

PHYSIOLOGICAL AND ANATOMICAL ADAPTATIONS INDUCED BY FLOODING IN *COTULA CORONOPIFOLIA*

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Cotula coronopifolia is a wild annual Asteraceae that grows in periodically-flooded prone environments and seems highly tolerant to periodic flooding. Seedlings of about 15 cm were collected directly from the edge of Soliman sabkha (N-E Tunisia, semi-arid stage) and grown under greenhouse conditions. Two treatments were considered: drainage and flooding. After 56 days of treatment, flooded plants showed a pronounced growth increase. This performance was essentially associated with significant increment in biomass production of both shoots and roots (about 220% of the control). The appropriate response to flooding was also characterized by the ability of the species to maintain its water status under such conditions. Neither water content nor water potential showed a significant variation as compared to those of non-flooded plants. However, transpiration rate decreased slightly but significantly in flooded plants (from 0.86 to 0.64 mmol H₂O m⁻² s⁻¹). Na⁺ and K⁺ concentrations were practically maintained under waterlogging conditions, except a significant increase of Na⁺ content in roots of flooded plants (157% of the control). These responses were concomitant with maintenance of photosynthetic rate. However, the contents of chlorophylls *a* and *b* increased to 167% and 295%, respectively. It seems that the enhancement in these photosynthetic pigments together with a significant improvement in water use efficiency (from 4.66 to 6.07 mmol CO₂ mol⁻¹ H₂O) allowed to the species to compensate the decrease in photosynthetic rate. At the anatomical level, this species responded to flooding by a significant development of its root aerenchyma (+63%) and an increase in the lignification of its stem xylem tissues (+37%). Based on the presented data, the plant fitness under flooding conditions was a result of dynamic readjustment of several morphological, physiological, and anatomical adaptive traits. Flood requirement together with salt tolerance are responsible for the predominance of *C. coronopifolia* in a large area in its natural biotope where most plants cannot tolerate interactive effects of flooding and salinity.

Keywords: Aerenchyma – *Cotula coronopifolia* – photosynthesis – waterlogging – water relations

INTRODUCTION

One of the most essential environmental factors for plants is water availability. Often land plants experience two extreme situations of water stress, drought or flooding [13]. In natural wetlands, seasonal fluctuations in water level are commonly registered and the tolerance of plants to these changes is the key factor for community

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composition and wetland species distribution [31, 32]. Water excess alters soil structure [14] and results in oxygen shortage as a consequence of the slow gas diffusion in water and oxygen consumption by microorganisms and plant roots [7]. The soil quickly becomes devoid of oxygen at depths below few millimeters. Indeed, in the floodwater, a broad unstirred boundary layer is formed around respiring tissues. This formation can lead to a tissue oxygen deficiency within few hours. Moreover, waterlogging leads to other changes in the soil that can influence plants; levels of ethylene [10, 24] and products of anaerobic metabolism by soil microorganisms (e.g. Mn^{2+} , Fe^{2+} , S^{2-} , H_2S and carboxylic acids) can accumulate [9]. Plant response to hypoxia associated with such kind of soils includes complex interactions between biochemical and genetic programs that involve differential expressions of a large number of genes [33]. However, waterlogging has a range of effects on plants; it can decrease growth, affect processes associated with solute transport across membranes such as inorganic nutrient uptake [1, 27], and cause a stomata closure and consequently a decline in photosynthesis. Many factors cumulate and determine the nature of flooding-induced responses and permit the classification of plants into tolerant and sensitive species. Some have evolved a wide range of specific responses that appear to help avoiding the effect of this stress. Two processes are critically associated with a plant species ability to tolerate flooding: internal oxygen transport to submerged organs to avoid anoxia and maintenance of root function to prevent inhibition of water and nutrient intake [8]. In periodically-flooded habitats, various adaptive responses allow tolerant plant species to perform these tasks, thus they not only can survive waterlogging periods [8], but also can take competitive advantage over flooding-sensitive neighbors [8, 30]. As oxygen concentrations decrease in flooded soils, internal oxygen supply to roots becomes critically important to plants [8, 15]. Several anatomical responses facilitate internal transport of oxygen by diffusion or sometimes by mass flow to allow submerged organs to avoid developing oxygen shortage, mainly the formation of gas spaces or aerenchyma. Several authors consider the development of aerenchyma as the most important trait for a plant to tolerate soil anoxia during extended flooding [3, 8].

