Invasive success of star weed (*Parthenium hysterophorus* L.) through alteration in structural and functional peculiarities

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Abstract

A study was conducted on fifteen distinct populations of the star weed (*Parthenium hysterophorus* L.) to investigate the factors contributing to its widespread distribution in diverse environmental conditions. The results revealed significant variations in growth performance, physiological traits, and internal structures among populations from different habitats. The populations from wastelands exhibited superior growth, with higher accumulation of soluble proteins (TSP) and chlorophyll content (chl a, b, Tchl, car, and chl a/b). They displayed increased root and stem area, storage parenchyma, vascular bundle area, metaxylem area, and phloem area. Noteworthy leaf modifications included thicker leaves, sclarification around vascular bundles, and widened metaxylem vessels. Roadside populations possessed larger leaf area, enhanced antioxidant activity, increased thickness of leaves in terms of midrib and lamina, and a higher cortical proportion. Populations found in agricultural fields depicted enhanced shoot biomass production, higher levels of chlorophyll b, and an increased total chlorophyll/carotenoid ratio. Additionally, they exhibited increased phloem area in their roots, stems, and leaves, with a thick epidermis only in the stem. In conclusion, the study revealed explicit structural and functional variations among *P. hysterophorus* populations collected from different habitats. These variations were attributed to the environmental variability and could contribute to the widespread distribution of this species.

Keyword: *P. hysterophorus*, growth behavior, microstructural and functional modifications, ubiquitous.

Introduction

Invasive species pose a significant threat to the diversity of native plant communities, leading to the loss of ecological and economic values (McGeoch et al., 2010). One prominent example of such an invasive species is *Parthenium hysterophorus* L., a perennial dicot herb belonging to the Asteraceae family. This species is widely distributed and thrives in open and disturbed environments. It is known to invade various habitats, including ~~river banks~~riverbanks, roadsides, railway tracks, dry and moist areas such as mountainous regions, water channels, drains, agricultural fields, open and barren lands, housing societies, and parking lots. Its ability to adapt to different conditions has made it an ecological disaster, particularly in agricultural fields, where it competes with crops for vital resources like water and minerals (Adkins and Shabbir, 2014).

Environmental stresses such as salinity and water deficit can have severe detrimental effects on plant morphology and anatomy (Abideen et al., 2019; Zulfiqar et al., 2020). The increasing scarcity of water in arid habitats is an alarming global issue that significantly limits viable agriculture (Alvarez-Flores et al., 2018; Ali et al., 2020). Scientists are exploring various techniques to promote resourceful and sustainable agricultural and horticultural practices (Zulfiqar et al., 2019a). In response to water scarcity and other stresses, plants adopt various survival strategies. They increase root biomass and reduce shoot growth, along with making changes in leaf orientation, size reduction, and shedding (Leukovic et al., 2009; Oliveira et al., 2018). At the anatomical level, these plants exhibit reduced cell size, enlargement in vascular tissues, alterations in the xylem/phloem ratio, and reductions in xylem and phloem vessel size (Makbul et al., 2011; Boughalleb et al., 2014). Additionally, under drought or salinity stress, plants significantly reduce xylem vessel diameter and increase the thickness of epidermis, phloem, and mesophyll tissues in aerial parts (El-Afry El Afry? et al., 2012). They also accumulate substantial amounts of protective compounds like glycine betaine, proline, and total soluble proteins to combat the adverse effects of these abiotic stresses. Ionic homeostasis is a crucial physiological mechanism in plants that contributes to their vitality and ~~vigor~~vigour even under harsh conditions (Siringam et al., 2011). This mechanism involves processes such as noxious ion accumulation, selective ion uptake, and excretion of toxic ions through specialized structures like leaf hairs, trichomes, leaf sheaths, and excretory organs (Hameed et al., 2009).

Parthenium hysterophorus L. is a widely spread and aggressive annual herbaceous weed. This weed is known for its robust growth and high reproductive capacity, particularly in warm climates.

It is native to northeast Mexico and endemic to America (Adkins and Shabbir, 2014). Over the past century, it has spread to Africa, Australia, Asia, and Pacific Islands, becoming one of the most destructive and hazardous weeds worldwide. It is commonly found in abandoned lands, residential areas near towns, along roadsides, railway tracks, dry mountains, scrub forests, and drainage and irrigation canals. It is often grown as an ornamental plant in gardens, plantations, and cultivated crops. The weed's high reproductive capacity allows a single plant to produce between 10,000 to 15,000 viable seeds, which can disperse and germinate, rapidly covering large areas (Maharjan et al., 2020).

