Physiological and Anatomical Response of Fragrant Rosa Species with Treated and Untreated Wastewater

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Abstract

The present study assessed the response of physiological and anatomical characteristics of four widely cultivated fragrant Rosa species. Water analysis showed that all minerals and chemicals were in permissible level in canal water and treated wastewater, whereas untreated wastewater contained higher EC, biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals like Cd, Co, Cu, Pb. There was considerable variations among different Rosa species regarding response to wastewater irrigations. Under treated wastewater, R. bourboniana showed highest photosynthetic rate, high transpiration rate and maximum chlorophyll contents than other Rosa species whereas stomatal conductance of R. gruss-an-teplitz was highest under treated wastewater. Leaf anatomical characteristics showed that R. Gruss-an-Teplitz under untreated wastewater showed large cortical cell area, vascular bundle area, large spongy cell area and thick midrib while large epidermal thickness of R. centifolia was recorded under treated wastewater. Large palisade cell and phloem area and thick leaves (lamina) were found in R. damascena under treated wastewater while large metaxylem area of R. bourboniana in untreated wastewater. The study showed that treated wastewater was most suitable and desirable irrigation treatment than canal water and untreated wastewater while R. bourboniana and R. Gruss-an-Teplitz was dominant Rosa species regarding physiological characteristics, while all species showed great diversity in leaf tissue architecture under treated and untreated wastewater. © 2016 Friends Science Publishers

Keywords: Physiology; Anatomy; Municipal wastewater effluent; Roses

Introduction

Rose is one of the most imperative ornamental crops in floricultural industry. It is woody perennial flowering plant which belongs to subfamily Rosoideae and family Rosaceae. Its genus Rosa encompasses more than 200 species and 20,000 cultivars, which are distributed globally (Younis et al., 2013). Many of these cultivars and species are cultivated for different sort of purposes and used in various industries e.g. garden and indoor plant, cut flower and making different kind of food stuffs (Nybon, 2009). There are four main fragrant species of roses grown for essential oil production in Pakistan with Rosa damascena as top ranked and extensively cultivated in Bulgaria (70–80%), China, Turkey, Russia and India (Nasir et al., 2007). Second one is Rosa centifolia, commonly grown in France, Egypt and Morocco. Later, Rosa bourboniana and Rosa Gruss-an-Teplitz were introduced in France and China respectively (Laurie and Ries, 1950).

Water is the most important need of plants which comprise 50–97% of plant body and also the most poorly managed reserve in the world (Khurana and Singh, 2012). Irrigation is by far the prime user of fresh water (70–90%) in both developed and developing countries (Ensink et al., 2002; Pedrero et al., 2010) with increasing continuously demand (FAOWATER, 2008). Industries and anthropogenic activities are main cause of exaggerated use of water resources to a crisis level (Rusan et al., 2007; Safi et al., 2007).

The use of wastewater for irrigation especially to woody plants and fruit vegetables exerts several anatomical (Ogunkune et al., 2013) and physiological modifications (Sun and Wang, 2005). Singh and Agrawal (2010) reported that photosynthetic rate, stomatal conductance and transpiration rate was high in wastewater irrigated plants than canal water irrigated ones. Petousi et al. (2013) observed that high pH of wastewater does not impose negative effects on carnation physiological characteristics. The supply of wastewater improves pigment concentration in leaves with clear increment in chlorophyll content in different plant species which ultimately resulted in increase of photosynthesis rate in plant leaves (Herteman et al., 2011). Due to heavy metals toxicity, stomatal size, epidermal size (Noman et al., 2012),
xylem and phloem cell size (Mahmood et al., 2005) are altered, while Ogunkune et al. (2013) found that wastewater posed severe hazardous impacts on vascular bundle areas of roots and stems of *Amaranthus hybridus* in the form of decreased vascular bundle area. Aldesuquy (2014) showed significant increment in lamina thickness, metaxylem vessel area, xylem area, vascular bundle area and number of closed stomata on both upper and lower epidermis occur under wastewater treatment in wheat plants.