Cotula coronopifolia (Asteraceae) is a non-woody wetland plant with yellow flowers and succulent leaves that occupies the edges of salt marshes on wet, muddy and saline soils. It is native to South Africa but has spread through the coasts of Europe, South and North America, New Zealand, Australia and Tasmania [4]. In Tunisia, *C. coronopifolia* is an annual, small (5 to 20 cm tall) plant spread on the borders of some salt marshes. On the edges of Soliman sabkha (N-E Tunisia, semi-arid stage), *C. coronopifolia* location seems to follow soil moisture. In this area, we found highly productive stands that dominate other species. Flooding was to or above the soil surface with temporal and spatial changes. Nevertheless, the soil was completely saturated during flooding episode. In rainy years, the population of *C. coronopifolia* establishes further than in dry years (personal observations). Hence, this species would show specific adaptations that allow it to survive flooding conditions. Moreover, *C. coronopifolia* is considered as an invasive halophyte that dominates plant communities and can form new phytosociological associations with a character-

istic species composition [4]. The aim of the present work was then to investigate whether *C. coronopifolia* occurrence in flooded ecosystems is due to a flood requirement for a better biomass production.

MATERIALS AND METHODS

Plant material and culture conditions

In winter 2008, *C. coronopifolia* seedlings of about 15 cm were collected from the flooded edges of Soliman sabkha. Seedlings were immediately transplanted into individual 5-liter plastic pots filled with sand and irrigated with diluted seawater (10%). The 21 pots were used 8 of which were not perforated. During the first week of the experiment (pretreatment period), the 8 non-perforated pots were irrigated with no water excess (with no flooding). After this period of pretreatment two treatments were started: 1) drainage with 10% seawater (non-flooded plants) and 2) waterlogging with 10% seawater (flooded plants).

Drained plants were watered to excess every 4 days and superfluous water allowed drainage through numerous holes at the bottom of each pot. Flooding was achieved by the use of pots without draining holes at the bottom and the maintenance of seawater level at 3 cm above the sand surface. For flooded pots, water losses through evapo-transpiration were constantly compensated by adding 10% seawater to maintain its level constant (at 3 cm above the sand surface). Eight replicate pots were used for each treatment. Before starting treatments, an initial harvest was made for 5 seedlings for shoot and root biomass determination and mineral analysis. The remaining plants were grown under greenhouse conditions. On the 56th day of treatment, gas exchange and water relations were measured. Then, plants were harvested and separated into shoots and roots for biomass and ion composition analyses. In addition, subsamples of roots and shoots were excised from each replicate for anatomical observations.

Ion analysis

After washing and fresh weight determination, shoot and root samples were oven-dried for 8 days at 60 °C then weighed, ground, and stored until analysis. For each sample, Na⁺ and K⁺ contents were determined by a Corning Flame photometer after digestion of the powder in HNO₃ (0.5%) solution.

Water potential and gas exchange measurements

Stem water potential was measured at the end of the treatment period using a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA) according to Scholander et al. [23]. Changes in photosynthesis rate and stomatal conductance were

determined by a portable gas-exchange system (Li-Cor 6200, Li-Cor Lincoln, NE). Measurements were performed on fully-expanded leaves ($n=5$ per treatment) between 10:30 am and midday under $1132 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

Chlorophyll contents

For each treatment, three leaves from different plants were collected for chlorophyll content determination. One hundred milligrams from each sample were incubated in 5 ml 80% acetone in darkness at 4°C over three days. Then, the absorbance of the extract was measured at 649 and 665 nm. Chlorophylls *a* and *b* contents were calculated using the equation of Grusak [6].

