Comparative anatomical studies of *Eichhornia crassipes* in different ecological zones of the Punjab, Pakistan

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Abstract

Eichhornia crassipes or water hyacinth has been identified as promising candidate for bioremediation of metal contaminated aquifers due to its thick root system. Knowledge of its developmental plasticity and structural modifications in different eco-zones of Punjab, Pakistan is vital to effectively utilize it for the said purpose. For study of ultra-structural variations in *E. crassipes*, samples were collected from three different areas of Punjab, Pakistan and changes in root and leaf were investigated. *E. crassipes* from Head balloki exhibited higher root diameter, thicker epidermis, parenchyma and metaxylem as compared to Head sullemanki and Renala khurd sites The anatomical traits of leaves displayed higher mesophyll thickness and improved vascular system then other ecozones. While no significant differences were observed in stomatal area. Thus, *E. crassipes* plants from Head balloki area have established an ability for stress tolerance via adaptations in roots and leaf anatomical features as compared to other areas.

Keywords: *Eichhornia crassipes*; Anatomical traits, Eco-zones

Introduction

Water hyacinth (*Eichhornia crassipes*) is a free floating aquatic weed and a member of Pontederiaceae family. It is originated in South America and has been spread around different continents including Asia, Africa and Europe due to its possible adaptability to different environmental conditions (Strange et al., 2004; Kriticos et al., 2016; Jones et al., 2018). It is reported that *E. Crassipes* morphological features vary depending upon growth conditions. In dense stands, petioles are generally elongated with circular leaves while plants are short with kidney shaped leaves in less dense mats (Singh et al., 2017). Also, presence of higher concentration of salts in aquifers can reduce water hyacinth growth and development (Sooknah and Wilkie, 2004).

It is important to mention here that *E. Crassipes* has been declared as an invasive and harmful plant sp. in many tropical countries (Laet et al., 2018). On the other hand, it has remarkable ability to accumulate toxic concentrations of different heavy metals such as Cr, Co, Cd, As, Pb, Zn, Cu and Ni without showing toxicity symptoms (Ugya et al., 2016; Saha et al., 2017; Ali et al., 2018; Goswami et al., 2018). Therefore, it is considered as a relatively cheap and environmental friendly tool for potential remediation of metal contaminated aquifers (Saha et al., 2017; Rezania et al., 2015). In water hayacinth, active transport of heavy metals take place from roots while in passive transport metals accumulate on upper parts of the plant during its direct contact with metal medium (Maine et al., 2004).

Increasing pollution levels in river Ravi and Satlaj impose serious threats both for aquatic and human lives of Pakistan. Especially, Mn, Pb, Fe and Zn metals predominate in Head sulemanki and Head baloki sites (Tabinda et al., 2013; Akhtar et al., 2011). The monitoring of river water quality is essential to examine the pollution status and to develop an appropriate remediation strategy (Shafi et al., 2018). As Water hyacinth has the advance tendency to remove metal contaminants from sludge ponds, domestic wastewaters and sewage effluents due to its gigantic biomass production and high tolerance and absorption rates for inorganic and organic contaminants (Jafari et al., 2010). Physiological and anatomical modifications in water hyacinth under metal contamination conditions have been reported by different researchers such as mesophyll thickness, increased stomatal density and higher number of xylem vessels (Pereira et al., 2017; Souza et al., 2009; Mahmood et al., 2005). Therefore, investigation of anatomical characteristics in different ecotypes of this invasive macrophyte could provide important insights to signify its use for remediation of contaminated aquifers in different zones.

Materials and methods

Sample collection and preservation

Samples of *E. crassipes* were collected from three different areas of Punjab including Head Balloki (31.2260° N, 73.8698° E), Head Sulemanki (30.3790° N, 73.8642° E) and Renala Khurd (30.8782° N, 73.5954° E) Punjab, Pakistan. Plant roots and shoots were immediately separated and preserved in FAA (Formalin acetic acid) solution as described by Johansen. (1940). Afterwards, samples were shifted to acetic alcohol solution (one-part acetic acid mixed with three parts ethyl alcohol) for log term preservation until analyses.

Anatomical studies

The anatomical analysis was executed on fully developed leaves and roots. Permanent cross sections of leaves and roots were prepared. For that purpose, leaf sections were cut from abaxial side using steel blades and root sections from maturation zone, then washed with sodium hypochlorite (50%) and rinsed in sterile water two to three times. The samples were stained with 1% safranine solution and then mounted on slides by using 50% glycerol (Johansen, 1940). All of the section cuttings were performed using steel blades manually. Double mounting method was used to prepare permanent slides by using Canada balsum in double mounted process. Olympus microscope (Olympus, Tokyo, Japan) was used to observe each slide at different powers objectives viz, X10, X40 and photographed with digital camera.

Statistical analysis

The data was subjected to analysis of variance using Statistix software (version 8.1) comparison among mean values was calculated using least significant difference (LSD).

