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COMPARISON OF WATER ADAPTABILITY BETREEN *COIX LACRYMA-JOBI* AND *COIX AQUATICA* BASED ON PHOTOSYNTHESIS

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Abstract

Coix lacryma-jobi is a traditional Chinese medicinal herbs, and it has a long history of eating and planting in China. This paper compares the photosynthetic physiology and morphological characteristics of *C. lacryma-jobi* and *C. aquatica,* according to their characteristics of the environment and natural distribution. In order to explain the photosynthetic physiology and morphological mechanism in adapting to the living environment, and make suggestions for planting of *C. lacryma-jobi*. The experiment designed four groups of treatment: *C. lacryma-jobi* planted in dry land (CL-D), *C. lacryma-jobi* planted in flooded land (CL-F), *C. aquatica* planted in dry land (CA-D), *C. aquatica* planted in flooded land (CA-F). Li-6400 portable photosynthesis device was used to measure photosynthesis, TTC method was used to measure root viability, and paraffin section method was used to observe root microstructure. The results showed that, the daily variation of net photosynthetic rate (P_N) of CA-D treatment group was slightly lower than that of other groups. P_N of CA-D was significantly lower than that of CA-F in August. The light response curve also showed that the P_N of CA-D was lower than other groups. Stomatal limitation was the main factor affecting P_N and E. Chlorophyll fluorescence parameter results showed that there was no obvious adversity in the four groups of treatments. However, the photochemical quenching (q_P) and apparent electron transfer rate (ETR) of the two species under dry cultivation were significantly lower than those of flooded cultivation. The adverse effect of dry cultivation on the electron transport capacity of *C. aquatica* was greater, and the chlorophyll content of *C. aquatica* in dry land was also significantly lower than in flooded land. Root vigor of the two plants under flooded culture was higher than that of dry land in August. The root cross-section structure of the two species was very similar. Parenchymatous parenchyma cells increased and formed ventilated tissue in flooded land. The vascular bundles number of *C. lacryma-jobi* was significantly more than that of *C. aquatica* under dry land. Adequate water could increase leaf area, tiller number, and fruiting rate of the two species. In summary, both types of the plants are more suitable for a well-watered environment, so sufficient water must ensure when planting *C. lacryma-jobi*. And the adaptability of *C. lacryma-jobi* to dry land is stronger than that of *C. aquatica,* which indicates that the *C. aquatica* has slowed down during the evolution process.

Key words: *Coix lacryma-jobi*, *Coix aquatica*, Photosynthesis, Microstructure, Plant leaf morphology.

Introduction

Coix lacryma-jobi (Job's tears) is a traditional Chinese medicinal material with high medicinal value (Anon., 2015). Ingredients such as esters extract from the seeds of Job's tears have a strong antitumor effect, and these substances have good effects on sarcoma and liver cancer (Fu *et al*., 2014; Wang *et al*., 2014; Qi *et al*., 2015). The ethyl acetate part (*AHE-ea*) can also inhibit the proliferation of primary human leiomyoma cells and prevent the smooth muscle hyperproliferation induced by sex hormones in mice (Lin *et al*., 2019). Due to its high nutritional value, Job's tears can be used as a feed supplement to regulate the intestinal flora of animals during livestock feeding (Li *et al*., 2019), so it has a good prospect in the development of functional foods (Zhang *et al*., 2019).

China is the largest producer and exporter of Job's tears currently, and it has a long history of edible and planting Job's tears. However, a strong genetic bottleneck appeared during the domestication of Job's tears. Especially the selection of caryophyll husk thickness was an important goal for domestication. This had also led to a loss of genetic diversity (Liu *et al*., 2019). The husk tissue sections and transcriptome sequencing data showed that the number of paper shell cells in the husks was small, and the expression of genes related to cell division and cell wall synthesis was suppressed (Guo *et al.,* 2019). *Coix* originates from tropical Asia and is an annual or perennial herb of the family Poaceae. Southwest China is a secondary central region of the genus *Coix* (Fu *et al*., 2019), which contains two species of *Coix aquatica* and *Coix lacryma-jobi*. Cai *et al.,* (2014) founded a kind of *Coix aquatica* in Guangxi Province, China. It was speculated that, this *C. aquatica* was a newly formed species of the *Coix* based on the analysis of the genome sequencing results. But its evolutionary process had been greatly hindered due to fertility issues. However, *C. lacryma-jobi* could evolve normally and undergo the expansion of transposons, making the genome expand and forming multiple variants. Because of human domestication and planting effects, and the characteristics of cross-pollination, the germplasm resources of *Coix* were very rich in China. There were great differences in plant leaf morphology (Li *et al*., 2019; Li *et al*., 2015), seed trait size (Jin *et al*., 2017) and nutritional composition (Li *et al*., 2018) among *Coix* plants. Combined with molecular marker technologies such as RAPD (Li *et al*., 2001), SSR (Guo *et al*., 2012), and SRAP (Xia *et al*., 2017), it was found in the study of *Coix* germplasm resources that the results of morphological character classification and molecular marker clustering showed the clustering of *Coix* had obvious regionality. Their morphological and physiological characteristics reflected the adaptability to temperature and moisture distribution (He *et al*., 2019).

To explore the process and mechanism of species formation, should base on the analysis of genomic data, comprehensive evidence of morphological development, ecology and biogeography, etc (Yang *et al*., 2017). *C. lacryma-jobi* and *C. aquatica* like moist soil and often grow by the stream. *C. aquatica* have the typical characteristics of aquatic plants that the root system must grow in streams. In contrast, *C. lacryma-jobi* have much lower requirement on water and are planted in hillsides or dry land. The soil water conditions have a great impact on the growth and development of plants, and plants also change on morphology and physiological metabolism to adapt to their environment (Osakabe *et al*., 2014). Are the plants of *Coix* more suitable for aquatic life or more suitable for land? What characteristics of their physiology, plant leaf morphology, and root morphology have change to fit the environment? In this paper, *C. lacryma-jobi* and *C. aquatica* are used as materials to compare the differences on morphology and photosynthetic physiology between flooded and dry cultivation. Try to explain the morphological and physiological mechanism of the adaptation of *Coix* plant to the living environment, and also to provide a theoretical basis for the artificial planting of *C. lacryma-jobi.*

