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RESEARCH ARTICLE

Identification of important morphology for waterlogging tolerance from developed mung bean F_2 population

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ABSTRACT

Waterlogging is severe abiotic stress during mung bean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) production. Waterlogging impacts yield loss for the legume crop by around 40%-70%, depending on the severity and duration of the waterlogging. In total, 107 collected mung bean varieties were evaluated under managed waterlogging. The F_2 population from crossing between waterlogging-tolerant and -susceptible mung bean varieties were screened under waterlogging conditions. Morphological responses to the waterlogging were recorded. Correlation and path coefficient analyses were used to identify essential traits for waterlogging tolerance and seed yield potential under water stress. Mung bean varieties in clusters 5 and 6 were high waterlogging tolerance with survival rates of 66.66% and 61.11%, respectively. Of 199 F_2 lines from crossings between the Kamphaeng Sean2 and W162, 20 lines presented high waterlogging tolerance. The injury score was negatively correlated with the number of leaves at the final date of waterlogging (-0.845) and 1 wk after terminating the waterlogging (-0.885). At the final date of waterlogging condition, the number of leaves and leaf greenness stability index directly affected leaf injury at -0.5928 and -0.4385. One week after waterlogging termination, only the number of leaves affected the leaf injury at -0.8599. The number of leaves directly affected seed yield components at 0.6534 and higher than the direct effect of the other traits. Thus, the number of leaves was a suitable morphological parameter along with the injury score for selecting waterlogging tolerance. The number of leaves was a suitable parameter for selecting high seed yield potential under waterlogging conditions.

Key words: Adventitious root formation, cluster analysis, correlation coefficient, injury score, leaf greenness, path coefficient analysis.

INTRODUCTION

Mung bean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is one of Southeast Asia's essential food legume crops. The legume crop is commonly used in South and Southeast Asia (Nair et al., 2020) because it is a short-duration crop with low water requirement compared with other crops and increases soil fertility. Mung bean has been considered a portion of healthy food that is a rich source of nutrition, especially protein and Fe (Dahiya et al., 2015). The demand for mung bean in Thailand was estimated at 113 000 t in 2019. Around 90% of the total yield of mung bean seeds were used in the country for consumption and in the food industry. The demand for mung bean in Thailand keeps increasing, while the mung bean production is only about 92 000 t. Mung bean productivity in Thailand is not high because of biotic and abiotic stresses. Consequently, increasing mung bean productivity is essential for sustainable cropping systems in the country.

Waterlogging is prolonged soil saturation with water at least 20% higher than the field capacity. It is usually caused by soil compaction, over-irrigation, rainfall occurring after irrigation, and poor surface and internal drainages in the soil, such as in heavy clay soil, claypan soil, or duplex soil (Kaur et al., 2020). Soil waterlogging affects over 20% of the total global irrigated area. Currently, waterlogging impacts around 10%-12% of agricultural soils. If current trends continue, they will negatively impact over 50% of the world's irrigated area by 2050 (Singh, 2021). Waterlogging affects on changing of morphological traits. Crops growing under waterlogged conditions are deficient in or completely lack oxygen which substantially affects plant growth, development, and survival. For mung bean production in Thailand, waterlogging is a significant abiotic stress which negatively affects pod and seed yield (Amin et al., 2016). Following waterlogging of the crop for over a week, mung bean grain yields were reduced by more than 50% of the total yield (Kumar et al., 2013; Shibly et al., 2020).

Four hundred fifteen mung bean accessions and 189 wild mung bean accessions were collected from Asia, Africa, and Australia (Sangiri et al., 2007). The accessions were evaluated, and 107 accessions were selected as a collection for mung bean breeding program. The collected mung bean accessions are available for selecting waterlogging-tolerant mung bean genotypes and use in breeding programs. Physiological and morphological traits can be used as criteria for the selection of waterlogging-tolerant mung bean genotypes. Example of morphological traits using as the parameters for waterlogging tolerance was as follows: Leaf chlorosis, plant height, 100-grain weight, high grain yield, dried leaves per plant, green leaves per the main stem, percentage of aerenchyma, DM production, adventitious root formation, and leaf injury (Ye et al., 2018; Kyu et al., 2021).

The presented study aimed to evaluate the 107-mung bean germplasm under waterlogging conditions and identify mung bean genotypes for waterlogging tolerance. The F_2 population from crossing between a cultivated variety of mung bean and the waterlogging-tolerant were screened under waterlogging conditions to select F_2 lines with high waterlogging tolerance, and the morphological traits which highly impact the waterlogging tolerance of mung bean were identified in the study.

MATERIALS AND METHODS

Plant materials

The mung bean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) samples from 107 varieties used in the study were taken from the mung bean collection. Out of 107 mung bean varieties, 103 were collected from Asian countries, and four were commercial mung bean cultivars in Thailand (KPS2, CN36, CN72, and CN84-1). After evaluation under waterlogging conditions, highly waterlogging-tolerant and -susceptible mung bean varieties were used to develop the F_2 population.