There is no literature available about physiological and anatomical characteristics of *Rosa* species under municipal wastewater irrigation. The present study was therefore carried out to investigate the physio-anatomical attributes of four widely cultivated fragrant *Rosa* species of Pakistan under untreated and treated municipal wastewater irrigation in peri-urban areas.

**Materials and Methods**

**Experimental Site**

The experiment was carried out at the Agronomy Research Area of University of Agriculture, Faisalabad (31°25’ N, 73°09’ E and altitude of 300 m above mean sea level) Pakistan, from first week of January, 2012 to January, 2014. This experimental region had a semi-arid climate with less rainfall annually. Soil of this experimental area is clay loam which collects sewage wastewater from the students living hostels of agriculture university and canal water from main canal of the city.

**Water Treatment and Analysis**

In this experiment, untreated wastewater was treated by natural purification process as discussed by Kiziloglu et al. (2008) to improve its physical and chemical quality using conventional method (Pescod, 1992). Water was treated in three large plastic tanks of 1500 gallons water storage capacity in three step process. First tank was filled by sewage wastewater through a pipe and opening of the pipe covered with 6 mm opening sieve as preliminary treatment to block the way of grits and removal of course solid and other materials often found in wastewater (Pescod, 1992). The upper portion of the tanks was kept to remain open for sun light penetration in wastewater as natural treatment process. These three tanks were connected with each other through plastic pipes and adjusted with valves to transfer water from one tank to another. Wastewater in first tank was kept for five days for the purpose of removal of settle able organic and inorganic solids by sedimentation and removal of floating materials by skimming. This water was shifted to second tank placed 2.5’ away and 2’ below than the first tank, water stayed there for next five days for further sedimentation of smaller particles. Then water was shifted to third tank (which was placed 2.5’ away and 2’ below than the second tank) and kept there for next 5 days. This water treatment process was completed in 15 days and water in third tank obtained after 15 days of treatment was applied to plants as treated wastewater treatment.

Physico-chemical properties of all irrigation water types were determined by standard methods of wastewater examination proposed by Eaton et al. (2005) and all heavy metals and some nutrients like P, K, Na and Ca concentration was determined with the help of inductively couples plasma (ICP-OES) (Optima 2100-DV Perkin Elmer) at Nuclear Institute of Agriculture and Biology (NIAB) Faisalabad.

**Soil Analysis**

Sixteen soil samples were randomly collected at the depth of 15 and 30 cm into the soil before the start of experiment. Composite soil samples of experimental sites were analyzed according to standard procedures (Table 1).

**Experimental Treatments and Measurement of Physiological Anatomical Characteristics of Plants**

There were two treatment factors in this experiment i.e., irrigation water and *Rosa* species. Two years old cuttings of four fragrant *Rosa* species like *Rosa centifolia*, *R. damascena*, *R. bourboniana* and *R. Gruss-an-Teplitz* were planted during first week of January 2012 and irrigated by canal water, treated and untreated wastewater. Treatments were set according to randomized complete block design (RCBD) with three replications. Data regarding physiological attributes including chlorophyll contents was estimated with the help of chlorophyll meter (CCM-200 plus) after its calibration and average was computed while photosynthetic rate, stomatal conductance and transpiration rate of five randomly selected leaves within each treatment were measured with the help of infra-red gas analyzer (IRGA) (LCl-SD, ADC-Bioscientific UK). For anatomical studies, one cm piece from the leaf center along the midrib was taken. The material was preserved in FAA (Formalin Acetic Acid) solution for fixation, which contained formalin 50%, ethyl alcohol 50%, acetic acid 10% and distilled water 35%. The material was transferred in acetic acid solution (one part acetic acid and three parts ethyl alcohol) for long term preservation. Samples were prepared and fixed in Formalin-Acetic acid-Alcohol (FAA). Dehydration, sectioning staining and mounting procedures was followed according to method described by Sass (1951). Sections were measured by the aid of light microscope. Measurement of each anatomical parameter was calculated in twelve sections in keel region in μm.

