

Annals of Agrarian Science

Journal homepage: http://journals.org.ge/index.php



Assessment of waterlogging tolerance in mungbean genotypes utilizing morphological traits and SSR markers

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Received: 09 March 2020; accepted: 12 May 2020

ABSTRACT

Some promising mungbean genotypes were employed to evaluate waterlogging tolerance and molecular characterization using SSR marker. Waterlogging treatment was applied to 25-d old plants maintaining 2-3 cm waterlogging depth for three days with extended seven days saturation period. It significantly reduced the growth and yield but the plants remarkably improved their depressed characters during the recovery period. The early response of waterlogging was the development of adventitious roots which is an important adaptive mechanism of plants under waterlogged situations. Based on waterlogging tolerance index calculated as the percent ratio of relative growth rate (RGR) in waterlogged plants and RGR in non-waterlogged plants of all plant components, the genotypes ACC12890054 and BUmug 4 appeared as the most tolerant to waterlogging. The genotypes ACC12890085 and ACC 12890054 that showed better tolerance to waterlogging gave the highest relative yield of 46% followed by BUmug 4 and VC 6173-A genotypes. Based on the correlation coefficient and relative values, the genotypes were grouped into four clusters using K-means cluster analysis. In SSR analysis, PIC values of the markers were above or almost equal to 0.5 indicating the used primers were effective to differentiate the genotypes at the molecular level. In analysis 16 pairs of mungbean genotypes showed 41.7% maximum dissimilarity. We grouped 12 genotypes into four clusters using unweighted pair group method with arithmetic mean (UPGMA). These four main clusters are distinctly dissimilar to each other on the based of genetic characters. Thus, the findings of this research could be used for envisaging promising mungbean genotypes and developing waterlogged-tolerant mungbean variety(s).

Keywords: Mungbean, Waterlogging tolerance, Genetic variability, SSR marker, Molecular level, Correlation coefficient.

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Introduction

Mungbean (Vigna radiata L. Wilczek) is recognized as one of the most promising pulse crops but its large-scale adoption is constrained by many biotic and abiotic stresses. Among abiotic stresses, waterlogging affects more than 1700 Mha of land worldwide [1]. It is anticipated that both the frequency and severity of floods will be increased in many places in the world due to climate change [2]. Possibly flooding or waterlogging will largely affect mungbean cultivation in the future, although some genotypes are found tolerant to waterlogging and capable of recovering from flooding injury [3,4].

Excess water generally causes hypoxia or even anoxia around roots due to the rapid consumption and slow diffusion of oxygen. As a result, plants suffer from devoid of energy [5] and eventually, uptake of water and nutrients is restricted [6]. A greater yield loss has been reported when the young plants are subjected to waterlogging [7]. Therefore, climate change-induced aggravation of waterlogging situations can further promote decreasing of mungbean production which is assumed an extraordinary challenge for its sustainable cultivation [8].

Many researchers conducted studies on the responses of mungbean genotypes to waterlogging and reported several morpho-physiological disturbances

[4, 9-11]. However, such responses are much pronounced in waterlogged-sensitive genotypes because of a slow recovery in photosynthesis and physiological traits, while a high photosynthetic rate and better physiological function were found in tolerant genotypes [10]. Therefore, searching waterlogged-tolerant genotypes and efforts to develop variety(s) capable of withstanding waterlogged situations are underway. Several molecular techniques are followed to develop crop variety(s) tolerant to many abiotic stresses but such techniques are hardly applied in mungbean due to a lack of genetic information of the crop. Developing the sequence of the mungbean genome would probably be an important source of genetic improvement of the crop [12].

DNA markers are needed for creating genetic maps and to locate the exact loci of the targeted gene(s) [13]. Some established molecular DNA markers are RFLP [14], RAPD [15] and SSR [16]. Simple sequence repeats (SSR) are repetitive DNA sequences that can represent the whole genome of an organism [17]. SSR marker is recognized as an influential tool for the evaluation of diverse plant genetic resources [18-19], species identification [20] and gene mapping [21]. Some SSR markers have been developed in mungbean [22] which does not prove to be adequate to fulfill the demand of the scientific community [23]. Moreover, comparatively better polymorphism has been observed between Vigna_species, while lower diversity was detected within the species.

Waterlogging for a few days can damage mungbean plants and results in significant yield losses. Therefore, it is important to understand the traits that can improve waterlogging tolerance, and the genes and proteins underlying these traits. Under global waterlogging nature accompanying climate change, it is evident to enhance our knowledge on waterlogging tolerance which will facilitate to development of flood-tolerant varieties [24]. Therefore, this study was undertaken to identify morphological traits for waterlogging tolerance under field conditions towards improvement and sustainable use of mungbean biodiversity and to characterize mungbean genotypes at a molecular level using SSR markers.