Evaluation of mung bean germplasm under waterlogging conditions

The 107 mung bean varieties were cultivated in plastic pots (diameter pot = 25.4 cm with a volume of 3.5 L) containing soil without added chemical fertilizers. Two seeds were planted in each pot; the more vigorous plant was used as a sample after germination. The planting pots were maintained at a pot spacing of 50 × 50 cm. The experiment was conducted in an experimental field using three replicates. Each replicate consisted of three pots for waterlogging and three pots for the control. At 18 d after emergence, the mung bean samples were subjected to waterlogging conditions for 4 wk, maintaining a water level depth of 1 cm above the soil surface. One week after terminating the waterlogging conditions, all the samples were investigated for morphological change and yield potential. Leaf scores 1-9 were used to describe the highest tolerance and the lowest susceptibility to waterlogging, respectively (Cornelious et al., 2005). The survival rate, plant height, injury score, leaf greenness, and yield per plant were recorded at the final date of waterlogging condition. The collected data for each trait from the mung bean germplasms were subjected to cluster analysis using the R software (R Core Team, 2019) and R Cluster package (Maechler et al., 2022).

Plant height stability index and yield per plant stability index were calculated by the equations (Singh et al., 2018):

$$\text{Plant height stability index} = Hs/Hp.$$

$$\text{Yield per plant stability index} = Ys/Yp.$$

where, Hs is the plant height of each genotype under waterlogging conditions, Hp is the plant height of each genotype under non-waterlogging conditions, Ys is the grain yield of each genotype under waterlogging conditions, and Yp is the grain yield of each genotype under non-waterlogging conditions.

Screening parent and F_2 population for survivability under waterlogged conditions

In total, 199 F_2 plants derived from crossings between 'W162' (waterlogging-tolerant from mung bean germplasm) and 'Kamphaeng Sean 2' (KPS2, waterlogging-susceptible from mung bean germplasm) were screened under waterlogging conditions in a greenhouse. Seeds were germinated in plastic pots (pot diameter 25.4 cm with a volume of 3.5 L) containing soil without adding chemical fertilizers, and one F_2 seed was planted in each pot. The planting pots were maintained at a pot spacing of 50 × 50 cm. After emergence for 18 d, F_2 plants were subjected for 6 wk to waterlogging conditions consisting of the water level maintained at a depth of 1 cm above the soil surface. All the F_2 plants were evaluated for injury scores on a 1-5 scale (1 = green plant with no sign of stress, 2 = 10%-30% chlorosis of whole leaves, 3 = 31%-70% chlorosis of whole leaves, 4 = 71%-99% chlorosis of whole leaves, 5 = dead plants) (Figure 1). First flowering dates of F_2 plants were investigated. The injury score, number of leaves, and leaf greenness were recorded after waterlogging condition terminated and 1 wk after the waterlogging condition terminated. The adventitious root formation score was recorded at the final date of waterlogging condition (Yeboah et al., 2008). The number of pods per plant, seed weight per plant, and 100 seeds weight were recorded after the harvesting (Figure 2).

Leaf greenness stability index was calculated by the equation (Singh et al., 2018):

$$\text{Leaf greenness stability index} = G_A/G_B$$

where, G_A is the leaf greenness of each F_2 plant under waterlogged conditions and G_B is the leaf greenness of each F_2 plant before waterlogging conditions.

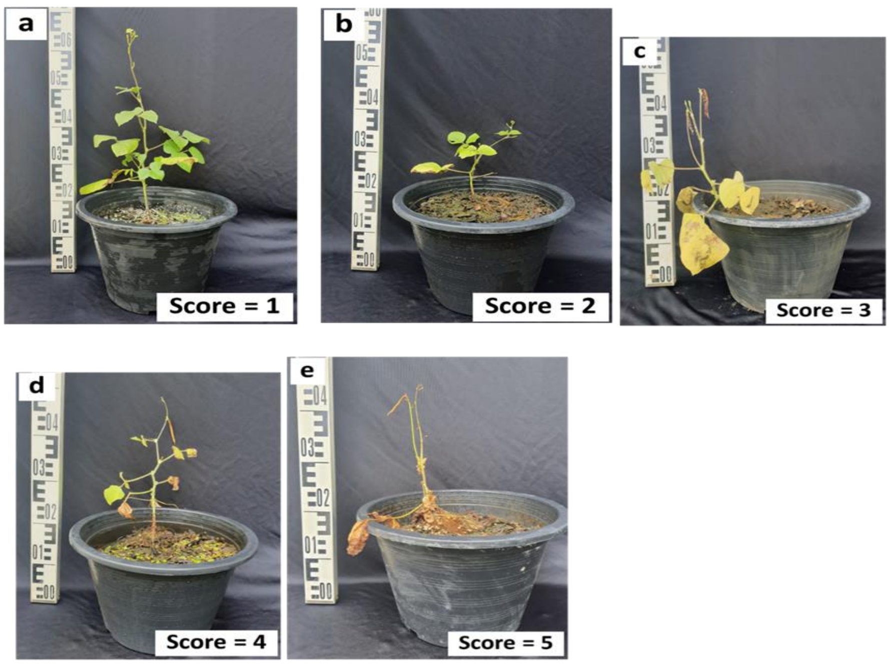

Figure 1. Visual scoring for mung bean leaf injury under waterlogging conditions of the F_2 population. Injury scored were evaluated from 1 (no sign of stress) to 5 (dead plant).