Anatomical analysis

Root and stem free-hand transverse sections were performed for drained and flooded plants. Subsamples were separately fixed in 70% ethanol and stained with carmine-green iodize dye. After that, they were observed with an Olympus BX 41 light microscope and photographed by an Olympus C4000 camera.

Measurement of morphometric parameters

Percentages of gas spaces and xylem lignification in stem and root sections were determined by Mesurim 2 software available at <http://pedagogie.ac-amiens.fr/svt/info/logiciels/Mesurim2/Telecharge.htm>.

Calculated parameters

Relative growth rate (RGR) was calculated according to the following equation:

$$\text{RGR} = (\ln \text{DW}_2 - \ln \text{DW}_1) / (t_2 - t_1),$$

with DW = total plant dry weight (mg), t = time (d), and the subscripts 1 and 2 = initial and final harvests.

Potassium absorption efficiency (KAE) was determined as the ratio of the whole K^+ quantity accumulated in each plant during the treatment period to its root dry weight.

Potassium use efficiency (KUE) was calculated as the ratio of shoot biomass production in each plant during the treatment period to the quantity of K^+ accumulated in these organs during the same period.

Table 1
Growth parameters in *C. coronopifolia* plants grown over 56 days under flooded and unflooded (drained) conditions

Treatments	Unflooded	Flooded
Total shoot length (m)	0.26 ± 0.02 ^a	0.31 ± 0.03 ^a
Total root length (m)	0.37 ± 0.04 ^a	0.20 ± 0.04 ^b
Specific root length (m g ⁻¹ DW)	0.21 ± 0.06 ^a	0.05 ± 0.01 ^b
Shoot dry weight (g)	3.77 ± 0.53 ^a	8.19 ± 1.46 ^b
Root dry weight (g)	1.80 ± 0.28 ^a	3.97 ± 0.41 ^b
RGR of the whole plant (mg g ⁻¹ day ⁻¹)	48.96 ± 6.87 ^a	63.06 ± 5.77 ^b
Root/shoot ratio	0.49 ± 0.08 ^a	0.50 ± 0.08 ^a

Values are means of 5 replicates ± Standard error. For each line, means with different letters are significantly different at $P \leq 0.05$.

Table 2
Water potential, transpiration rate, water use efficiency, and shoot and root water contents of *C. coronopifolia* plants grown over 56 days under unflooded (drained) and flooded conditions

Treatments	Unflooded	Flooded
Shoot water content (ml g ⁻¹)	5.80 ± 0.49 ^a	5.49 ± 0.14 ^a
Root water content (ml g ⁻¹)	9.80 ± 0.19 ^a	11.33 ± 1.33 ^a
Water potential (MPa)	-12.17 ± 0.65 ^a	-12.33 ± 0.65 ^a
Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)	0.86 ± 0.10 ^a	0.64 ± 0.11 ^a
Water use efficiency: WUE (mmol CO ₂ mol ⁻¹ H ₂ O)	4.66 ± 0.45 ^a	6.07 ± 0.68 ^b

Values are means of 5 replicates ± Standard error. For each line, means with different letters are significantly different at $P \leq 0.05$.

Table 3
Shoot and root Na⁺ and K⁺ concentrations and potassium absorption (KAE) and use efficiencies (KUE) in *C. coronopifolia* plants grown over 56 days under unflooded (drained) and flooded conditions

Treatments	Unflooded	Flooded
Shoot Na ⁺ content (mmol g ⁻¹)	2.41 ± 0.12 ^a	2.75 ± 0.46 ^a
Root Na ⁺ content (mmol g ⁻¹)	2.62 ± 0.34 ^a	4.11 ± 0.67 ^b
Shoot K ⁺ content (mmol g ⁻¹)	0.54 ± 0.04 ^a	0.62 ± 0.09 ^a
Root K ⁺ content (mmol g ⁻¹)	0.58 ± 0.10 ^a	0.58 ± 0.12 ^a
KAE (mg mg ⁻¹ root DW)	4.74 ± 0.54 ^a	6.64 ± 1.01 ^b
KUE (g DW g ⁻¹ K)	2.18 ± 0.23 ^a	1.79 ± 0.24 ^a