Materials and Methods

Materials and planting: Collected some wild caryopses of *C. lacryma-jobi* (Huaihua, Hunan Province, China) and *C. aquatica* (Sanjiang, Guangxi Province, China). The experimental site was located in Huaihua, Hunan Province, China. Caryopses were selected randomly, then soaked in clear water for 24 hours and planted in soil in April. The seedlings were transplanted into potted (dry) and non-porous (flooded) pots, when they grew to about 14 cm high. One seedling per pot. The soil content in the pots was the same. The non-porous pots were always filled with water. The perforated pots must be watered from time to time to avoid excessive drought. Applied bio-organic fertilizer every 15 days to ensure sufficient sunlight and nutrients to allow the plants growing healthy. There were four treatment groups in the two species: *C. lacryma-jobi* planted in perforated pots as dry land group (CL-D), *C. lacryma-jobi* planted in non-porous pots as flooded land group (CL-F), *C. aquatica* planted in perforated pots as dry land group (CA-D), *C. aquatica* planted in non-porous pots as flooded land group (CA-F). *Coix* entered the vigorous vegetative growth period in June after transplanting, August was the blooming period, and October was the yellow ripening period of the fruit. Therefore, these three months were selected for subsequent experiments to determine the indicators.

Determination of daily variation in gas exchanges: The sunny weather were selected in June, August, and October respectively, and measured leaves every 1.5 hour from 7:30 am to 18:00 pm, used Li-6400 portable photosynthetic analyzer (Li-COR, USA). Ten healthy leaves of *C. lacryma-jobi* and *C. aquatica* were selected randomly from each group. Natural conditions such as light and CO_2 were used, and each material measurement was completed within 15 minutes. The net photosynthetic rate (P_N, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (G_s, mmol m^{-2} s^{-1}), and intercellular CO_2 concentration (C_i, μmol m^{-2} s^{-1}) were recorded.

Determination of P_N and light response curve: The plants were induced under natural light for 2 hours, and then the light response curve was measured by Li-6400 portable photosynthetic analyzer from 9 to 11 am. Ten leaves from each group were determined. CO_2 concentration and temperature under natural conditions was used. Gradient set the photosynthetically active radiation (PAR) to 2100, 1700, 1300, 1100, 900, 700, 500, 300, 150, 100, 50, 20, and 0 μmol m^{-2} s^{-1}, which generated by the red and blue light source equipped with the instrument, and automatically recorded parameters such as net photosynthetic rate. The measured data was imported into computer, and the light response curve parameters such as Rd (Dark breathing rate), AQY (apparent quantum efficiency), LCP (light compensation point), LSP (leaf light saturation point) and $P_{N\ max}$ (light-saturated net photosynthetic rate) were fitted through the light response curve fitting software (v1.0 green version).

Determination of chlorophyll fluorescence parameters: The Li-6400 portable photosynthetic analyzer and its supporting fluorescence measuring chamber were used to measure the chlorophyll fluorescence parameters of the leaves, and ten leaves of each treatment group were measured. Wrap the leaves with tin foil for dark adaptation for 30 minutes. Weakened modulated measurement light to measure primary fluorescence (Fo), plus saturated intense light pulses to measure maximum fluorescence (Fm) in the dark. Subsequently, the plants were exposed to light for 30 min, and the fluorescence parameters, such as Fs, Fo', Fm', and Fv'/Fm' were measured. Calculate parameters according to the formula: photochemical quenching coefficient (q_P) = (Fm'-Fs)/(Fm'-Fo'); photochemical quenching coefficient (q_N) = (Fm-Fm')/(Fm-Fo'); actual Photochemical efficiency ($\Phi_{PS\,II}$) = (Fm'-Fs)/Fm'; electron transfer rate (ETR) = $\Phi_{PS\,II}$×0.5×0.84×1000.

Determination of chlorophyll content: Healthy leaves were picked randomly, the main vein of leaves was removed and cut, and 0.2 g of fresh leaves was weighed, grounded with 95% ethanol to homogenize, centrifuge, then the supernatant to 25 mL volume with 95% ethanol was taken. Determined the light absorption of the solution at 665 nm, 649 nm, and 470 nm, and calculated the chlorophyll content. Chloroplast pigment content (mg/g FW) = (C×V×N)/m ×1000, where: C was the pigment content (mg/L), V was the volume of the extract (ml), and N was the dilution factor, m was the sample mass (g), 1000 was 1 L = 1000 mL. Each treatment was repeated in triplicate.

Determination of root vigor: Root vigor was measured by TTC method at a wavelength of 485 nm. Determined the reduction amount of TTC from the standard curve. Root vitality = C/(1000*W*h) [mg(g/h)$^{-1}$], where: C was the reduction amount of TTC (mg), and W was the weight of the root (g) , H was time (h). Each treatment was repeated in triplicate.

Observing root microstructure of paraffin sections: Fresh and robust root tips were taken as materials, washed and fixed with FAA fixative for more than 24 hours, and then washed, stained, dehydrated, transparent, immersed in wax, embedded, sliced, and dewaxed, and then observed for coverslip. The apical cross sections were observed and photos were taken with Olympus BX-60 optical microscope (Japan), then PhotoShop 13.0.1 image processing software was used to process and measure data. Ten replicates for each group.

Determination of plant leaf morphology: The grains to be harvested and the relevant morphological indicators to be determined in mid-to-late October, when 80% of them were yellow. Area of all functional leaves on single plant was determined with Li-3000C leaf area meter (Li-COR, USA). The number of tillers and fruits of single plant was counted. The number of angles between the tiller and the vertical line on the ground were measured with protractor. The root of the whole plant was taken, washed and dried until there was no obvious water retention, weighed with an electronic balance, and the fresh weight of the root was recorded. The dry weight of each root was determined after drying the roots to constant weight in a drying box at 45℃. Content of root moisture calculation method: moisture content (%) = [(fresh weight - dry weight) / fresh weight]×100%. Ten plants were determined per group.

Statistical analysis

All data were analyzed with Excel 2010 and SPSS 21.0, and the mean and standard deviation were taken. Duncan's new multple range test was used on the data among four treatment in the same month.

