BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 333-340

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240140

Waterlogging and salinity stress affecting growth and morphological character changes of *Limnocharis flava*

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Manuscript received: 24 October 2022. Revision accepted: 12 January 2023.

Abstract. *Putra SP, Santosa, Salsinha YCF. 2023. Waterlogging and salinity stress affecting growth and morphological character changes of* Limnocharis flava. *Biodiversitas 24: 333-340.* Salinity stress and waterlogging affect plant growth response. This study aims to determine the effect of variations in salinity concentration (NaCl) and water depth on the growth and morphological characters of Genjer (*Limnocharis flava*) plants. The study used a completely randomized design (CRD) with 5 replications with a variable depth of water (W) consisting of W0 = 0 cm from the soil surface, W1 = 5 cm from the soil surface, W2 = 10 cm from the soil surface, and treatment of variations in salt concentration. (NaCl) (S) as many as 4 levels, namely S0 = 0 ppm, S1 = 25 ppm, S2 = 50 ppm and S3 = 75 ppm. Statistical analysis with Analysis of Variance (ANOVA) showed the interaction of high NaCl concentration and higher water depth decreased wet weight, dry weight, height, length and width of leaves of *L. flava*. The concentration of NaCl significantly affects the growth morphology and anatomy of *L. flava.* The high concentration of NaCl reduced the wet weight, dry weight, height, length and width of the leaves of *L. flava*. The high concentration of NaCl causes changes in the anatomical structure of *L. flava.* Water depth significantly affects the growth morphology and anatomy of *L. flava*. The higher water depth reduces the wet weight, dry weight, height, length and width of the leaves of *L. flava.* There is an interaction between NaCl concentration and water depth. The high concentration of NaCl and high level of water depth reduced the wet weight, dry weight, height, length and width of the leaves of *L. flava*.

Keywords: Abiotic stress, *Limnocharis flava*, physiology, salinity stress, waterlogging

INTRODUCTION

Tidal swamp land along the coastal area has various marginal conditions such as waterlogging. Saltwater flooding was known as a most potential condition reduce plant growth and productivity in this area (Pezeshki 2001; Mielke 2010). The flooding phenomenon is common in the northern coastal area of Java Island, Indonesia. This phenomenon mostly caused by the changes in sea level due to global warming (Marfai 2011). The increase in sea levels accompanied by the increase in water salinities affects plant growth in the area (Ahmad et al. 2017).

One type of hydro macrophyte that lives in tidal areas is Genjer (*Limnocharis flava* L.). This plant belongs to the perennial herbaceous group that can grow up to one meter in height (Nishan and George 2018; Zakaria et al. 2018). The main characteristics of this plant are glabrous stems and leaves, containing a scapigerous substance. The wide leaves of this plant have various shapes including rounded, broadly elliptic, or ovate, sheathing with wavy edges, and appear in clusters above the water surface. The plant petiole is triangular with thick lacunae (air spaces) (Nishan and George 2018). According to Lakitan (Lakitan et al. 2019), this aquatic plants are clumps in the aquatic environment rice fields and rivers. *L. flava* habitats are found along ditches, flooded rice fields, and swamps (Serang and Laili 2021)

Limnocharis flava can reproduce vegetatively and generatively. In addition to using rhizomes, *L. flava* produce seeds that can quickly expand their population distribution (Ranawakage et al. 2013; Sundari et al. 2013). Based on previous research (Juhaeti 2013; Zakaria et al. 2018) it is known that the shoot yield from seed propagation is more than that from plantlets. This plant can be grown for 5 months cropping time. Based on research on the effect of nutrient water (Noorasmah et al. 2016), plants selectively absorb more than one type of nutrient from the water. There is no specific nutrient that is responsible for changes in growth parameters. Until now, the yield of *L. flava* biomass is still low compared to crop crops such as rice in Indonesia (Zakaria et al. 2018).

Previous research (Wardani et al. 2017) showed that *L. flava* has potential as a phytoremediation plant. The test results showed that *L. flava* has the potential to reduce nitrate and orthophosphate levels up to 90% after 6 days of incubation. This plant is resistant to heavy metal toxicity conditions. Research by Rijal et al. (Rijal et al. 2016) showed that *L. flava* can absorb heavy metals in the form of lead, mercury, and cadmium and has the potential for phytoremediation.