Values are means of 5 replicates ± Standard Error. For each line, means with different letters are significantly different at $P \leq 0.05$

RESULTS

Morphological adaptations and growth responses

At the end of the treatment period, flooded plants not only survived the 56-day period of constant flooding with no damage symptoms, but also showed much higher growth levels than the control. Flooding significantly increased shoot and root dry weights by about 120% as compared to the control, keeping constant root/shoot ratio at almost 0.5 (Table 1). Hence, whole plant RGR was significantly improved under waterlogging conditions, reaching 129% of the control. Roots of flooded plants were noticeably thicker and shorter than those of unflooded plants showing a decrease of 46% in their total length. Thus, specific root length (i.e. total root length/root dry weight) decreased by 76.2% under flooding. By contrast, waterlogging enhanced total shoot length by 19.2%, but difference with the control was not statistically significant. These traits seem to be presumably adaptive responses to waterlogging in *C. coronopifolia*.

Water relation adaptations

Flood-treated plants exhibited no significant variations in shoot and root water contents as well as in water potential and transpiration rate (Table 2). On the contrary, they showed a significant increase in their water use efficiency (WUE) from 4.66 to 6.07 mmol CO₂ mol⁻¹ H₂O (Table 2).

Nutritional adaptations

Flooding did not significantly alter Na⁺ concentration in shoots, whereas it significantly increased it in roots (Table 3). Indeed, Na⁺ root concentration was at least 1.5 times higher in flooded than in unflooded plants. K⁺ tissue concentration did not significantly vary with treatment. This was probably due to a significant increase (+40%) in potassium absorption efficiency (KAE). By contrast, potassium use efficiency (KUE) experienced a decline of 18%, but difference with the control was not statistically significant (Table 3).

Photosynthetic adaptations

Photosynthesis performance was not significantly affected by flooding (Table 4). Photosynthetic rate (P_N) was 3.90 and 3.43 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively in unflooded and flooded plants. Nevertheless, waterlogging resulted in a significant enhancement in chlorophyll concentrations (from 0.45 to 0.75 mg g⁻¹ FW in the case of chlorophyll *a* and from 0.19 to 0.56 mg g⁻¹ FW in the case of chlorophyll *b*).

Table 4
Net CO₂ assimilation (P_N), stomatal conductance (g_s) and chlorophyll *a* and *b* concentrations of *C. coronopifolia* plants grown over 56 days under unflooded (drained) and flooded conditions

Treatments	Unflooded	Flooded
P_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	3.90 ± 0.29^a	3.43 ± 0.31^a
Chlorophyll <i>a</i> content (mg g^{-1} FW)	0.45 ± 0.01^a	0.75 ± 0.15^b
Chlorophyll <i>b</i> content (mg g^{-1} FW)	0.19 ± 0.01^a	0.56 ± 0.06^b

Values are means of 5 replicates \pm Standard error. For each line, means with different letters are significantly different at $P \leq 0.05$.

Anatomical adaptations

Stem cross-sections of both unflooded and flooded plants (Fig. 2A and B) exhibited a relatively-small vascular cylinder and a well-developed cortex. This aspect is unusually observed in stems of land plants. The cortical parenchyma is occupied by a well-developed aerenchyma. Small gas spaces were distributed near the epidermis (Fig. 1A and B), while large ones were localized in the middle part of the cortical parenchyma. Gas spaces were bordered by intact cell walls. This suggests a genetic origin of this aerenchyma. Conducting tissues were relatively thin reminding the stem