Correlation analysis

The correlation analysis of this study was investigated in two periods. First, correlations were investigated among seed weight per plant, 100 seeds weight, number of pods per plant, first flowering date, leaf injury, number of leaves, leaf greenness stability index, and adventitious root formation from the termination date of waterlogging conditions. Second, the correlations were investigated among seed weight per plant, 100 seed weight, number of pods per plant, leaf injury, number of leaves, and leaf greenness stability index from 1 wk after the termination date of waterlogging conditions. The correlations were calculated according to the standard method.

Path analysis

In the present study, the relative importance of the direct and indirect effects of collected morphological traits and seed yield were determined using path analysis. The path analysis was used to calculate the direct and indirect effects of various traits on injury score and seed yield. For path analysis, injury score and seed yield were set as the dependent variable, and the other traits were considered independent variables. The path analysis was carried out using R Studio version 1.2 1335 (R Core Team, 2019) and the R Agricolae package (Mendiburu, 2021).

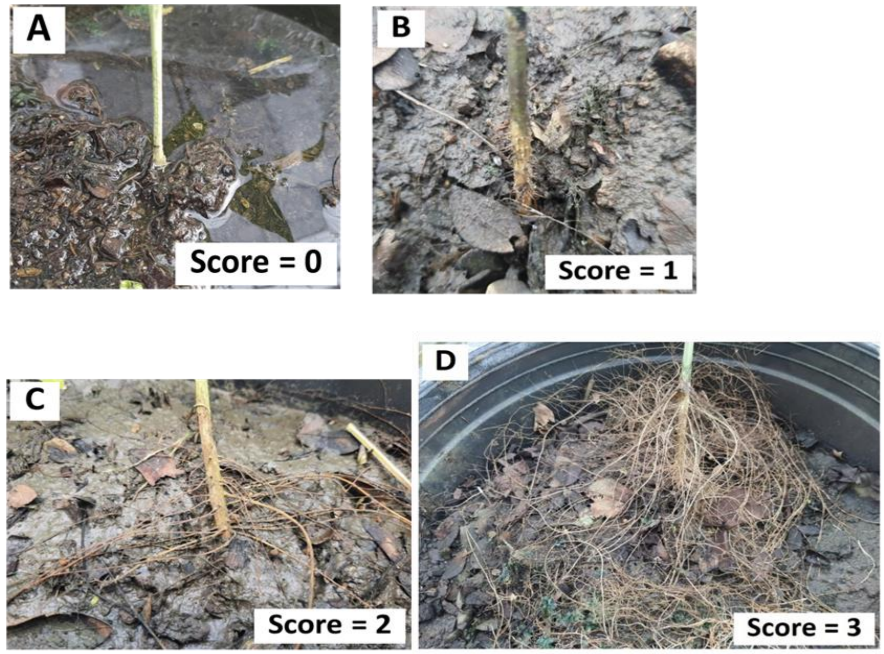

Figure 2. Visual scoring for adventitious root formation under waterlogging conditions of the mung bean F_2 population. Adventitious root formation was scored from 0 (no adventitious roots) to 3 (high numbers of adventitious roots).

RESULTS

Cluster analysis of mung bean varieties for waterlogging tolerance

The 107 mung bean varieties were grouped into 6 clusters based on agronomic traits (germination rate, survival percentage, leaf greenness, injury score, plant height stability index, and yield stability index), as shown in Figure 3. Clusters 1, 2, 3, 4, 5, and 6 consisted of 39, 24, 30, 8, 4, and 2 mung bean varieties, respectively. A list of the mung bean varieties in each cluster is provided as follows. Cluster 1, regarded as a susceptible group, contains cvs. H022, H023, H027, H110, H205, H211, H215, H229, H230, H241, H243, H273, H296, H306, H307, H357, M135, M151, W039, W055, W063, W073, W077, W104, W115, W116, W128, W130, W131, W134, W14,1 W158, W165, W174, W175, W176, W191, W203, and W204. Cluster 2, regarded as a moderately tolerant group, contains cvs. Chai-Nat36, Chai-Nat84-1, H090, H095, H150, H152, H192, H219, H242, H250, H262, H274, H280, H370, H384, H412, H417, M146, W001, W058, W065, W111, W133, and W169. Cluster 3, regarded as a susceptible group, contains cvs. H011, H030, H102, H157, H162, H248, H285, H287, H351, M136, M153, W010, W026, W046, W053, W083, W101, W105, W109, W138, W147, W159, W164, W168, W172, W177, W179, W182, W190, and Kamphaeng Sean 2. Cluster 4, regarded as a moderately tolerant group, contains cvs. H050, H151, H234, H279, H33H, H366, H377, and W080. Cluster 5, regarded as a tolerant group, contains cvs. Chai-Nat72, H209, H337, and W162. Cluster 6, regarded as a tolerant group, contains cvs. H071 and H149.

The averages of all the collected traits in each cluster are presented in Table 1. The average injury scores from the 6 clusters were 3.0-8.0. Clusters 2, 5, and 6 had average injury scores of 4.0, 4.0, and 3.0, respectively, while cluster 1 had an average injury score of 8.0. The survival rate of the 6 clusters was 3.4%-66.6%. Clusters 5 and 6 had survival rates of 66.6% and 66.1%, respectively, while the survival rate of cluster 1 was 3.4%. Only cluster 1 had very low leaf greenness (2.2 SPAD units), while the other clusters had leaf greenness rankings in the 26.99-34.33 SPAD units.