Materials and methods Study location

The experiment was carried out at the Field Research Site and Genetics and Plant Breeding Lab of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur from February 2015 to June 2016. The experimental site is located

at 24°02′15.06″N latitude and 90°23′45.80″ E longitude. The area belongs to high terrace of Madhupur Tracts of Bangladesh.

Experimental layout

Twelve mungbean genotypes and two waterlogging treatments (waterlogging and non-waterlogging control) were the treatment variables. A total of 72 plots were prepared to assign all the treatment combinations. The experiment was a randomized complete block design and replicated three times. The experimental unit size was 1.2×1.2 m. They were surrounded by raised boundaries covered with polythene sheets to prevent water leakage from the waterlogging treated plots.

Plant materials

Twelve mungbean genotypes viz. GK48, GK65, BARI mung 4, BARI mung 6, ACC 12890085, ACC 12890054, BU mug 4, VC 1160-A, VC 6173-A, IPSA-13, GK63 and IPSA-15 were used in this experiment. All the genotypes showed different degrees of tolerance in the previous studies.

Raising of seedlings and treatment imposition

Three seedlings were raised per hill maintaining a distance from the line to line 30 cm and plant to plant 10 cm. To maintain a uniform size of the seedlings, the seedlings were reduced two times keeping vigorous healthy ones. Waterlogging treatment was applied at 25 days after emergence (DAE) maintaining waterlogging depth of 2-3 cm for three days. Thereafter, the excess water was drained out from the waterlogged plots. These three days of waterlogging with seven days prolonged saturated periods (25-35 DAE) were considered as the waterlogging period. The period 35-45 DAE was considered as first recovery period and that of 45-55 DAE as second recovery period. On the contrary, optimal soil moisture was provided to the plant retained as a control. The first sampling was done on the day of waterlogging (25 DAE) and continued the sampling at 10 days intervals up to 55 DAE in both waterlogged and non-waterlogged plants.

Estimation of RGR and WT:

Relative growth rate (RGR) of plant components i.e. root, stem and leaf etc. were calculated accord-

ing to Gardner *et al.* [25]. Waterlogging tolerance (WT) of each plant component was calculated according to Chen and Burton [26]: WT= RGR (waterlogged)/ RGR (control)*100.

Yield attribute and seed yield

The maturity stage, pods were harvested from the plant and data regarding the branches per plant, number of pod per plant, number of seeds per pod, 1000-seed weight and seed yield and harvest index were recorded for waterlogged and control plants in each genotype.

SSR markers and DNA extraction

Four SSR markers (VR 188, VR 225, VR 276 and VR 304) with clear amplifications were selected for genetic diversity analysis of twelve mungbean genotypes. One gram young leaf tissue collected from 2-weeks old seedlings was powdered under liquid nitrogen in a mortar and pestle, and the DNA was extracted employing modified CTAB method [27]. DNA quantification and quality measurement were done as per procedures described by Huda *et al.* [19] and a working concentration of 25 ng/μl was made.

Polymerase chain reaction (PCR) amplification

A 25 µl mixture was prepared for the PCR reaction containing 3 µl template DNA, 2.5 µl of 10x buffer, 2.5 mM dNTPs and 25 mM MgCl₂, respectively, 1.25 µl for both forward and reverse primers, and 0.3 µl of Taq polymerase. PCR fragment size was assessed using DNA molecular weight marker. The PCR reaction was performed at 95°C for 5 min and then for 42 cycles of 95°C for 45 sec, 55°C for 45 sec, 72°C for 1 minute and finally 72°C for 5 min. The products were electrophoresed through 1% agarose gel and subjected to photography on a UV transilluminator. Scoring of genomes was done considering the presence or absence of polymorphic bands. A UPGMA method was followed to indexing genetic variation and constructing a dendrogram.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) by using Statistix 10 program. Besides, Microsoft Excel was used to estimate standard deviation (SD) and standard error (SE).

For cluster analysis, computer software SPSS 16 was used. The Analysis of Variance (ANOVA) was performed for various plant traits and means were separated by the Duncan's Multiple Range Test (DMRT). For molecular characterization, computer software DARwin was used.

Results and discussion Waterlogging tolerance in root

The relative growth rate (RGR) of the plant roots both waterlogged and non-waterlogged plants of 12 mungbean genotypes during waterlogging, first recovery period and second recovery period have been illustrated in Table 1. Waterlogging affected the RGR of the roots in all the genotypes and showed waterlogging tolerance (WT) values much low. However, most of the genotypes showed higher WT values during 35-45 DAE indicating a remarkable recovery in root growth after the termination of waterlogging. During the period 45-55 DAE, the genotypes ACC12890085, ACC12890054, BUmug 4, VC 1160-A, VC 6173-A, GK 63 and IPSA-15 showed much recovery in root growth and showing WT values more than 100. The greater increase in RGR of waterlogged plant roots indicated the development of adventitious roots after damaging the original ones. A faster formation of adventitious roots at the early stage is a common response of waterlogged-tolerant crop species [28, 29].