Results

Root anatomy of *E. crassipes* in different Ecozones

The roots of water hyacinth plants collected from different eco-zones displayed modified anatomical characteristics (Figure 1). Statistically significant differences were examined in root diameter (Table 1). The plants of H. balooki (946µm) and H. sulemanki (946µm) sites showed higher root diameter as compared to Renala site (857µm). Similarly, different ecozones influenced significantly on thickness and cell area of root epidermis (Fig 2b,c). The higher values of epidermal thickness and cell area were noted in roots collected from H. balloki area (24.510µm & 471.57µm^2) respectively, as compared to other two sites. Furthermore, thickness of parenchyma and its cell area was also measured and it showed significant differences among three collection sites. Parenchyma thickness was higher in plants of Head Balloki site (31.720µm) as compared to plants from other two sites. The parenchyma area was found higher in samples of H.sullemanki site (213µm^2) then H.balloki (208µm$^{2)}$ and R.khurd site (53µm^2). Furthermore, significant differences were observed in thickness and cell area of metaxylem among three collection sites. Mainly, H. balloki site showed higher metaxylem thickness (114µm) and cell area (1203µm^2) while minimum metaxylem thickness (39.60µm) and cell area (1203µm^2) was recorded in plants of H. Sulemanki site. Furthermore, statistically significant differences were observed in phloem thickness and cell area among all samples collected from different ecozones. The maximum thickness of phloem was observed in the samples of H.balloki (16µm) and H. sulemanki

(18µm) respectively, while lowest thickness of Phloem was observed in plants of Renala site (8 µm). Similarly, in case of phloem cell area, higher values were observed in the samples of H. balloki (209 $µm^2$) and H.sulemanki (206$µm^2$) as compared to plants of R.khurd site (53 $µm^2$).

Leaf anatomy of *E. crassipes* in different Ecozones

The leaves of water hyacinth plants collected from different eco-zones displayed modified anatomical characteristics (Figure 2, Table 2). No significant differences were observed in the thickness of leaf epidermis but the samples of R. khurd site displayed higher leaf epidermis area (11293) as compared to H. sullemanki (10262) and H.balloki (9790) sites. Furthermore, significant differences are found in mesophyll thickness and cell area. It was assessed from studies, that increase in mesophyll thickness (126.58 µm) and cell area (10652 $µm^2$) were recorded in samples of Head Balloki site as compared to other two sites. To investigate the changes in anatomy of stomata in different ecozones, cell area of stomata was calculated and statistically no significant differences are found in stomatal area. Similarly, non-significant differences are found in the mataxylem thickness and metaxylem area among samples of three sites. The phloem thickness was significantly higher in plants of H. balloki site (15µm) as compared to H. sullemanki (11µm) and R.khurd (9.00µm) sites. Plant samples from H. balloki site also displayed higher phloem area (229 $µm^2$) as compared to R.khurd (121 $µm^{2)}$ and H.sullemanki site (125 $µm^2$).

DISCUSSION

Removal of different contaminants and heavy metals by application of aquatic flora is the most profitable and proficient method (Guittonny-Philippe et al., 2015). To enhance the phytoremediation process, it is very important to wisely select the aquatic plant spp for accumulation of heavy metals (Galal et al., 2018). In this regard, water hyacinth acquires the unique ability to flourish in heavily polluted water and rapidly extract pollutants (Maine et al., 2001). In our study, we collected samples of water hyacinth from three aquatic ecozones namely H. balloki, H. sulemanki and R. khurd in Punjab region due to presence of heavy metals such as Mn, Pb, Fe and Zn etc and other contaminants as reported previously (Tabinda et al., 2013; Akhtar et al., 2011). The anatomical analysis displayed significant variations in root and leaf anatomical characteristics collected from these ecozones. Previously, water hyacinth plants have been exhibited increased stomatal density, and parenchyma thickness of stems under heavy metal stress conditions while roots did not exhibit any vascular system improvement (Pereira et al., 2017). In our study, samples from H. balloki site appeared with increase root diameter, epidermis thickness and area as well as parenchyma thickness as compared to other two sites. The exodermis, endodermis and epidermis of roots are considered as apoplastic barriers to different substances (Ribeiro et al., 2015). It suggests that increase in epidermis area and thickness can block the entry of toxic substances towards shoots which can ultimately improve stress tolerance in H.balloki site plants. The increase in root diameter, epidermis and parenchyma from H. balloki samples is also an indicator of higher contents of metals in this region as compared to other two sites. Xylem and phloem tissues are mainly related to an important phenomenon of biological compound formation known as photo-assimilation transport, which ultimately leads towards better development of root system as well as improved food supply in daughter plants (Pereira et al., 2016). As thickness of vascular tissues (metaxylem and phloem) have been observed in root and leaf samples of H. balloki and H. sullemanki sites, which suggests improvement of vascular systems under stress conditions. Furthermore, thicker mesophyll was noted in samples of H. balloki region as compared to R. khurd site which strengthen our hypothesis that plants under heavy metals stress conditions increase these parameters and ultimately improve CO_2 flow in to the leaf. In contrary, Qaisar et al. (2005) reported the reduction in cell sizes of different plant organs such as in roots, rhizomes and leaves of water hyacinth under textile water treatment as compared to control plants which ultimately arrests plant growth. However, root and leaf anatomical studies showed significant variations from all three sites, specifically H. balloki sites which suggests higher levels of stress tolerance in plants of this ecozone as compared to other two sites. The increase in stomatal density as well as guard cells length and width of guard cells are considered as an important contributing factors to improve CO_2 uptake under heavy metals stress conditions as plants mostly exhibit this trait under different type of stresses such as Cd and waste water treatment (Pereira et al., 2014; Souza et al., 2010; Qaisar et al., 2005). In our study non-significant differences were observed in stomatal area from all three sites.