Results

The daily variation of gas exchanges: The net photosynthetic rate (P_N) peak of *C. lacryma-jobi* and *C. aquatica* both appeared at 10:30 am in June (Fig. 1), and there was a "lunch break" phenomenon at 12:00 noon under the influence of strong light and high temperature. P_N of both species under flooded treatment was higher than that under dry treatment. The P_N of *C. lacryma-jobi* was higher than that of *C. aquatica* under drought conditions, especially the P_N of CL-D had a small peak at 13:30 pm, while the P_N of CA-D failed to rise. The P_N of *C. lacryma-jobi* and *C. aquatica* under flooded culture were higher than those under dry culture in August with high temperature and little rainfall. The daily variation of P_N of the two species were very close under flooded conditions. But the daily variation of P_N of *C. lacryma-jobi* were higher than those of *C. aquatica* under dry conditions. The daily variation of P_N all showed a single-peak curve in October, which was low overall.

The transpiration rate (E) in flooded culture was higher than that in dry culture, especially in the CL-F group, which the E was higher than other groups in June and August. E could maintain high level under flooded culture in August, and E of *C. lacryma-jobi* was higher than that of *C. aquatica* under dry culture. The diurnal variation curves of E of the four groups were very close in October, and the peaks all appeared at 10:30 AM. The diurnal variation curves of G_s were similar with P_N and E. The daily variation of sub-stomatal CO_2 concentration (C_i) of the four groups had little change, and only increased at 18:00 pm in October. P_N of four groups were significantly and positively correlated with E and G_s in October by correlation analysis, while P_N were not significantly correlated with C_i (Table 1).

Comparison of P_N and light response curve: P_N increased rapidly with the increase of photosynthetically active radiation (PAR) when the PAR was less then 500 μmol (photon) m^{-2} s^{-1}, and then the increase of P_N gradually slowed down to no more (Fig. 2). The light response curve of the CA-D group was the lowest overall, and the processes of other three groups were highly overlapping in June. The light response curves of the two treatment groups of *C. lacryma-jobi* were higher than those of *C. aquatica* in August. Compared with the same species, the flooded treatment group was higher than the dry treatment group. There was little difference in the light response curves of the four groups in October. The P_N curve of the *C. aquatica* was the highest under flooded treatment, but that under the dry treatment was the lowest. The fitting parameters by Photosynthesis.exe software showed that, CA-D had the lowest apparent electron efficiency (AQY) in June, which was only 0.027 μmol (CO_2) m^{-2} s^{-1}, and the maximum photosynthetic rate was only 17.92 μmol (CO_2) m^{-2} s^{-1}, which was significantly lower than the other three groups (Table 2). The same pattern appeared in August and October. Except for August, there was no significant difference in the maximum net photosynthetic rate ($P_{N\ max}$) of *C. lacryma-jobi* by fitting the salamanders under flooded conditions and dry conditions.

Comparison of chlorophyll fluorescence parameters: Fv/Fm is the maximum light energy conversion efficiency when the PSII reaction center is fully open. A higher value indicate that it is less likely to cause photoinhibition, and a lower value indicate a higher degree of photoinhibition. There was no significant difference in the Fv/Fm of the four groups in different months (Table 3). q_N is a non-photochemical quenching coefficient, reflecting the part of light energy that is not used for the transfer of photosynthetic electrons and is dissipated in the form of thermal energy. The q_N value of *C. aquatica* was higher than that of *C. lacryma-jobi* in June and August. The q_N value of *C. aquatica* under dry conditions was significantly higher than that under flooded condition in October. While there was no significant difference under flood and drought cultivation of *C. lacryma-jobi*. The photosynthetic electron transfer rate (ETR) represent the apparent electron transfer efficiency under actual light intensity conditions, and reflect the electron capture efficiency of the PSII reaction center. The ETR of the two species under flooded treatment were significantly higher than those under dry treatment in June and August. The ETR of CL-D was the highest and CA-D was the lowest in October, and their difference reached a significant level.