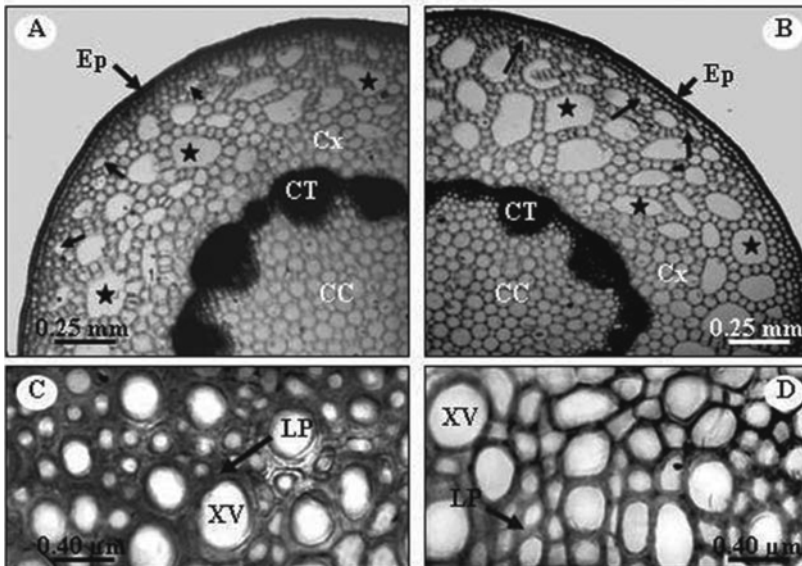


Fig. 1. Stem transverse sections in drained (A) and flooded (B) plants of *C. coronopifolia*. C and D illustrate their respective details of xylem tissues. Large and small gas spaces are shown in the micrographs by stars and arrows, respectively. VC: vascular cylinder, CC: Central cylinder, CT: conductive tissues, Cx: cortex, Ep: epidermis, LP: lignified parenchyma, XV: xylem vessel

