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Characteristics of physiology, phenology, and drought susceptibility index of two varieties of Job's tears under water deficit stress

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Abstract. *Wicaksono FY, Sinniah UR, Ruminta, Sumadi, Nurmala T. 2021. Characteristics of physiology, phenology, and drought susceptibility index of two varieties of job's tears under water deficit stress. Biodiversitas 23: 381-387.* Job's tears or hanjeli (*Coix lacryma-jobi* L.) is a functional food crop with less popularly cultivated and longer life cycle than paddy and maize, so that it is potentially exposed to drought stress in the dry season in tropical areas. This study aims to evaluate the physiology, phenology, and drought susceptibility index lack of both Job's tears varieties under water deficit stress. The study was conducted in quantitative method, by using 2 populations of *ma-yuen* and *stenocarpa* varieties which were treated with two levels of watering treatment during the vegetative phase (6 to 12 weeks after sowing), i.e. every day watering and every three days watering. Observations were made on physiological traits, phenology, and drought susceptibility index. The results showed that water deficit decreased stomatal conductance and chlorophyll content, but it could increase electrolyte leakage and proline content. Increasing proline content had a significant correlation with crop yields improvement. Flowering time was prolonged as the effect of drought stress in both varieties. Based on drought susceptibility index, *stenocarpa* was more tolerant than *ma-yuen*. However, some farmers might still cultivate the *ma-yuen* due to the soft seed shell character, therefore, this study finding could be used as a basis for developing further plant irrigation techniques for sensitive variety amid a prolonged dry season.

Keywords: Climate change, drought stress, functional food, hanjeli, *ma-yuen*, *stenocarpa*

INTRODUCTION

Job's tears (*Coix lacryma-jobi* L.) is a tropical crop that has potential to be used as functional food crop due to its nutrients rich seed (Grubben et al. 1996; Tensiska 2017). Job's tears leaf extracts are reported to have pharmacological activity such as antimicrobial effects, anti-cancer, and lowering cholesterol (Ragasa et al. 2014). This plant is also easy to cultivate, resistant to pests and diseases (Nurmala and Irwan 2007). Job's tears varieties that are commonly cultivated in Indonesia are *stenocarpa* and *ma-yuen* (Iamsupasit 1996). Both varieties can be distinguished from the seed character. The *stenocarpa* variety has hard seed shell so that popularly used as ornaments rather than food, while *ma-yuen* variety has soft seed shell and is generally used as cereals (Xi et al. 2016; Gupta et al. 2018). The harvest time of both varieties is longer than paddy and maize, i.e., ranging from 5 to 7 months (Nurmala and Irwan 2007; Liao et al. 2019).

In general, Job's tears have wide adaptation to various environmental conditions, so that it is potentially developed as functional food crop amid global warming (Nurmala and Irwan 2007). Global warming that occurred in the past decade has resulted in weather anomalies and shifting seasons (Abiodun et al. 2013; Mishra and Liu 2014). About 70% of the decline in yields worldwide is due to drought as a result of climate change, and substantial yield reductions are expected among major cereals (Nawiri et al. 2017). Previous studies have reported the longer dry season and the shorter rainy season leading to water shortage in some areas in tropic (Nur'utami and Hidayat 2016; Supari et al. 2018). The long harvest life of Job's tears may increase the occurrence of drought exposure during the extended duration of dry season. An earlier study reported that Job's tears require at least 4 wet months (based on the Oldeman agro-climatic zone) to obtain the maximum yield (Ruminta et al. 2017; Wicaksono et al. 2021), therefore the shorter wet month may damage the crop yield.

Drought stress induces plant physiological disruption, such as oxidative stress occurrence, increasing electrolyte leakage in leaves, damaged chloroplasts, reduced chlorophyll content and stomatal conductance leading to the drop of assimilation rate (Ahmadizadeh et al. 2011; Demidchik et al. 2014). To deal with oxidative stress that occurred during drought stress conditions, plants produce proline to counter generated reactive oxygen species (ROS) and also accelerate the recovery process (Hayat et al. 2012; Ali et al. 2013).

Drought stress is also previously reported to cause phenological changes both in vegetative and reproductive phases (Morales et al. 2013; Li et al. 2017). Plants reacted to drought stress by accelerating their life cycle, however, several studies on cereals have also reported the delays in flower initiation as the effect of drought stress (Mattioli et al. 2009; Mattioli et al. 2020).