To investigate the hypothesis regarding *P. hysterophorus* response to environmental stresses, a study was conducted to explore various aspects. The study aimed to answer the following questions: a) how does *P. hysterophorus* respond to heterogeneous environmental conditions at the levels of growth, anatomy, and physiology? b) What types of micro-structural, physiological, and morphological adaptations enable *P. hysterophorus* to mitigate the detrimental effects of abiotic stresses? c) Are the induced micro-structural and physiological modifications specific to certain environmental conditions? d) Can the resistance mechanisms and alterations be classified based on the population's ~~behavior~~behaviour in relation to their respective environments? e) Do all populations of *P. hysterophorus* exhibit both internal and external responses to the prevailing climatic conditions? The study aimed to shed light on the mechanisms and adaptations employed by *P. hysterophorus* to cope with environmental stresses. By examining the responses and modifications at different levels, the researchers sought to gain a comprehensive understanding of the weed's ability to thrive in diverse environmental conditions.

Materials and Methods

Study surveys, ~~samplings~~sampling, and collection sites

Parthenium hysterophorus populations were sampled from ecologically distinct regions of Punjab province to determine the growth, physiological and anatomical response towards heterogenic environmental conditions (Fig. 1 α 2, Table 1). The sampling was done during the peak of flowering season (March to April) in year 2021. Each study site was thoroughly search in radius of 1km and total 50 plants were ear marked. Ten plants (n=10) per population were finalized for the measurement of morpho-anatomical and physiological parameters. The populations were collected from five prominent ecological regions such as i) near wasteland (RYK-Rahim Yar

Khan, SDK-Sadiqabad, KHP-khanpur), ii) along water channels (BWP-Bahawalpur, LAP-Liaqatpur, AHP-Ahmadpur, MUL-Multan), iii) along roadside (VEH-Vehari, DGK-DG Khan, RJP-Rajanpur, JHG-Jhang), iv) near agriculture fields (MUZ-Muzaffargarh, SAR-Sargodha, FSD-Faisalabad, LYH-Layyah). Coordinates were measured with the help of google positioning system (GPS, model: Garmin E-Trex 20, GPS accuracy ± 1 m) (Table 2). Climatic data was taken from meteorological department situated in each district.

Soil physiological parameters

The soil texture was assessed using the USDA textural triangle, which categorizes soils into distinct textural classes according to the relative proportions of sand, silt, and clay present in the soil sample. The Walkley method (1947) was employed to measure the organic matter content in the soil. This method involves oxidation of organic matter by dichromate in the presence of sulfuric acid. A combined pH and EC meter (WTW series InoLab pH/Cond 720, USA) was used to measure the soil pH and ECe. Saturation paste, prepared by saturating the soil with water and extracting the solution, was used for these measurements. The saturation paste was ~~analyzed~~analysed to determine the concentrations of different ions, including Na^+, K^+, and Ca^{2+}, utilizing a flame photometer (Jenway, PFP–7, UK). The nitrogen content in the soil was assessed using the micro-Kjeldahl method, which involves digesting the soil sample with sulfuric acid. The resulting ammonia was then distilled and titrated using a semi-automatic ammonia distillation unit (UDK-132, NIB-B (3)-DSU-003 Italy). The soil phosphorus content was measured following the protocol described by Wolf in 1982. This method typically involves extracting the available phosphorus from the soil using a suitable extractant, followed by colorimetric analysis. The chloride content in the soil was assessed using the Mohrs' titration method. This method, developed by Mohrs in 1856, involves titrating a solution containing the extracted chloride ions with a silver nitrate solution to determine the chloride concentration. To determine the soil saturation percentage, the soil samples were dried in an oven at 70 °C, and 200 g of the dried soil was used to prepare a composite saturation paste, which was then analyzed. Saturation percentage assayed by following formula:

$$SP\ (\%) = \frac{Amount\ of\ water\ added(g)}{Oven\ dried\ soil\ (g)}\ x\ 100$$

Where SP % is saturation percentage.

Morphological parameters

To collect the necessary measurements, a meter rod was utilized to directly measure the height of the plant, as well as the lengths of both the shoot and root. A digital loading balance was employed to determine the fresh weights of the shoot and root. Immediately after harvesting, the plant parts were weighed to obtain their fresh weights. For dry weight analysis, the plant samples were subjected to oven-drying at a temperature of 65 °C until a constant weight was achieved. This ensured the complete removal of moisture from the samples. The dry weights of the shoot and root were then measured using a digital loading balance. To assess the leaf characteristics, the number of leaves on each plant was manually counted. The leaf area was determined using cm-graph paper, providing a quantitative measurement of the area occupied by the leaves. The leaf area was calculated using a formula provided by Lopes et al. (2016).