The rapid growth of the basal meristem petiole makes this plant has the ability to escape from submersion conditions for at least 12 days. This plant can survive as long as at least 1 leaf is left above the water surface (Ranawakage et al. 2014). In addition, the petiole character which is composed of air spaces similar to aerenchyma helps plants to transfer oxygen internally from the leaves to other parts of the organ that are below the water surface (Lakitan et al. 2019; Serang and Laili 2021). The growth ability of this plant has the potential to support its resistance to other stress conditions such as salinity and flooding.

According to study (Parida et al. 2016), salinity (NaCl) treatment with low concentrations is known to increase plant tolerance. Previous research (Grattan 2005; Arif et al. 2020) salt accumulation can occur due to the movement and evaporation of water from the groundwater surface so that salt is left in the soil due to low leaching. In tomatoes, salt pretreatment at a certain growth phase can increase the plant's ability to adapt to salinity so that it becomes more tolerant (Cuartero et al. 2006). Several treatments and practical management can be done to reduce the level of salt in the soil. The treatment given to plants can increase their tolerance of plants to salinity (Reddy et al. 2017; Buffington et al. 2020).

In several places in Indonesia, salinity stress poses a threat to decreased crop production due to seawater intrusion (Arif et al. 2020). Research Aldrian et al. (Aldrian et al. 2011) showed that the salinity level in Indramayu, West Java, was moderate to very high at 0-30 cm and 30-70 cm depth, respectively. According to Grattan (Grattan 2005), salinity levels below 2.0 dS/m did not affect rice yields. If salinity increases above 2.0 dS/m the yield will decrease by about 10% for every 1.0 dS/m increase.

This study aims to determine the effect of variations in salinity (NaCl concentration) and water depth on the growth, morphology, and anatomy of *L. flava*, as well as the effect of interactions between variations in salinity (NaCl concentration) and water depth on the growth, morphology, and anatomy of *L. flava* which can be the basis for physiological analysis of salinity and waterlogging tolerant plants.

MATERIALS AND METHODS

Experimental site and plant materials

This research was conducted at Universitas Gadjah Mada, Yogyakarta, Indonesia with greenhouse facilities at the Sawitsari Research Station (7°45′22″ S, 110°23′18″ E). The physicochemical parameters at the research site include: sunlight intensity ranging from 5600-12000 lx during daytime with temperatures ranging between 24 ºC and 34 ºC.

The material used in this study was genjer (*Limnocharis flava* L.) seeds in the early vegetative period obtained from rice fields being planted with rice from the Seyegan area, Sleman, Yogyakarta. The salinity treatment used a salt solution (NaCl), while the soil medium was paddy soil obtained in the Seyegan area, Sleman, Yogyakarta.

Experimental design and treatment

The 72 buckets filled with 1 kg growing media consisted of 1:3 compost and soil were prepared. The soil used comes from the Sleman, Indonesia area and is classified as regosol soil with a composition of clay, sand and volcanic material. The soil surface is leveled, the planting medium is ready for *L. flava*. The stock of *L. flava* with a height of 3 cm and leaves of two ribbons was taken placed in each bucket. Each bucket contains individual *L. flava*. This experiment was conducted with the arrangement of a randomized-complete blocked design consisting of two factors (salinity and waterlogging) with five replications each.

The treatments given in this study were 4 levels of salinity and 3 levels of water depth. The salinity treatment group consisted of S0 (Salinity NaCl 0 ppm), S1 (Salinity NaCl 25 ppm), S2 (Salinity NaCl 50 ppm) S3 (Salinity NaCl 75 ppm) (Rasouli et al. 2022). The different water depth levels consist of W0 (0 cm water height from the surface of the growing media), W1 (5 cm water height from the surface of the growing media) and W2 (10 cm water height from the surface of the growing media). The water level of each treatment was kept constant every day by increasing the volume of well water.

Plant morphological characters such as plant height, leaf blade length, and leaf width as well as plant biomass characters in the form of dry weight and wet weight were measured when the plant had reached 35 days after planting (DAT).

Plant morphological analysis

Measurements of growth were made at the age of 35 DAP by measuring the height of the plant clump measured from the soil surface to the tip of the highest leaf. Leaf width measurements were carried by measuring the average leaf width of the second leaf. Leaf length measurements were carried out by measuring the average leaf length. *L. flava* clumps and their roots were harvested at 35 DAP. After washing and cleaning, the samples were weighed using a digital scale as the fresh weight of the plant. Fresh weight measurements were divided into total fresh weight, fresh weight of shoots and fresh weight of roots. The *L. flava* clumps and their roots, which had been weighed fresh, were dried in an oven at 80 °C until the weight was constant, then the dry weight was weighed again until it’s stabil. The dry weight measurement was divided into total dry weight, shoot dry weight and root dry weight.