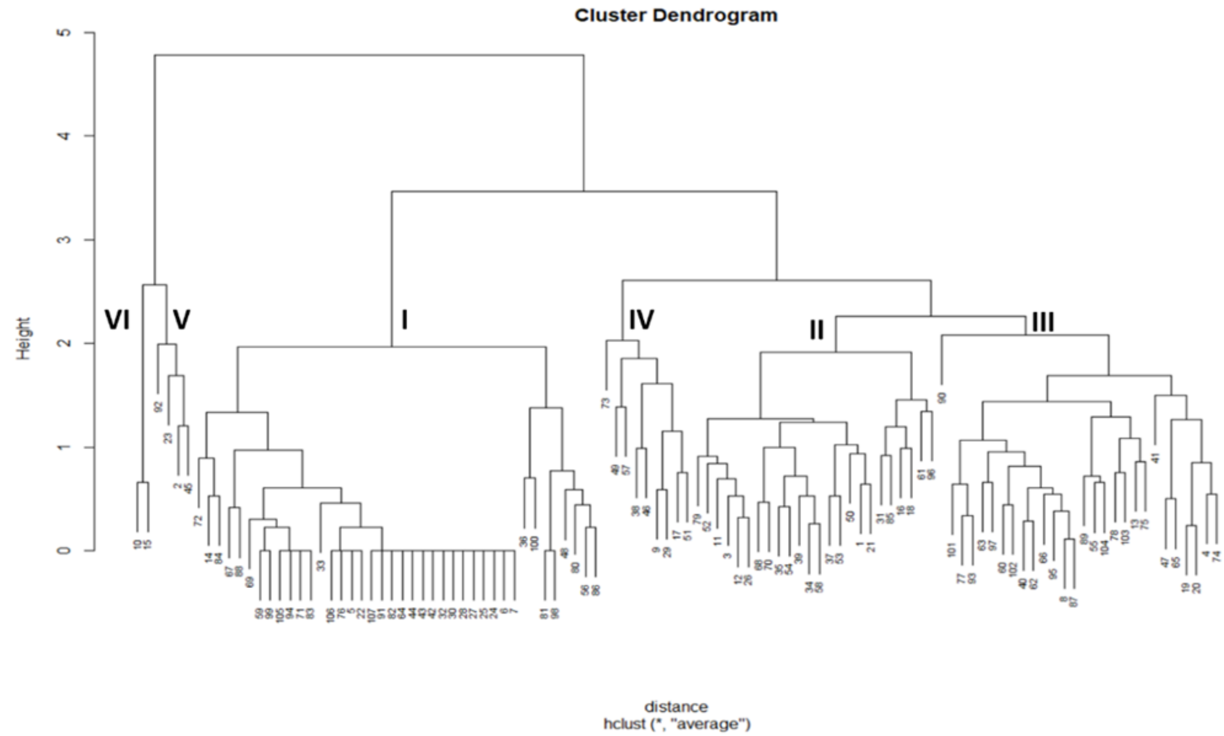

Figure 3. Grouping of mung bean varieties based on morphological characteristics responding to waterlogging conditions.

Table 1. Morphological characteristics under waterlogging conditions of six mung bean clusters based on hierarchical clustering. Data presented as mean ± SD. Plant height stability index = Hs/Hp. Yield stability index = Ys/Yp. Ys: Grain yield of each genotype under waterlogging conditions; Yp: grain yield of each genotype under non-waterlogging conditions; Hs: plant height of each genotype under waterlogging conditions; Hp: plant height of each genotype under non-waterlogging conditions.

	Cluster					
Morphological characteristic	1	2	3	4	5	6
Nr of genotypes	39	24	30	8	4	2
Germination, %	26.92 ± 32.07	88.71 ± 9.39	57.23 ± 26.68	92.71 ± 9.35	91.66 ± 6.81	100.00 ± 0.00
Survival, %	3.42 ± 8.51	56.71 ± 14.83	15.92 ± 9.08	58.33 ± 11.49	66.66 ± 27.22	61.11 ± 7.86
Leaf greenness (SPAD unit)	2.17 ± 8.23	31.88 ± 5.17	26.99 ± 10.45	32.21 ± 6.41	31.33 ± 4.51	34.33 ± 4.65
Plant height stability index	0.01 ± 0.03	0.21 ± 0.01	0.24 ± 0.13	0.53 ± 0.12	0.29 ± 0.07	0.29 ± 0.01
Injure score	8.00 ± 3.76	4.00 ± 0.78	5.00 ± 1.77	4.25 ± 0.46	4.00 ± 0.82	3.00 ± 0.00
Yield stability index	0.01 ± 0.04	0.04 ± 0.13	0.00 ± 0.00	0.01 ± 0.02	0.06 ± 0.01	0.04 ± 0.02

Morphology of F_2 population response to the managed waterlogging condition

Injury score at 1 wk after the waterlogging condition terminated. The F_1 hybrids were crossed between susceptible (KPS2), from cluster 3, and tolerant (W162), from cluster 5, mung bean varieties. From KPS2×W162, 37 F_1 seeds were obtained. The F_1 plants were raised under normal conditions and were self-pollinated to produce F_2 seeds. In total, 199 F_2 plants were cultivated in a greenhouse and screened under managed waterlogging conditions. One week after terminating the waterlogging conditions, the injury scores of W162 (P1) and KPS2 (P2) were 2 (tolerance) and 5 (very susceptible), respectively. The average injury score of the F_2 populations was 4.02 (Figure 4a). The average injury score of the F_2 population derived from crossing between KPS2 and W162 was higher than for the female parent (KPS2).