**Statistical Analysis**

Data collected for all parameters were analyzed by performing Fisher’s analysis of variance technique (ANOVA) using Statistica soft 5.5 and treatment means were compared according to least significant difference test (LSD) at 5% level of probability (Steel et al., 1997).
Table 1: Composition of soil before experiment

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>Texture</th>
<th>pH</th>
<th>EC</th>
<th>OM (%)</th>
<th>N (%)</th>
<th>P (ppm)</th>
<th>K (ppm)</th>
<th>Pb (ppm)</th>
<th>Cd (ppm)</th>
<th>Ni (ppm)</th>
<th>Zn (ppm)</th>
<th>Cu (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00–15 cm</td>
<td>Clay loam soil</td>
<td>8.20</td>
<td>2.54</td>
<td>1.12</td>
<td>0.041</td>
<td>10.50</td>
<td>194</td>
<td>3.16</td>
<td>0.04</td>
<td>0.36</td>
<td>5.28</td>
<td>3.04</td>
</tr>
<tr>
<td>16–30 cm</td>
<td>Clay loam soil</td>
<td>8.20</td>
<td>2.49</td>
<td>1.18</td>
<td>0.041</td>
<td>9.50</td>
<td>134</td>
<td>3.32</td>
<td>0.05</td>
<td>0.34</td>
<td>3.60</td>
<td>2.30</td>
</tr>
<tr>
<td>IASS</td>
<td></td>
<td>4.85</td>
<td>4.00</td>
<td>&gt;0.86</td>
<td>--</td>
<td>&gt;7</td>
<td>&gt;80</td>
<td>&gt;500</td>
<td>1.00</td>
<td>20</td>
<td>250</td>
<td>100</td>
</tr>
</tbody>
</table>

EC= Electrical conductivity, OM= Organic matter
1IASS=International Agricultural Soil Standards, Source: Alloway (1990)

Results

Before experiment, canal water, treated and untreated wastewater was physically and chemically analyzed. Water analysis showed that EC of untreated wastewater was above the standard limit of international irrigation water quality standards (IWQS) and national environmental quality standards (NEQS) for municipal wastewaters of Pakistan. Untreated wastewater also contained higher biological oxygen demand (BOD), chemical oxygen demand (COD), heavy metals (Cd, Pb, Cu), sodium and nitrogen while treated wastewater and canal water contained all physical and chemical values within permissible limits (Table 2).

Physiological Characteristics of Rosa Species

Photosynthetic rate (μmol m⁻² s⁻¹): Maximum photosynthetic rate was recorded in *R. bourboniana* followed by *R. centifolia* under treated wastewater treatment while minimum in *R. damascena* under canal water treatment. Mean values showed that *R. bourboniana* gained highest photosynthetic rate among *Rosa* species and treated wastewater attained highest value regarding irrigation water treatments in 2012. During 2013, *R. bourboniana* had highest photosynthetic rate followed by *R. Gruss-an-Teplitz* under treated wastewater and minimum of it was found in *R. centifolia* under canal water treatment. Mean values showed that *R. bourboniana* and treated wastewater was at the top regarding *Rosa* species and irrigation water treatment respectively (Table 3).

Transpiration rate (mmol m⁻² s⁻¹): Higher transpiration rate was recorded in *R. damascena* under treated wastewater treatment followed by *R. bourboniana* and *R. centifolia* under same irrigation treatment respectively during 2012 while minimum in *R. damascena* under canal water treatment. Mean values regarding *Rosa* species treatment showed that *R. bourboniana* and *R. centifolia* had highest and lowest transpiration rate, respectively while treated wastewater had maximum of it under irrigation water treatment. During 2013, maximum transpiration rate was recorded in *R. bourboniana* while minimum in *R. centifolia* under canal water treatment. Mean values revealed that treated wastewater showed maximum value and canal water showed minimum regarding irrigation water treatment and *R. bourboniana* attained highest transpiration rate in case of *Rosa* species treatment (Table 3).