The disruption of plant physiological and phenological conditions may influence the final crop yield. In general, there is a yield drop under water deficit stress. Drought susceptibility index is important variable to know the yield reduction effect of drought stress (Widyastuti et al. 2016). However, studies on the physiology, phenology and drought susceptibility index of Job's tears are still limited. Therefore, present study aimed to evaluate the physiology, phenology, and drought susceptibility index lack of both Job's tears varieties under water deficit stress.

MATERIALS AND METHODS

Study area

This research was carried out from April to October 2018 (dry season) at the Ciparanje experimental field (750 m above sea level), Sumedang, Indonesia. Growing location was categorized as C3 climate type (Oldeman rainfall classification), with an average temperature of about 23.8ºC (Wicaksono et al. 2021). The type of soil in growing location was inceptisol with a soil pH of about 5.83.

Procedure

The research used quantitative methods without an experimental design. The study used 2 populations of *ma-yuen* and *stenocarpa* varieties which were treated with two levels of watering treatment during the vegetative phase (6 to 12 weeks after sowing), i.e. every day watering and every three days watering. Each population has consisted of 300 plants. Irrigated water was equivalent to 57.6 L per plant. Water was given using sprinklers with a discharge of 0.19 m^3 $hour^{-1}$ within 2 hours (Wicaksono et al. 2021). To maintain the accuracy of watering, electrode sensors were installed which were read by the Gardsense soil moisture meter at soil depth of 20 cm. Treatment of everyday watering caused the formation of field capacity condition, while another treatment, every three days watering, caused the drop of soil moisture content and available water percentage and even results to be at a permanent wilting point on third day (Table 1).

Job's tears plants were planted with a spacing of 40 x 60 cm for *ma-yuen* and 80 x 60 cm for *stenocarpa*. The maintenance carried out were: NPK (15-15-15) fertilizer application at a dose of 200 kg ha^{-1} and hand weeding at 15 and 30 days after sowing. Harvesting was done when the seeds reached their physiological maturity, as indicated by the plants starting to dry out, the seeds were pithy, shiny white, and the maximum seed moisture content was 25%. Observations were made on physiological traits (chlorophyll content index, electrolyte leakage, stomatal conductance, and proline content), phenology (days to form unproductive tillers and flowering time), and drought susceptibility index.

Measured variables

Chlorophyll content index

The chlorophyll content index was measured using a SPAD chlorophyll meter in the middle of the fully developed and opened leaf (Ling et al. 2011). Leaf samples for physiological observations were taken on the 4^{th} to 6^{th} position from the apical bud.

Electrolyte leakage

Observation of electrolyte leakage was followed Thalhammer et al. (2014) with a slight modification. The 5^{th} leaves were rinsed with distilled water and then put into a jar containing 200 mL of water and incubated at 25ºC on a rotary shaker for 24 hours. The electrodes of the EC meter were then inserted into the solution to measure the electro-conductivity (L1). The sample was then heated in autoclave at a temperature of 120ºC for 20 minutes. Then, the electro-conductivity of the solution was measured by using an EC meter (L2). The electrolyte leakage (EL) was expressed in % and calculated by following question: EL = (L1/L2) x 100%.

Stomatal conductance

Stomatal conductance (SC) was measured by leaf porometer in the 5^{th} leaf (Toro et al. 2019).

Proline content

The proline content was measured by following Bates et al. (1973). Approximately 0.25 g leaves were crushed and extracted with 20 mL of 3% sulfosalicylic acid. Then 2 mL of ninhydrin solution and 2 mL of glacial acetic acid were added and then heated at 100ºC for one hour. After that, 4 mL of toluene was added and the chromophore was measured by using a spectrophotometer at a wavelength of 520 nm.

Days to form unproductive tiller

Days to form unproductive tillers were counted manually. Job's tears tillers were labeled after their emergence and then observed day by day whether these tillers formed panicles or not. This variable was expressed weeks after sowing.

Flowering time

The mean flowering time was the average number of weeks needed by plant to bloom flower and it was expressed in weeks after sowing. The median flowering time was determined when 50% of plants began to flower and also expressed in weeks after sowing (WAP).

Table 1. Results of soil water content analysis

Frequency of watering	Moisture content^a (%)	Moisture content in (%)		Available water^b (%)
		pF 2.54	pF 4.2	
Everyday watering	44.95	37.7	23.65	14.05
Every 3 days watering	19.8	28.9	21.5	7.4

Note: ^{a} is Water content based on laboratory analysis, ^{b} is the difference between water content in pF 2.54 and pF 4.2

Drought susceptibility index

The harvested seeds were dried to reach moisture content of 12%, then weighed using an analytic balance. Drought susceptibility index was calculated based on seed weight per plant (Widyastuti et al. 2016), with the following formula:

$$DSI = (1-Yp/Y)/(1-Xp/X)$$

Where: Yp is average seed weight per plant of a variety under water deficit stress condition, Y is average seed weight per plant of a variety in normal irrigated condition, Xp is average seed weight per plant for all varieties under water deficit stress condition, and X is average seed weight per plant for all varieties in normal irrigated condition.

