation. The chemical mutagens like of alkylating agents, sulphur mustards, nitrogen mustards, epoxides, imines, ethyl methane sulphonate etc., dyes and others nitrous acid, hydroxyl amine and sodium azide. From these commonly used mutagens are the gamma rays, ethyl methane sulphonate and sodium azide (Ahloowalia and Maluszynski, 2001). The important role of mutants in breedings is reported by Girija and Dhanavel (2009). With the improvement, there are many mutagenic agents discovered by Gustaffson, (1940). The proteases are known as peptidyl peptide hydrolases, it has indus-trial application as its 65% contribution in the indus-trial world (Johnvesly et al., 2001; Mei *et al*., 2005; Olsson *et al*., 1992) from the 25% of the total global enzyme production (Layman *et al*., 1986; Joo *et al*., 2005). The other applications of proteases have been reported like detergent additives, in waste treat-ment process, etc (Godfrey *et al.*, 1996; Gupta *et al*., 2002; Mei *et al*., 2005).

MATERIALS AND METHODS

The present study was carried out at Medical and Environment Research Laboratory, Institute of Biotech-nology and Genetic Engineering, University of Sindh, Jamshoro Hyderabad, Pakistan.

Plant material: The cotton seed bought from Rahim-yar Khan seed corporation Punjab, Pakistan.

Treatment of seed: First removed cotton fiber from cotton seed by use of 70% Sulphuric acid (H_2SO_4), soaked in distilled water for one hour then treated by UV rays for 1 and 2 hours. The 15 treated seeds were sowed in field (pH of field was 6-7). After germination, number of plants and bolls were counted from every plant respectively. After maturation, cotton was sepa-rated from plants, seeds and weighed.

Preparation of aqueous extract: The seed Separated from cotton fibers and removed seed coat. When seed coat is removed, remaining seed part was crushed in a homogenizer to fine powder and fine powder dipped in diethyl ether about 3 hours for oil remove and dried it. 5 gram of powder sample was dissolved in distilled water and centrifuged at 6,000 rpm for 20 minutes; the supernatant was filtered through a What-man No.1 filter paper. The final volume was made up to 25 ml using distilled water.

Determination of protease activity: Protease activity was determined by method of Penner and Ashton (1967). The casein was used as a substrate. One ml of the supernatant was mixed with 2ml of 2% casein (w/v) in 0.2M sodium phosphate buffer (PH 6.5). The reaction was incubated at 50^{O}C for 15 min, after that 5ml of 5% (w/v) trichloro acetic acid was added to the residue solution. The reaction mixture was centrifuged at 4000rpm for 15minutes. 1ml supernatant was trans-ferred to test tube followed by 4ml NaOH (0.5N),1ml Folin-Ciocalteu reagent (1:1 v/v) and 4ml distilled water was added in each tube. The tyrosine concen-tration of supernatant was read at 625nm.

A unit of protease activity is defined as the amount of protease required to catalyze the liberation of 1µg of tyrosine per 1ml under the assay conditions.

Statistical analysis: The standard error was calculated by using Microsoft excel.

RESULTS AND DISCUSSION:

The seed of cotton K1 was grown in field at the garden of Institute if Genetic Engineering and Biotech-nology, University of Sindh Jamshoro, Pakistan. Total 15 seeds were used each for control, UV and Sodium azide treatment and were sowed in the field. It is clear from Table 1 that germination rate was higher in control and 1 hour UV treatment in comparison to 2 hours UV treatment. Whereas weight of cotton and

seeds were observed in Control 29.5 g and 26g. The weight of cotton 37g and seeds 32g were higher in control and 1 hour UV treatment. The prolong uv treatment reduces the rate of germination as well as growth (Teramur and Sullivan, 1994).

Table-1: Germination rate and yield if cotton, boul and seeds.

Treatment	Number of seed sowed	Germinated Seeds	% of germination	Total weight of cotton (g)	Number of bouls	Weight of seeds (g)
Control	15	9	60	29.5	22	26
1 hr UV	15	9	60	37	22	32
2 hr Uv	15	5	33.33	23.7	13	20.7

After two and half month cotton was produced by plant. The control and UV treated plant were further carried out for experiment. The bolls were counted in control, UV1 hour and UV2 hours treated plants and cotton separated from bolls and weights were measured in three samples. Higher weight of cotton was observed in 2hours UV treated seed then in control and one hours UV treated plants as shown in figure-1. Present observation confirmed by Islam et al. (2013), Farooq et al., (2013) and Rao et al., (2013) and observed significant variations for boll weight and showed its positive effect on seed cotton yield.