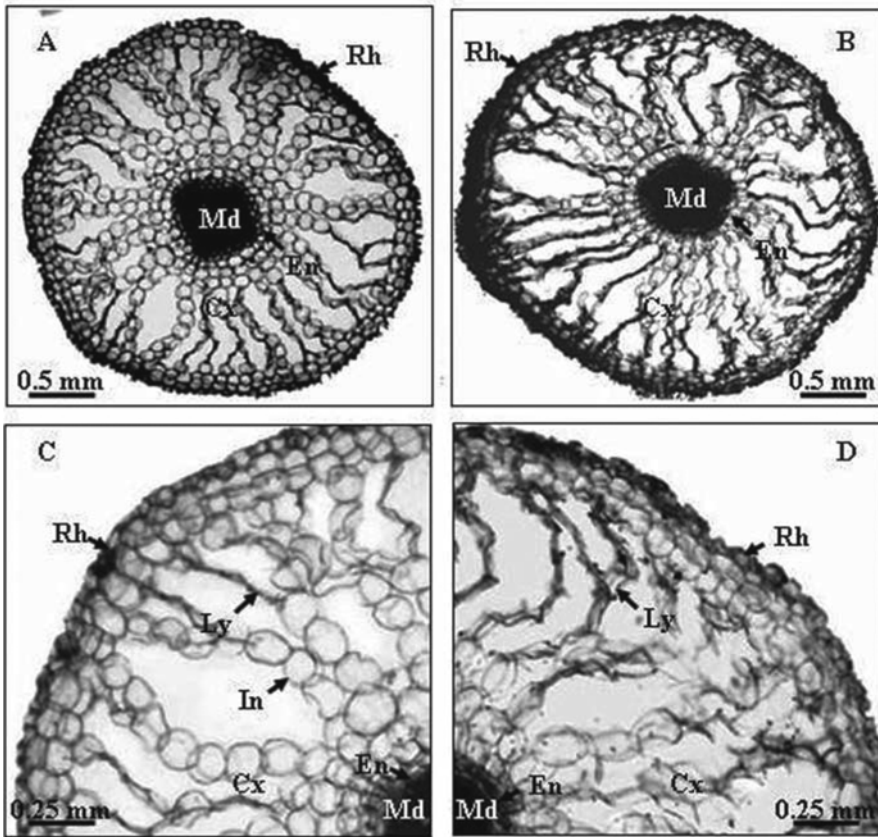


Fig. 2. Root transverse sections in drained (A and C) and flooded (B and D) plants of *C. coronopifolia*. Notice the fewer lacunas in the cortical area of drained plants comparing to those of submerged ones. Cx: cortex, En: endodermis, LP: lignified parenchyma, In: intact cells, Ly: Lysed cells, Md: medulla, VC: vascular cylinder, Rh: rhizodermis

Table 5

Percentages of gas spaces and lignification of xylem tissue in stem and root sections of *C. coronopifolia* plants grown over 56 days under unflooded (drained) and flooded conditions

Treatments	Unflooded	Flooded
Stem gas spaces (%)	14.8 ± 1.5 ^a	16.5 ± 1.2 ^a
Root gas spaces (%)	14.2 ± 1.8 ^a	23.2 ± 2.1 ^b
Stem xylem lignification (%)	34.6 ± 1.8 ^a	47.3 ± 1.5 ^b
Root xylem lignification (%)	39.2 ± 2.2 ^a	31.5 ± 0.9 ^b

Values are means of 3 replicates ± Standard error. For each line, means with different letters are significantly different at $P \leq 0.05$.

structure in herbaceous plants. In submerged plants, wood seems more developed than in drained plants. Lignification of secondary xylem was more developed in flooded (47.3% of stem section area) than in unflooded plants (34.6%) with large vessels and less-thick walls (Fig. 1C and D; Table 5). Leaf transverse sections showed no apparent differences between flooded and unflooded plants (non-shown data).

Transverse sections of roots of unflooded plants showed less aerenchyma (14.2% of total area section) in the cortex than those of flooded plants (23.2%; Table 1). Some of these gas spaces were bordered by broken cells of the root parenchyma (Fig. 2C and D). The gas spaces of the non-submerged roots were separated from the endodermis by two layers of parenchyma cells, whereas those of submerged roots reached the endodermis. Some of these lacunas were bordered by parenchyma broken cell. This confirms the lysigenous origin of these gas spaces. In the vascular cylinder of flooded roots (Table 5), lignification of conductive tissues was less important than in those of unflooded roots (39.2 and 31.5% of xylem total area, respectively).

DISCUSSION

The present study showed *C. coronopifolia* as an obligate wetland plant since it experienced a significant increase (+29%) in its relative growth rate under waterlogging conditions, which indicates that flooding enhanced whole plant carbon balance [18]. This results from a combination of life-history traits [2] and by means of physiological and anatomical adaptations allowing avoidance of oxygen-deficiency by internal aeration [12]. In addition, roots were thicker and shorter under flooding conditions. Indeed, roots of many wetland species showed a tendency to be shallow and limited to the upper soil horizon [16]. The high capacity of *C. coronopifolia* to tolerate such a constant flooding can be related to several factors.

First, the presence of aerenchyma in the massive roots certainly facilitates internal aeration. Videmsk et al. [28] reported that the aerenchyma can develop either as part of the normal development of the plant or in response to a stress by two mechanisms: schizogeny and lysigeny. Schizogenous gas-spaces result from cell separation during tissue development, while lysigenous spaces are due to cell lysis. Lysigenous aerenchyma can be formed in both mature and newly-developing roots, whereas older roots lose the capacity of gas-space formation [24, 28]. Aerenchyma can be formed over virtually the entire body of a plant. Nevertheless, its site formation depends on the species and environmental conditions, with the exception of meristems, vascular bundles, sclerenchyma, and covering tissues [21, 29]. Several authors showed that ethylene synthesis by roots growing under oxygen deficiency is strongly promoted by hypoxia. Ethylene had been widely reported to influence root morphology and growth and to trigger aerenchyma formation. This tissue type furnishes plants with an interconnected network of intercellular gas-filled spaces that permits a relatively unhindered internal diffusion or mass flow of oxygen and other gases down concentration or pressure gradients between submerged and emerged parts [9]. This system allows the transport of the needed oxygen to the submerged roots which enables them to

