

Effects of cattail intercropping with maize under different phosphorus concentrations and waterlogging

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Abstract

This work evaluated maize anatomy and physiology, under waterlogging, interacting or not with *Typha domingensis* at different phosphorus concentrations. Plants were transplanted to pots containing vermiculite waterlogged by a nutrient solution of different phosphorus concentration (0, 12.4, 24.8 mg P L⁻¹) interacting or alone. The gas exchange parameters were analysed, leaves and roots collected and prepared accordingly to the usual methods of plant microtechnique. The slides were mounted and prepared for evaluation in an image analysis software. The experimental design was completely randomized in a factorial 3 x 2, and data were submitted to analysis of variance by Scott-Knott test for 5% probability. The presence of *T. domingensis* decreased root aerenchyma proportion and increased the gas exchange parameters, stomatal size and root apoplastic barriers under 24.8 mg P L⁻¹. The intercropping is favourable under 24.8 mg P L⁻¹.

Keywords: Apoplastic Barriers, IRGA, Photosynthesis, Root Aerenchyma, *Typha domingensis*, *Zea mays*.

Efeitos do consrcio entre taboa e milho sob encharcamento e diferentes concentraes de fsforo

Resumo

Este trabalho avaliou a anatomia e fisiologia do milho sob encharcamento, interagindo ou no com a *Typha domingensis* em diferentes nveis de fsforo. As plantas foram transplantadas

para bandejas contendo vermiculita encharcada por solução nutritiva modificada em diferentes concentrações (0, 12.4, 24.8 mg P L⁻¹), com interação ou não. Avaliou-se as trocas gasosas, folhas e raízes foram coletadas e preparadas de acordo com os métodos usuais de microtécnica vegetal, as lâminas montadas e preparadas para avaliação em software de análise de imagens. O delineamento foi inteiramente casualizado em fatorial 3 x 2, e dados foram avaliados por meio da análise de variância com teste de Scott-Knott a 5% de significância. A *Typha domingensis* diminuiu a proporção de aerênquima radicular e aumentou os parâmetros de trocas gasosas, tamanho estomático e barreiras apoplásticas radiculares a 24.8 mg P L⁻¹. O consórcio é benéfico para o milho sob 24.8 mg P L⁻¹.

Palavras-chave: Aerênquima radicular, Fotossíntese, IRGA, Barreiras apoplásticas, Encharcamento, *Typha domingensis*, *Zea mays*.

Introduction

Although maize (*Zea mays* L.) is very susceptible to anoxia, there are some waterlogging tolerant genotypes such as the BRS-4154 'Saracura' which can survive under intermittent waterlogging conditions. Likewise, the waterlogging tolerant maize is relevant since all crop areas on the planet suffer periods of flooding, with approximately 15% of Southeast Asia and 8.5 million ha in India specifically suffering with low oxygen conditions (Souza et al., 2011). Maize tolerance to waterlogging is related to increased aerenchyma development in the roots, thickening of root epidermis and endodermis as well as reduced cortex thickness (Pereira et al. 2010; Souza et al. 2009).

However, wetland agriculture can suffer losses by the proliferation of invasive plants with higher growth rates and competition capacity. The presence of weeds in sites used for cultivation is one of the major reasons for the decline in wetland productivity (Dai et al. 2014). Although Kloss et al (2021) reports that *T. domingensis* benefit maize growth under waterlogging by increasing dissolved oxygen, the competition between macrophyte species (aquatic higher plants) is influenced by many factors such as dissolved oxygen, eutrophication, and water level which promotes spatial segregation (Cunha et al. 2012). Therefore, as crops can overcome competition with macrophytes it may depend on unknown environmental variables, resulting in unclear results from previous literature.