Waterlogging tolerance in total plants

Waterlogging affected the RGR of total plants in all the genotypes and showed much low or even negative WT values (Table 2). However, RGR of the waterlogged plant either increased or decreased to some extent depending on the genotypes during the recovery period of 35-45 DAE that indicated the genotypic differences in WT were not pronounced immediately after termination of waterlogging. However, a remarkable recovery in RGR of total plants was found during 45-55 DAE in almost all genotypes. The genotypes BU mug 4 and GK 63 showed the negative FT indexes during 35-45 DAE had the FT indices 200 and 141 respectively during 45-55 DAE. Other genotypes also performed similarly. A plausible explanation of such a rapid increase in RGR can be explained by the fact that the genotypes expended the accumulated dry matter quickly produced through adventitious roots and then eventually utilized that in producing shoot dry matter during the second recovery period.

Yield attributes and seed yield

Yield contributing characters and seed yield of twelve mungbean genotypes as affected by waterlogging are presented in Table 3. The number of branch plant⁻¹, number of pods per plant⁻¹, seed yield plant and harvest index was significantly affected by waterlogging, where seed weight was not significantly affected. The number of branches plant-1 was more vulnerable to waterlogging and showed 17-64% reduction. There was great variation among genotypes in producing pods plant⁻¹ that ranged from 3.03 to 11.58 in waterlogged plants and 5.75 to 21.25 in control plants. The genotype ACC12890054 produced the highest number of pods plant-1 under waterlogging situation and control conditions. The variation of seed weight due to waterlogging was not comparable for both waterlogging and non-waterlogged plants. However, genotypes and GK 63 produced bolder seeds in waterlogged situations. 11 Ahmed et al. (2002) found that waterlogging reduced seed yield by reducing the number of pods plant-1 rather than reduced the number of seeds pod-1 or seed weight.Irrespective of waterlogging treatment, seed yield showed a significant variation across the genotypes. The genotypes produced 0.34 to 1.56 ton ha-1 under waterlogged and 2.33 to 3.88 ton ha-1 under control condition. Waterlogging induced reduction in seed yield ranged between 54 to 87% depending on genotypes. The genotypes ACC12890085 and ACC 12890054 that showed better tolerance to waterlogging gave the highest yield (46% relative to control) followed by BUmug 4 and VC 6173-A. From Table 3, the harvest index HI was changed remarkably due to waterlogging treatment. The genotype GK 48 had the lowest harvest index which indicates that it had the lowest economic yield due to the negative effect from waterlogging. The genotype ACC12890054 had and the highest HI (90% of control). It means

that this genotype showed tolerance to waterlogging and gave the highest economic yield. Therefore, ACC12890054 is the best among the genotypes in respect of yield performance under waterlogging situations.

K-means cluster analysis

K-means non-hierarchical cluster analysis was performed using eight quantitative plant characters i.e. waterlogging tolerance of stem, leaf, root and total biomass, relative root-shoot ratio, pods per plant, harvest index and grain yield for grouping 12 mungbean genotypes. The correlation coefficient values with grain yield were low for other plant characters and they were excluded from multivariate analysis. A dendrogram was prepared on the basis of cluster analysis (Figure 1). The tree was cut at the rescaled distance of 5.0 to produce classes that were maximally related to other specific variables of interest. Thereafter, the genotypes were grouped into four clusters. Cluster 1 is comprised of genotype GK 48 which is characterized by the lowest relative value in all the eight plant characters (Table 4). Cluster 2 contains five genotypes viz. ACC12890085, ACC 12890054, VC 1160-A, VC 6173-A and GK 63 those are characterized by the highest relative root-shoot ratio (47.2) and harvest index (73.5). All other plant characters performed well and the genotypes gave better grain yield relative to control. Cluster 3 includes genotype BU mug 4 having the highest waterlogging tolerance of stem, leaf, root and total biomass as well as pods per plant (65.4) and concurrently gave the highest relative grain yield (41.9). Cluster 4 genotypes viz. GK 65, BARImung 4, BARImung 6, IPSA-13, IPSA-15 were mainly characterized by the moderate plant characters which were higher than that of cluster 1 genotypes. In the clustering pattern, cluster 3 genotype performed better followed by cluster 2 genotypes.