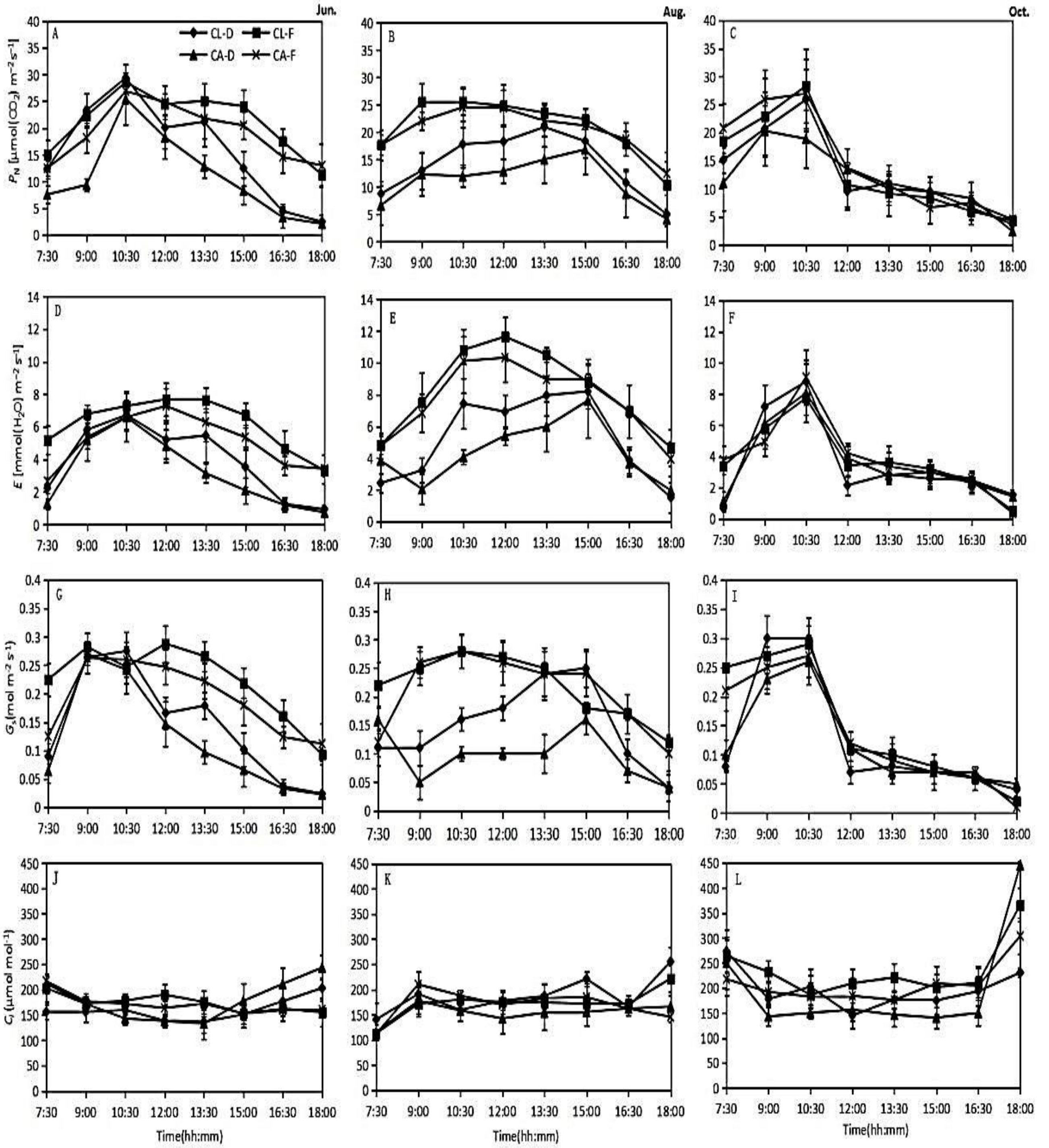

Fig. 1. The daily variation of gas exchanges of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation
Error bars reflect the standard deviation of biological duplicates (n=9).
A. was daily variation of P_N (net photosynthetic rate) in Jun.; **B.** was daily variation of P_N in Aug.; **C.** was daily variation of P_N in Oct.; **D.** was daily variation of E(transpiration rate) in Jun.; **E.** was daily variation of E in Aug.; **F.** was daily variation of E in Oct.; **G.** was daily variation of G_s(stomatal conductance) in Jun.; **H.** was daily variation of G_s in Aug.; **I.** was daily variation of G_s in Oct.; **J.** was daily variation of C_i(sub-stomatal CO_2 concentration) in Jun.; **K.** was daily variation of C_i in Aug.; **L.** was daily variation of C_i in Oct.

Comparison of chlorophyll content: Chlorophyll content in leaves is an important indicator of plant photosynthetic capacity. In general, higher chlorophyll content is more beneficial for plants to capture more light energy, which is used by photosynthesis. The chlorophyll content of two species under flood and drought treatments had significant differences (Table 4), according to the Duncan's new multiple range test results. Dry treatment could increase total chlorophyll content of *C. lacryma-jobi* in June. While flooded treatment could increase chlorophyll content of *C. aquatica* during the entire growth period. The total chlorophyll content of the two species was highest in August, of which CL-F was 1.862 mg g^{-1} and CA-F was 2.526 mg g^{-1}. The increase of total chlorophyll content mainly depended on the increase of chlorophyll a, according to the ratio of chlorophyll content.

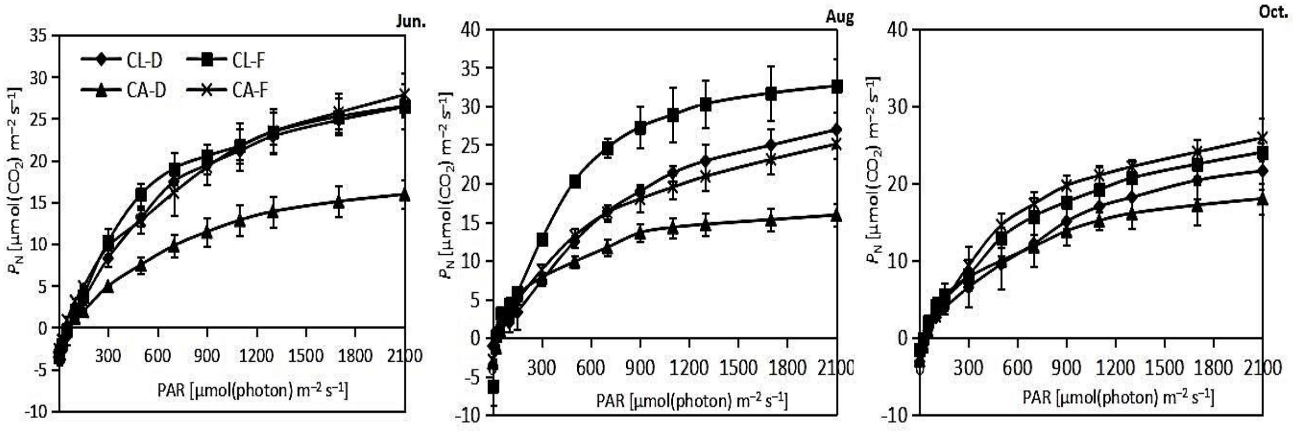

Fig. 2. The P_N (net photosynthetic rate) and light response curve of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month
Error bars reflect the standard deviation of biological duplicates (n=9).
PAR – Photosynthetically active radiation

Table 1. The correlation coefficient between P_N and E, GS, Ci based on diurnal changes in photosynthesis.