Aquatic macrophytes are of significant importance to wetlands because they are primary producers, participate in cycling and storage of nutrients, act on the control of the pollution and hypertrophication (O'Brien et al. 2014). These plants also serve as food and shelter for animals. In addition, the study of aquatic environments is of essential relevance for their conservation and management (Cervi et al. 2009). *Typha domingensis* Pers. is a

cosmopolitan aquatic emergent macrophyte belonging to the Typhaceae family. Its underground part is constituted by roots and a horizontal rhizome, and the shoot is comprised only by leaves and reproductive structures (Correa et al. 2023). Studies show that *T. domingensis* when competing with other species is not affected by low nutrient concentration being treated as a highly competitive species (Correa et al. 2017).

The management of *T. domingensis* depends on the water level, nutrient availability, and disturbances in the environment, either for propagation or weed control. The limitation of phosphorus is limiting to *T. domingensis* spread while eutrophication is accompanied by dense stands (Surratt, Shinde & Aumen, 2012). In addition, maize is a crop with high phosphorus demand. Thus, limiting P content to constrain *Typha spp.* growth may also limit maize productivity. Kloss et al. (2021) shows that *T. domingensis* benefits maize under waterlogging, however this benefit was not verified in a condition of nutrient restriction, therefore the study about maize under P restriction and waterlogging while intercropped with *T. domingensis* shall better elucidate this relationship.

Despite the literature showing *T. domingensis* higher competitive capacity with other macrophyte species, few studies exist concerning its interaction with crops. In addition, the competitiveness may be related to increased phosphorus level which enhances *T. domingensis* growth. Thus, the objective of this work was to analyse the intercropping between maize and *T. domingensis* under different concentrations of phosphorus and waterlogging to better understand how these conditions affect maize gas exchange, root, and leaf anatomy.

Materials and Methods

The seeds, from the flooding tolerant maize genotype BRS4154, were obtained from the National Maize and Sorghum Research Center, located in Sete Lagoas, MG, Brazil. The seeds were germinated in plastic trays with 2.8 L of vermiculite and 1.6 L of a nutrient solution at 40% of its ionic strength (Hoagland & Arnon 1950). Seeds were kept in a germination chamber at 22 °C and constant light for one week. Further, seedlings were transferred to plastic pots in a greenhouse at the Federal University of Lavras.

The *T. domingensis* plants were collected from the natural populations in wetlands located at the Federal University of Lavras containing rhizomes with about 25 cm in length and 3 cm in diameter and approximately ten leaves (1.5 m tall). The *T. domingensis* plants were treated with sodium hypochlorite 50% [commercial NaClO solution and distilled water ($v v^{-1}$)] with a final concentration of 3% ($v v^{-1}$)] for 10 min and then washed in tap water before further cultivation in the greenhouse (Correa et al., 2017). The plants were grown in plastic pots with 10 L of a nutrient solution (Hoagland & Arnon 1950) at 40% ionic strength for 60 days to obtain acclimatized clone plants.

Experimental plants were comprised of maize and *T. domingensis* individuals of similar size (about 15 cm tall), The aquatic plants were selected because they are common in

wetlands worldwide, rooted, competitive and dominant at soil where the P concentration is 900 mg kg⁻¹ or higher (Surratt, Shinde & Aumen, 2004; Carvalho et al. 2014).

Four plants of Maize were grown alone or under intercropping with one *T. domingensis* plant in plastic pots containing 2.8 L of vermiculite and 1.6 L of a Hoagland and Arnon (1950) nutrient solution modified at 40% of its ionic strength. This nutritive solution had the NH₄H₂PO₄ (the only phosphorus source of the solution) concentrations changed. The nutrient solution was replaced in regular intervals fortnightly, and the water lost by evapotranspiration refilled daily, the volume of nutrient solution used was 60% of the vermiculite volume, enough to cause waterlogging by water saturation and the water level to rise above the substrate surface.

Three P concentrations were used: 0, 12.4 and 24.8 mg L⁻¹. These concentrations were selected because 12.4 mg P L⁻¹ is the ideal concentration proposed by Hoagland and Arnon (1950); and the 24.8 mg P L⁻¹ being considered excessive by Santos et al. (2015). Vermiculite was used as a substrate to grant P availability and control of source and concentration of this nutrient according to the methods used in Diaz et al. (2018).

The experimental design was completely randomized in a factorial 3x2 design (phosphate concentrations x intercropping) with four replicates (n= 24).