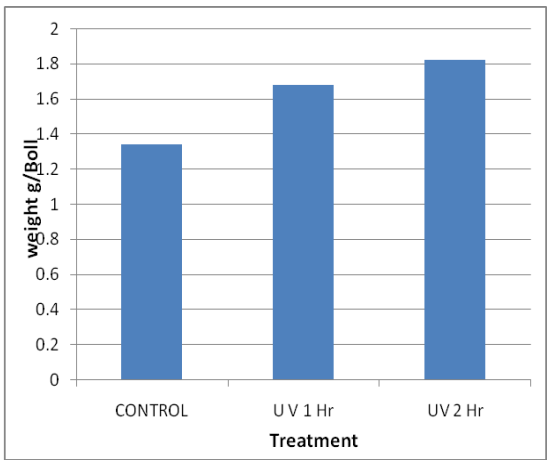

Figure-1: Weight of cotton per boul.

The 20% aqueous extract of cotton seed were prepared and pH was checked at the time of extraction. The control sample pH was observed higher than UV treated samples as shown in figure-2. The pH of 2 hours UV treated sample was higher than 1 hours UV treated seed sample.

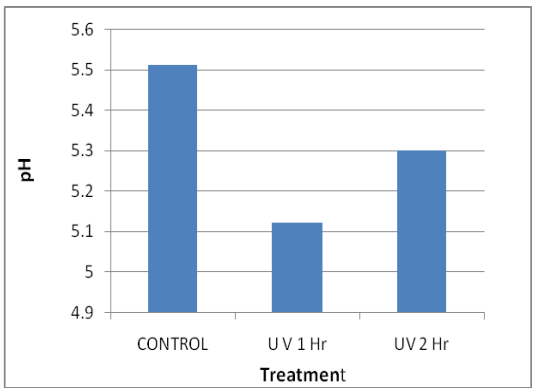

Figure-2: pH of 20% water extract of cotton seeds control, UV treatment for 1hr. and UV2 hrs.

The protease activity was determined from the 20% aqueous extract of UV induced mutated cotton seed. The highest protease activity was observed in control followed by 2 hours UV tread seeds samples as shown in Figure-3. Protease activity was also affected by UV irradiations of seeds in both *Portulaca grandiflora* and *Portulaca oleracea* (Babak et al., 2012)

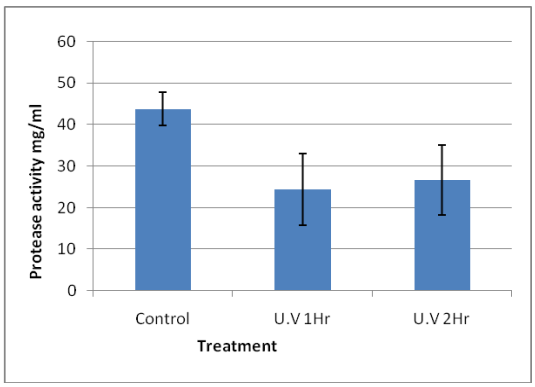

Figure-3: Protease activity of Control and UV treated seed samples.

Present results have shown the regulation in plants by proteolysis. They are involved in protein maturation, degradation and protein rebuilt in response to different external stimuli and to remove abnormal, misfolded proteins. It is concluded that Protease may be helpful in early assessment of effectiveness and superiority of irradiation dose

REFERENCES:

Ahloowalia, B. S. and M. Maluszynski, Induced mutations A new paradigm in plant breeding. *Euphytica* **118**: 167-73 (2001).

Babak, P., M. Seify, M.S. Fadaei and M. Hatim, UV Irradiation Effects on Seed Germination and Growth, Protein Content, Peroxidase and Protease Activity in *Portulaca grandiflora* and *Portulaca oleracea.* World Applied Sciences Journal 17 (7): 802-808 (2012)

Cherry, J.P., and H.R. Leffler, Seed *In*: R.J. Kohel and C.F. Lewis (eds.). Cotton No. 24 in Agronomy series. ASA, CSSA, SSSA, Madison, Wisc. PP. 511 – 569 (1984).