Time	Item	*E* [mmol (H_2O) m^{-2} s^{-1}]	G_s (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})
Jun.	CL-D	0.981**	0.972**	-0.687
	CL-F	0.936**	0.778*	0.109
	CA-D	0.880**	0.691	-0.827*
	CA-F	0.958**	0.851**	-0.332
Aug.	CL-D	0.959**	0.924**	-0.209
	CL-F	0.815*	0.883**	-0.252
	CA-D	0.769*	0.376	0.157
	CA-F	0.915**	0.945**	0.636
Oct.	CL-D	0.837**	0.918**	0.043
	CL-F	0.916**	0.977**	-0.381
	CA-D	0.860**	0.916**	-0.659
	CA-F	0.835**	0.992**	-0.486

"**" means significantly correlated at 0.01 level; "*" means significantly correlated at 0.05 level
P_N – net photosynthetic rate; *E* – transpiration rate; G_s – stomatal conductance; C_i – sub-stomatal CO_2 concentration

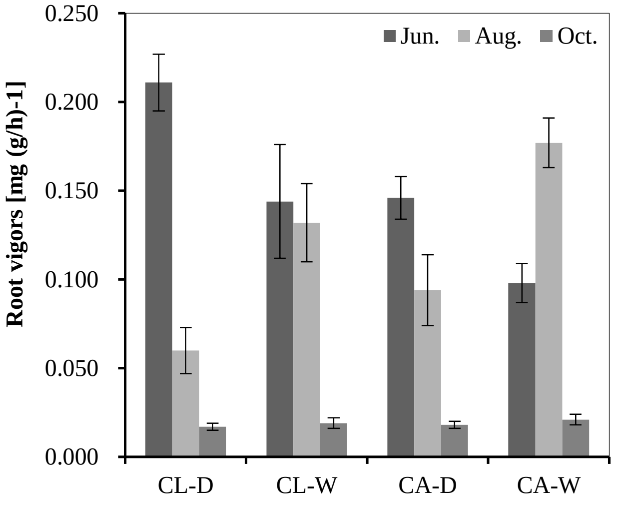

Fig. 3. Comparison on root vigor of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.
Error bars reflect the standard deviation of biological duplicates (n=3).

Comparison of root vigor: Root vigor varied with the period changed (Fig. 3). CL-D had the highest root activity in June, reached to 0.211 mg (g/h) $^{-1}$. The root activity under flooded treatment was higher than that under dry treatment in August, and CA-F had the highest root activity, reached to 0.177 mg (g/h) $^{-1}$. The root activity of the four groups were very low in October, when the *Coix* had entered into mature stage, and the metabolic activity of root was very weak.

Comparison of root microstructure: The roots cross-section structure of *C. lacryma-jobi* and *C. aquatica* is very similar, mainly including epidermis, outer cortex, cortex, inner cortex, marrow and other structures. Parenchyma cells of cortical are lateral grooming pathway for water and solutes from root hairs to vascular columns, and are also the site for storing nutrients and ventilation. Cortical parenchymatous cells accounted for the largest proportion of materials in each group (Fig. 4), and the cortex of *C. lacryma-jobi* was thicker than that of *C. aquatica*. The cortex under flooded cultivation was significantly thicker than under dry cultivation in the same specie, which dissociated air cavities and air passages were common. The roots parenchymal cells of both species were gradually thickened as the time went (Table 5).

Root epidermal cells and outer cortex cells arrange closely can effectively reduce the loss of water in the root, maintain its own water demand, and have mechanical protection. The thickness of epidermis and outer cortex under dry cultivation was greater than that under flooded cultivation in the same species, and the difference in thickness of epidermal reached a significant level. The root epidermis of *C. lacryma-jobi* under flooded cultivation gradually became thinner as the planting time flowed. The vascular bundles number of two species under flooded cultivation was more than that under dry cultivation in June. But as time went, the number of vascular bundles had gradually increased under dry cultivation, while that showed a decreasing trend under flooded cultivation in August, that phenomenon was especially obvious on *C. lacryma-jobi*.

Table 2. Photosynthetic parameters of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.

Time	Item	Rd [µmol (CO_2) m^{-2} s^{-1}]	AQY [µmol (CO_2) m^{-2} s^{-1}]	LCP [µmol (photon) m^{-2} s^{-1}]	LSP [µmol (photon) m^{-2} s^{-1}]	$P_{N\,max}$ [µmol (CO_2) m^{-2} s^{-1}]
Jun.	CL-D	-3.541a	0.047b	75.56b	2006a	29.95a
	CL-F	-3.448a	0.053a	67.20c	1688b	29.12a
	CA-D	-2.622b	0.027d	84.04a	1651b	17.92b
	CA-F	-2.249c	0.041c	62.97c	2070a	29.92a
Aug.	CL-D	-1.083d	0.033c	33.58c	2049a	28.97b
	CL-F	-5.246a	0.059a	12.62d	1851b	35.51a
	CA-D	-3.242b	0.023d	142.8a	1489d	18.75d
	CA-F	-2.854c	0.049b	58.81b	1526c	25.97c
Oct.	CL-D	-1.557c	0.039b	42.02d	1764a	24.60a
	CL-F	-3.312a	0.043a	71.40a	1701a	25.37a
	CA-D	-3.243a	0.047a	63.30b	1218b	19.35b
	CA-F	-2.846b	0.045a	54.57c	1763a	27.16a

Values in table are obtained by Nonlinear Fitting by *SPSS* 21.0

Means followed by the same letter do not differ based on the Duncan's New Multple Range Test at $p \leq 0.05$

Rd – Dark breathing rate; AQY – apparent quantum efficiency; LCP – light compensation point; LSP – leaf light saturation point; $P_{N\,max}$ – light-saturated net photosynthetic rate

Table 3. Comparison on chlorophyll fluorescence parameters of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.

Time	Item	F_v/F_m	F_v/F_0	q_P	q_N	$\Phi_{PS\,II}$	ETR
Jun.	CL-D	0.779±0.009a	3.115±0.101c	0.562±0.015ab	0.663±0.019b	0.217±0.015b	90.89±3.906b
	CL-F	0.777±0.001a	3.481±0.031b	0.572±0.023a	0.738±0.060b	0.256±0.016a	107.4±6.832a
	CA-D	0.784±0.040a	3.555±0.019b	0.507±0.027b	0.833±0.014a	0.213±0.019b	89.24±8.281b
	CA-F	0.790±0.002a	3.730±0.037a	0.553±0.009ab	0.791±0.009a	0.262±0.001a	110.0±0.275a
Aug.	CL-D	0.781±0.004a	3.610±0.074b	0.485±0.006b	0.837±0.016ab	0.201±0.007bc	81.78±1.112b
	CL-F	0.778±0.002a	3.531±0.025b	0.577±0.007a	0.800±0.008b	0.260±0.006a	109.1±2.395a
	CA-D	0.796±0.003a	3.915±0.060a	0.503±0.019b	0.877±0.008a	0.194±0.011c	81.25±4.701b
	CA-F	0.795±0.003a	3.875±0.019ab	0.596±0.033a	0.838±0.028ab	0.240±0.017ab	101.8±7.614a
Oct.	CL-D	0.766±0.004a	3.275±0.032a	0.363±0.036a	0.939±0.030ab	0.106±0.047a	44.58±11.29a
	CL-F	0.737±0.001a	2.819±0.031bc	0.359±0.045a	0.913±0.017ab	0.097±0.012a	40.52±3.949ab
	CA-D	0.730±0.012a	2.711±0.174b	0.234±0.038c	0.964±0.003a	0.074±0.011c	30.88±3.866c
	CA-F	0.721±0.005a	2.609±0.053c	0.264±0.009b	0.872±0.011b	0.086±0.001b	35.98±0.244b

