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ABSTRACT

Citrus and Fig were analyzed for different physiological and biochemical parameters along with the determination of heavy metal accumulation. Fig showed maximum plant fresh weight while minimum plant fresh weight was observed for Citrus. Citrus showed more dry plant weight, higher concentrations of proline, DNA with more DNA purity, proteins, Cd, Cr, and Pb as compared to Fig.

Key Words: Heavy Metals, Fig, Citrus, analysis

RESULTS AND DISCUSSION

The present study describes the various physiological, biochemical and heavy metal concentrations of two different orchid crops i.e. Citrus and Fig. These parameters are presented and discussed below.

Physiological parameters:

Data concerning plant fresh and dry weight is presented in Table 1. The data indicated in Table 1 revealed that maximum plant fresh weight of 8.72 g was recorded by

fig followed by citrus with plant fresh weight of 8.55 g. This difference may be due to differences in their genetic makeup. Data recorded for plant dry weight as indicated in Table 1 revealed that maximum plant dry weight of 3.89 g was noted for Citrus followed by Fig revealing 2.22 g plant dry weight.

Biochemical parameters:

Different biochemical characteristics investigated were proline, protein and DNA concentration of two different orchid crops i.e. Citrus and Fig (Table 2). It is clear from the data shown Table 1 that higher concentration of 0.029 µg/g fresh weight proline was noted in citrus while lowest proline concentration of 0.001 µg/g fresh weights was noted in Fig. Data regarding DNA concentration and quality is shown in Table 1. The data revealed that maximum DNA concentration was noted in citrus (10 mg/ml) followed by fig with DNA concentration of 7.05 mg/ml. It is also clear from the data shown in Table 1 that citrus had the highest DNA purity (0.261) followed by fig (0.186). Data concerning protein concentration is indicated in Table 1. The results revealed that highest concentration of 3.058 mg/ml protein was recorded in citrus while lowest protein concentration of 2.794 mg/ml was noted for fig (Table 1).

Table 1: Physiological and biochemical characters of orchid crops Citrus and Fig

Plants	Plant Fresh Wt. (g)	Plant Dry Wt. (g)	Proline (µg/g)	Protein (mg/ml)	DNA (mg/ml)	DNA Purity
Citrus	8.55	3.89	0.029	3.056	10.00	0.261
Fig	8.72	2.22	0.001	2.794	7.05	0.186

Heavy Metals concentration:

Table 2 presents data regarding different heavy metal concentration in citrus and fig plants collected from Malakand Research Farm of KPK Agricultural University

Peshawar. The data showed that citrus recorded maximum Cd concentration (11.5 µg/g and minimum Cd concentration was noted in fig (1.725 µg/g). The data shown in Table 2 further revealed that highest concentration of Cr was noted in citrus (82.475 µg/g) followed by fig with Cr concentration of 48.5 µg/g. The data regarding Pb levels revealed that maximum levels of Pb was accumulated by citrus (45.6 µg/g) followed by fig with Pb levels of 36.275 µg/g. (Table 2).

Table 2: Heavy metal concentrations (µg/g) of orchid crops Citrus and Fig

Plants	**Cadmium (Cd)**	**Chromium (Cr)**	**Lead (Pb)**
Citrus	11.5	82.475	45.6
Fig	1.725	48.5	36.275

MATERIALS AND METHODS

The present research was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE) Khyber Pukhtunkhwa Agricultural University Peshawar during 2011. The aim of the study was to investigate heavy metal accumulation in Fig (*Ficus*) and Citrus genotypes and other physiological and biochemical parameters affected by these heavy metals. For this purpose three genotypes of fig were collected from the field grown crops at Malakand Research Farms of Khyber Pukhtunkhwa Agricultural University Peshawar. Plant materials were analyzed for different physiological and biochemical parameters along with the determination of heavy metal accumulation by the collected fig and citrus genotypes. The following parameters were studied during the course of the study.

Plant materials

Two crops Fig and Citrus were used in the experiment, and following parameters were studied:

Plant fresh weight, plant dry weight, proline content, protein content, DNA quantification and purity and heavy metals (Cd, Cr and Pb).

Procedures for data recording

1. Plant fresh weight

Plant fresh weight was recorded by taking fresh weight of ten plants with the help of electric balance and their averages was then calculated.

2. Plant dry weight

Plants taken for fresh weight data was dried at 80 ºC for 48 hours in oven and their dry weight was noted with the help of an electronic balance and averaged.

3. Proline Content

Proline was measured as describe by Bates et al. [1] with minor modification. For this purpose, 100 mg of frozen plant material will be homogenized in 1ml of sterilized iron free water; the debris was removed by centrifugation at 5000 rpm. 250µl of the extract was reacted with 1ml of Acid Ninhydrin and 1ml of Glacial Acetic Acid. The mixture was then placed in water bath for I hour at 100ºC, and the reaction was terminated in an ice bath. The reaction mixture was mixed with 4 mL of Toluene and its Optical Density was measured at 520 nm. The amount of Proline was determined from standard curve.

4. Protein Extraction and Quantification

Protein was extracted by grinding about 800 mg lyophilized plant material pre-cooled mortar and pestle. The slurry was homogenized with 2ml buffer containing 100 mM Tris HCl (pH 6.8), 1% SDS and 0.1% β-marceptoethanol and centrifuged at 15000 rpm for 10 minutes at 4 ºC. The supernatant was collected and protein was quantified through Bradford method [2] using Bovine Serum Albumin as standards.

5. Genomic DNA extraction from leaves using Fig and Citrus plants

Leaf samples were grinded into fine powder and transferred it into the eppendorf tube. Hundred mg of grinded samples were added to 600 µl pre-warmed DNA 2X CTAB extraction buffer (Table 3). Then 0.6 volumes of chloroform iso amyl alcohol (24:1) was added, mixed by shaking for 15 minutes and centrifuged at 15000 rpm for 10 minutes. The supernatant was transferred to fresh tubes and added 0.6 volumes of iso-propanol to precipitate the DNA. The samples were then centrifuged at 12000 rpm for 10 minutes.

The pellet was washed with 90, 80 and 70% ethanol and then dried by putting the tubes upside down for 10 minutes. The dried pellet was then dissolved in distilled water at 60 ºC in water bath to facilitate dissolution. DNA samples were then stored at -80 ºC until used. The samples were then quantified through UV-spectrophotometer at 260 and 280 nm.

Table 3.Composition of 2X CTAB DNA Extraction Buffer

Preparation of 2X CTAB DNA Extraction Buffer	
CTAB	2%
NaCl	1.4 M
EDTA	20 mM (pH 8)
Tris-HCl	100 mM
β-mercaptoethanol	2µl/ml of buffer

6. Procedures for heavy metal analysis

Samples collected were dried at 80 ℃ for 48 hours and then finely grinded by electric grinder. Then the dried and crushed shoot sample (1g) was prepared for atomic absorption spectrophotometer analysis. For this purpose samples were digested with 15 ml of concentrated nitric acid overnight. Digested samples were then heated to 250 ℃ till when white fumes appeared, and the heating was continued for another one hour. The samples were then cooled down to room temperature and diluted to 25 ml with distilled water and then filtered. Concentration of Pb, Cr and Cd was determined by atomic absorption spectrophotometer.

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