Conclusion

Water hyacinth plants display anatomical modifications in different ecozones due to presence of heavy metals, contaminants and water quality. Samples collected from Head balloki site exhibited higher root diameter including thicker epidermis, parenchyma and metaxylem cells while plants from Head sullemanki site displayed thicker phloem. Similarly, in study of leaf anatomical characteristics, the samples of Head balloki site showed more thickness in leaf mesophyll, metaxylem and phloem cells as compared to other two sites. Whereas, stomatal area did not exhibit any significant difference. It suggests that plants from Head balloki site have developed stress-mediated anatomical modifications in roots and leaves as compared to other areas. However, further studies are required regarding water quality, nature of contaminants and concentrations of heavy metals in this ecozone.

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Habitats	**Root diameter (μm)**	**Epidermis thickness (μm)**	**Epidermis Area (μm)**	**Parenchyma thickness (μm)**	**Parenchyma area (μm)**	**Metaxylem thickness (μm)**	**Metaxylem Area (μm²)**	**Phloem Thickness (μm)**	**Phloem Area (μm²)**
Renala Khurd	857^{b}	15.61^{c}	200^{b}	18.97^{b}	53.03^{b}	105.8^{a}	6000^{b}	8.30^{ab}	53.70^{b}
H.Baloki	946^{a}	24.51^{a}	471.5^{a}	31.72^{a}	208.6^{a}	114.3^{a}	10589^{a}	16.34^{a}	209.7^{a}
H.Sulemanki	946^{a}	20.00^{b}	154.8^{c}	25.57^{ab}	213.5^{a}	39.60^{b}	1203^{c}	18.14^{a}	206.9^{a}

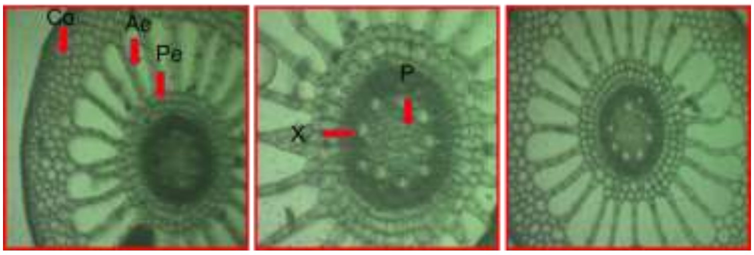

Fig.1 Anatomical study of root samples collected from (a) Renala (b) Head baloki (c) Head sulemanki sites co, Cortex; Ae, Aerenchyma; Pe, Pericycle, X, Xylem; P, Phloem

Table.1 Anatomical characteristics of root tissues in *E. crassipes* from different eco-zones

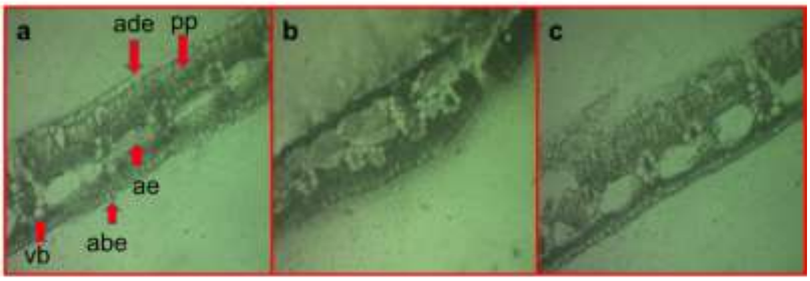

Fig.2 Anatomical study of leaf samples collected from (a) Renala (b) Head baloki (c) Head sulemanki sites; ade: Abaxial epidermis; ade: Adaxial epidermis; ae: Aerenchyma; vb: Vascular bundle; pp: Palisade parenchyma

Habitats	**Epidermis thickness (μm)**	**Epidermis area (μm)**	**Mesophyll. thickness (μm)**	**Mesophyll area (μm)**	**stomatal area (μm)**	**Metaxylem thickness (μm)**	**Metaxylem Area (μm^2)**	**Phloem Thickness (μm)**	**Phloem Area (μm^2)**
Renala	119.8^{a}	11293^{a}	73.61^{b}	2040^{b}	1428^{a}	45.16^{a}	1518^{a}	9.32^{b}	121^{b}
H.Baloki	120^{a}	9790^{b}	126^{a}	10652^{a}	15381^{a}	37.97^{a}	1172^{a}	15.80^{a}	229^{a}
H.Sulemanki	111^{a}	10262^{ab}	123^{a}	8630^{a}	2814.6^{a}	32.68^{a}	910^{a}	11.30^{ab}	125^{ab}

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