Stomatal conductance (mmol m⁻² s⁻¹): *Rosa* Gruss-an-Teplitz had maximum stomatal conductance under treated wastewater followed by *R. centifolia* under same irrigation water treatment while minimum stomatal conductance was recorded in *R. centifolia* under canal water treatment during 2012. Mean values showed that *R. Gruss-an-Teplitz* and treated wastewater treatment showed high values in *Rosa* species and irrigation wastewater treatments respectively. In 2013, high stomatal conductance was recorded in *R. Gruss-an-Teplitz* under treated wastewater treatment, while minimum in *R. centifolia* under canal water treatment. Mean values showed that treated wastewater and *R. Gruss-an-Teplitz* showed maximum values under irrigation water and *Rosa* species treatment respectively (Table 3).

Chlorophyll contents (mg g⁻¹): *Rosa bourboniana* under untreated wastewater contained highest chlorophyll contents while minimum in *R. damascena* under canal water treatment in 2012. Mean values showed that *R. bourboniana* in *Rosa* species with irrigation of canal water contained maximum chlorophyll contents. During 2013, high value was recorded in *R. bourboniana* under treated wastewater treatment followed by *R. Gruss-an-Teplitz* in same irrigation treatment while minimum in *R. damascena* under canal water treatment. Mean values showed similar trend of highest chlorophyll contents in *R. bourboniana* and lowest in *R. damascena* in *Rosa* species and treated wastewater contained maximum value under irrigation water treatments (Table 3).

Anatomical Characteristics of Rosa Species

Cortical cell area (μm²): *Rosa* Gruss-an-Teplitz was dominated in all irrigation water treatments as compared to other *Rosa* species during both years of experiment (Fig. 1). In 2012, maximum cortical cell area was recorded in untreated wastewater, treated wastewater and canal water treatment with values of 851.75 μm², 815.96 μm² and 728.64 μm² respectively. Cortical cell area (μm²) of *R. bourboniana* and *R. centifolia* was in medium range and minimum value was recorded in *R. damascena* (373.05 μm²) in canal water treatment. During 2013, *R. Gruss-an-Teplitz* (721.72 μm², 620.13 μm² and 618.79 μm²) in treated wastewater, canal water and untreated wastewater treatment had maximum values, respectively while *R. damascena* (425.75 μm²) in canal water treatment exhibited minimum cortical cell area.

Epidermal thickness (μm): Results showed that all *Rosa* species showed significant variations but *R. centifolia* was dominant in all irrigation water treatments during 2012
Treatments sharing similar statistical letters are significantly not different from each other

**IIWQS: International Irrigation Water Quality Standards; NEQS: National Environmental Quality Standards for municipal wastewater of Pakistan; †: Standard value of NEQS; ††: Standard value of IIWQS; EC: Electrical conductivity; DO: Dissolved Oxygen; BOD: Biological Oxygen Demand; COD: Chemical Oxygen Demand; TDS: Total Dissolved Solids; SS: Settle able Solids; TSS: Total Suspended Solids**