The 28 F_2 lines had green leaves and no dry leaf areas. Leaf injury of the 28 F_2 lines was scored at 1 (high tolerance), as shown in Figure 4a. In total, 15 F_2 lines and W162 presented chlorosis on around 10%-30% of all leaves with injury scored at 2 (tolerance). Eight F_2 lines had 31%-70% chlorosis of all leaves, with injury scored at 3 (moderate tolerance). Twenty-three F_2 lines were classified as waterlogging-susceptible with an injury score of 4, and these 23 lines did not survive 1 wk after terminating the waterlogging conditions. The Kamphaeng Sean 2 and 125 F_2 lines were considered very susceptible under waterlogging conditions because these varieties had an injury score of 5 and died during the waterlogging evaluation process (Figure 4a).

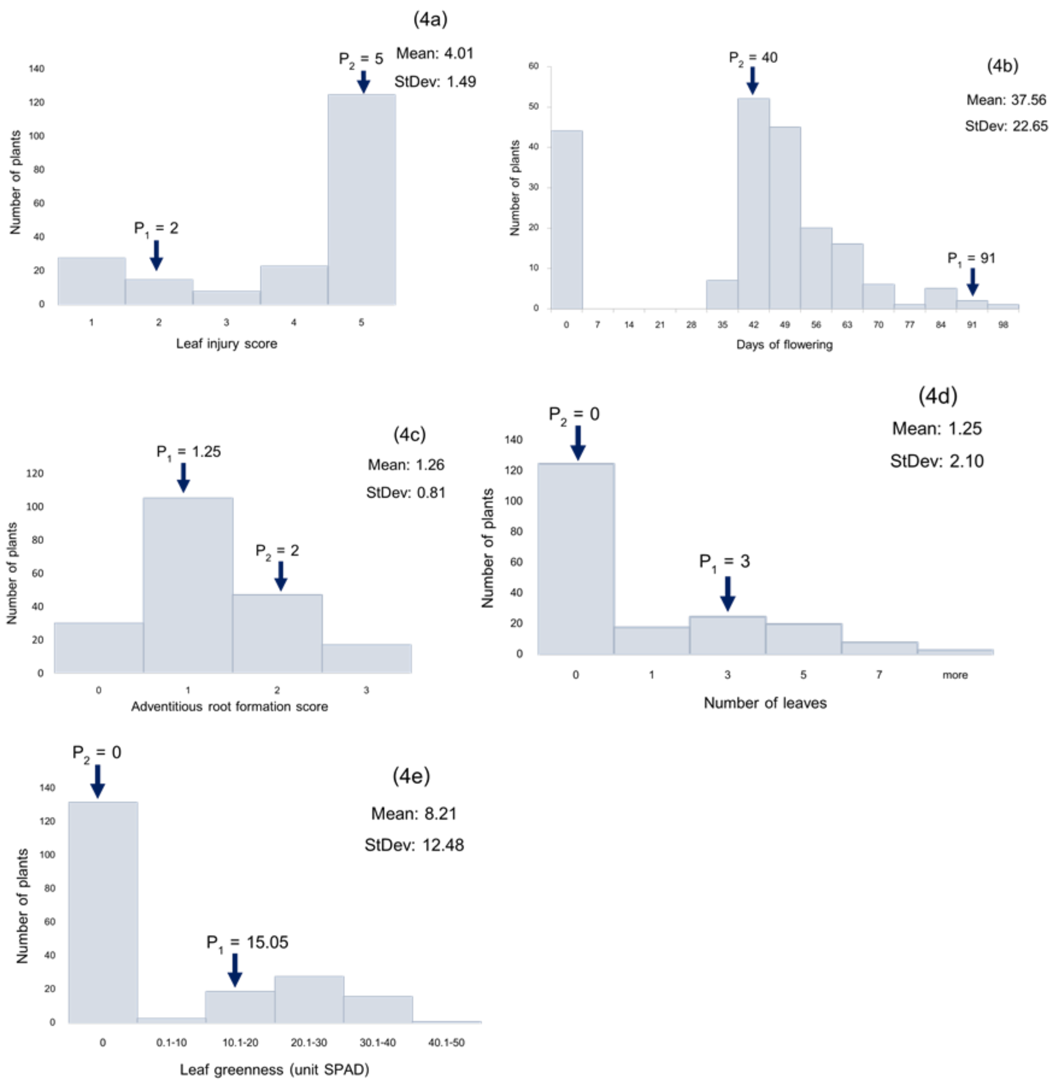

Figure 4. Frequency distribution of 199 mung bean F_2 populations at 1 wk after termination of waterlogging condition: Injury score (4a), first flowering date (4b), adventitious root formation score at termination of waterlogging conditions (4c), number of leaves (4d), and leaf greenness (4e).