Statistical analysis

The significance of the data affected salinity and waterlogging levels were calculated and tested using ANOVA followed by Duncan multiple range test (DMRT) with a 95 % confidence level in statistical software (IBM-SPSS Ver. 25.00.US).

RESULTS AND DISCUSSION

Plant morphology

The results of the measurement of morphological parameters including plant height, length and width of small water hyacinth leaf blades aged 35 DAP with water depth and salinity treatments are shown in Table 1. The data (Table 1) shows the decrease in plant height, length and width of *L. flava* leaves due to variations in salinity concentration and water depth at 35 days after planting. The higher the salinity, the lower the plant height, length and leaf width. The treatment of deeper water depth showed a decrease in plant height.

From the results of the analysis of variance, the variation of salinity and water depth treatment had a significant effect ($p \leq 0.05$) on the height of *L. flava*. The results of the DMRT further test on the data in Table 1. showed that the variation in salinity and water depth was different between the treatment groups ($p \leq 0.05$). From the results of the DMRT analysis, it was shown that the higher the salinity concentration and the higher the water depth, the plant height, length and width of the harvested *L. flava* leaves at 35 DAP could be reduced. There is an interaction between salinity and water depth treatment on plant height, length and width of the *L. flava* leaf blade, so that the treatment of 10 cm water depth and 75 ppm salinity (W2S3) caused the plant height, length and width of the *L. flava* leaf to be the lowest.

The decrease in plant height can also be caused by the limited supply of water and organic matter in the tissues. The decrease in the amount of water causes the cells to lose turgor so that there is a tendency for plasmalemma to separate from the cell wall (plasmolysis). In the process of cell elongation, plants need an appropriate water balance because the strength of cell elongation is the result of turgor pressure (Salsinha et al. 2020). Scoffoni (Scoffoni et al. 2014) stated that the presence of water will increase the turgor of the cell wall which causes the cell wall to stretch so that the bonds between the cell walls weaken. This is what pushes the cell walls and membranes to increase in size, so that the lack of water availability will inhibit plant growth. Limited organic matter also inhibits plant height growth causing the plants to become stunted.

The decrease in leaf length and width in *L. flava* grown on saline soil indicated that *L. flava* was a salt sensitive plant. This decrease was due to the presence of NaCl solution in the soil media which resulted in the amount of water and nutrients in plants decreasing so that the metabolic process was hampered. The decrease in the amount of water causes a decrease in photosynthesis so that the availability of carbohydrates decreases. Carbohydrates are needed for the initial process of tissue formation such as roots, stems, and leaves, so that the decrease in carbohydrates causes the formation of plant tissue to be inhibited (Chandran and Ramasamy 2015; Lakitan et al. 2018).

Table 1. Plant height, leaf length and width at the age of 35 from *Limnocharis flava* treated with salinity and waterlogging

Parameters	**Water depth**	**Salinity (ppm)**			
		S_0	**S_1**	**S_2**	**S_3**
Plant height (cm)	W_0	33.07^{l}	24.02^{jk}	15.60^{ef}	13.62^{cd}
	W_1	23.98^{jk}	20.04^{i}	14.18^{ef}	7.74^{b}
	W_2	18.36^{gh}	16.20^{gh}	12.06^{cd}	1.48^{a}
Leaf length (cm)	W_0	9.22^{k}	6.56^{i}	4.58^{fg}	3.78^{e}
	W_1	7.40^{j}	5.36^{h}	2.80^{d}	1.98^{bc}
	W_2	5.04^{fg}	3.90^{e}	2.16^{bc}	0.94^{a}
Leaf width (cm)	W_0	5.04^{l}	2.96^{jk}	1.74^{h}	0.88^{de}
	W_1	3.08^{jk}	2.34^{i}	1.02^{fg}	0.36^{bc}
	W_2	1.34^{fg}	0.70^{de}	0.32^{bc}	0.12^{a}

Note: Means (n=5) followed by the same letter in the same column and row shows no significant difference ($p \geq 0.05$), with S_0 (NaCl salinity 0 ppm), S_1 (NaCl salinity 25 ppm), S_2 (NaCl salinity 50 ppm) and S_3 (NaCl salinity 75 ppm). The different water depth levels consist of W_0 (0 cm water depth on the surface of the growing media), W_1 (5 cm water depth on the surface of the growing media) and W_2 (10 cm water depth on the surface of the growing media)

