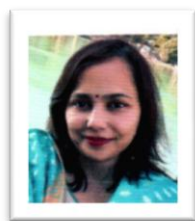


# Changes in Biochemical and Molecular Parameters of *Vigna Radiata* (L) Wilczek by Irrigation of Yamuna River Water



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## Abstract

In the present study we investigate the effect of different concentrations of Yamuna River water on different biochemical and molecular parameters of *Vigna radiata*. Following three different concentrations (25%, 50% & 100%) of Yamuna river water were tested in the experiment in which 100% concentration showed maximum inhibitory effect on the biochemical parameters of *Vigna radiata* over control. Minimum inhibition in the growth parameters was recorded under the influence of 25% of Yamuna river water in comparison to control (Tap Water). Molecular parameters were also affected from the above concentrations.

**Keywords:** Nitrogen, Protein, DNA, *Vigna Radiata*; Yamuna River Water.

## Introduction

In India mostly all the important cities are located along the bank of the major rivers and their pollution poses a serious threat to the secure urban water supply. Water is one of the most vital infrastructures in the city support system and this finite resource is going to be more scarce in the years to come. It has both quality and quantity dimensions Singh et al., 2014. The Yamuna River is a major tributary in northern India which flows through many major Indian provinces into the Ganges. This river has become an important cultural symbol in the Hindu tradition, representing the goddess Yami and the powers attributed to her. The culture that has evolved around this river have become threatened in the past century due to the effects of pollution. Yamuna is considered the most sacred among all the rivers as per Hindu mythology. Its source is at Yamunotri, in the Uttarakhand (Mishra, 2010). In the Himalayan Mountains it flows through the states of Delhi, Haryana and Uttar Pradesh, before merging with the Ganges at Allahabad. The cities of Delhi, Mathura and Agra lie on its banks. Originating in the Yamunotri glacier in the Himalayas, it is estimated that about 92% of Yamuna river water is used for irrigation. In the entire Yamuna basin the irrigated land is about 12.3 million hectares and approximately half of it (about 49%) is irrigated exclusively from surface water. Irrigated agriculture is dependent on an adequate water supply of usable quality. In irrigation water evaluation, emphasis is placed on the chemical and physical characteristics of the water. Industrial wastes are major sources of pollution in all environments and require on-site treatment before discharge into sewage system (Paliwal et al., 2007). The analysis of wastewater for trace and heavy metal contamination is an important step in ensuring human and environmental health. Wastewater is regulated differently in different countries, but the goal is to minimize the pollution introduced into natural waterways. In recent years, metal production emissions have decreased in many countries due to heavy legislation, improved production and cleaning technology. A variety of inorganic techniques can be used to measure trace elements in waste water including flame atomic absorption spectrometry (FAAS) and graphite furnace (or electrothermal) atomic absorption spectrometry (GFAAS or ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Depending upon the number of elements to be determined, expected concentration range of analytes and the number of samples to be run, the most suitable technique for business requirements can be chosen. Several industrial wastewater streams may contain heavy metals such as Sb, Cr, Cu, Pb, Zn, Co, Ni, etc. The toxic metals, existing in high or even in low concentrations, must be effectively treated/removed from the wastewaters

(Rani et al., 2013). In view of this, the present investigation was undertaken to evaluate the effect of Yamuna river water on the growth parameters of *Vigna radiata*.

#### **Aim of the Study**

In the present study we try to investigate the effect of different concentrations of Yamuna river water on biochemical and molecular parameters of *Vigna radiata* crop, so that we can know the deleterious effects of water samples collected at different locations from Agra (Kailash ghat, Pohiya ghat and near Taj Mahal).

#### **Material and Methods**

The healthy seeds of *Vigna radiata* variety, i.e. PUSA- VISHAL were surface sterilized with 0.1 per cent mercuric chloride for 2 minutes and washed thoroughly with tap water and then with distilled water. Forty seeds of *Vigna radiata* were arranged equispacially in plastic trays lined with filter paper. They were irrigated uniformly with three different concentrations i.e. 25%, 50% and 100% of Yamuna river water which were taken from three different locations in Agra (Kailash ghat, Pohiya ghat and near Taj Mahal) and with tap water (Control). They were allowed to grow for 10th days. Three replications were maintained for this varietal screening experiment. The number of seeds germinated in each treatment was counted on each and every day up to 10th day after sowing. Twenty seedlings were randomly selected on 10<sup>th</sup> day from each treatment to record the biochemical parameters

#### **Estimation of protein (Lowry et al., 1951)**

One ml of the plant extract was taken in a 10 ml test tube and 5 ml of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the UV-Spectrophotometer. The protein contents were expressed in mg/g fresh weight.

#### **Preparation of Reagents**

##### **Reagent A**

0.4 g of sodium hydroxide was dissolved in 100 ml of distilled water. To this solution, 2 g of sodium carbonate was added.

##### **Reagent B**

One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartarate.