maintain aerobic respiration and to oxidize reducing compounds in the rhizosphere [20, 28]. In addition to the enhancement of internal aeration, lysigenous air spaces, found in the root cortex of *C. coronopifolia* in our study, are also thought to reduce the number of oxygen consuming cells [22, 28].

Second, the maintenance of a non-affected photosynthetic rate can ensure a good biomass production. This is a characteristic of some tolerant species that have developed adaptive mechanisms to maintain high photosynthetic capacity [5, 26]. However little is known about the involved response mechanisms [20]. In our study, the maintenance of the photosynthetic rate seems to be due to a significant increase in chlorophyll concentrations and WUE. Ye et al. [33] noticed also that a 12-week waterlogging treatment significantly improved chlorophyll *a* and *b* concentrations in the mangrove *Bruguiera gymnorhiza*. According to Matsumoto et al. [17], chlorophyll concentration is a very effective parameter expressing seasonal variability in stomata behavior. Indeed, a reduction in their concentration induces a hyperbolic decrease in stomatal conductance [17].

Third, the maintenance of an adequate nutrient status such as potassium uptake and management could be responsible for both growth and photosynthesis responses to waterlogging in *C. coronopifolia*. The absence of significant differences in shoot and root K^+ concentrations between flooded and unflooded plants suggests that K^+ uptake systems were not altered by waterlogging. Indeed, a significant increase in KAE was noticed in flooded plants. This physiological response ensured a sufficient supply of these plants with potassium. Hence, it was no need to improve KUE for a better management of the absorbed potassium quantity. Jackson and Drew [11] noticed that root aerenchyma may increase nutrient absorption. In addition, Kozłowski [14] reported that flood-tolerant species often absorb more mineral nutrients in response to soil inundation than unflooded well-watered plants. Several adaptations had been suggested to be involved in the high nutrient absorption efficiency of flood-tolerant plants such as the production of hypertrophied lenticels, aerenchyma tissue, and adventitious roots [14].

Finally, the conservation of adequate water status could be responsible for the maintenance of the previously-described physiological functions (growth, nutrient uptake and photosynthesis). Indeed, factors associated with flooding may influence plant water relations, and water intake under such conditions can be slower than under aerated conditions [20]. Flooded plants of *C. coronopifolia* showed a quite similar pattern of water content and potential as the control. However, many authors noted that the maintenance of an adequate water status may not necessarily reflect sustained root conductivity but is likely due to stomatal closure and low transpiration rates. Thus, a slow water absorption rate would sufficiently compensate transpired water since many wetland species close stomata as initial response to soil flooding [20]. But, exceptions have been noticed depending on species, treatment period, and soil reduction intensity [20]. Based on our findings, the maintenance of a relatively constant transpiration rate together with the improvement of WUE in flooded plants of *C. coronopifolia* could explain their healthy water status.

CONCLUSIONS

Our study showed that *C. coronopifolia* is an obligate wetland plant since it grew much better under flooding than under drainage conditions, which was related to its ability to avoid the flooding-induced oxygen deficiency stress. This was essentially achieved by a development of massive aerenchymatous roots, maintenance of photosynthetic activity and nutrient supply, and a regulation of water status during flooding period. It seems that the main plant strategy to survive extended flooding is an internal oxygen transport to submerged roots to maintain aerobic respiration in their tissues. This flood requirement can explain why the distribution of this species follows wet sites. In addition, *C. coronopifolia* plants were found on the borders of salt marshes where salinity can also constitute another abiotic stress that limits the occurrence of the majority of plant species.

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