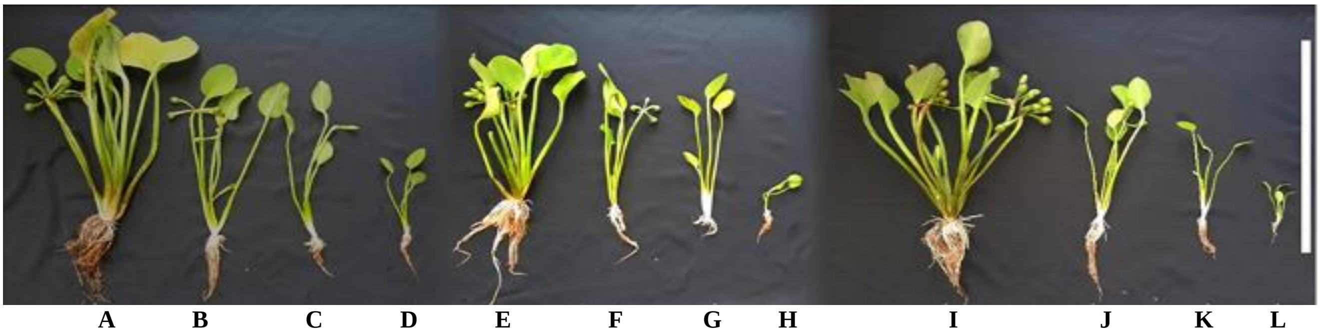


Figure 1. Morphology of *Limnocharis flava* treated with 3 levels of salinity and 3 levels of waterlogging stress with: A. W0S0, B. W0S1, C. W0S2, D. W0S3, E. W1S0, F. W1S1, G. W1S2, H. W1S3, I. W2S0, J. W2S1, K. W2S2, L. W2S3. The letter S stands for S_0 (NaCl salinity 0 ppm), S_1 (NaCl salinity 25 ppm), S_2 (NaCl salinity 50 ppm) and S_3 (NaCl salinity 75 ppm), while the letter W stands for the different water depth levels consist of W_0 (0 cm water depth on the surface of the growing media), W_1 (5 cm water depth on the surface of the growing media) and W_2 (10 cm water depth on the surface of the growing media). Bar = 30 cm

The decrease in leaf length and width of *L. flava* is also caused by an imbalance of ions in the roots. Watering the NaCl solution on the media causes the accumulation of excessive Na^+ ions so that it will damage the cell wall permeability by replacing Ca^{2+} ions in the root cell walls. Kumar et al. (2017) stated that calcium acts as a cell wall reinforcement, increases cell division in meristem areas, helps nitrate absorption and regulates the availability of water in cells. So reduced calcium causes stunted leaf growth.

Fresh weight

Plant growth can be measured from the increase in volume and mass of tissues (Afzal et al. 2019). *L. flava* growth recorded from total fresh weight showed a decrease in total fresh weight with increasing water depth and salinity. Fresh weight is measured by harvesting all parts of the plant, and weighing it directly before too much water evaporates.

Table 1. shows that the total fresh weight, shoots and roots of *L. flava* decreased due to the increasing in salinity concentration and water depth variations at 35 days after planting. The higher salinity treatment resulted in the decrease of the total fresh weight of shoots and roots. The treatment of deeper water depths showed a decrease in total fresh weight.

From the results of statistical analysis, variations in salinity and water depth had a significant effect ($p \leq 0.05$) on the total fresh weight of the shoots and roots of *L. flava*. From the results of the DMRT analysis, it was shown that the higher the salinity concentration and the higher the water depth, the total fresh weight of shoots and roots of *L. flava* harvested at 35 DAP was reduced. There is an interaction between salinity and water depth treatment on the total fresh weight of the shoots and roots of *L. flava*. The treatment of water depth of 10 cm and salinity of 75 ppm (W_2S_3) caused the total fresh weight of the shoots and roots of *L. flava* to be the lowest. The results of total fresh weight of shoots, and roots of small water hyacinth shoots aged 35 DAP with water depth and salinity treatments are shown in Table 2.