Table 2: Composition of canal water, treated and untreated wastewater utilized in the experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Canal Water</th>
<th>Treated Water</th>
<th>Untreated Water</th>
<th>IIWQS/NEQS††</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC (μS/L)</td>
<td>1.13</td>
<td>1.44†</td>
<td>2.11†</td>
<td>1.5†</td>
</tr>
<tr>
<td>pH</td>
<td>7.42</td>
<td>7.58†</td>
<td>8.31†</td>
<td>6.92†</td>
</tr>
<tr>
<td>Color</td>
<td>---</td>
<td>Rust Brown</td>
<td>Greyish</td>
<td>---</td>
</tr>
<tr>
<td>Turbidity (mg/L)</td>
<td>43</td>
<td>29.12</td>
<td>155</td>
<td>--</td>
</tr>
<tr>
<td>Hardness (mg/L)</td>
<td>184</td>
<td>416</td>
<td>536</td>
<td>--</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4</td>
<td>2.38</td>
<td>1.36</td>
<td>--</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>---</td>
<td>267</td>
<td>432</td>
<td>300†</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>---</td>
<td>481</td>
<td>669</td>
<td>500†</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>218</td>
<td>1281</td>
<td>1678</td>
<td>2500†</td>
</tr>
<tr>
<td>SS (mg/L)</td>
<td>0.9</td>
<td>0.15</td>
<td>1.1</td>
<td>--</td>
</tr>
<tr>
<td>Total Solids (mg/L)</td>
<td>218</td>
<td>982</td>
<td>1372</td>
<td>--</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>24</td>
<td>63</td>
<td>194</td>
<td>400†</td>
</tr>
<tr>
<td>Chlorides (mg/L)</td>
<td>138</td>
<td>290</td>
<td>436</td>
<td>1000†</td>
</tr>
<tr>
<td>Cadmium (mg/L)</td>
<td>0.001</td>
<td>0.010</td>
<td>0.013</td>
<td>0.01†</td>
</tr>
<tr>
<td>Nickel (mg/L)</td>
<td>0.10</td>
<td>0.08</td>
<td>0.12</td>
<td>0.2†</td>
</tr>
<tr>
<td>Arsenic (mg/L)</td>
<td>ND</td>
<td>0.004</td>
<td>0.005</td>
<td>0.1†</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.18</td>
<td>2.62</td>
<td>3.48</td>
<td>5.0†</td>
</tr>
<tr>
<td>Chromium (mg/L)</td>
<td>30.41</td>
<td>17.61</td>
<td>40.73</td>
<td>--</td>
</tr>
<tr>
<td>Lead (mg/L)</td>
<td>0.021</td>
<td>0.42</td>
<td>0.66</td>
<td>0.5†</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>0.32</td>
<td>3.47</td>
<td>4.82</td>
<td>5.0†</td>
</tr>
<tr>
<td>Cobalt (mg/L)</td>
<td>0.17</td>
<td>0.029</td>
<td>0.079</td>
<td>0.05†</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>0.05</td>
<td>0.13</td>
<td>0.24</td>
<td>0.2†</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>88.46±5.3</td>
<td>83.5±5.1</td>
<td>86.5±5.3</td>
<td>86.5±5.3</td>
</tr>
<tr>
<td>Sodium (mg/L)</td>
<td>76.74±3.7</td>
<td>77.78±3.7</td>
<td>77.71±3.7</td>
<td>77.71±3.7</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>0.39</td>
<td>1.76</td>
<td>2.49</td>
<td>15†</td>
</tr>
<tr>
<td>Phosphorus (mg/L)</td>
<td>4</td>
<td>5.72</td>
<td>8.0</td>
<td>5.0†</td>
</tr>
<tr>
<td>Total Nitrogen (mg/L)</td>
<td>9.44±0.4</td>
<td>9.44±0.4</td>
<td>9.44±0.4</td>
<td>9.44±0.4</td>
</tr>
</tbody>
</table>

Table 3: Physiological characteristics of *Rosa* species under different irrigations