Data analysis

Differences in each treatment were tested using the student's T-test at 5% significance level which had previously been tested for analysis of variance (Kim 2015), except median of flowering time and drought susceptibility index. The regression test on continuous data was carried out using the Chow test at 5% significance level (Lo and Newey 1985). Analysis of correlation was performed using SPSS v.20.0 software (IBM Corp 2011).

RESULTS AND DISCUSSION

Physiological characters

The result showed that the frequency of watering during vegetative phase affected the physiology of Job's tears (Table 2). The chlorophyll content index and stomatal conductance decreased significantly at the water deficit condition (every 3 days watering) compared to the normal irrigated condition (everyday watering). Water stress resulted the reduction of (i) chlorophyll content index by 15.9% for *ma-yuen* and 13.5% for *stenocarpa*; and (ii) stomatal conductance by 39.4% for *ma-yuen* and 65.5% for *stenocarpa*. The opposite result was found in variables of electrolyte leakage and proline content. Water deficit condition caused significant improvement of (i) electrolyte leakage by 81.5% for *ma-yuen* and 153.3% *stenocarpa*; and (ii) proline content by 35.8% for *ma-yuen* and 79.5% for *stenocarpa*. Underwater deficit conditions, *ma-yuen* variety showed a significantly lower EL, CCI and proline but higher SC than *stenocarpa*.

Phenological characters

The result of regression analysis of the percentage of unproductive tillers was described as the linear regression model (Figure 1). Chow test at 5% significance level revealed that the linear regression model for water deficit treatment had different slope from those for sufficient ones. It means that the pattern of unproductive tillers production under water deficit was different from sufficient water.

Flowering time of *ma-yuen* variety was longer in treatment of water deficit than normal irrigated condition. In opposite, the flowering time of *stenocarpa* variety was not significantly different from the effect of watering levels (Table 3). However, *ma-yuen* variety showed a significantly faster flowering time than *stenocarpa* both underwater sufficient and deficit condition. In addition, the median flowering time (50% of plants began to flower) at both varieties was longer in water deficit conditions rather than normal irrigated ones.

Table 2. Job's tears physiological condition under different watering treatment during vegetative stage

Watering treatments	Variety	
	Ma-yuen	***Stenocarpa***
Electrolyte leakage (%)		
Everyday watering	6.428 ± 1.783 a A	9.577 ± 2.432 a B
Every 3 days watering	11.665 ± 1.994 b A	24.236 ± 4.333 b B
Chlorophyll content index		
Everyday watering	34.680 ± 4.173 b A	41.444 ± 4.791 b B
Every 3 days watering	29.179 ± 3.022 a A	35.850 ± 4.262 a B
Stomatal conductance (mmol m^{-2} s^{-1})		
Everyday watering	414.425 ± 93.658 b A	488.035 ± 108.332 b B
Every 3 days watering	251.245 ± 62.198 a B	168.495 ± 26.755 a A
Proline content (μmol g^{-1})		
Everyday watering	2.562 ± 0.440 a A	4.205 ± 0.704 a B
Every 3 days watering	3.447 ± 0.658 b A	7.547 ± 0.652 b B

Note: the different lowercase letters in the same column that followed the mean and standard deviation showed significant differences according to the student's t-test at 5% significance level. The different uppercase letters below the mean in the same row showed significant difference according to the student's t-test at 5% significance level