The decrease in total fresh weight of the shoot and roots of *L. flava* was influenced by the level of water depth (Table 2). *L. flava* growing at a depth of 10 cm (W_2) had the smallest total fresh weight compared to *L. flava* growing at a depth of 5 cm (W_1) and a depth of 0 cm (W_0). According to Chen (Chen 2009), the depth and duration of inundation can affect the decrease in the number of primary branches, secondary branches, canopy biomass and total plant biomass. Waterlogged soils are substantially different from well-drained soils. In well-drained soil, oxygen available from the atmosphere is sufficient to supply the needs of microorganisms and plants. Diffusion of oxygen in stagnant water is usually much slower, so oxygen can be reduced in the soil (Norman 1956).

Limnocharis flava growth is inhibited by increased inundation, this can occur because the soil is flooded with low oxygen levels. Low oxygen levels cause anaerobic organisms to perform anaerobic respiration. Anaerobic respiration causes the accumulation of phytotoxic compounds H_2S, CO_2, C_2H_5OH and methane. In addition, anaerobic respiration reduces the redox potential of the soil, lowers soil pH, decreases the decomposition of organic matter and produces various toxic organic chemicals that can inhibit growth (Phule et al. 2019).

Salinity is a universal threat for crop plants, negatively affecting in many ways specifically reducing their yield (Afzal et al. 2019). NaCl is a compound containing the element sodium which is an essential micronutrient for plants. The main role of sodium in plants is to replace some of the potassium needed for maximum growth (Iswadi 2004). Chlorine is absorbed by plants in the form of ions, these ions are micro nutrients needed in the process of photosynthesis. The function of chlorine is directly related to the regulation of osmotic pressure in plant cells (Mommer 2005).

Salinity in general has an effect on reducing plant growth as a result of a decrease in leaf area and leaf number. Salinity at low to moderate concentrations affects the osmotic value in the root area of plants (Mommer 2005; Kumar et al. 2017). Salinity also affects the roots by shortening the roots and making the roots thinner.

Table 2. Total fresh weight of *Limnocharis flava* treated by salinity and waterlogging at the 35 DAP

Parameters	Water depth	Salinity (ppm)			
		S_0	S_1	S_2	S_3
Total fresh weight (g)	W_0	11.564^{l}	6.390^{jk}	3.278^{hi}	0.340^{bc}
	W_1	5.862^{jk}	3.316^{hi}	2.082^{fg}	0.226^{bc}
	W_2	1.850^{fg}	1.156^{de}	0.740^{de}	0.098^{a}
Shoot fresh weight (g)	W_0	7.694^{l}	3.964^{jk}	2.238^{hi}	0.196^{bc}
	W_1	3.858^{jk}	2.288^{hi}	1.610^{fg}	0.144^{bc}
	W_2	1.258^{fg}	0.698^{de}	0.492^{de}	0.058^{a}
Root fresh weight (g)	W_0	3.870^{l}	2.426^{jk}	1.040^{hi}	0.144^{bc}
	W_1	2.004^{jk}	1.028^{hi}	0.472^{fg}	0.082^{bc}
	W_2	0.592^{fg}	0.458^{de}	0.248^{de}	0.040^{a}

Note: Means (n=5) followed by the same letter in the same column and row shows no significant difference ($p \geq 0.05$), with S_0 (NaCl salinity 0 ppm), S_1 (NaCl salinity 25 ppm), S_2 (NaCl salinity 50 ppm) and S_3 (NaCl salinity 75 ppm). The different water depth levels consist of W_0 (0 cm water depth on the surface of the growing media), W_1 (5 cm water depth on the surface of the growing media) and W_2 (10 cm water depth on the surface of the growing media)

The osmotic effect of salinity causes a decrease in plant growth rate, changes in leaf color, and the development of characteristics such as root and canopy ratios (Buffington et al. 2020). The decrease was caused by the low water supply to the leaves during growth (Mielke 2010).

Dry weight

Parameters of plant growth in addition to measuring the fresh weight can also measure the dry weight of the plant. According to Mielke & Schaffer (Mielke 2010), the generally accepted measure of growth is dry weight, either from the whole plant or from other parts. The dry weight of the plant is 90% the result of photosynthesis. Growth analysis by calculating dry weight is intended to measure the ability of plants to produce photosynthate. The results of measurements of total dry weight, shoots, and roots of small water hyacinth shoots aged 35 DAP with water depth and salinity treatments are shown in Table 3.