Physiological parameters

Osmolytes and soluble proteins

Fresh samples were taken in falcon tubes and stored (-80 ◦C) for chlorophyll pigments, osmoprotectants, and antioxidants activity. For the analysis of proline, fresh leaf samples were thoroughly homogenized in sulfo-salicylic acid. Then was transferred into cuvette containing ninhydrin solution. After subjected to water bath (100 °C) toluene was added for extraction of proline. Lastly, readings were taken on a spectrophotometer (Model 220, Hitachi, Japan) at 520 nm wavelength (Bates et al., 1973).

$$Proline\ (\mu mol\ g^{-1} fresh\ weight) = \frac{\mu g\ proline\ ml^{-1} \times\ ml\ of\ toluene/115.5}{sample\ weight\ (g)}$$

To measure the glycine betaine content in the leaf samples, fresh leaf samples weighing 0.5 g were soaked in 20 ml of deionized water (H_2O) at a temperature of 25 °C for a duration of 24 hours. Following the soaking period, an extract was prepared from the soaked samples and assayed using the established protocols outlined by Grattan and Grieve (1998). For the analysis of total soluble proteins, fresh leaf samples weighing 0.2 g were sliced and thoroughly crushed in 5 ml of phosphate buffer at a pH of 7.0. The buffer facilitated the extraction of proteins from the crushed leaf samples. The mixture of crushed leaf samples and buffer was then subjected to centrifugation at 5000 rpm for 5 minutes. This centrifugation step effectively separated the solid components of the mixture from the liquid supernatant. The supernatant, containing the soluble proteins, was

collected for further analysis. To quantify the protein content in the supernatant, the method developed by Lowry et al. (1951) was employed. This method relies on a colorimetric assay to measure the protein concentration present in the sample

Photosynthetic parameters

To estimate the photosynthetic pigments, including chlorophylls (chla, chlb, and total chl.) and carotenoids, the methods described by Arnon in 1949 and Davis in 1979 were followed. A spectrophotometer (Hitachi-220, Japan) was used for the measurements. The formulas used for calculations were:

$$Chl.a\ (mg\ g^{-1}\ f.wt.) = [12.7(\mathrm{OD663}) - 2.69(\mathrm{OD645})]\mathrm{x}\frac{\mathrm{V}}{1000}\mathrm{x\ W}]$$

$$Chl.b\ (mg\ g^{-1}\ f.wt.) = [22.9(\mathrm{OD645}) - 4.68(\mathrm{OD663})]\mathrm{x}\frac{\mathrm{V}}{1000}\mathrm{x\ W}]$$

$$Total\ chl.\ (mg\ g^{-1}\ f.wt.) = [20.2(\mathrm{OD645}) - 8.02\ (\mathrm{OD663})]\mathrm{x}\frac{\mathrm{V}}{1000}\mathrm{x\ W}]$$

$$Carotenoids\ (mg\ g^{-1}\ f.wt.) = [12.7(OD480) - 0.114\ (OD663)] - 0.638\ (OD645)]/2500$$

Total antioxidant activity

For the measurement of total antioxidant activity, a dried leaf sample weighing 0.5 g was placed in a test tube. To facilitate the extraction of antioxidants from the leaf tissue, 20 mL of a 0.45% salt solution was added to the test tube. The sample was then subjected to heating in a water bath at 40°C for a duration of 20 minutes. After the heating process, the test tube was centrifuged at 3000 rpm for 30 minutes, enabling the separation of the supernatant from the solid residue. The supernatant, which contained the extracted antioxidants, was carefully separated and stored at -20°C until further analysis. To measure the total antioxidant activity, the FTC (Ferric Thiocyanate) method described by Rahmat et al. (2003) was employed. This method involves assessing the ability of the antioxidants to inhibit lipid peroxidation by reacting with ferric ions.