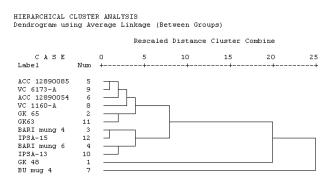


Fig 1. Graphical illustration of hierarchical cluster analysis of mungbean genotypes using dendrogram

TM	Clusters						
Plant traits	1	2	3	4			
No. of genotypes	1	5	1	5			
Waterlogging tolerance of stem	22.5	114.8	179.4	79.5			
Waterlogging tolerance of leaf	27.1	132.1	161.0	100.2			
Waterlogging tolerance of root	2.5	148.3	239.1	66.0			
Waterlogging tolerance of total biomass	32.1	129.7	198.6	86.5			
Root shoot ratio	28.0	47.2	25.0	41.6			
Pods per plant	29.8	57.0	65.4	53.5			
Harvest index	31.1	73.5	72.7	70.7			
Grain vield	14.5	37.1	41.9	32.2			

Table 4. Comparison profile of the genotypes grouped under four clusters

Molecular characterization through SSR markers

Four SSR markers were used in this study. The details of the used primers and molecular diversity present among mungbean genotypes are presented in Table 5. VR188 and VR 225 primers produced three bands. VR276 and VR 304 primers produced two and four bands, respectively. The selected four primers generated twelve bands in total where all the twelve bands were polymorphic. PIC (Polymorphism Information Content) value indicates primer effectiveness. All PIC values of primers were above or almost equal to 0.5 indicating that the primers were effective. Maximum PIC value was found for VR 276, while the minimum was for VR 225.

A dissimilarity matrix was constructed using the binary data obtained through SSR analysis with a view to observe the genotypic relatedness. The lowest pair-wise estimate of dissimilarity was found to be 0.000 while the highest was 0.417 (Table 6). The highest value was observed for 16 pairs of mungbean genotypes (0.417). Each pair showed 41.7% maximum dissimilarity in their genotypic

characters. The lowest dissimilarity (0%) was found for four pairs of mungbean genotypes such as GK 65 and ACC12890085, BARI mung 6 and BARI mung 4, IPSA-13 and VC 1160-A, GK 63 and VC 6173-A. They bear the same genotypic character in each pair of genotypes. A significant amount of genetic divergence was found within the mungbean genotypes as exposed by the dissimilarity matrix.

Genetic similarities served as the source of creating the cluster diagram. Nei's similarity coefficients clustered the 12 genotypes into four different groups (Figure 2). These four main clusters are distinctly dissimilar to each other. Cluster (I) divided into subcluster A and B (Table 7). Subcluster A further divided into subcluster AA and AB. Subcluster AA is also divided into subcluster I and II, which have some similar genotypic characters. Subcluster I involved two mungbean genotypes as IPSA-13, VC 1160-A. Genotypic characters of these genotypes are mostly similar to each other. Subcluster II includes one mungbean genotype IPSA -15. Subcluster AB includes GK 63, VC 6173-A genotypes; they have some similar characteristics but also showed some dissimilar genotypic characters.

Table 5. List of				

SSR Primers	1		Polymorphic bands		Monom- orphic bands		PIC value
		bands	No.	%	No.	%	- '
VR 188	F ATACAAGGGCAGGTGTAGCATC R CAGAAAACTTCATCCCCAGCTA	3	3	100	0	0	0.6287
VR 225	F CAGCAACAGAACTACAATCCCA R CGGCAATCCTCCTATATTCATT	3	3	100	0	0	0.4910
VR 276	F TTGATCCTTGTATTGGATGGTG R GTGGGATTTCTGGTTTTGT	2	2	100	0	0	0.6832
VR 304	F GAAGCGAAGAAGCCATAGAAAA R CCTCACACACAACACAACAGAA	4	4	100	0	0	0.5065

Cluster II has one genotype BU mug 4. Cluster III is divided into subcluster A2 and B2. Subcluster A2 is also divided into subcluster I and II, based on their genotypic characters, which are dissimilar to each other. Subcluster I has two genotypes as BARImung 6, BARImung 4 and subcluster II has two genotypes ACC 12890085 and GK 65, they have same genotypic character. Subcluster B2 in the cluster III has GK 48. Cluster IV has ACC12890054 mungbean genotype. The genotype bear distinctly different character compared to other genotypes.

The distinct clusters were constructed based on morphological and molecular data. Although the total number of clusters is the same the genotypes included in the clusters for morphological and molecular data were not the same. The dendrogram obtained from the SSR markers must be more discriminatory and highly polymorphic and thus, more informative than the one obtained from morphological characterization. Although, the dendrogram generated from the morphological data has provided an overall pattern of variation as well as the degree of relatedness among the genotypes, variation in environmental conditions should be taken into consideration. Moreover, SSR markers are sequence-specific. The targeted region may not control the morphological traits studied. Including more morphological traits and SSR markers representing the whole genome of mungbean may provide a similar dendrogram pattern.