Farooq, J., M. Anwar, M. Riaz, A. Mahmood, A. Farooq, M.S. Iqbal and M.S. Iqbal, Association and path analysis of earliness yield and fibre related traits under cotton leaf curl virus (CLCuV) intensive conditions in *G. hirsutum* L. Plant Knowledge J. 2(1): 43-50 (2013).

Godfrey, T. and S. West, Introduction to industrial enzymology. In: Godfrey T, West S (eds) Industrial enzymology, 2nd edn. Macmillan Press, London, pp 1–8 (1996).

Gupta, R., Q.K. Beg, S. Khan and B. Chauhan, An overview on fermentation, downstream processing and properties of microbial alkaline proteases. Appl. Microbiol. Biotechnol. **60**: 381-395 (2002).

Girija, M. and D. Dhanavel, Mutagenic effectiveness and efficiency of gamma rays ethyl methane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp). *Global J. Molec. Sci.* **4**: 68-75 (2009).

Gustaffson, A. The mutation system of the chlorophyll apparatus. *Lunds Univ. Arrskr NF Adv*. **36**:1-40 (1940).

Hu, X.-P. and Y. -L. Hsieh, Crystalline structure of developing cotton fibers. J. Polym. Sci. Part B: Polym. Phys. 34 (8):1451–9 (1996)

Islam, M.K., M. Akhteruzzaman and D. Sharmin, Multivariate and genetic component analysis of new cotton (*Gossypium hirsutum* L.) genotypes. Bangladesh J. Prog. Sci. Technol. 11(2): 185190 (2013)

Joo, H.S. and C.S.Chang, Production of protease from a new alkalophilic Bacillus sp I-312 grown on soybean meal: optimization and some properties. Proc. Biochem. **40**: 1263-1270 (2005).

Johnvesly, B. and G.R.Naik, Studies on production of thermostable alkaline protease from thermophilic and alkalophilic Bacillus s JB-99 in a chemically defined medium. Proc. Biochem. **37**: 139-144 (2001)

Kharkwal, M. C., R.N. Pandey and S.E. Pawar, Mutation Breeding for Crop Improvement. *In. Plant Breeding – Mendelian to Molecular Approaches (Eds. Jain, H.K. and M.C. Kharkwal.)*, Narosa Publishing House, New Delhi, Pp. 601-645 (2004).

Layman, P.L., Industrial enzymes: battling to remain specialties. Chem. Eng, News **64**: 11-14 (1986).

Mei, C. and X. Jiang, A novel surfactant and oxidation-stable alkaline protease from Vibrio metschnikovii DL 33–51. Process Biochemistry **40**: 2167-2172 (2005).

Olsson, J.C., A. Westerdahl, P.L. Conway and S. Kjelleberg, Intestinal colonization potential of turbot (*Scophthalmus maximus*) and dab (*Limanda limanda*) associated bacteria with inhibitory effects against *Vibrio anguillarum*. Appl. Environ. Microbiol. **58**:551–556 (1992).

O'Brien, R.D., L.A. Jones, C.C. King, P.J. Wakelyn, and P.J. Wan, Cotton seed oil. *In:* F. Shahidi (ed.). Bailey's Industrial Oil and Fat Products. 6th Edition. John Wiley & Sons, Inc. Pp. 173-279 (2005).

Penner, D. and F.M. Ashton, Hormonal Control of Proteinase Activity in squash Cotyledons. Plant Physiol. **42**: 791-796 (1967).

Rao, P.J.M. and M. Gopinath, Association analysis of yield and fibre quality characters in upland cotton (*Gossypium hirsutum* L.). Aust. J. Basic Appl. Sci. 7(8): 787-790 (2013).

Song, G. and K. Yamaguchi. Efficient Agroin filtration mediated transient expression system of assaying different parameters in rice. Plant Biotechnol. 20: 235-239 (2003).

Teramura, A.H. and J.H. Sullivan, Effects of UV-B radiation on photosynthesis and growth of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynth. Res. 39: 463-473 (1994)

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535 (1990).

Welsh, J. and M. McClelland, Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18:7213-7218 (1990)