Table 3. shows the decrease in total dry weight, shoots and roots of *L. flava* due to variations in salinity concentration and water depth at 35 days after planting. The higher the salinity, the lower total dry weight, shoots and roots dry weight recorded. The treatment of deeper water depth showed a greater decrease in total dry weight. Waterlogging reduces the growth of plants by decreasing photosynthesis and plant dry weight (Ashraf 2012; Sun et al. 2016).

From the results of the analysis of variance, the variation of salinity and water depth treatment had a significant effect ($p \leq 0.05$) on the total dry weight, shoots and roots of *L. flava*. The results of the DMRT further test on the data in Table 3. showed that the variation in salinity and water depth was different between the treatment groups ($p \leq 0.05$). From the results of the DMRT analysis, it was shown that the higher the salinity concentration and the higher the water depth, the total dry weight, shoots and roots of *L. flava* harvested at 35 DAP were reduced. There was an interaction between salinity and water depth treatment on the total dry weight of the shoot and roots of *L. flava*, so that the treatment of water depth of 10 cm and salinity of 75 ppm (W_2S_3) resulted in the lowest total dryness, shoots and roots of *L. flava*.

According to Kundur (Kundur et al. 2015), soil that is waterlogged for 35 days will reduce the dry weight of the plant because in flooded soil the aeration level is low. Previous study (Lakitan et al. 2018) stated that due to inundation, the soil is anaerobic and has a detrimental effect on shoot growth by inhibiting plant internode elongation, leaf formation and expansion, causing chlorosis, premature aging and premature abortion.

According to Wu (Wu et al. 2022), when the soil is flooded, there is a lack of oxygen around the roots which hinders electron transfer in the mitochondria, namely NADH oxidation and ATP synthesis. Inhibition of mitochondrial respiration reduces the efficiency of energy production. Hipoxic conditions (lack of oxygen) cause low metabolic activity, thereby reducing total dry weight, shoots and roots. Waterlogging can inhibit root growth, because roots only appear and extend to the soil surface layer. It also inhibits the total growth of plants (Ranawakage et al. 2013; Phule et al. 2019).

Limnocharis flava showed a decrease in plant dry weight with the increasing of salinity concentration. The decrease in plant dry weight was due to the administration of NaCl solution at various concentrations which caused a higher concentration of the soil solution so that the amount of water entering the plant roots would decrease.

The presence of salts in the soil affects the decrease in the ability of plants to absorb water so that the amount of water in plant cells decreases and can increase the wilting point of plants (Hasana and Miyake 2017; Kumar et al. 2017). This is supported by Pangaribuan (Pangaribuan 2001) who stated that the presence of NaCl resulted in an increase in transpiration. An increase in the rate of transpiration will reduce the amount of plant water so that the plant wilts.

NaCl treatment causes the amount of water in the plant to decrease so that the turgor of the stomatal guard cells decreases. The decrease in stomata turgor causes the photosynthesis process to be hampered so that the amount of assimilate produced by plants decreases. Pangaribuan (Pangaribuan 2001), states that high salinity causes an imbalance in the processes of respiration and photosynthesis. If the respiration rate is greater than photosynthesis, the dry weight of the plant decreases.

Table 3. Total dry weight, shoot dry weight and root dry weight of *Limnocharis flava* treated with salinity and waterlogging at the age of 35 DAP

Parameters	Water depth	Salinity (ppm)			
		S_0	S_1	S_2	S_3
Total dry weight (g)	W_0	0.636^{l}	0.286^{jk}	0.163^{hi}	0.041^{bc}
	W_1	0.345^{jk}	0.148^{hi}	0.093^{fg}	0.017^{bc}
	W_2	0.146^{fg}	0.051^{de}	0.020^{de}	0.003^{a}
Shoot dry weight (g)	W_0	0.473^{l}	0.181^{jk}	0.100^{hi}	0.028^{bc}
	W_1	0.221^{jk}	0.104^{hi}	0.068^{fg}	0.012^{bc}
	W_2	0.099^{fg}	0.032^{de}	0.014^{de}	0.003^{a}
Root dry weight (g)	W_0	0.163^{l}	0.103^{jk}	0.063^{hi}	0.013^{bc}
	W_1	0.124^{jk}	0.044^{hi}	0.025^{fg}	0.005^{bc}
	W_2	0.047^{fg}	0.019^{de}	0.006^{de}	0.001^{a}