Table 6. Dissimilarity matrix of mungbean genotypes analyzed using Nei's original measures of genetic identity

Genotype	GK	GK	BARI	BARI	ACC	ACC	BU	VC	VC	IPSA	GK	IPSA
	48	65	mung	mung	128900	128900	mug 4	1160-A	6173-	-13	63	-15
			4	6	85	54			A			
GK 48	1.000											
GK 65	0.167	1.000										
BARImung 4	0.250	0.084	1.000									
BARImung 6	0.250	0.084	0.000	1.000								
ACC12890085	0.167	0.000	0.084	0.084	1.000							
ACC12890054	0.167	0.167	0.250	0.250	0.167	1.000						
BUmug 4	0.250	0.250	0.167	0.167	0.250	0.084	1.000					
VC 1160-A	0.417	0.417	0.333	0.333	0.417	0.250	0.167	1.000				
VC 6173-A	0.417	0.417	0.333	0.333	0.417	0.417	0.333	0.167	1.000			
IPSA -13	0.417	0.417	0.333	0.333	0.417	0.250	0.167	0.000	0.167	1.000		
GK 63	0.417	0.417	0.333	0.333	0.417	0.417	0.333	0.167	0.000	0.167	1.000	
IPSA -15	0.333	0.333	0.417	0.417	0.333	0.167	0.250	0.084	0.250	0.084	0.250	1.000

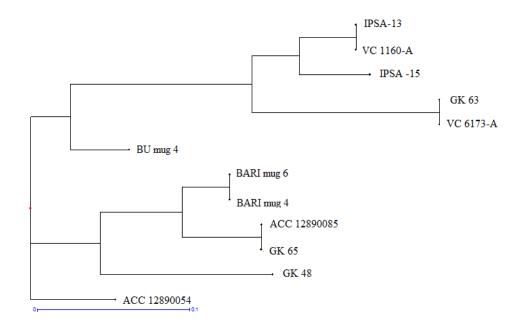


Fig 2. Dendrogram (UPGMA) pattern of SSR analysis in different mungbean genotypes

IV

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Cluster Genotypes included in different clusters genotypes in cluster Sub cluster Sub IPSA -13, VC 1160-A 2 Sub cluster ${f I}$ cluster 1 Sub cluster ${f II}$ IPSA -15 AAGK 63, VC 6173-A 2 Sub cluster AB $_{\rm II}$ BUmug 4 1 III Sub cluster BARImung 6, BARImung 4 2 Sub cluster ${f I}$ A2 ACC12890085, GK 65 2 Sub cluster ${f II}$ Sub cluster B2 GK 48 1

Table 7. Distribution of twelve mungbean genotypes in different clusters

Table 1. Relative growth rate and waterlogging tolerance of plant root in twelve mungbean genotypes subjected to soil waterlogging

ACC12890054

Genotype	Relative growth		Root					
	rate (RGR, g/g/day)	Waterlogging Period (25-35DAE)	Recovery period (35-45 DAE)	Recovery period (45-55DAE)				
GK 48	RGR waterlogged	0.047(27)	0.065(57)	0.003(3)				
	RGR control	0.176	0.114	0.122				
GK 65	RGR waterlogged RGR control	0.082(55) 0.150	0.053(60) 0.088	0.053(69) 0.07				
BARImung 4	RGR waterlogged	0.019(14)	0.062(72)	0.067(94)				
	RGR control	0.131	0.085	0.072				
BARImung 6	RGR waterlogged	0.038(35)	0.055(45)	0.004(10)				
	RGR control	0.109	0.123	0.041				
ACC12890085	RGR waterlogged	0.031(30)	0.105(88)	0.099(161)				
	RGR control	0.102	0.119	0.062				
ACC12890054	RGR waterlogged	0.079(71)	0.040(41)	0.120(157)				
	RGR control	0.112	0.099	0.076				
BUmug 4	RGR waterlogged	0.017(9)	0.035(32)	0.110(238)				
	RGR control	0.184	0.111	0.046				
VC 1160-A	RGR waterlogged	0.023(20)	0.044(37)	0.165(178)				
	RGR control	0.115	0.120	0.093				
VC 6173-A	RGR waterlogged	0.071(73)	0.043(63)	0.087(125)				
	RGR control	0.097	0.069	0.069				
IPSA-13	RGR waterlogged	0.063(61)	0.058(74)	0.027(50)				
	RGR control	0.104	0.079	0.054				
GK 63	RGR waterlogged	0.064(51)	0.020(24)	0.065(119)				
	RGR control	0.126	0.080	0.054				
IPSA-15	RGR waterlogged	0.080(58)	0.049(75)	0.103(108)				
	RGR control	0.138	0.066	0.096				

The numerical values in the parenthesis indicate waterlogging tolerance (WT) calculated as the percent ratio of RGR of waterlogged and RGR of control plants.