Table 2. Correlation among first flowering date, number of leaves, adventitious root formation score, leaf greenness, injury score, number of pods per plant, hundred seeds weight, and seed weight per plant of mung bean at final date of waterlogged conditions. *Data were collected at 1 wk after waterlogging terminated. Leaf greenness stability index = G_A/G_B. G_A: Leaf greenness of each F_2 plant at finale date of waterlogged conditions; G_B: leaf greenness of each F_2 plant before waterlogging conditions. Correlation = 0.0-0.2 (weak to no correlation), 0.21-0.40 (weak), 0.41-0.60 (moderate), 0.61-0.80 (strong), and 0.81-1.00 (very strong).

Parameters	First flowering date	Number of leaves	Adventitious root formation score	Leaf greenness stability index	Injury score	Number of pods per plant	Hundred seeds weight, g	Seed weight per plant, g
First flowering date	1							
Number of leaves	0.184	1						
Adventitious root formation score	0.113	0.557	1					
Leaf greenness stability index	0.037	0.694	0.337	1				
Injury score	-0.173	-0.845	-0.401	-0.821	1			
Number of pods per plant*	0.272	0.401	0.313	0.125	-0.331	1		
Hundred seeds weight, g*	0.268	0.218	0.228	0.003	-0.182	0.609	1	
Seed weight per plant, g*	0.254	0.398	0.319	0.170	-0.348	0.901	0.633	1

First flowering dates. The first flowering dates of W162 (P1) and KPS2 (P2) were 91 and 40 d after planting (DAP) (Figure 4b). The first flowering dates of the F_2 population were between 35 to 98 DAPs. The average first flowering date of the F_2 population was 37.56 DAP. The first flowering dates of the F_2 population under waterlogging conditions had the highest frequency at 42 DAP. However, 44 F_2 lines did not flower, and the F_2 lines did not survive during waterlogging evaluation. The average first flowering dates of the F_2 population were lower than for the male parent (W162).

Adventitious root formation. At the last date of waterlogging conditions, the adventitious root formation scores of the W162, KPS2, and F_2 lines were split in the range of 0-3 (Figure 4c). W162 and KPS2 showed average adventitious root formation under waterlogging of 1.25 and 2, respectively. The average adventitious root formation of the F_2 populations was 1.26. The average adventitious root formation of the F_2 population was lower than for the female parent (KPS2).

Number of leaves at 1 wk after the waterlogging condition terminated. W162 (P1) had three leaves per plant, whereas there were no leaves on the KPS2 (P2) plants because they all died during waterlogging evaluation (Figure 4d). The average leaf number of the F_2 populations under waterlogging conditions was 1.25 leaves per plant. The 125 F_2 lines of the F_2 populations had no leaves during waterlogging evaluation. The average leaf number of the F_2 population was higher than for KPS2.

Leaf greenness at 1 wk after the waterlogging condition terminated. The leaf greenness of W162 was 15.05 SPAD units. There was no leaf greenness information for the KPS2 (P2) and 132 F_2 lines because the varieties died during the waterlogging evaluation before the sampling date. The average leaf greenness of the F_2 lines was 8.22 SPAD units. However, some F_2 lines had leaf greenness values in the 20-50, which was higher than for W162 (Figure 4e).

Correlation between morphological traits of F_2 population responding to waterlogging conditions

At the final date of waterlogging, number of leaves, adventitious root formation, and leaf greenness stability index significantly correlated with the injury score (Table 2). The injury score had a solidly negative correlation with the number of leaves (r = -0.845) and leaf greenness (r = -0.821). The injury score negatively correlated with adventitious root formation (r = -0.401). Seed weight per plant showed a weak correlation with day to flowering (r = 0.254), number of leaves (r = 0.398), adventitious root formation score (r = 0.319), but a weak negative correlation with injury score (r = -0.348). Seed yield per plant at the final date of waterlogging was a weak correlation with leaf greenness stability index (r = 0.170).

Table 3. Correlation among number of leaves, leaf greenness, injury score, number of pods per plant, hundred seeds weight, and seed weight per plant of mung bean at 1 wk after waterlogging terminated. Leaf greenness stability index = G_A/G_B. G_A: Leaf greenness of each F_2 plant at 1 wk after termination of waterlogged conditions; G_B: leaf greenness of each F_2 plant before waterlogging conditions. Correlation = 0.0-0.2 (weak to no correlation), 0.21-0.40 (weak), 0.41-0.60 (moderate), 0.61-0.80 (strong), and 0.81-1.00 (very strong).

Parameter	Number of leaves	Leaf greenness stability index	Injury score	Pods per plant at harvesting date	Hundred seeds weight, g	Seed weight per plant, g
Number of leaves	1					
Leaf greenness stability index	0.119	1				
Injury score	-0.885	-0.153	1			
Number of pods per plant at harvesting date	0.673	0.065	-0.607	1		
Hundred seeds weight, g	0.449	0.064	-0.458	0.609	1	
Seed weight per plant, g	0.654	0.057	-0.574	0.901	0.633	1

Table 4. Path coefficient analysis showing direct and indirect effects of various characters on mung bean leaves injury score at the final date of waterlogging and 1 wk after termination of waterlogging conditions. Bold: Direct effect; normal: indirect effect.