Anatomical parameters

To examine the anatomy of the root, stem, and leaf, the largest ramet from each replicate was selected. For leaf anatomy, a 2 cm section was obtained from the leaf base of fully mature and sun-exposed leaves. For stem anatomy, a section was taken from the base of the internode of the main tiller. Similarly, for root anatomy, a section was obtained from the thickest adventitious root near the junction of the root and shoot. The collected plant material was fixed using a formaldehyde

acetic alcohol solution consisting of 10% formaldehyde, 5% acetic acid, 50% ethanol, and 35% distilled water. The plant material was immersed in the fixative solution for 48 hours, followed by transfer to an acetic alcohol solution containing 25% acetic acid and 75% ethanol for long-term storage. To prepare the sections for microscopic analysis, free-hand sections were made from the fixed plant material. These sections underwent a series of dehydration steps using ethanol. For staining, the sections were subjected to the standard safranin and fast green double-staining technique, as outlined by Ruzin (1999). Measurements of the sections were taken using a light microscope (Nikon SE Anti-Mould, Japan) equipped with an ocular micrometer that was calibrated using a stage micrometer. Micrographs of the stained sections were captured using a digital camera (Nikon FDX-35) mounted on a stereomicroscope (Nikon 104, Japan).

Statistical analysis

The morphological, physiological, and anatomical trait data were subjected to statistical analysis using a One-way analysis of variance (ANOVA) in a complete randomized design with ten replicates. Mean values were compared using the least significant difference (LSD) test at a significance level of 5%. The statistical analysis was conducted using the Minitab software package (version 17.1.0, Pennsylvania State University, USA). To examine the relationships between the different morphological, physiological, and anatomical traits and the soil physicochemical parameters of the collection sites, Principal Component Analysis (PCA) was conducted. The analysis was carried out using the R-studio software, and the data were plotted to visualize the patterns and associations. Furthermore, heatmaps were constructed using the ~~pheatmap~~heatmap? package in R-studio. These heatmaps were used to cluster the selected groups based on (i) soil physicochemical attributes and morphophysiological parameters, (ii) soil physicochemical attributes and root anatomy, (iii) soil physicochemical attributes and stem anatomy, and (iv) soil physicochemical attributes and leaf anatomy. The heatmaps provide a visual representation of the relationships and similarities among the different variables.

Results

Soil physicochemical characteristics

The soil in most of the habitats was sandy (Table 2). The loamy soil was observed in four habitats RYK (near the wasteland), KHP (near waste deposit), VEH (near the roadside), FSD (along rice field) and LYH (wheat field) whereas loamy sand was observed in two habitats such as MLN

(along river Chenab) and SAR (along sorghum field). Clayey loam was seen in MUZ habitat (near cotton field). The soil electrical conductivity of soil ranged from 0.76 to 6.73 dSm^{-1}, the maximum value of soil ECe was recorded at RYK (near the wasteland) and KHP (near waste deposit) sites and the minimum was observed at SDA (along barren land) and RJP (near M5 motorway). Habitats like water channel (LAP), along ~~road side~~roadside (VEH) and near agriculture field (FSD) showed exceptionally highly level of soil ECe than rest of the populations. Most of the habitat comprised of alkaline pH, ranging from 7.8 to 8.9. The acidic pH was observed only in one habitat RYK (near the wasteland). The soil organic matter was varied from 0.21 to 0.56%. the maximum OM was noted in soil of ~~Chenab river~~Chenab River (MLN) and the minimum was measured in soil of roadside population (VEH). The soil saturation percentage ranged from 15 to 42%. The maximum SP was observed in soil of wheat filed (FSD) population. It was the minimum in soil of water canal (LAP) and rice filed (FSD) populations. The soil Phosphate concentration varied from 1.6 mg L^{-1} in the LAP and FSD habitats to 3.6 mg L^{-1} in the LYH habitat. The nitrate content in the LYH habitat exhibited the highest value, while the DGK habitat recorded the lowest value. The soil chloride ion (Cl^{-}) content reached its maximum (567.8 mg L^{-1}) in the RYK habitat, while the minimum (72.1 mg L^{-1}) was observed in both the MLN and MUZ habitats. The soil's calcium ion (Ca^{2+}) concentration ranged from 54.2 to 156.1 mg L^{-1}. The RYK habitat showed the highest soil calcium concentration, while the SDA habitat exhibited the lowest. The soil sodium ion (Na^{+}) content ranged between 54.2 and 398.9 mg L^{-1}, with the RYK population having the highest value and the SDA habitat recording the lowest. The maximum soil potassium ion (K^{+}) concentration was observed in the MLN and LYH habitats, while the minimum was found in the SDA habitat.