Table 2. Relative growth rate and waterlogging tolerance of total plant in twelve mungbean genotypes subjected to soil waterlogging

Genotype	Relative growth rate	Total plant					
	(RGR, g/g/day)	Waterlogging Period (25-35DAE)	Recovery period (35-45 DAE)	Recovery period (45-55DAE)			
GK 48	RGR waterlogged	0.074(55)	0.045(45)	0.044(32)			
	RGR control	0.135	0.099	0.137			
GK 65	RGR waterlogged	0.076(59)	0.040(53)	0.067(122)			
	RGR control	0.127	0.075	0.055			
BARImung 4	RGR waterlogged	0.079(54)	0.024(36)	0.085(89)			
	RGR control	0.145	0.065	0.095			
BARImung 6	RGR waterlogged	0.064(52)	0.020(22)	0.036(64)			
	RGR control	0.124	0.091	0.056			
ACC12890085	RGR waterlogged RGR control	0.004(4) 0.090	0.077(68) 0.113	0.098(114) 0.086			
ACC12890054	RGR waterlogged	0.081 (65)	0.042(52)	0.118(115)			
	RGR control	0.125	0.081	0.102			
BUmug 4	RGR waterlogged	0.049(39)	-0.010(-11)	0.141(200)			
	RGR control	0.126	0.090	0.071			
VC 1160-A	RGR waterlogged	-0.007(-5)	0.025(40)	0.183(153)			
	RGR control	0.143	0.062	0.120			
VC 6173-A	RGR waterlogged RGR control	0.047(47) 0.100	0.032(37) 0.087	0.101(125) 0.081			
IPSA-13	RGR waterlogged	0.045(34)	0.046(51)	0.058(79)			
	RGR control	0.132	0.090	0.074			
GK 63	RGR waterlogged	0.030(26)	-0.004(-5)	0.099(141)			
	RGR control	0.117	0.082	0.070			
IPSA-15	RGR waterlogged	0.036(33)	0.035(63)	0.083(79)			
	RGR control	0.109	0.056	0.106			

The numerical values in the parenthesis indicate waterlogging tolerance (WT) calculated as the percent ratio of RGR of waterlogged and RGR of control plants.

Table 3. Effect of waterlogging on yield and yield attributes of mungbean genotypes

Genotype	Waterlogging level	Branch Plant ⁻¹ (no)	Pods Plant ⁻¹ (no)	1000-Seed Weight (g)	Seed Yield Plant ⁻¹ (ton ha ⁻¹)	Harvest Index
GK 48	Waterlogged	0.50(58)	3.33(31)	30.92(93)	0.34(13)	0.14(32)
	Control	0.83	11.17	33.40	2.34	0.45
GK 65	Waterlogged	1.42(64)	7.96(65)	33.86(88)	1.05(30)	0.26(61)
	Control	2.17	14.00	39.33	3.27	0.42
BARImung 4	Waterlogged	0.98(36)	5.23(32)	30.40(94)	0.68(23)	0.20(55)
	Control	2.83	18.92	32.30	3.22	0.37
BARImung 6	Waterlogged	0.92(50)	4.56(55)	48.35(103)	0.97(39)	0.31(88)
	Control	1.92	9.25	47.00	2.48	0.35
ACC12890085	Waterlogged	0.83(53)	5.07(92)	49.59(98)	1.07(46)	0.29(77)
	Control	1.67	5.75	50.87	2.33	0.37
ACC12890054	Waterlogged	1.67(78)	11.58(54)	32.76(96)	1.56(46)	0.33(90)
	Control	2.17	21.25	34.16	3.37	0.37
BUmug 4	Waterlogged	1.25(51)	7.68(72)	36.46(95)	1.12(43)	0.32(72)
	Control	2.33	11.75	38.27	2.67	0.44
VC 1160-A	Waterlogged	1.75(83)	6.84(41)	30.67(99)	1.18(30)	0.30(76)
	Control	2.25	16.75	31.07	3.88	0.40
VC 6173-A	Waterlogged	1.75(80)	7.21(76)	39.24(85)	1.08(42)	0.30(75)
	Control	2.25	10.00	46.65	2.54	0.40
IPSA-13	Waterlogged	0.67(58)	5.06(84)	48.44(84)	0.91(34)	0.31(70)
	Control	1.17	6.58	58.04	2.70	0.44
GK 63	Waterlogged	0.67(61)	3.03(30)	37.26(103)	0.48(20)	0.20(49)
	Control	1.08	10.33	36.48	2.33	0.40
IPSA-15	Waterlogged	1.25(64)	7.06(86)	34.25(96)	1.04(36)	0.33(77)
	Control	2.08	12.42	35.58	2.97	0.42