<table>
<thead>
<tr>
<th><em>Rosa</em> species</th>
<th>2012</th>
<th>2013</th>
<th>Photosynthetic rate</th>
<th>Stomatal conductance</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.B.</td>
<td>8.75±0.4</td>
<td>6.85±0.4</td>
<td>6.85±0.4</td>
<td>6.85±0.4</td>
</tr>
<tr>
<td>R.C.</td>
<td>7.02±0.7</td>
<td>5.86±0.6</td>
<td>5.86±0.6</td>
<td>5.86±0.6</td>
</tr>
<tr>
<td>G.T.</td>
<td>8.44±0.3</td>
<td>7.30±0.2</td>
<td>7.30±0.2</td>
<td>7.30±0.2</td>
</tr>
<tr>
<td>R.D.</td>
<td>6.77±0.4</td>
<td>5.64±0.3</td>
<td>5.64±0.3</td>
<td>5.64±0.3</td>
</tr>
<tr>
<td>Average</td>
<td>7.17±0.4</td>
<td>6.04±0.3</td>
<td>6.04±0.3</td>
<td>6.04±0.3</td>
</tr>
</tbody>
</table>

- Treatments sharing similar statistical letters are significantly not different from each other
- CW= Canal water; TW= Treated wastewater; UTW= Untreated wastewater
- RB = *R. bourboniana*; RC = *R. centifolia*; GT = Gruss-an-Teplitz; RD = *R. damascena*
and maximum epidermal thickness was recorded under treated wastewater treatment (359.47 μm) followed by untreated wastewater with value of 309.47 μm and minimum in *R. bourboniana* (100.52 μm) in untreated wastewater treatment. During 2013, *R. centifolia* (334.18 μm) in treated wastewater was at the top followed by same *Rosa* species in canal water and untreated wastewater treatments with values of 332.31 μm and 317.03 μm respectively while minimum epidermal thickness was recorded in *R. bourdoniana* (100.52 μm) in untreated wastewater treatment.

**Midrib thickness (μm):** Results showed that *R. Gruss-an-Teplitz* dominated in all irrigation water treatments than other *Rosa* species (Fig. 1). Maximum midrib thickness during both years was found in *R. Gruss-an-Teplitz* (737.01 μm and 823.61 μm) in untreated wastewater followed by same species (656.08 μm and 702.98 μm) in treated wastewater and *R. centifolia* (570.90 μm and 644.68 μm) in untreated wastewater treatment while minimum value was recorded in *R. bourdoniana* (398.47 μm and 431.63 μm) in canal water treatment. All species during 2013 showed comparatively larger midrib thickness than 2012 in all irrigation treatments.

**Vascular bundle area (μm²):** *Rosa Gruss-an-Teplitz* contained highest vascular bundle area than other species and maximum value was recorded under untreated wastewater (64924.9 μm²) followed by same *Rosa* species in treated wastewater (57953.6 μm²) and canal water (50285.7 μm²) during 2012, while minimum of it in *R. bourdoniana* (15882.6 μm²) in canal water treatment. During 2013, maximum vascular bundle area was recorded in *R. Gruss-an-Teplitz* (60638.8 μm²) in treated wastewater treatment followed by same *Rosa* species (57207.9 μm²) in untreated wastewater treatment while minimum in *R. bourdoniana* (21016.3 μm²) in canal water treatment. Cortical cell area of *R. centifolia* and *R. bourdoniana* was in medium range between *R. Gruss-an-Teplitz* and *R. bourdoniana* during both years (Fig. 2).

**Metaxylem area (μm²):** There was significant variation in metaxylem area in *Rosa* species and *R. bourdoniana* was dominant in all irrigation treatments during both years (Fig. 2). Maximum metaxylem area was recorded in untreated wastewater treatment (435.53 μm² and 379.8 μm²) followed by treated wastewater (399.27 μm² and 366.05 μm²) during 2012 and 2013, respectively while minimum in *R. damascena* (85.80 μm² and 78.44 μm²) for untreated

![Fig. 1: Leaf anatomical characteristics (cortex, epidermis, midrib thickness) of *Rosa* species under different irrigations](image)

Note: RB = *R. bourboniana*; RC = *R. centifolia*; GT = *Gruss-an-Teplitz*; RD = *R. damascena*
wastewater treatment. Metaxylem area of *R. Gruss-an-Teplitz* and *R. centifolia* was in medium range between *R. bourboniana* and *R. damascena* in all irrigation treatments during both years of experiment.