Growth characteristics

Plant height was the maximum (56.5cm) in BWP population and the minimum (16.3 cm) in FSD population (Fig. 2, Table 3). The maximum shoot length (44.7 cm) was recorded in BWP population while the minimum (11.3 cm) of this parameter was noted in FSD population. Three populations, KHP, VEH and SAR showed maximum shoot fresh (11.5 g $plant^{-1}$) and dry weight (5.8 g $plant^{-1}$), while population FSD had least shoot fresh (3.0 g $plant^{-1}$) and dry weight (1.2 g $plant^{-1}$). Root length was the maximum (11.5 cm) in BWP and the minimum (4.5 cm) in VEH population. Four populations namely RYK, KHP, LAP and SAR showed maximum root fresh weight (1.5 g $plant^{-1}$), while the population RJP exhibited low value of dry weight (0.4 g $plant^{-1}$). Population RYK showed the maximum dry weight (1.2 g $plant^{-1}$) and populations BWP, AHP,

RJP and LYH possessed the minimum dry weight (0.2 g plant^{-1}). The maximum number of leaves (29.5) were recorded in RYK population, while their minimum value (9.0) was observed in FSD population. Two populations, BWP (65.3 cm^2) and VEH (65.4 cm^2) showed the maximum value of leaf area, while the minimum (14.9 cm^2) of that parameter was measured in RYK population.

Physiological characteristics

The population from RYK exhibited the highest total soluble protein content (47.9 µg g^{-1} d.wt.), while the population from VEH had the lowest (9.4 µg g^{-1} d.wt.) (Table 3). Population BWP showed the maximum proline content (19.8 µmol g^{-1} d.wt.), whereas populations AHP and LYH possessed the minimum (1.6 µmol g^{-1} d.wt.). Glycine betaine content was highest in the BWP population (10.2 µmol g^{-1} d.wt.) and lowest in the FSD population (1.3 µmol g^{-1} d.wt.). For chlorophyll a content, the SDA population had the highest value (2.4 mg g^{-1} f. wt.), while populations MUL, DGK, RJP, JHG, and FSD had the lowest value (1.3 mg g^{-1} f. wt.). Four populations, SDA, JHG, MUZ, and FSD, showed the highest chlorophyll b content (2.0 mg g^{-1} f. wt.), whereas the RYK population showed the lowest value (0.3 mg g^{-1} f. wt.). The SDA population had the maximum total chlorophyll content (4.4 mg g^{-1} f. wt.), while the RYK and DGK populations had the minimum (2.1 mg g^{-1} f. wt.). The LAP population had the highest carotenoid content (2.8 mg g^{-1} f. wt.), and the MUZ population had the lowest (1.0 mg g–1 f. wt.). The chlorophyll a/b ratio was highest in the RYK population (6.3) and lowest in the SAR population (0.3). The MUZ population had the maximum total chlorophyll/carotenoid ratio (3.7), whereas the VEH population had the minimum (0.3). Antioxidant activity was the maximum (9.9 %) in three populations, MUL, VEH and DGK, whereas it was the minimum (3.5%) in LAP population.

Anatomical characteristics

Root anatomy

The maximum root area (400.4 µm^2) was recorded in two populations, SAR and RYK, whereas the minimum (259.1 µm^2) was in FSD population (Fig. 3, Table 4). The population from MUL had the maximum epidermal thickness (31.4 µm), while the population from LYH had the minimum epidermal thickness (9.4 µm). Population RYK showed the maximum cortical thickness (94.2 µm), and population FSD did the smallest (31.4 µm). The maximum value of cortical cells (41.1 µm) were recorded in RYK and KHP populations, whereas their minimum value (7.4 µm^2) was seen in two populations, MUZ and SAR. Population BWP possessed the largest vascular

bundles (121.3 μm^2) than rest of the populations. On the other hand, population MUL had smallest vascular bundles (55.0 μm^2). Three populations namely KHP, BWP and MUZ exhibited widened metaxylem vessels (15.7 μm^2), whereas the populations of VEH and SAR had the narrowest vessels (9.4 μm^2). Phloem area was the maximum (2.5 μm^2) in four populations, KHP, LAP, MUZ and FSD, but the minimum (0.5 μm^2) was recorded in BWP and JHG.