	Character	Number of leaves	Adventitious root formation sore	Leaf greenness stability index	Residual effect
Final date of waterlogging	Number of leaves	**-0.5928**	0.0454	-0.3025	0.1689
	Adventitious root formation score	-0.3319	**0.0810**	-0.1491	
	Leaf greenness stability index	-0.4090	0.0276	**-0.4385**	
One week after termination of waterlogging	Number of leaves	**-0.8599**	-0.0147	-0.0052	0.2228
	Adventitious root formation score	-0.4042	**-0.0314**	-0.0043	
	Leaf greenness stability index	-0.1032	-0.0031	**-0.0436**	

One week after waterlogging termination, the number of pods per plant and seed weight per plant strongly correlated with the number of leaves (r = 0.654 and 0.673). The number of pods per plant, hundred seed weight, and seed weight per plant showed a negatively moderate correlation with injury score (r = -0.607, r = -0.458, r = -0.574). The leaf greenness stability index was not correlated with seed weight per plant (r = 0.057) (Table 3).

Path coefficient analysis

The number of leaves presented the most direct effect on injury score at the final date of waterlogging condition (-0.5928), followed by leaf greenness stability index (-0.4385) and adventitious root formation (0.0810) (Table 4). At 1 wk after the termination of waterlogging conditions, the number of leaves showed the most considerable negative direct effect on injury score (-0.8599), followed by leaf greenness stability index (-0.0436) and adventitious root formation score (-0.0314) (Table 4).

Table 5. Path coefficient analysis showing direct and indirect effects of characters at the final date of waterlogging on mung bean seed yield per plant. Bold: Direct effect; Normal: indirect effect.

Characters	First flowering date	Number of leaves	Adventitious root formation sore	Leaf greenness stability index	Injury score	Residual effect
First flowering date	**0.1494**	0.0397	0.0169	-0.0125	0.0564	
Number of leaves	0.0269	**0.2206**	0.0859	-0.2157	0.2822	
Adventitious root formation score	0.0164	0.1236	**0.1535**	-0.1063	0.1328	0.7622
Leaf greenness stability index	0.0059	0.1523	0.0522	**-0.3127**	0.2722	
Injury score	-0.0254	-0.1875	-0.0614	0.25641	**-0.3320**	

Table 6. Path coefficient analysis showing direct and indirect effects of various characters at 1 wk after termination of waterlogging conditions on mung bean seed yield per plant. Bold: Direct effect; Normal: indirect effect.

Characters	Number of leaves	Leaf greenness stability index	Injury score	Residual effect
Number of leaves	**0.6534**	-0.0011	-0.0352	
Leaf greenness stability index	0.0784	**-0.0096**	-0.0060	0.5707
Injury score	-0.5749	0.0014	**0.0400**	

At the final date of waterlogging conditions, the number of leaves, adventitious root formation, and days to flowering traits showed a positive direct effect on seed yield at 0.2206, 0.1535, and 0.1494, respectively. Furthermore, the leaf greenness stability index and injury score at the final date of waterlogging presented direct adverse effects on the seed yield (-0.3127 and -0.3320, respectively) (Table 5).

At 1 wk after the termination of waterlogging, the number of leaves was the highest positive direct effect (0.6534) on seed weight, followed by the injury score (0.0400) and leaf greenness stability index (-0.0096) (Table 6). In addition, the number of leaves presented a high indirect effect on seed weight through the leaf greenness stability index (-0.5749).

DISCUSSION

The waterlogging condition negatively influences the development of mung beans, such as seedling establishment, shoot and root development, healthy plant, and yield performance which agree with the previously reported (Ding et al., 2020; Kyu et al., 2021). Mung bean varieties in clusters 1 and 3 showed low performance under waterlogging conditions, which revealed their susceptibility to waterlogging stress. Mung bean varieties in clusters 5 and 6 were classified as waterlogging-tolerant because these varieties presented high values for survival rate, leaf greenness, and seed yield compared with other clusters (Islam et al., 2007; Shibly et al., 2020).

Mung bean varieties for waterlogging susceptibility and waterlogging tolerance were crossed to develop F_1 and F_2 populations, and the cross between Kamphaeng Sean 2 (cluster 3) and W162 (cluster 5) successfully developed the F_1 and F_2 populations for breeding mung bean with high waterlogging tolerance. W162 was a waterlogging-tolerant mung bean variety. The variety presented adapt potential to avoid waterlogging conditions better than other varieties to survive under waterlogging. The survival rate of the W162 after waterlogging termination was 100%, and the flowering date could be extended. Plant varieties that survive under waterlogging can adapt by delaying the flowering date (Celedonio et al., 2015; Asami et al., 2021). The W162 developed a lot of adventitious roots under waterlogging, which was a good adaptation in low-oxygen environments (Yamauchi et al., 2017; Fukao et al., 2019; Qi et al., 2019). Moreover, the W162 could maintain leaf number and greenness under waterlogging conditions. After waterlogging termination, the W162 maintained balance in the plant to survive and produce seeds. Transgressive segregations were found in the F_2 lines under managed waterlogging for the first flowering date, adventitious root formation, leaf greenness, number of leaves, and injury score. The additive effect might have increased the expression of the phenotypes in the F_2 lines. The transgressive segregation in the F_2 lines creates the opportunity for the selection of the F_2 lines with high waterlogging tolerance and good agronomic traits (Ye et al., 2018; Pabuayon et al., 2021).