The photon flux density for actinic light (PPFD) is 1000 µmol (photon)m^{-2} s^{-1}

Values in table are presented as mean ± standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multple Range Test at $p \leq 0.05$

F_v/F_m – maximal quantum yield of PSII photochemistry; F_v/F_0 – potential activity of PSII photochemistry; q_P – photochemical quenching coefficient; q_N – nonphotochemical quenching coefficient; Φ_{PSII} – effective quantum yield of PSII photochemistry; ETR – electron transport rate

Table 4. Comparison on chlorophyll content [mg g^{-1} (FM)] of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.

Time	Item	Chl a	Chl b	Chl a/b	Total Chl
Jun.	CL-D	0.828 ± 0.008b	0.380 ± 0.023a	2.189 ± 0.152b	1.207 ± 0.015b
	CL-F	0.641 ± 0.007c	0.263 ± 0.003b	2.435 ± 0.005b	0.904 ± 0.010c
	CA-D	0.579 ± 0.002d	0.252 ± 0.002b	2.302 ± 0.009b	0.831 ± 0.004d
	CA-F	0.970 ± 0.002a	0.352 ± 0.001a	2.755 ± 0.002a	1.321 ± 0.002a
Aug.	CL-D	0.857 ± 0.050c	0.354 ± 0.014c	2.423 ± 0.046c	1.211 ± 0.064c
	CL-F	1.452 ± 0.055ab	0.410 ± 0.011b	3.541 ± 0.039b	1.862 ± 0.067b
	CA-D	1.314 ± 0.194b	0.376 ± 0.067bc	3.516 ± 0.109b	1.689 ± 0.260b
	CA-F	2.013 ± 0.214a	0.514 ± 0.059a	3.924 ± 0.033a	2.526 ± 0.273a
Oct.	CL-D	0.459 ± 0.024d	0.236 ± 0.013c	1.946 ± 0.011c	0.695 ± 0.037d
	CL-F	0.849 ± 0.016a	0.356 ± 0.001a	2.387 ± 0.046a	1.204 ± 0.016a
	CA-D	0.582 ± 0.031c	0.262 ± 0.015bc	2.223 ± 0.003b	0.844 ± 0.046c
	CA-F	0.738 ± 0.022b	0.299 ± 0.008b	2.472 ± 0.014a	1.037 ± 0.029b

Values in table are presented as mean ± standard deviation (n=3)

Means followed by the same letter do not differ based on the Duncan's New Multple Range Test at $p \leq 0.05$