##### **Reagent C**

Fifty ml of reagent A and 1 ml of reagent B were taken and mixed and it was prepared freshly at the time of experiment.

##### **Folin-Phenol Reagent**

One ml of folin-phenol reagent was diluted with 2 ml of distilled water.

##### **Total Nitrogen (Khalil and Manan (1990).**

Solution was prepared by using sample of dried plant in Kjeldhal flask. This solution was titrated against 0.05 N HCl. Nitrogen content was estimated using the following formula,  
Nitrogen (%) = (Sample titrate - blank titre) N of HCl  $\times 14 \times 100 / 1000 \times$  Sample weight.

#### **Molecular Analysis**

Twenty four promising genotypes of mungbean selected randomly from three different locations

#### **DNA Extraction and Quantification**

The genomic DNA was extracted from fresh leaves of each of the 24 genotypes, crushed with pestle and mortar in liquid nitrogen. The plant genomic DNA purification spin kit "Hi Pura" of Himedia Company and protocols suggested as per protocol described by Birren et al., 1989 and Sambrook et al., 1989.

#### **Molecular Analysis of RAPD**

The RAPD amplification products were scored as present (1) or absent (0) for each primers genotype combination. Whereas, diversity in the frequency of fragment size of RAPD patterns was apportioned within and among moong bean genotypes.

#### **Results**

##### **Location (A) – (Kailash Ghat)**

In the case of 25% treatment the protein was 38.16 $\pm$ 0.42, in 50% treatment it was 26.77 $\pm$ 0.81, in 100% treatment it was 18.96 $\pm$ 0.30 and in the case of control it was 44.80 $\pm$ 0.19. In the case of 25% treatment the nitrogen was 3.4 $\pm$ 0.2, in 50% treatment it was 2.6 $\pm$ 0.5, in 100% treatment it was 1.8 $\pm$ 0.1 and in the case of control it was 3.8 $\pm$ 0.4.

##### **Location (B) – (Pohiya Ghat)**

In the case of 25% treatment the protein was 39.90 $\pm$ 0.10, in 50% treatment it was 28.02 $\pm$ 0.42, in 100% treatment it was 19.40 $\pm$ 0.24 and in the case of control it was 45.02 $\pm$ 0.20. In the case of 25% treatment the nitrogen was 3.8 $\pm$ 0.1, in 50% treatment it was 2.9 $\pm$ 0.8, in 100% treatment it was 2.0 $\pm$ 0.4 and in the case of control it was 4.2 $\pm$ 0.2.

##### **Location (C) – (Near Taj Mahal)**

In the case of 25% treatment the protein was 36.02 $\pm$ 0.12, in 50% treatment it was 25.24 $\pm$ 0.28, in 100% treatment it was 17.08 $\pm$ 0.10 and in the case of control it was 42.06 $\pm$ 0.60. In the case of 25% treatment the nitrogen was 3.0 $\pm$ 0.1, in 50% treatment it was 2.2 $\pm$ 0.4, in 100% treatment it was 1.4 $\pm$ 0.6 and in the case of control it was 3.5 $\pm$ 0.2.

The perusal of phenotypic characterization data of 22 moong bean genotypes showed significant variations in most of the characters. The number of PCR amplified products formed ranged from nine to twenty two with an average of about 14 bands per primer. The primer OPA-09 was most informative primer which exhibited 100% polymorphism in RAPD banding patterns. The depiction of principal coordinate analysis of RAPD data clearly delineated all the 22 genotypes of *V. radiata* and showed that first three principal coordinates contributed in variance. The maximum phenotypic diversity in morpho-agronomic characters of population is validated by the molecular analysis of RAPD. The analysis validates the existence of higher genetic diversity within population because of rich genetic diversity in mungbean varieties. Based on obtained results, the elite mung bean genotypes showing significant phylogenetic distances in RAPD analysis. Whereas, the number of pods/plant and number of

seeds/plant as dependable phenotypic markers for improving yield potentials and mung bean improvement due to highly significant and positive correlation with seed yield. We report existence of reasonable genetic variability in mung bean

genotypes that may be useful in crop improvement. The RAPD profiles generated by two informative primers (OPA-09 and OPA-02) are shown vide Figs. 1 & 2

Fig. 1 RAPD profiles of 1–22 genotypes of *Vigna radiata* amplified by OPA-9 primer