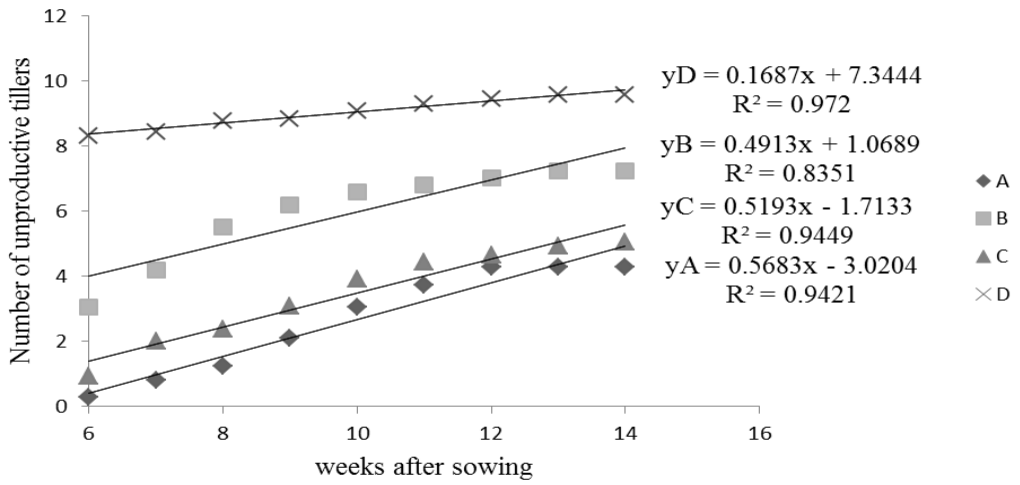

Figure 1. Graph of the percentage of unproductive tillers after watering treatment. Note: Line A intersects with Line B, while Line C intersects with Line D according to the Chow Test at 5% significance level. The 'A' is everyday watering treatment for the *ma-yuen* variety, the 'B' is every 3 days watering treatment for the *ma-yuen* variety, the 'C' is everyday watering treatment for the *stenocarpa* variety, and 'D' is every 3 days watering treatment for the *stenocarpa* variety

Table 3. Job's tears flowering time (weeks after sowing) under different watering treatment during vegetative stage

Watering treatments	Variety	
	Ma-yuen	***Stenocarpa***
Mean of flowering time		
Everyday watering	12.37 ± 0.85 a A	14.43 ± 1.25 a B
Every 3 days watering	13.07 ± 1.57 b A	15.13 ± 2.01 a B
Median of flowering time		
Everyday watering	12.0	14.5
Every 3 days watering	14.0	16.0

Note: the different lowercase letters in the same column that followed the mean and standard deviation showed significant differences according to the student's t-test at 5% significance level. The different uppercase letters below the mean in the same row showed significant difference according to the student's t-test at 5% significance level

Drought susceptibility index

The drought susceptibility index (DSI) of both varieties was calculated based on crop yield, thus the result of DSI might be highly associated with the crop yield obtained. In general, the crop yield of both varieties was significantly reduced as the effect of water deficit condition resulted by every 3 days watering treatment (Table 4). Underwater deficit condition, the reduction of crop yield in *ma yuen* was higher than those observed in *stenocarpa*, i.e., 51% and 38%, respectively. The higher crop yield under stressed conditions, the lower its DSI. The DSI of *ma yuen* was significantly higher than *stenocarpa*. In similar to DSI, leaf morphological characteristics of *ma yuen* and *stenocarpa* also changed in response to water stress, i.e., normal open leaf in water sufficient condition of both *ma yuen* (Figure 2.A) and *stenocarpa* (Figure 2.B), folded leaf in stressed *ma yuen* (Figure 2.C), rolled leaf in stresses *stenocarpa* (Figure 2.D).

There were significant correlations between physiological traits and yield (Table 5). Electrolyte leakage had significant negative correlation with chlorophyll content index, stomatal conductance, and crop yield, but it showed positive and significant correlation with proline content.

Discussion

The lack of irrigation as indicated by watering every 3 days could form water deficit condition. Numerous studies have been reported the negative effect of water deficit for plant by damaging the integrity and permeability of cell membranes leading to the membrane failure to regulate ion transport and then eventually causing electrolyte leakage (Ahmadizadeh et al. 2011; de Faria et al. 2013; Demidchik et al. 2014). In similar to that argument, present finding also highlighted the increasing electrolyte leakage in Job's tears under water deficit conditions was significantly and negatively correlated to the crop yield (Table 5).

Figure 2. The leaves characteristic of *ma-yuen* (A) and *stenocarpa* (B) underwater sufficient condition; *ma-yuen* (C) and *stenocarpa* (D) under water deficient condition at a week after treatment

Table 4. Crop yield (g) and drought susceptibility index of Job's tears under different watering treatment during vegetative stage

Watering treatments	Variety	
	Ma-yuen	***Stenocarpa***
Crop yield (g)		
Everyday watering	333.30 ± 65.52 b A	521.01 ± 107.12 b B
Every 3 days watering	164.45 ± 37.25 a A	320.50 ± 73.68 a B
Drought Susceptibility Index	0.81 ± 0.20 A	0.57 ± 0.38 B