Under the managed waterlogging conditions, the 20 F_2 lines were classified as high waterlogging tolerance. Waterlogging-tolerant plants were expected to maintain a high photosynthetic rate, chlorophyll content, membrane stability, and rapid photosynthetic recovery under waterlogging conditions (Kumar et al., 2013). The 148 F_2 lines died during the waterlogging condition evaluation. Waterlogging can reduce photosynthesis, plant growth, grain yield, function and survival of nodules, and biological N fixation and cause plant death of legume crops during or after the end of waterlogging. Under waterlogging, the enzyme activities related to photosynthesis were inhibited. The chlorophyll synthesis ability of leaves decreased, leading to leaf senescence, yellowing, and peeling, and the formation of new leaves was blocked, leading to plant death (Wu and Yang, 2016; Pan et al., 2021). If mung bean genotypes did not have a waterlogging-tolerant mechanism, they were not able to survive under severe waterlogging conditions.

In the present study, the F_2 lines were classified as waterlogging-tolerant or -susceptible based on their values for injury score, number of leaves, leaf greenness, adventitious root formation, number of pods per plant, and seed weight per plant. Twenty F_2 lines were identified as waterlogging tolerance and selected for a mung bean breeding program. The F_2 lines possibly maintain photosynthesis, chlorophyll content, and membrane stability under waterlogging conditions (Kumar et al., 2013). Moreover, the 20 F_2 lines were selected for the next breeding program by the selection intensity from selecting 10% of superior plants (Ejara et al., 2016; Mahdy et al., 2022).

The correlations ecoefficiency of leaf injury with the number of leaves and leaf greenness were high at the final date of waterlogging and after waterlogging termination. The correlation ecoefficiency was more than the correlation between leaf injury and adventurous root formation scored (almost double). The correlation revealed that the number of leaves and leaf greenness were essential for waterlogging tolerance. Moreover, the F_2 population of mung bean was under waterlogging conditions for 6 wk, which is longer than other studies on mung bean. The number of leaves and leaf greenness may be the critical mechanism for long waterlogging. The correlation results revealed that the number of leaves is essential for mung bean recovery after waterlogging conditions and affects seed yield. The mung bean lines that maintain their leaves can absorb the oxygen from their leaves and decrease the impact of reduced oxygen uptake from roots under waterlogging conditions. The leaf greenness is a key, as well, to maintain the photosynthetic rate under waterlogging conditions (Ren et al., 2016; Sathi et al., 2022). Based on the results, the number of leaves and leaf greenness were essential indicators for selecting waterlogging-tolerant mung bean lines, and the number of leaves was an essential trait for seed yield potential under waterlogging conditions.

Path coefficient analysis revealed that the number of leaves and leaf greenness had direct and indirect effects on leaf injury at the final date of waterlogging condition and 1 wk after waterlogging termination. The direct effects of the traits on leaves injury at the final date of waterlogging condition were consistent with the leaf injury 1 wk after waterlogging termination. Then, the selection of mung bean lines for waterlogging tolerance can start at the final date of waterlogging condition. Moreover, the number of leaves and leaf greenness had a substantial direct effect on leaf injury. The path coefficient analysis results agreed with the correlation analysis, with the number of leaves and leaf greenness having a higher correlation with leaf injury. Thus, the number of leaves and leaf greenness were suitable parameters for selecting mung bean varieties for waterlogging tolerance (Kumar et al., 2013).

Path coefficient analysis between morphological traits and leaf injury at a final date of waterlogging revealed that the number of leaves and leaf greenness had a high direct effect on waterlogging tolerance. One week after the waterlogging termination, the number of leaves, directly and indirectly, affected the mung bean seed yield. At this stage, the number of leaves had a greater direct effect on seed yield than the direct effect on leaf greenness by around 16.25 times. The path-coefficient analysis confirmed that the number of leaves and leaf greenness were highly affecting waterlogging tolerance for an extended period, and the number of leaves has the highest impact on seed yield potential under waterlogging conditions (Fukao et al., 2019). Thus, the number of leaves was a suitable parameter for selecting mung bean varieties for seed yield under waterlogging conditions (Huang et al., 2022).

CONCLUSIONS

Mung bean varieties were evaluated under waterlogging conditions and grouped into 6 clusters based on morphological traits responding to the water stress. Mung bean varieties in clusters 5 and 6 showed waterlogging tolerance, while mung bean varieties in clusters 1 and 3 showed susceptibility to waterlogging. Screening of 199 F_2 populations developed from Kamphaeng Sean 2 and W162 genotypes under waterlogging conditions could identify 28 F_2 lines with low leaves injury scores under the water stress conditions. Based on the path coefficient analysis, the number of leaves and leaf greenness were suitable parameters for the selection of mung bean for waterlogging tolerance. In addition, the number of leaves was a suitable parameter for selecting mung bean for seed yield potential under waterlogging conditions.

Author contributions

Conceptualization: T.C., P.S. Methodology: N.T., T.C. Validation: T.C., N.T. Formal analysis: N.T. Investigation: N.T., T.C., P.K. Resources: P.S., T.C. Data curation: N.T., T.C. Writing-original draft: N.T., T.C. Writing-review & editing: N.T., T.C., P.K. Visualization: N.T., T.C., P.K. Supervision: T.C., P.S. Project administration: T.C., P.S. Funding acquisition: T.C., P.S. All co-authors reviewed the final version and approved the manuscript before submission.

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