Note: Means (n=5) followed by the same letter in the same column and row shows no significant difference ($p \geq 0.05$), with S_0 (NaCl salinity 0 ppm), S_1 (NaCl salinity 25 ppm), S_2 (NaCl salinity 50 ppm) and S_3 (NaCl salinity 75 ppm). The different water depth levels consist of W_0 (0 cm water depth on the surface of the growing media), W_1 (5 cm water depth on the surface of the growing media) and W_2 (10 cm water depth on the surface of the growing media)

It was also previously stated by Shahzad et al (Shahzad et al. 2020) that the dry weight yield of plants is a balance between CO_2 uptake for photosynthesis and CO_2 expenditure from respiration. Salinity affects plants in several ways and induces dissimilar but distinctive stresses such as osmotic, ionic and oxidative stress (Coelho et al. 2018; Guimarães et al. 2020).

NaCl causes an imbalance of ions, so that plants lack nutrients, especially NPK. This is supported by studies (Haryati et al. 2012; Kumar et al. 2017) that the accumulation of Cl- ions in roots results in reduced NO$_3$-absorption, so that fewer amino acids are formed. Nitrogen plays an important role as a constituent of leaf chlorophyll so that nitrogen deficiency causes yellow and curly leaves as symptoms that appear in plants grown on soil media with NaCl treatment.

Salt stress will cause a decrease in the synthesis of hormones that promote growth and an increase in hormones that inhibit growth. IAA is a hormone that stimulates cell division, elongation and enlargement. The presence of high salinity causes a decrease in amino acids such as tryptophan which is needed as a precursor in the synthesis of the IAA hormone so that the concentration of the existing IAA hormone decreases. The decrease in the IAA hormone will inhibit the growth of *L. flava* (Ryu and Cho 2015). The excessive levels of salt stress will reduce IAA in salt-sensitive plants. Previous study (Chaves et al. 2011) shows that the content of the hormone ABA increased under stress conditions. ABA has an antagonistic role with the IAA hormone, which inhibits growth.

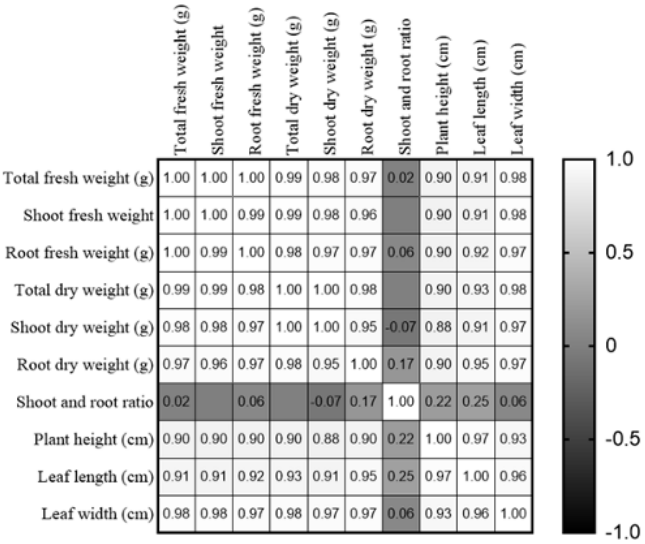
Ratio of root and shoot

From the analysis of variance, the variation of salinity and water depth treatment had a significant effect ($p \leq 0.05$) on the ratio of *L. flava* roots and shoots. The results of the DMRT further test on the data in Table 4. showed that the variation in salinity and water depth did not differ between treatment groups ($p \leq 0.05$). The variations in salinity concentration have the same effect on the ratio of *L. flava* roots and shoots. This also occurred in variations in water depth which showed the same effect on the ratio of *L. flava* roots and shoots.

In general, it can be said that the growth of roots and shoots is influenced by NaCl concentration and water depth. This is due to the possibility of a decrease in root dry weight with an equivalent shoot dry weight. There was the same comparison between the decrease in root dry weight and the decrease in shoot dry weight in each treatment group. The decrease in root dry weight between treatment variations was the same, as did the decrease in shoot dry weight between treatment variations.

A good root system allows plants to get water and nutrients for photosynthesis in sufficient quantities because root depth greatly affects the amount of water absorbed. High soil moisture content will reduce root elongation, penetration depth and root diameter (Haryati 2006; Haryati et al. 2012). The rate of root elongation is influenced by internal factors, such as the supply of photosynthate from leaves and environmental factors such as temperature and soil water content (Salsinha et al. 2021).