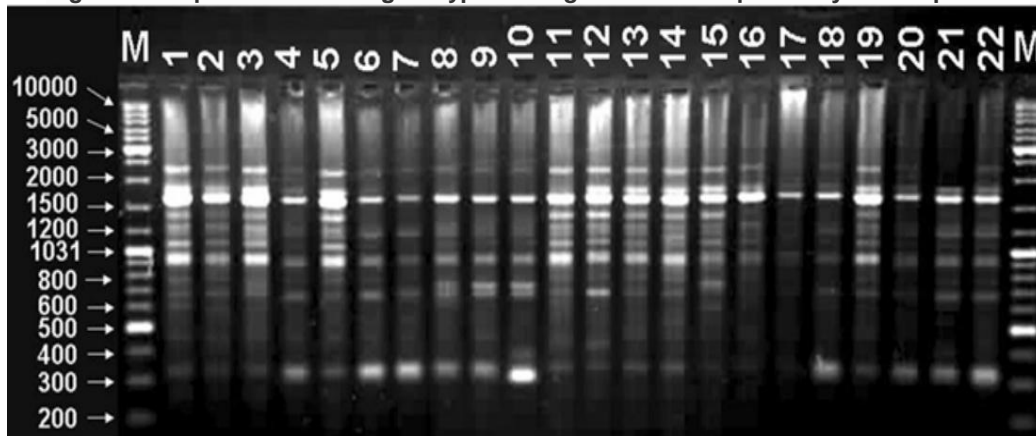
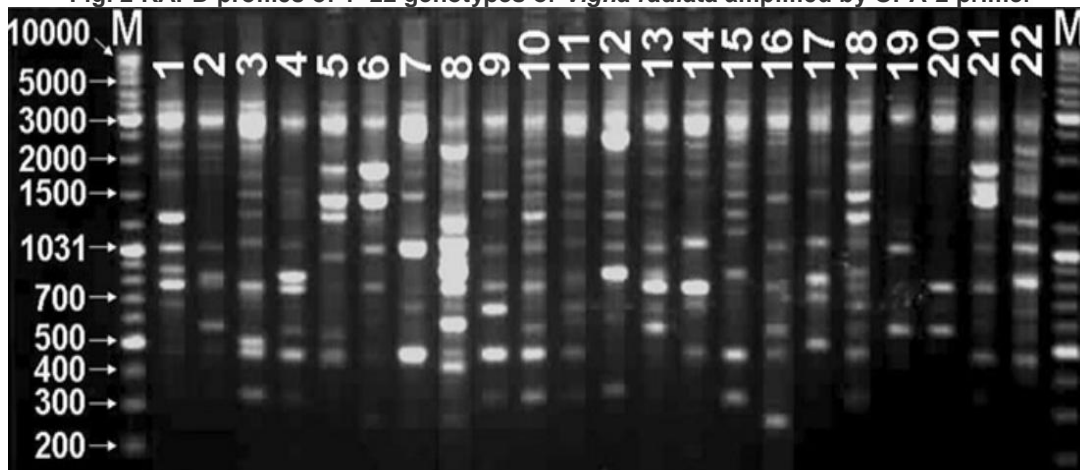


Fig. 2 RAPD profiles of 1–22 genotypes of *Vigna radiata* amplified by OPA-2 primer



**Discussion**

Sehgal et al., 2012, worked on maize seeds which were germinated in soil treated with various concentrations (0- 100%) of paper mill effluent. Both the germination percentage and seedling growth increased upto 25% effluent concentration and decreased at the higher concentrations. nitrogen contents increased with 50-75% concentration. It was suggested that paper mill effluent after proper dilution (25%) can be used for irrigation of crops. Observations further indicated that seeds of control sets contain 49% of total nitrogen, while in seeds of plants grown on zinc amended soil, the relative amount was 43% of total plant nitrogen. Singh et al., 2014 pointed out that there is some inhibition in transfer of total N from vegetative parts of developing seeds in the presence of zinc. He further showed that like total N, total P level also decreased which were about 62.5% and control. He concluded that zinc, which is otherwise a micronutrient, could become a pollutant if present in more than the necessary amount. the elite mungbean genotypes showing significant phylogenetic distances in RAPD analysis. Whereas, the number of pods/plant and number of

seeds/plant as dependable phenotypic markers for improving yield potentials and mungbean improvement due to highly significant and positive correlation with seed yield. We report existence of reasonable genetic variability in mungbean genotypes that may be useful in crop improvement.

**Table-1**

**Effect of Different Concentrations of Yamuna River Water on Biochemical Content of Vigna Radiata (Location Pohiya Ghat)**

Treatments	Protein Content	Nitrogen %
25%	38.16±0.42	3.4±0.2
50%	26.77±0.81	2.6±0.5
100%	18.96±0.30	1.8±0.1
Control	44.80±0.19	3.8±0.4

**Table-2**

**Effect of Different Concentrations of Yamuna River Water on Biochemical Content of Vigna Radiata (Location Kailash Ghat)**

Treatments	Protein Content	Nitrogen %
25%	39.90±0.10	3.8±0.12
50%	28.02±0.42	2.9±0.08
100%	19.40±0.24	2.0±0.18
Control	45.02±0.20	4.2±0.32

**Table-3**  
**Effect of Different Concentrations of Yamuna River Water on Biochemical Content of Vigna Radiata (Location near Taj Mahal)**

Treatments	Protein Content	Nitrogen %
25%	36.02±0.12	3.0±0.10
50%	25.24±0.28	2.2±0.24
100%	17.08±0.10	1.4±0.16
Control	42.06±0.60	3.5±0.42

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