The gas exchange analysis was performed after 20 days from the beginning of the experiment. These measurements were performed in two fully expanded, pathogen-free leaves from one plant per replicate, and measured using an infrared gas analyser (IRGA) model LI-6400XT (Li-COR Bio-sciences, Lincoln, Nebraska USA), a 6 cm² cuvette and a red/blue LED source of light (LI-6400-02B Li-COR Bio-sciences, Lincoln, Nebraska USA).

The net photosynthesis (*A*), stomatal conductance (*g_s*) and transpiration rate (*E*) were evaluated. In addition, the water use efficiency (WUE) was calculated by dividing the photosynthetic rate by the transpiration rate (*A/E*). All the measurements were taken between 8 and 10 a.m. with the Photosynthetic Photon Flux Density (PPFD) standardized at 1000 μmol m⁻² s⁻¹ in the cuvette of the equipment with vapor pressure deficit at 2.66 kPa, pump flow at 500 μmol s⁻¹ (Pereira et al., 2016).

After 30 days from the beginning of the experiment, fully expanded leaves and root fragments from the same placement in root were sampled and fixed in F.A.A. 70% [formaldehyde, acetic acid and 70% ethanol (ethanol and distilled water v v⁻¹) 1:1:18] for 72 hours then preserved in ethanol 70% v v⁻¹ for further analysis (Johansen 1940; Kraus & Arduim, 1997). Fragments of the median region of leaves and the pilifer zone of the roots were dehydrated in a crescent ethanolic series from 70, 80, 90 to 100% v v⁻¹. The material was then transferred to a solution composed of ethanol 100% and base resin 1:1 for 24 hours. Further, the samples used for transversal sections were embedded in Histo-resin (Leica Microsystems,

Wetzlar, Germany) as instructed by the manufacturer. The infiltration of the base resin was accomplished after 24 hours at 4 °C.

Morphoanatomy followed the methodology described by Pereira et al. (2010) and Souza et al. (2011) with transversal sections of the root were made at 3 cm away from the apex at the pilifer zone and in the median region of the leaf. The paradermic sections were handmade in both abaxial and adaxial surfaces of the leaves using steel blades. Transversal sections were performed in a semi-automated rotary microtome Yidi YD-335 (Jinhua Yidi Medical Appliance CO., LTD, Zhejiang, China). The transversal sections were stained with toluidine blue 1% (w v⁻¹) at 4.7 pH for 5 minutes then mounted on slides with Canada balsam while the paradermic sections were stained with aqueous 0.1% safranin (w v⁻¹) (Pereira).

The slides were photographed using an AxioCam Erc 5s (Zeiss, Oberkochen, Germany) attached to the microscope Axio Lab.A1 (Zeiss, Oberkochen, Germany) the images obtained were then analysed in the UTHSCSA Image Tool 3.0 (University of Texas, San Antonio, United States of America). One slide and 15 fields were evaluated for each replicate.

The data was submitted to the statistical analysis on the SISVAR 5.0 software (Ferreira 2011). The data was submitted to two-way analysis of variance (ANOVA) and the Scott-Knott test for means comparison at 5% probability.

Results and Discussion

There was significant interaction between sources of variation (P concentrations and cattail presence) for net photosynthesis (*A*) (Table 1). When grown together with *T. domingensis*, *A* of maize plants was reduced only in the absence of phosphorus. However maize alone *A* was higher at 12.4 mg P L⁻¹ (Table 1). In addition, regarding *A*, no competition with cattail was observed in the solution lacking P whereas under 12.4 mg P L⁻¹ *T. domingensis* reduced the *A* of maize but increased this parameter under 24.8 mg P L⁻¹ (Table 1).

Table 1. Net Photosynthesis of Maize submitted to different phosphorus concentrations, grown alone or under intercropping with *T. domingensis*.

Net Photosynthesis (µmolCO ₂ m ⁻² s ⁻¹)		
Phosphorus	Intercropping	Alone
0	5.32 bA	6.91 bA
12.4	9.91 aB	12.84 aA
24.8	9.33 aA	5.14 bB

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-

Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} .

The stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) showed no significant intercropping between P and the *T. domingensis* presence. Phosphorus concentration promoted no effect in the g_s and WUE whereas higher transpiration rate was found on plants from the 12.4 mg P L^{-1} (Table 2). In addition, when grown together with *T. domingensis*, maize plants had higher g_s and E, but no effects were found to WUE (Table 2).

Table 2. Stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) of Maize submitted to different phosphorus concentrations, grown alone or under intercropping with *T. domingensis*.

Phosphorus	g_s ($\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	E ($\text{mmolH}_2\text{O m}^{-2} \text{ s}^{-1}$)	WUE ($\mu\text{molCO}_2 \text{ mmolH}_2\text{O}^{-1}$)
0	0.08 a	1.06 b	8.98 a
12.4	0.18 a	2.23 a	7.71 a
24.8	0.12 a	1.45 b	8.98 a
Growth Model	g_s ($\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	E ($\text{mmolH}_2\text{O m}^{-2} \text{ s}^{-1}$)	WUE ($\mu\text{molCO}_2 \text{ mmolH}_2\text{O}^{-1}$)
Intercropping	0.20 a	2.39 a	5.51 b
Alone	0.04 b	0.77 b	10.31 a

Means followed by the same lower-case letters in the columns do not differ by the Scott-Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} .

The mesophyll thickness was not changed by P in maize plants interacting with *T. domingensis*, however, it was reduced by higher P concentrations when maize was grown alone (Table 3). Likewise, the cultivation of maize and *T. domingensis* together reduced mesophyll thickness of maize in the treatment lacking phosphorus (Table 3). In addition, larger bundle sheath's cells were found when maize were grown together with cattail at 12.4 mg P L^{-1} whereas, when grown alone, the largest means were found on maize cultivated without P supply (Table 3).

The bundle sheath percentage in the mesophyll was not changed by P in maize plants grown with *T. domingensis*, however, it was reduced in plants at higher P concentrations (Table 3). In addition, maize plants grown together with *T. domingensis* showed lower bundle sheath percentage in the mesophyll in the absence of P (Table 3).

Thicker mesophyll is often related to increased net photosynthesis for C3 plants (Santos et al. 2015; Pereira et al. 2016). However, maize has a C4 photosynthetic metabolism, where PEPcase is found in the mesophyll and Rubisco in the bundle sheath cells (Edwards et al. 2001) meaning that increased bundle sheath percentage is prone to increase the area available for photosynthetic active tissue in the mesophyll as well.

The intercropping with *T. domingensis* has favoured net photosynthesis and transpiration of maize plants, as well as increased photosynthetic tissues area (chlorenchyma and bundle sheath). Therefore, under higher P concentrations, no competition between maize and *T. domingensis* can be found instead, as also evidenced by Kloss et al (2021), our work shows that the intercropping with *T. domingensis* is favourable to maize under waterlogging condition whereas the P limitation may cause some minor reduction in some parameters.

Table 3. Anatomical parameters of Maize leaves submitted to different phosphorus concentrations grown alone or under intercropping with *T. domingensis*.

Mesophyll Thickness (μm)		
Phosphorus	Intercropping	Alone
0	105.84 aB	122.32 aA
12.4	109.86 aA	108.16 bA
24.8	109.99 aA	112.57 bA
Diameter of the Vascular Bundle Sheath Cells (μm)		
Phosphorus	Intercropping	Alone
0	20.24 bB	21.57 aA
12.4	21.74 aA	18.15 cB
24.8	20.37 bA	19.69 bA
Bundle Sheath Percentage in the Mesophyll (%)		
Phosphorus	Intercropping	Alone
0	16.96 aB	20.38 aA
12.4	16.84 aA	17.71 bA
24.8	16.13 aA	15.43 bA

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} .