The values in parenthesis indicate percent relative to control

Conclusion

The different morpho-physiological traits of mungbean were found susceptible to waterlogging, although genotypic variation in improving the waterlogging tolerance during recovery stages was highly evident. The recovery of the depressed plant traits was satisfactory and correlated well with yield and yield contributing characters and hence gave better yield in some genotypes. The dendrogram obtained from the SSR markers was more discriminatory and highly polymorphic and thus, more informative than the one obtained from morphological characterization. Further systematic studies are needed under field conditions to improve waterlogging tolerance of the selected genotypes for sustainable cultivation. A series of molecular lab experiments with more primers representing the wholegenome are essential as a step towards the genetic improvement of mungbean under soils waterlogging environment.

Acknowledgements

The authors wish to thank the Bangladesh Bureau of Educational Information and Statistics (BANBEIS), Ministry of Education, Government of the People's Republic of Bangladesh for providing the fund under the Higher Educational Research Program.

References

- [1] L.A.C.J. Voesenek, R. Sasidharan, Ethylineand oxygen signaling- drive plant survival during flooding. Plant Biol. 15 (2013) 426-435. https://doi.org/10.1111/plb.12014.
- [2] Y. Hirabayashi, R. Mahendran, S. Koirala, L. Konoshima, D. Yamazaki and S. Watanabe, H. Kim, S. Kanae, Global flood risk under Climate change. Nature Climate Change, 3 (2013) 816- 821. http://dx.doi.org/10.1038/nclimate1911.
- [3] D.P. Singh, B.B. Singh, Breeding for tolerance to abiotic stresses in mungbean, J. food legumes (2011) 83-90.
- [4] M. R. Islam, A. Hamid, Q.A. Khaliq, J.U. Ahmed, M.M. Haque, M.A. Karim. Genetic variability in flooding tolerance of mungbean (*Vigna radiata* L. Wilczek) genotypes. Euphytica, 156 (2007) 247-255. DOI: 10.1002/ldr.2339.
- [5] J. Gibbs, H. Greenway, Mechanisms of anoxia

- tolerance in plants. I. Growth, survival and anaerobic catabolism. Funct. Plant Biol. 30 (2003) 1-47. DOI: 10.1071/PP98095 ER.
- [6] T.D. Colmer, H. Greenway, Ion transport in seminal and adventitious roots of cereals during O2 deficiency, J. Exp. Bot. 62 (2011) 39-57. DOI: 10.1093/jxb/erq271.
- [7] M.R. Islam, A. Hamid, M.A. Karim, M.M. Haque, Q.A. Khaliq, J.U. Ahmed, Gas exchanges and yield responses of mungbean (*Vigna radiata* L. Wilczek) genotypes differing in waterlogging tolerance, Acta Physiol. Plant, 30 (2008) 697-707. DOI: 10.1007/s11738-008-0168-0.
- [8] M. Sarkar, S. Datta, S. Kundagrami, Global climate change and mungbean production: A roadmap towards future sustainable agriculture. In: Pandey, D and Sarkar, A (eds). Sustainable Future Food Security in Changing Environments. Nova Science Publishers, Inc. 99-120 (2017).
- [9] A. Sharma, S. Dhanda, Abiotic Stress Response in *Vigna radiata* L. (Mungbean), Int.
 J. Life Sci. Biotechnol. Pharma Res. 3 (2014)
 14. http://www.ijlbpr.com/currentissue.php.
- [10] P.Kumar, M. Pal, R. Joshi, R.K. Sairam, Yield, growth and physiological responses of mungbean [*Vigna radiata* (L.) Wilczek] genotypes to waterlogging at vegetative stage, Physiol. Mol. Biol Plants, 19 (2013) 209-220. DOI: 10.1007/s12298-012-0153-3.
- [11] S. Ahmed, E. Nawata, T. Sakuratani, Effects of waterlogging at vegetative and reproduction growth stage on photosynthesis, leaf water potential yield in mungbean, Plant Prod. Sci. 5 (2002) 117-123. DOI: 10.1626/pps.5.117.
- [12] Y.J.Kang, S.K. Kim, M.Y. Kim, P. Lestari, K.H. Kim, B.K. Ha, T.H. Jun, W.J. Hwang, T. Lee, J. Lee, S. Shim, M.Y. Yoon, Y.E. Jang, K.S. Han, P. Taeprayoon, N. Yoon, P. Somta, P. Tanya, K.S. Kim, J.G. Gwag, J.K. Moon, Y.H. Lee, B.S. Park, A. Bombarely, J.J. Doyle, S.A. Jackson, R. Schafleitner, P. Srinives, R.K. Varshney, S.H. Lee, Genome sequence of mungbean and insights into evolution within *Vigna* species, Nat. Commu. 5 (2014) 5443. DOI: 10.1038/ncomms6443.
- [13] Q.J Song, J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, P.B. Cregan.. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110 (2005) 550-560. DOI: 10.1007/s00122-004-1871-x.
- [14] P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. V. D. Lee, M. Hornes, M. Zabeau, AFLP: a new