Stem anatomy

The maximum value of stem area (440.4 μm^2) was observed in populations KHP and MUZ, while their minimum value (182.6 μm^2) was noted in JHG (Fig. 4, Table 4). Epidermal thickness was the maximum (23.6 μm) in SAR and KHP, and the minimum (9.4 μm) in RYK and RJP. Population KHP showed the highest cortical proportion (70.7 μm), whereas the populations of RYK and MUL had lowest region (18.8 μm) of that character. Cortical cells area was the maximum (14.1 μm^2) in population AHP, FSD and LYH, and the minimum (6.3 μm^2) was in BWP. Population AHP and KHP showed largest vascular bundles (164.9 μm^2) as compared to other populations, while populations of RYK, SDA and FSD represented smallest vascular regions (94.2 μm^2). The maximum value of metaxylem vessels (18.8 μm^2) were recorded in KHP and MUL, and their minimum value (9.4 μm^2) was noted in BWP, VEH, MUZ and LYH populations. Phloem area was the maximum (69.1 μm^2) in population LYH, and the minimum (14.1 μm^2) in SDA.

Leaf anatomy

Leaf thickness greatly varied in all populations of *P. hysterophorus* (Fig. 5, Table 4). Midrib thickness was the maximum (420.8 μm) in SDA, and the minimum (235.5 μm) in FSD population. The maximum value of lamina thickness (38.1 μm) was observed in population VEH, while the minimum value (11.0 μm) was observed in RJP. Thicker epidermis (23.6 μm) was measured in three populations, KHP, LAP and AHP, whereas the thinner (10.6 μm) of this parameter was noted in MUL. Enhanced cortical region (185.3 μm) was observed in VEH, and their reduced (100.1 μm) was in FSD. The population from roadside habitats (VEH) exhibited the largest cortical cells, while the populations from FSD and LYH had the smallest cortical cells. The vascular bundle area was highest in the SDA population, whereas the RYK population had the lowest vascular bundle area. Among the populations, SDA had the highest number of metaxylem vessels, while BWP had the fewest. The phloem area was greatest in the SAR ~~population, but~~population but was minimal in the KHP and AHP populations.

Multivariate analysis

Principal component analysis (PCA)

Principal component analysis (PCA1) exhibited 27.4% and 21.2% (48.6%) variability among morpho-physiological and soil physicochemical characteristics of *P. hysterophorus*. The Chl a, TChl/Car, TChl, RDW, RFW, SFW and GB showed strong influence of soil NO3, SP, PO4 and pH, whereas Chl b, Chl a/b, TSP, SDW, SL, RL and LA represented least influence of soil OM (Fig. 6A). Principal component analysis revealed significant influence of soil physiochemical characters on anatomical traits of species. PCA2 represented the variability of 33.2% and 18.4% (51.6%) among root anatomy and soil physicochemical attributes, as the CCA showed close influence of soil Ca, ECe, Cl and Na, while MA, RA, CT, EpT, PhA and VBA had least influence of soil OM (Fig. 6B). PCA3 indicated 36.5% and 18.9% (55.4%) variations between stem anatomy and soil parameters, for example the MA represented very close influence of soil K and NO3, whereas the CCA showed with soil ECe and VBA with soil pH (Fig.6C). PCA4 exhibited 28.8% and 19.9% (58.8%) variability amid leaf anatomy and soil attributes, as the LMT, Mrt and CCA showed strong influence of soil N03, K, SP and PO4, while the EPT with soil Ca and Cl, and the VBA and PhA with soil pH (Fig. 6D).

Clustered heatmaps

Heatmap between soil physicochemical characters and morpho-physiological attributes exhibited six major clusters (Fig. 7A). In first cluster, soil attribute, OM form cluster with Ca and Car content. The second cluster indicated the clustering of soil ECe, Cl and Na with LN, RFW and RDW. In third cluster, RL form cluster with chlb, Tchl, TSP and GB. The fourth group showed clustering of soil attributes K, NO3, SP and PO4. The fifth cluster exhibited the clustering of LA and soil pH, and the sixth cluster showed the clustering of Chl a/b, Chla and TChl/Car. The seventh cluster indicated the clustering of SDW, SFW, PH and SL. Heatmap between root anatomical characteristics and soil attributes indicated four major clusters (Fig. 7B). The first cluster indicating the clustering of OM and MA. In the second cluster, soil pH form cluster with RA, CT, PhA, EpT and VBA. In the third cluster, soil attributes like NO3, K, SP and PO4 form clustering. In the fourth cluster, soil Ca, Na, ECe and Cl showed clustering with CCA.