Both stomatal width and length showed significant statistical interaction (between phosphorus concentrations and the presence of *T. domingensis*) at both epidermal sides while the stomatal density only showed significant interaction for the adaxial epidermis. The stomatal width on both sides of the epidermis and the stomatal length on the abaxial epidermis were larger in maize plants grown together with *T. domingensis* under 0 and 24.8 mg P L^{-1} (Table 4). However, for maize plants grown alone, the larger means for the stomatal length and width were found under P limitation (Table 4). Furthermore, both stomatal length and width were increased in maize plants cultivated together with *T. domingensis* (Table 4). In addition, the stomatal length on the adaxial side showed higher means at all P concentrations (Table 4).

Table 4. Stomatal parameters in Maize submitted to different phosphorus concentrations, grown alone or under intercropping with *T. domingensis*.

Stomatal Width on Abaxial Epidermis (μm)		
Phosphorus	Intercropping	Alone
0	30.59 aA	30.32 aA
12.4	25.75 bA	27.19 bA
24.8	31.95 aA	26.37 bB
Stomatal Width on Adaxial Epidermis (μm)		
Phosphorus	Intercropping	Alone
0	29.90 aA	29.49 aA
12.4	28.58 bA	24.18 cB
24.8	31.17 aA	27.08 bB
Stomatal Length on Abaxial Epidermis (μm)		
Phosphorus	Intercropping	Alone
0	41.07 aA	41.17 aA
12.4	39.35 bA	34.80 cB
24.8	41.94 aA	39.79 bB
Stomatal Length on Adaxial Epidermis (μm)		
Phosphorus	Intercropping	Alone
0	34.27 bA	30.08 bB
12.4	38.80 aA	31.09 bB
24.8	37.52 aA	35.77 aB

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} .

Table 5. Stomatal density on the abaxial epidermis of Maize plants submitted to different phosphorus concentrations, grown alone or in intercropping with *T. domingensis*.

Phosphorus	Stomatal Density
0	62.11 a
12.4	62.25 a
24.8	55.98 b
Growth Model	Stomatal Density
Intercropping	57.39 b
Alone	62.84 a

Means followed by the same letters in the columns do not differ by the Scott-Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} . Stomatal density in stomata mm^{-2} .

The stomatal density from plants grown under 24.8 mg P L^{-1} was 10.07% lower compared to other P concentrations, in addition, maize grown interacting with *T. domingensis* had the stomatal density reduced by 8.67% (Table 5). Furthermore, the stomatal density of the adaxial epidermis at 12.4 mg P L^{-1} showed higher means in maize cultivated together with *T.*

domingensis, however, this concentration reduced the stomatal density for plants grown alone (Table 6). In addition, the intercropping with *T. domingensis* slightly reduced the stomatal density of maize plants grown at 0 and 24.8 mg P L⁻¹ (Table 6).

Table 6. Stomatal density on the adaxial epidermis of Maize plants submitted to different phosphorus concentrations, grown alone or in intercropping with *T. domingensis*.

Phosphorus	Stomatal Density	
	Intercropping	Alone
0	43.85 bB	56.68 aA
12.4	53.02 aA	38.21 bB
24.8	37.79 bB	58.80 aA

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-Knott test at $P < 0.05$.

Phosphorus concentration in mg L⁻¹. Stomatal density in stomata mm⁻².

Stomata are related to CO₂ uptake and the regulation of the transpiration rate. In addition, it is known that higher water uptake by root systems may increase stomatal opening and conductance (Hubbard et al. 2001). In this work, the stomatal conductance of maize plants was increased when grown under intercropping with *T. domingensis* and this may be related to increased stomatal size. According to Hubbard et al. (2001) and Berry et al. (2010) bigger stomata remain open for longer times, increasing transpiration, gas uptake and photosynthesis. In addition, Franks and Beerling (2009) state that smaller stomata improve water-use efficiency by showing faster response times and closing. It is important to note that, as the ‘Saracura’ maize is grown in well-watered (or waterlogged) conditions, no water limitation is relevant, and the larger stomata may have contributed to a higher stomatal conductance.