**Phloem area (μm²):** Results showed that *R. damascena* (236.67 μm²) in treated wastewater had maximum phloem area followed by *R. Gruss-an-Teplitz* (225.48 μm²) in same irrigation water treatment during 2012 while minimum value was recorded in *R. bourboniana* (109.62 μm²) in untreated wastewater. During 2013, *R. Gruss-an-Teplitz* (238.44 μm²) in treated wastewater obtained highest phloem area followed by *R. damascena* in treated wastewater with value of 223.47 μm² while minimum phloem area was recorded in *R. bourboniana* (129.84 μm²) in untreated wastewater treatment (Fig. 3).

**Palisade cell area (μm²):** Results showed that during 2012, *R. damascena* (495.41 μm²) in treated wastewater obtained maximum palisade cell area followed by *R. centifolia* (409.44 μm²) and *R. damascena* (406.08 μm²) in treated wastewater and canal water treatment respectively while minimum of it was found in *R. bourboniana* (136.11 μm²) for untreated wastewater. During 2013, *R. damascena* (492.2 μm²) in treated wastewater treatment again showed maximum palisade cell area while minimum value was recorded in *R. bourboniana* (139.68 μm²) in untreated wastewater (Fig. 3).

**Spongy cell area (μm²):** Results showed that *R. Gruss-an-Teplitz* was at the top during both years as compared to other *Rosa* species in all irrigation waters. Maximum spongy cell area was recorded in untreated wastewater (459.44 μm²) followed by treated wastewater and canal water treatment with values of 386.8 μm² and 341.89 μm² respectively while minimum of it in *R. bourboniana* (119.44 μm²) under canal water treatment. During 2013, *R. Gruss-an-Teplitz* in treated wastewater (385.92 μm²) had maximum spongy cell area followed by same species (355.68 μm²) in canal water while minimum value was recorded in leaves of *R. bourboniana* (153.68 μm²) in canal water (Fig. 3).

**Lamina thickness (μm):** Data showed that *R. damascena* (193.28 μm) in treated wastewater was at the top followed by same *Rosa* species in canal water and untreated wastewater with leaf thickness values of 177.31 μm and 171.01 μm respectively during 2012, while minimum of it was found in *R. bourboniana* (104.16 μm) in untreated wastewater. Results during 2013 illustrated that

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**Fig. 2:** Leaf anatomical characteristics (vascular bundle, xylem, phloem) of *Rosa* species under different irrigations

Note: RB= *R. bourboniana*; RC= *R. centifolia*; GT= Gruss-an-Teplitz; RD= *R. damascena*
R. damascena (198.56 μm) in treated wastewater was at the top followed by same species (191.42 μm) and R. centifolia (183.00 μm) in untreated wastewater and treated wastewater treatment respectively whereas minimum of it was recorded in R. bourboniana (125.88 μm) under canal water. All species during 2013 showed comparatively higher lamina thickness than 2012 (Fig. 3). Transverse sections of leaf anatomical characteristics of fragrant Rosa species under canal water, treated and untreated wastewaters were presented in Fig. 4.

**Discussion**

Water used in this experiment was basic in nature as its pH was more than 7 and EC of untreated wastewater was more than standard values set by IIWQS and NEQS for municipal wastewaters of Pakistan while all other minerals and chemicals in treated wastewater and canal water treatment were in permissible range. Untreated wastewater contained high concentration of some minerals and toxic heavy metals (Cd, Pb, Co, Cu) and for this reason its BOD and COD were high (Kakar et al., 2011). Plants were silent sufferers and their response against untreated wastewater reduces growth and physiological disturbance as compared to canal water and treated wastewater treatments.