Heatmap between soil physicochemical attributes and stem anatomical features exhibited three clusters (Fig. 7C). In the first cluster, soil pH and OM form clustering with VBA, EpT and PhA. In the second cluster, soil ECe, Cl, Na and Ca showing clustering with CCA and CT. The third cluster indicated the clustering of K, PO4, SP and NO3 with MA. Heatmap between soil

physicochemical attributes and leaf anatomical features exhibited four clusters (Fig. 7D). In the first cluster, soil OM and pH form cluster with PhA and VBA, whereas in the second cluster, soil NO3, SP and PO4 form cluster with LMT and CCA. The third cluster indicating the clustering of EPT and CT, while the fourth cluster showing clustering of soil ECe, NA, Cl and Ca with MrT and MA.

Discussion

The evaluation of morpho-anatomical and physio-biochemical adaptive markers is crucial for understanding the underlying mechanisms of adaptation in differently adapted populations to multiple stresses (Hameed et al., 2011). In the face of severe drought conditions or physiological drought induced by other environmental stresses, water conservation becomes a primary strategy (Sun et al., 2018). In water-scarce conditions, water conservation in plants is achieved through mechanisms such as water storage in parenchymatous tissues like pith and cortex (Alvarez et al., 2008), efficient water translocation facilitated by widening of vessels, and reduction of water loss through the presence of mechanical tissues and a thick cuticle on the surface of plant organs (Micco and Aronne, 2012). To evaluate the strength of adaptation and the extent of these adaptations in plant survival, populations of *P. hysterophorus* were sampled from a wide range of habitats.

The investigation revealed significant variations in morphological characteristics among the populations of *P. hysterophorus*, which can be attributed to the diverse environmental conditions in which these populations were originally adapted. Under the controlled conditions of the study, the genetically fixed characteristics of each population were expressed, reflecting their adaptation to their respective habitats (Mojica et al., 2012; Paccard et al., 2013). The population from the BWP site, which is located along a water channel with relatively soft soil texture, exhibited the maximum growth (as shown in Table 3). This type of habitat seems to be more ~~favorable~~favourable for the growth and development of *P. hysterophorus*, as reported for other hydrophytes (Qadir et al., 2008; Hasanuzzaman et al., 2014). The compactness of the soil directly influenced the growth and propagation of the species, with habitats consisting of compact soil showing shorter plants, such as the FSD and VEH populations. Similar findings were reported by Hamza and Anderson (2005), who observed shorter stature plants in compact soil. Biomass production, both in roots and shoots, is a reliable criterion for assessing tolerance potential of a species (Khosroshahi et al., 2014). The RYK and KHP populations demonstrated good overall growth response, indicating

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their potential for stress tolerance. The SAR population also exhibited vigorous growth, suggesting its complete adaptation to its specific habitat. Root and shoot parameters, such as length, number, fresh and dry weights, have been previously associated with abiotic stresses like drought or physiological drought in other plant species (Talukdar, 2013; Ye et al., 2015). The RYK population displayed a high number of leaves per plant, although they were smaller in size. Having a large number of leaves can enhance a plant's photosynthetic efficiency (Weraduwage et al., 2015), while smaller leaves can increase water use efficiency by reducing transpiration rates (Medrano et al., 2015). This adaptation is particularly important for survival in harsh saline desert conditions.

Chlorophyll pigments serve as sensitive indicators of the metabolic state under salt stress conditions (Chattopadhyay et al., 2011). In the present study, the least saline population SDA and moderately saline population KHP showed an increase in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. Similar findings have been reported by Amirjani (2011) and Sarabi et al. (2017). Conversely, the highly saline population RYK exhibited lower amounts of chlorophyll pigments and carotenoids. This decrease in pigment content aligns with other studies that have reported a significant reduction in photosynthetic pigments under highly saline conditions, such as López-Millán et al. (2009) in *Lycopersicon esculentum*, Peng et al. (2013) in Elsholtzia splendens, and Sytar et al. (2013) in various plant species. In the present study, the BWP population showed an increasing trend in organic osmolytes. The accumulation of osmolytes is an effective strategy employed by plants to endure prevailing, which serves as a defensive mechanism for plants to maintain turgor pressure and prevent tissue collapse due to desiccation (Kholodova et al., 2010; Sun et al., 2010). Elevated levels of total antioxidant activity were observed in *P. hysterophorus* populations inhabiting roadside areas, such as VEH and DGK. These findings align with previous studies conducted by Nadgorska-Socha et al. (2013), Zemanova et al. (2013), and Almohisen (2014), which demonstrated that dust pollution stimulates the production of various metabolites in plants. These metabolites play a crucial role in mitigating stress by activating the plants' defense systems (Sharma & Dietz, 2006).