The vascular cylinder percentage in maize roots grown at 12.4 mg P L⁻¹ and in intercropping was increased (Table 7). However, this parameter was reduced in maize grown under intercropping at 0 and 24.8 mg P L⁻¹. Whereas maize grown alone at 12.4 mg P L⁻¹ had the proportion of the vascular cylinder reduced (Table 7). Maize interacting with *T. domingensis* showed lower means in aerenchyma percentage at all P concentrations (Table 7). The cortex thickness was higher for maize plants grown at 0 and 24.8 mg P L⁻¹ in intercropping; however, 0 and 12.4 mg P L⁻¹ increased this parameter in maize grown alone (Table 7).

Plants under waterlogging benefit from aerenchyma development by decreasing hypoxia stress, therefore decreasing reactive oxygen species damage, by storing and diffusing O₂ (Souza et al. 2009; Rakhmankulova 2014). This tissue is a ubiquitous waterlogging

tolerance trait on maize genotypes (Pereira et al. 2010), likewise it also develops under phosphorus deficiency, decreasing root hydraulic conductivity (Diaz et al. 2018).

The O₂ liberation through the root and rhizosphere is well reported on *T. domingensis* plants and is called radial oxygen loss (ROL) (Chabbi et al. 2000). As described by Jackson et al. (1985) the ethylene production in Maize roots is completely halted when there is enough oxygen in the nutrient solution, preventing the aerenchyma formation, however, when oxygen is present at very low concentrations it stimulates the aerenchyma development suggesting that *T. domingensis* supplied oxygen in the solution is able to limit aerenchyma development stimulated by ROS.

The main benefit of aerenchyma percentage reduction is increased hydraulic conductivity and nutrient uptake (Diaz et al. 2018) which in our study has favoured maize gas exchange. Thus, the lower root aerenchyma percentage in maize roots for plants grown under intercropping with *T. domingensis* is related to the higher O₂ concentration which is shown to be promoted by *T. domingensis* in maize cultivation by Kloss et al. (2021) and by decreasing ROS damages, such as those caused by hydrogen peroxide, decreases signalling for ethylene mediated aerenchyma formation.

Therefore, although at lower P concentrations, maize grown with *T. domingensis* may not be suitable for an intercropping model, at higher concentration the intercropping benefits maize roots by increasing its capacity for water and nutrient uptake under waterlogging by decreasing apoplastic barriers and increasing hydraulic flow (Kloss et al., 2021).

The xylem parameters showed significant interaction between sources of variation, with 24.8 mg P L⁻¹ promoting higher means for xylem percentage in the vascular cylinder independent of the intercropping (Table 8). The vessel element diameter showed higher means for maize grown at 24.8 mg P L⁻¹ and intercropping with *T. domingensis*; however, for maize grown alone this concentration promoted the lowest means (Table 8). In addition, maize grown under intercropping showed the highest means at 24.8 mg P L⁻¹ for the vessel element diameter and decreased with the 12.4 mg P L⁻¹ and 0 P concentrations (Table 8).

The endodermis thickness was increased at 24.8 mg P L⁻¹ in maize under intercropping with *T. domingensis*, however, this variable was not significantly affected in maize plants grown alone (Table 8). In addition, the endodermis was thicker in the plants under 24.8 mg P L⁻¹ (Table 8). According to Pereira et al. (2008) 'Saracura' Maize roots under hypoxia shows reduced xylem vessel element diameter but higher aerenchyma development. Likewise, these results corroborate that decreased aerenchyma proportion and increased xylem vessel diameter in maize could be promoted by the intercropping with *T. domingensis* that alleviates hypoxia by releasing O₂ by ROL (Kloss et al., 2021).

Table 7. Root anatomical parameters of Maize plants submitted to different phosphorus concentrations, grown alone or in intercropping with *T. domingensis*.

Vascular Cylinder (%)		
Phosphorus	Intercropping	Alone
0	15.34 cB	23.99 aA
12.4	23.23 aA	20.37 bB
24.8	18.25 bB	24.28 aA
Aerenchyma (%)		
Phosphorus	Intercropping	Alone
0	7.27 aB	14.28 aA
12.4	1.43 bB	2.83 bA
24.8	0.16 bB	11.71 aA
Cortex Thickness (μm)		
Phosphorus	Intercropping	Alone
0	185.87 aA	164.35 bB
12.4	163.48 bB	182.42 aA
24.8	181.14 aA	109.20 cB

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-Knott test at $P < 0.05$. Phosphorus concentration in mg L^{-1} .