All physiological parameters considered in this experiment increased under treated wastewater treatment due to optimum concentration of nutrients in treated water as compared to canal water and untreated wastewater treatment. Due to higher concentrations of EC, BOD, COD, Pb, Cd, Co and Na in untreated wastewater, photosynthetic rate, transpiration rate and stomatal conductance were reduced in all Rosa species during both years while chlorophyll contents slightly increased in R. bourboniana and R. Gruss-an-Teplitz during second year of experiment. Cornic (2000) and Flexas and Medrano (2002) reported that due to chemical stress, stomatal closure takes place which limits photosynthesis. Stomatal limitation reduces photosynthetic rate which ultimately results in inhibition of metabolism in plants (Cornic, 2000). Copper and nickel are essential metals for higher plants particularly for physiological processes i.e., photosynthesis (Chatterjee et al., 2006; Mahmood and Islam, 2006) and its toxicity leads to distance in metabolic pathways and damage to
macromolecules (Neelima and Reddy, 2002; Singh and Tiwari, 2003; Demirevska-kapova et al., 2004). Kannaiyan (2001) and Saravanamoorthy and Kumari (2007) reported that optimum mineral nutrient status resulted in higher concentrations of chlorophyll in plants and elevated metals and salts concentration in irrigation water reduces the chlorophyll contents in *Rosa* species (Niu et al., 2008).

Photosynthesis inhibition in landscape plants was also reported by Azza et al. (2007) and Munns and Tester (2008) due to high salt and metal concentration. Singh and Agrawal (2010) found that in *Beta vulgaris*, photosynthetic rate and stomatal conductance were higher in wastewater irrigated sites as compared to ground water irrigated ones and concluded that these positive response of physiological characteristics of the plants at wastewater irrigated site suggest that the concentration of toxic metals may not be up to the extent causing adverse effects on photosynthetic apparatus. Khaleel et al. (2013) confirmed the results of this experiment and argued that treated wastewater treatment elevated the chlorophyll contents in *Abelmoschus esculentus*. Enhancement of chlorophyll could be due to enhanced nutrient uptake, synthesis and translocation probably facilitated by optimum availability of some of the beneficiary plant nutrients and also due to reduction in phenol compounds due to the dilution effect. Results of this study are also similar with the findings of Kakar et al. (2010) who argued that optimum chemical concentration in irrigation water increased stomatal conductance, transpiration rate and photosynthetic rate while untreated wastewater with high load of contamination strongly reduced the physiological parameters.

Large cortical cell area, epidermal thickness, midrib thickness, vascular bundle area, metaxylem area and spongy cell area in leaves increased in all *Rosa* species as
contamination increased in irrigation waters and R. Gruss-an-Teplitz produced highest cortical cell area as compared to other species. It could be due to the reason that most of salts and heavy metals in polluted waters were in permissible range to apply for irrigation to plants. Aldesuquy (2014) reported that wastewater increased vascular bundle area and xylem area in leaves of wheat plants. Tyagi et al. (2013) reported that anatomical characteristics were reduced as pollution increased in irrigation water treatments. Nawaz et al. (2011) showed similar results and argued that R. Gruss-an-Teplitz had larger cortical cell area, thick midrib, larger vascular bundles and metaxylem area as compared to other Rosa species. Plants shows gradual changes in leaf structure with an upturn in heavy metals concentration (Maruthi et al., 2005; Shanker et al., 2005; Valerie et al., 2006). Optimum concentration of nutrients and heavy metals delayed the entry of metals into the roots by stimulating a lignified exodermis and thereby subsidized to a higher metal tolerance (Cheng et al., 2012).

Conclusion

From this study, it can be concluded that there were substantial variations among Rosa species regarding response to wastewater irrigations. Treated wastewater regarding irrigation water and R. Gruss-an-Teplitz among Rosa species was dominant treatment and plants grown under treated wastewater produced highest values of physiological characteristics while leaf structural features of Rosa species showed diversity in their response to treated and untreated wastewater irrigation treatments.

References


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