Chl – Chlorophyll; FM – Fresh mass

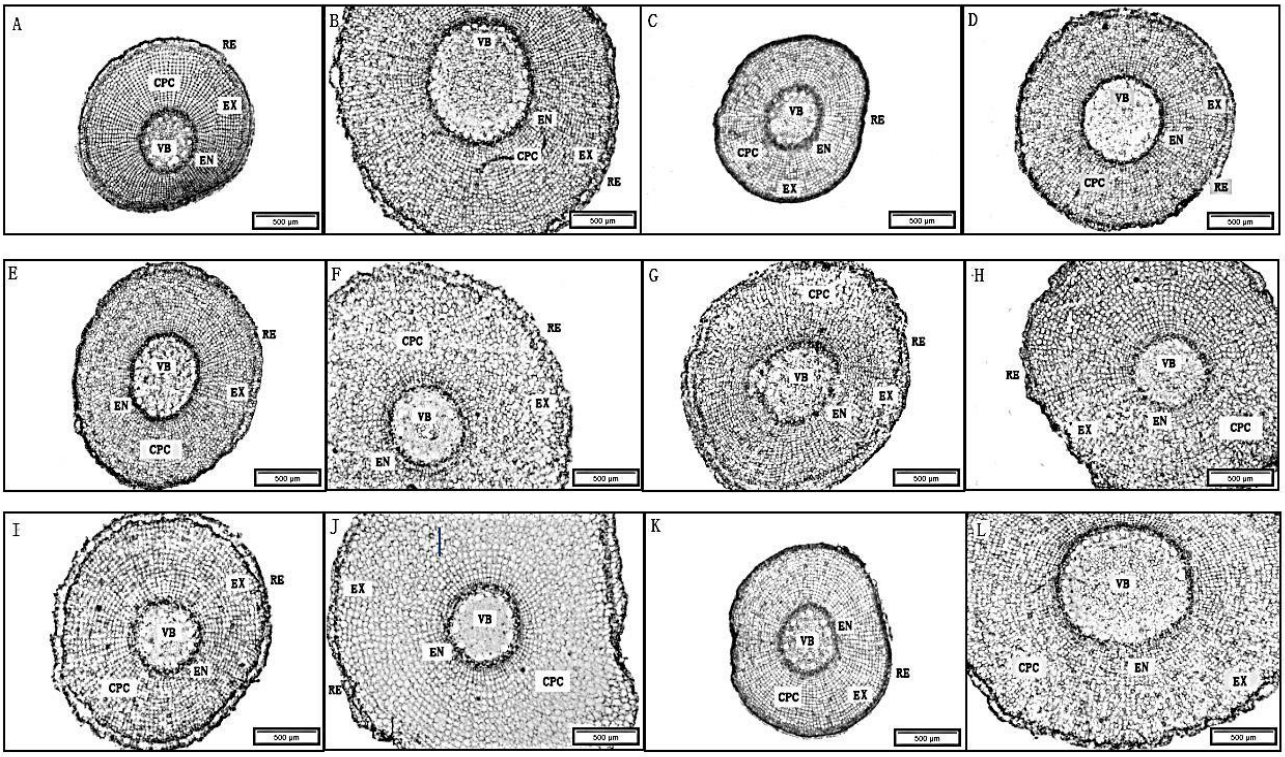

Fig. 4. Root cross-section microstructure of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation
A was root cross-section of *C. lacryma-jobi* under dry land treatment in Jun.; B was root cross-section of *C. lacryma-jobi* under flooded cultivation treatment in Jun.; C was root cross-section of *C. aquatica* under dry land treatment in Jun.; D was root cross-section of *C. aquatica* under flooded cultivation treatment in Jun.; E was root cross-section of *C. lacryma-jobi* under dry land treatment in Aug.; F was root cross-section of *C. lacryma-jobi* under flooded cultivation treatment in Aug.; G was root cross-section of *C. aquatica* under dry land treatment in Aug.; H was root cross-section of *C. aquatica* under flooded cultivation treatment in Aug.; I was root cross-section of *C. lacryma-jobi* under dry land treatment in Oct.; J was root cross-section of *C. lacryma-jobi* under flooded cultivation treatment in Oct.; K was root cross-section of *C. aquatica* under dry land treatment in Oct.; L was root cross-section of *C. aquatica* under flooded cultivation treatment in Oct.
RE – root of epidermis; EX – outer cortex; CPC – cortex; EN – inner cortex; VB – vascular bundle.

Comparison on plant leaf morphology: The leaf area of *C. aquatica* was larger than that of *C. lacryma-jobi*, and the leaf area under flooded cultivation was larger than that of dry cultivation (Table 6). Flooded culture was good for tillering of species, but dry culture could increase the angle of branches. That meant, dry culture could promote the scattered growth of the plants to use more space and capture more sunlight. Flooded culture made the plants root system developed, and under these conditions, the fresh and dry weights of the roots of two species were significantly higher than those of dry culture. Meanwhile, the water content in the roots was also significantly higher than under dry treatment. Water content in root of *C. aquatica* was significantly higher than that of *C. lacryma-jobi* under dry cultivation. Flooded culture could significantly increase the seed rate of both species. The seed rate of *C. lacryma-jobi* was much higher than that of *C. aquatica*, regardless of flood or dry cultivation, and the seed rate of CL-F could reach 417.3 per plant.

Discussion

Photosynthesis of plants can be significantly affected under water stress (Chadha *et al*., 2019). The daily variation of P_N in the four groups were similar in June and October in this experiment, but the P_N of CA-D group was slightly lower than that of other groups. The P_N of the dry culture group (especially the CA-D) was significantly lower than that of the flooded culture in August, and the light response curve showed similar variation. This means that the effects of flooded culture and humid dry culture have a similar effect on their growth and development. But *C. lacryma-jobi* can adapt to terrestrial environment better, and *C. aquatica* have a higher demand for water, which the root system is completely immersed in water just like flooded culture. Plant photosynthesis can be affected by environmental factors such as light intensity, air carbon dioxide concentration, and also by physiological factors such as stomatal conductance and intercellular carbon dioxide concentration. The factors that reduce the photosynthetic rate of different plants under different adversities will also be different (Zhang *et al*., 2010; Lang *et al*., 2018). The value of stomatal conductance (G_s) and the sub-stomatal CO_2 concentration (C_i) can be observed at the same time to judge the factors affecting the photosynthetic rate, that is, the photosynthetic rate is restricted by stomatal or non-stomatal (Flexas & Medrano, 2002). The P_N and G_s in the four groups were all significantly and positively correlated, and the correlation between P_N and C_i was not significant. That means photosynthetic rate was mainly limited by stomatal.

Table 5. Comparison on root cross-section microstructure of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.

Time	Item	Epidermis (μm)	Outer cortex (μm)	Cortex (μm)	Inner cortex (μm)	Number of vascular bundles
Jun.	CL-D	57.48 ± 0.40b	47.54 ± 0.34a	411.43 ± 1.34c	46.33 ± 0.22b	13.42 ± 0.04c
	CL-F	37.19 ± 0.49c	47.49 ± 0.48a	614.43 ± 0.83a	59.95 ± 0.52a	21.55 ± 0.07a
	CA-D	71.61 ± 1.19a	42.29 ± 0.85b	346.83 ± 0.78d	49.09 ± 0.25b	10.94 ± 0.12d
	CA-F	28.08 ± 0.32d	40.27 ± 0.34c	425.62 ± 0.30b	38.19 ± 0.25c	16.70 ± 0.10b
Aug.	CL-D	61.90 ± 0.38b	51.98 ± 0.33a	476.48 ± 1.26b	50.83 ± 0.21a	14.42 ± 0.04a
	CL-F	34.94 ± 0.35c	48.40 ± 0.29bc	684.56 ± 0.51a	53.13 ± 0.25a	9.74 ± 0.07c
	CA-D	71.22 ± 0.56a	43.64 ± 0.44cd	408.59 ± 0.33c	52.53 ± 0.29a	11.25 ± 0.07b
	CA-F	28.66 ± 0.21d	41.15 ± 0.32d	668.40 ± 0.60a	49.61 ± 0.30a	9.97 ± 0.09c
Oct.	CL-D	55.27 ± 0.41b	45.33 ± 0.35a	564.42 ± 1.17c	59.87 ± 0.19a	15.42 ± 0.04a
	CL-F	29.79 ± 0.33c	42.30 ± 0.36ab	799.76 ± 1.05a	60.28 ± 0.26a	10.66 ± 0.08c
	CA-D	67.06 ± 0.56a	39.56 ± 0.43bc	499.29 ± 0.30d	61.48 ± 0.27a	12.25 ± 0.07b
	CA-F	24.21 ± 0.22d	36.72 ± 0.34c	758.89 ± 0.56b	58.55 ± 0.28a	10.96 ± 0.08c

Values in table are presented as mean ± standard deviation (n=9)
Means followed by the same letter do not differ based on the Duncan's New Multple Range Test at $p \leq 0.05$

Table 6. Comparison on plant leaf morphology of *C. lacryma-jobi* and *C. aquatica* under dry land and flooded cultivation in different month.