Note: the different lowercase letter in the same column that followed the mean and standard deviation showed significant difference according to the students' t-test at 5% significance level. The different uppercase letter below the mean in the same row showed significant difference according to the students' t-test at 5% significance level

Table 5. The result of correlation analysis of physiological traits and crop yield of Job's tears under different watering treatments during vegetative stage

Variables	EL	CCI	SC	Proline
CCI	-0.562*			
SC	-0.559*	0.405*		
Proline	0.788*	-0.401*	-0.542*	
Yield	-0.781*	0.488*	0.691*	-0.658*

Note: * is significant according to the F test at 5% significance level, EL – electrolyte leakage, CCI – chlorophyll content index, SC – stomatal conductance

In addition, water deficit stress could also stimulate the stomatal closure, leading to the drop of stomatal conductance (de Faria et al. 2013; Li et al. 2017). The response of stomatal conductance might vary in response to genetic factors. The lower stomatal conductance observed in the *stenocarpa* variety rather than *ma-yuen*, implied that more stomatal closure in *stenocarpa* variety. Present finding also reported the negative correlation between stomatal conductance as the effect of water deficit stress to crop yield of both varieties of Job's tears (Table 5). The higher stomatal conductance, the more stomatal closure, the severe water deficit level. This strategy is adopted by plant in order to reduce the transpiration but in longer time it could also reduce the net assimilation rate and eventually crop yield. However, the reduction level of assimilation rate and crop yield might differ in various genotypes. This finding highlighted the success of *stenocarpa* to be able to maintain more final yield than *ma-yuen*.

Leaves pigmentation was also affected by water deficit stress, i.e., lowering chlorophyll content under drought stress (de Souza et al. 2013; Salehi et al. 2016; Miftahudin et al. 2020; Reyes et al. 2020). The reduction of chlorophyll content index (CCI) of both varieties in treatment of every 3 days watering implied the presence of water deficit stress nearby the plant. The CCI is positively and significantly correlated to crop yield, thus the lower CCI caused the lower yield harvested (Table 5). The low chlorophyll content was resulted from the activation of the chlorophyllase enzyme (Batra et al. 2014; Chakhchar et al. 2018). The lack of chlorophyll potentially caused the lowering of photosynthetic capacity (Batra et al. 2014; Kim et al. 2017) leading to the lowering net assimilation rate.

In general, plant with a sufficient assimilation rate could have optimum number of tillers. While water deficit might inhibit the tillers formation (Tirtana et al. 2021). Present study found that water deficit conditions could stimulate the formation of more unproductive tiller rather than normal irrigated conditions, in both varieties. The higher rate of unproductive tillers formation could lead to a decrease in crop yield (Fioreze et al. 2020; Wang et al. 2020).

To adapt to drought stress, plant produced proline, an amino acid to minimize the ROS, adjust cellular osmotic adjustment and accelerate recovery process (Hayat et al. 2012; Ali et al. 2013; Salehi et al. 2016; Ghaffari et al. 2019; Salsinha et al. 2019; Norouzi et al. 2020). The increasing proline content was also as found as adaptation strategy when plant experienced lowering chlorophyll content also associated with the chlorophyll content (Zali and Ehsanzadeh 2018); and increase stomatal closure (Reddy et al. 2015; Tian et al. 2016). The increase of electrolyte leakage would force the plant to boost proline production (Jungklang et al. 2017). Therefore, the proline content in *stenocarpa* was higher than *ma-yuen*. The accumulation of proline in leaves as the effect of drought stress might cause the delay of flowering (Mattioli et al. 2009; Mattioli et al. 2020) and this argument occurred in present study. Interestingly, that delay did not follow by flowering failure, since the plant could bloom flower once they passed the unfavorable environmental condition (Schmalenbach et al. 2014).

All in all, *ma-yuen* variety was more sensitive to water deficit than *stenocarpa* variety (Table 4), although a previous study reported that water requirements of *ma-yuen* were lower than *stenocarpa* (Wicaksono et al. 2021). This finding emphasized that low water requirements did not always follow higher drought tolerance. Proline content seemed to be important variable rather than water requirement for determining Job's tears tolerance to drought stress, since higher proline content of *stenocarpa* resulted in less yield reduction. The drought susceptibility index was also confirmed a similar result, i.e., *stenocarpa* was more tolerant than *ma-yuen*. Flowering time was prolonged as the effect of drought stress in both varieties. However, some farmers might still cultivate the *ma-yuen* due to the soft seed shell character, therefore, this study finding could be used as a basis for developing further plant irrigation techniques amid a prolonged dry season.

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