Maize-weed interaction at the low P availability found on tropical soils becomes a major problem when plants compete for this nutrient (Bloomfield et al. 2014). In fact, aquatic macrophytes can be favoured by the hypertrophication of wetlands and show excessive population growth (Webb & Zhang 2013; Santos et al. 2015).

Thus, crop plants may become endangered by this behaviour of macrophytes when competing for the same resources. Despite the obvious competition between maize and invasive plants, *T. domingensis* did not compete under hypertrophic condition. However, maize is considered as a high sensitivity crop under competition and low P conditions (Maddonna & Otegui, 2006; Zhang et al. 2014) and our work corroborate these previous results; however, some traits at 12.4 mg P L^{-1} were favoured in maize in intercropping with *T. domingensis* which is unprecedented. These results may be related to alleviation of hypoxia by the intercropping with *T. domingensis* and its ROL, decreasing damages caused by hypoxia such as ROS production and consequently cell membrane collapse as shown by Kloss et al. (2021).

Table 8. Anatomical parameters of the root vascular cylinder and endodermis of Maize plants submitted to different phosphorus concentrations, grown alone or in intercropping with *T. domingensis*.

Xylem in the Vascular Cylinder (%)		
Phosphorus	Intercropping	Alone
0	78.42 bA	69.82 bB
12.4	66.66 cB	71.45 bA
24.8	81.93 aA	78.19 aB
Xylem Vessel Diameter (μm)		
Phosphorus	Intercropping	Alone
0	42.64 bB	53.20 aA
12.4	42.20 bB	53.80 aA
24.8	52.11 aA	45.82 bB
Endodermis Thickness (μm)		
Phosphorus	Intercropping	Alone
0	5.77 bB	6.93 aA
12.4	5.92 bB	6.23 aA
24.8	7.38 aA	6.77 aB

Means followed by the same lower-case letters in the columns and the capital letters in the rows do not differ by the Scott-Knott test at 5% of significance level. Phosphorus concentration in mg L^{-1} .

Areas colonized by *T. domingensis* often show high P concentrations, therefore, lowering P concentration and increasing water depth are both ways of managing *T. domingensis* populational growth (Surratt, Shinde & Aumen, 2012; Lagerwall et al. 2016). The intra-specific competition increases with the population density of *T. domingensis* which is related to nutrients availability, plant growth and anatomical parameters (Corrêa et al. 2015, 2017).

In fact, *T. domingensis* shows strong invasive characteristics that can even affect local biodiversity (Surratt, Shinde & Aumen, 2012). Furthermore, P eutrophication by agricultural runoff greatly increases *T. domingensis* population growth, promoting expansive domination of such environments (Surratt, Shinde & Aumen, 2012). In addition, the use of an intercropping system consisting of both *T. domingensis* and maize for biomass production could be a viable sustainable alternative, as Nkemka et al. (2015) states the enormous potential of these two plants for lignocellulosic energy.

Conclusions

As shown by Kloss et al. (2021) maize under waterlogged is favoured by *T. domingensis* intercropping, however, when nutrients are restricted, the following points are to be considered:

- Maize plants grown interacting with *T. domingensis* under waterlogging show improved net photosynthesis and transpiration rate, which are promoted by higher hydraulic conductance related to a lower aerenchyma development in roots.
- Competition between Maize and *T. domingensis* is found only under low phosphorus concentrations.
- Maize anatomy and leaf gas exchange parameters are improved under waterlogging in intercropping with *T. domingensis* at hypertrophic phosphorus concentrations.

For future studies we should investigate the intercropping under drought, as aerenchyma formation happens under drought conditions too, however for varied reasons. Also, other aquatic plants such as rice are good candidates for an intercropping model based on O₂ supply.

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