- technique for DNA fingerprinting, Nucleic Acids Res. 23 (1995) 4407-4414.
- [15] J.G.K. Williams, A.R. Kubelik, K.J. Livak, J.A. Rafalski, V.T. Scott, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Res. 18 (1990) 6531-6535. https://doi.org/10.1093/nar/18.22.6531.
- [16] D. Tautz, Hypervariablity of simple sequences as a general source of polymorphic DNA markers, Nucleic Acids Res. 17 (1989) 6463-6471. https://doi.org/10.1093/nar/17.16.6463.
- [17] G. Toth, Z. Gaspari, J. Jurka, Microsatellites in different eukaryotic genome, survey and analysis, Genome Res. 10 (2000) 1967-1981. DOI: 10.1101/gr.10.7.967.
- [18] J. Tian, T. Isemura, A. Kaga, D.A. Vaughan, N. Tomooka, Genetic diversity of the rice bean (*Vigna umbellata*) gene pool as assessed by SSR markers, Genome 56 (2013) 717-727https://doi.org/10.1139/gen-2013-0118.
- [19] M.N. Huda, M. Hasan, H.M. Abdullah, U. Sarker, Spatial distribution and genetic diversity of wild date palm (*Phoenix sylvestris*) growing in coastal Bangladesh, Tree Genetics & Genomes, 15 (2019): 3. DOI: 10.1007/s11295-018-1310-9.
- [20] F. Martin, An application of kernel methods to variety identification based on SSR markers genetic fingerprinting, BMC Bioinform, 12 (2011) 177. DOI: 10.1186/1471-2105-12-177.
- [21] Y.H. Wang, D.D. Poudel and K.H. Hasenstein, Identification of SSR markers associated with saccharification yield using pool-based genome-wide association mapping in sorghum, Genome, 54 (2011) 883-889. https://doi.org/10.1139/g11-055.
- [22] K.T. Moe, J.W. Chung, Y.I. Cho, J.K. Moon, J.H. Ku, J.K. Jung, J. Lee, Y.J. Park, Sequence information on simple sequence repeats and single nucleotide polymorphisms through transcriptome analysis of mungbean, J Integr Plant Biol. 53 (2011) 63-73. DOI: 10.1111/j.1744-7909.2010.01012.x.
- [23] T. Isemura, A. Kaga, S. Tabata, P. Somta, P. Srinives, T. Shimizu, U. Jo, D.A, Vaughan and N. Tomooka. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS ONE 7 (2012) e41304. https://doi.org/10.1371/journal.pone.0041304.
- [24] P. Perata, W. Armstrong and L.A. Voesenek, Plants and flooding stress, New Phytologist. 190 (2011) 269-273. https://doi.org/10.1111/

- j.1469-8137.2011.03702.x.
- [25] F. P. Gardner, R.B. Pearce, R.L. Mitchell, Physiology of Crop Plant. Iowa State University Press. (1985) 187-208.
- [26] J.W.Y. Chen, R.S. Burton, Variation in alcohol dehydrogenase activity and flood tolerance in white clover (*Trifolium repens*), Evolution 46 (1992) 721-734. DOI: 10.1111/j.1558-5646.1992.tb02078.x.
- [27] G.C. Allen, M.A. Flores-Vergara, S. Krasynanski, S. Kumar, W.F. Thompson, A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide, Nat Protoc. 1 (2006) 2320–2325. DOI: 10.1038/ nprot.2006.384.
- [28] X. Zhang, S. Shabala, A. Koutoulis, L. Shabala, P. Johnson, D. Hayes, D.S. Nichols, M. Zhou, Waterlogging tolerance in barley is associated with faster aerenchyma formation in adventitious roots, Plant Soil, 394 (2015) 355-372. DOI:10.1007/s11104-015-2536-z.
- [29] M. Sauter, Root responses to flooding. Curr. Opin. Plant Biol. 16 (2013) 282–286. DOI: 10.1016/j.pbi.2013.03.013.