In disturbed land, the root weight was higher than the shoot weight, while in undisturbed land the shoot weight was higher than the root weight. Each plant has a character relationship between the shoot and roots.



	Total fresh weight (g)	Shoot fresh weight	Root fresh weight (g)	Total dry weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot and root ratio	Plant height (cm)	Leaf length (cm)	Leaf width (cm)
Total fresh weight (g)	1.00	1.00	1.00	0.99	0.98	0.97	0.02	0.90	0.91	0.98
Shoot fresh weight	1.00	1.00	0.99	0.99	0.98	0.96		0.90	0.91	0.98
Root fresh weight (g)	1.00	0.99	1.00	0.98	0.97	0.97	0.06	0.90	0.92	0.97
Total dry weight (g)	0.99	0.99	0.98	1.00	1.00	0.98		0.90	0.93	0.98
Shoot dry weight (g)	0.98	0.98	0.97	1.00	1.00	0.95	-0.07	0.88	0.91	0.97
Root dry weight (g)	0.97	0.96	0.97	0.98	0.95	1.00	0.17	0.90	0.95	0.97
Shoot and root ratio	0.02		0.06		-0.07	0.17	1.00	0.22	0.25	0.06
Plant height (cm)	0.90	0.90	0.90	0.90	0.88	0.90	0.22	1.00	0.97	0.93
Leaf length (cm)	0.91	0.91	0.92	0.93	0.91	0.95	0.25	0.97	1.00	0.96
Leaf width (cm)	0.98	0.98	0.97	0.98	0.97	0.97	0.06	0.93	0.96	1.00

Figure 2. The correlation between each parameters in *Limnocharis flava* treated with 3 levels of salinity and 4 levels of waterlogging treatments. The value of 1 indicating significant positive correlation between parameters compared, 0 indicating no correlation between parameters and -1 value indicating significant negative correlation between parameters. This data shows that most of the parameters given is positively correlated, indicating the increasing in one parameters affect the increase in another parameters, except the correlation value of shoot dry weight and shoot and dry root ratio with negative correlations

Table 4. Shoot and root ratio from *Limnocharis flava* treated with salinity and waterlogging at the age of 35 DAP

Treatments		Salinity			
		S0	S1	S2	S3
Water depth	W0	0.345a	0.569a	0.630a	0.464a
	W1	0.561a	0.423a	0.368a	0.417a
	W2	0.475a	0.594a	0.429a	0.333a

Means (n=5) followed by the same letter in the same column and row shows no significant difference ($p \geq 0.05$), with S0 (NaCl salinity 0 ppm), S1 (NaCl salinity 25 ppm), S2 (NaCl salinity 50 ppm) and S3 (NaCl salinity 75 ppm). The different water depth levels consist of W0 (0 cm water depth on the surface of the growing media), W1 (5 cm water depth on the surface of the growing media) and W2 (10 cm water depth on the surface of the growing media)

Homeostasis of shoots and roots is an effort of these plant organs to maintain physiological balance, so that each plant organ can perform its function normally. Hormones play a role in maintaining the balance of shoots and roots.

Root growth will stimulate shoot growth because of the homeostatic properties to maintain the balance of roots and shoots. The ratio of shoot and root depends on the species, age, environmental conditions and growing season. The shoot/root ratio increased, because the distribution of assimilate was more in the direction of shoot growth (Makbul et al. 2011; Studer et al. 2017). The low shoot and root weight ratio was due to the assimilate being translocated only to leaves and roots.

Correlation of morphological and biomass characters

Water depth and salinity affect plant height, leaf length and width. The deeper the inundation, the shorter the plant and the smaller the leaf size (Ranawakage et al. 2013). Morphological response to waterlogging, plants become shorter and leaf size decreases compared to those that are not flooded. The concentration of NaCl significantly affects the growth morphology and anatomy of *L. flava*.

The high concentration of NaCl reduced the wet weight, dry weight, height, length and width of the leaves of *L. flava*. The high concentration of NaCl causes changes in the anatomical structure of *L. flava*. Water depth significantly affects the growth morphology and anatomy of *L. flava*. The high water depth reduces the wet weight, dry weight, height, length and width of the leaves of *L. flava*. There is an interaction between NaCl concentration and water depth. The high concentration of NaCl and high water depth reduced the wet weight, dry weight, height, length and width of the leaves of *L. flava*.

ACKNOWLEDGEMENTS

Authors extend our gratitudes towards Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia for facilitation support for this research and publication.

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