The anatomical characteristics of plants have been recognized as highly responsive to climatic conditions (Caemmerer and Evans, 2015). This adaptability enables plants to thrive and survive in challenging environment (De Micco and Aronne, 2012). The size of the root cross-sectional area is predominantly determined by the relative proportions of the cortical region and the vascular bundle area, as indicated in Table 4. An expansion in root area not only enhances the capacity for

water storage but also strengthens the mechanical integrity of the plant's soft tissues, enabling efficient transport of water and minerals. The observed increase in root cross-sectional area indicates better growth in the population inhabiting waste land (RYK). Roots, being underground plant parts, are relatively less affected by environmental conditions compared to other plant organs (Fitter and Hay, 2012). Epidermis is an outermost protective layer of roots, and under harsh condition it strong friction of rhizospheric soil (McKenzie et al., 2013). In resulting, this may be damaged, mainly in grasses and herbs (McCully, 1999). *P. hysterophorus* showed a significant increase of this parameter in MUL population (along water channel). Thicker epidermal layers play vital role in resisting the friction of soil compaction as well as impede the excessive water and solute translocation inside root tissues (Chimungu et al., 2015). The water storage parenchyma (cortex) and vascular region (metaxylem vessels and phloem) in the roots play a crucial role, especially during water deficit or saline conditions. These adaptations are particularly significant for the survival of arid zone species such as *P. hysterophorus* (Hsiao and Xu, 2000). A significantly increased storage parenchyma and vascular region has been observed in populations of KHP (along waste deposit) and MUZ (along agriculture field).

The plants growing in wastelands (KHP) demonstrated the highest values for the majority of stem anatomical characteristics, as shown in Table 4. These characteristics encompass dermal, vascular, and storage tissues, indicating favorable growth conditions and enhanced biomass production, as evidenced by the shoot fresh weight (Table 4). These findings are consistent with previous studies conducted by Engloner (2009) and Guo and Miao (2010). The presence of sclerified tissues in the stems is a notable adaptation to dry conditions (Nikolova and Vassilev, 2011). It was recorded in stems from almost all habitats, but in populations from roadsides (VEH and DGK), there was higher lignin deposition compared to the other populations. Under extreme dry and hot condition, tissue sclerification is beneficial for preventing from collapse of internally metabolically active tissues during desiccation (Ahmad et al., 2016; da Cruz Maciel et al., 2015).

In arid zone species like *P. hysterophorus*, the leaf blade plays a vital role as it needs to withstand harsh environmental conditions for the plant's survival. Among the studied populations, the plants from roadside habitats (VEH) exhibited the highest values for various leaf anatomical characteristics, including leaf thickness in terms of midrib and lamina thickness, as well as mechanical and storage tissues such as cortical thickness and its cells area. These adaptations are indicative of the plant's ability to protect the leaf blade from the challenging environmental

conditions encountered in roadside habitats. Three populations namely KHP (near wasteland), AHP and LAP (along agriculture field) possessed thick epidermis and spare surface hairiness. Both are effective for evapo-transpiration loss when population surviving in dry environmental condition (Gonzáles et al., 2008).

Conclusion

In conclusion, *P. hysterophorus* displays significant variations in both structural and functional attributes, enabling it to tolerate diverse environmental adversities. The wide distribution of this species can be attributed to its specific adaptations along environmental gradients. It exhibits a range of adaptations, including changes in growth parameters, microstructural features, and functional traits. These adaptations, such as enhanced biomass production, long and numerous roots, thicker epidermis, development of storage parenchyma tissues, lignification of cortical region and vascular bundles, and increased levels of organic osmolytes and antioxidants. Overall, the structural and functional adaptations of *P. hysterophorus* contribute to its resilience, competitive ability, and ability to colonize a wide range of habitats, making it a successful and problematic invasive species.

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Author Contribution:

ZU: The principal researcher responsible for conducting the experimental work.

UI: The principal supervisor of the second author, providing guidance in statistical analysis, data visualization, modeling, and interpretation.

UI and KSA: Conducted a thorough review of the article to correct any language errors.

AA and HA: Contributed to the research by carrying out the practical aspects, including biochemical analysis, anatomical photography, and data collection.

Availability of Data and Material:

All the data and relevant information is present in the manuscript.

Declarations

Ethics Approval: Since the study did not involve animal or human subjects, specific ethical approval was not required. However, all necessary guidelines provided by The Islamia University of Bahawalpur, Rahim Yar Khan Campus for handling plant material in the laboratory were strictly

adhered to. Following the completion of the study, proper measures were taken to dispose of all materials in order to prevent any potential bio-contamination.

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