Item	Leaf area (cm^2)	Fresh weight of roots (g)	Dry weight of roots (g)	Water content of roots (%)	Number of tillers	Angle of branch (°)	Number of fruits
CL-D	64.14 ± 14.30c	109.9 ± 15.54d	52.60 ± 2.980b	51.13 ± 10.12c	8.331 ± 1.532c	8.071 ± 0.503c	362.3 ± 13.42b
CL-F	87.06 ± 15.40b	382.3 ± 65.10a	85.37 ± 8.172a	77.67 ± 5.143a	10.03 ± 2.586ab	5.251 ± 0.314d	417.3 ± 15.71a
CA-D	95.99 ± 14.04ab	132.8 ± 23.52c	48.67 ± 4.837d	63.35 ± 9.252b	9.160 ± 2.353bc	10.50 ± 0.759a	83.00 ± 9.012d
CA-F	104.9 ± 21.94a	247.0 ± 67.06b	63.23 ± 4.261c	74.41 ± 7.376a	10.25 ± 2.651a	9.133 ± 0.726b	231.7 ± 10.55c

Values in table are presented as mean ± standard deviation (n=9).
Means followed by the same letter do not differ based on the Duncan's New Multple Range Test at $p \leq 0.05$.

Chlorophyll fluorescence parameters can reflect a series of important adaptation processes in photosynthetic apparatus. We can get information about the use of light energy by analyzing various fluorescence parameters (Maxwell & Johnson, 2000). These parameters can indicate the absorption, utilization and conversion of light energy during photosynthesis from a microscopic perspective. It contains a wealth of information on changes in photosynthesis processes and is considered as an internal probe of the relationship between plant photosynthesis and the environment (Roháek, 2002; Razavi *et al*., 2008). There was no significant difference in the maximum photochemical efficiency (Fv/Fm) of the four groups in this experiment, indicating that neither flooded cultivation nor dry cultivation would cause significant adverse effects on *C. lacryma-jobi* and *C. aquatica*. The values of Fv/Fm and potential photosynthetic capacity (Fv/Fo) in the same group showed an upward trend and then a downward trend in different months. That was, the values of Fv/Fm and Fv/Fo were the highest in August, indicating that the two species had the strongest capacity of potential photosynthetic in August. The photochemical quenching (q_P) and apparent electron transfer rate (ETR) of both species showed a decreasing trend as time flowed under dry cultivation. The ETR of *C. aquatica* dropped significantly especially in October. The CA-D group of *C. aquatica* was significantly lower than other groups. These results indicate that dry cultivation will affect the ability of the two species to convert light energy into chemical energy and electron transfer in the PSII reaction center, and the adverse effects on the electron transport capacity of *C. aquatica* under dry cultivation are greater.

As an important material for photosynthesis, Chlorophyll is an important biochemical parameter reflecting the growth stage and nutritional status of plants, and is also an important indicator for studying the stress conditions of plants (Hong *et al*., 2019). Chlorophyll content in new leaves is low, and the content increases with the development of the leaves, at last the content will gradually decrease as the leaves entering the senescence stage (Shi *et al*., 2006). The total chlorophyll content was highest in August when *C. lacryma-jobi* and *C. aquatica* were in vigorously growing by comparison of three months. The chlorophyll content of *C. aquatica* under dry cultivation was significantly lower than that of flooded cultivation during the same period. But the chlorophyll content of *C. lacryma-jobi* under dry cultivation was higher than that under flooded cultivation in June. This shows that sufficient water should need throughout the growing period on *C. aquatica*, and proper droughts are more favorable for chlorophyll synthesis at the seedling stage on *C. lacryma-jobi*.

Root vitality is a physiological indicator that objectively reflects activity of root life (Wu *et al*., 2017). The root vigor of both species under dry cultivation was higher than that of flooded cultivation in June. But the root vigor under flooded cultivation was higher than that of dry cultivation in August, that reflected the greater demand for water in August of the two species. Plants of the two species were in senescence in October and the root vigor was very low. Water-tolerant plants will form a large amount of aeration tissue under flooding conditions, which facilitates the transport of oxygen required for aerobic respiration in the root system (Tang *et al*., 2008). In this experiment, the root systems of both species could form aeration structures in the cortex of parenchyma cells over time that was the performance to adapt to the flooded environment of *Coix*. The contrast here was, the thickness of the epidermis and outer cortex which played a protective and mechanical support role became thicker under dry cultivation, and the number of grooming vascular bundles also increased. It shows that both species can adapt to the dry cultivation on morphology structure. That is similar to the change in phenotype of some drought-tolerant plants such as *Ziziphus jujuba* (Zhu *et al*., 2018). It is found that *C. lacryma-jobi* shows stronger adaptability in morphological under dry cultivation by comparing the specific data of the two species. The plant leaf morphology and the yield of the experimental material can also be known, that sufficient water can increase leaf area and tiller number, and the fruiting rate per plant will increase significantly. The dry cultivation can effectively expand the angles of tillering, and the plant leaf morphology can make full use of space, which is beneficial for collecting light.

Conclusions

The *C. lacryma-jobi* and *C. aquatica* can maintain high photosynthetic rate, photochemical conversion ability, and have higher chlorophyll content, root vigor, and form a lot of aeration tissue, increase leaf area under flooded culture. The performance of two species from photosynthetic physiology to morphological structure under flooded cultivation is better than that under dry cultivation, indicating that the two species are more suitable for a water-rich environment. It can also be seen from the comparison of the experimental results that the adaptability of *C. lacryma-jobi* to dry land is stronger than that of *C. aquatica*. This shows that the pace of *C. aquatica* has slowed down during the evolution process. After adapting to the terrestrial environment successfully, *C. lacryma-jobi* changes more in the morphological structure and physiological functions. However, sufficient water supply should still be ensured during artificial planting of *C. lacryma-jobi*, and paddy field planting mode should be adopted as much as possible especially in the vigorous summer.

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