Physiological responses and expression of VrDREB2A gene at different growth stages of mungbean (*Vigna radiata* L. Wilczek) under drought stress

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Physiological responses and expression of *VrDREB2A* gene at different growth stages of mungbean (*Vigna radiata* L. Wilczek) under drought stress

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Abstract

Mungbean is an important pulse crop and commonly grown in Asia. Drought affects mungbean growth and yield at different growing stages and various levels through physiological traits and gene expression. In this study, two mungbean cultivars, DX208 and Tam Thanh Hoa, were exposed to drought at the vegetative and flowering stages and assessed for various morphophysiological traits at 8, 12, 15 and 15 days post withholding water and the plant recovery 7 days after re-watering. Differential expression of *VrDREB2A* gene was observed in leaf and root of two mungbean cultivars under drought condition. Plants used up water more quickly at the flowering stage than the vegetative stage. Drought adversely affected the plant height, leaf number, above-ground plant biomass and root weight with relative reduction to the control by 4.0 – 85%. Yield components and individual yield reduced significantly by around 50 – 60% compared to the control. Relative expression of *VrDREB2A* gene was varied, with stronger expression in leaves and roots when drought imposed at the flowering and vegetative stages respectively. Increase in *VrDREB2A* expression occurred earlier at 8 days compared with 12 days for drought imposed at the flowering and vegetative stages respectively, resulting in more tolerance of plants to drought at the flowering stage. The results indicate that *VrDREB2A* functioned as an important transcriptional activator and might help increase the drought stress tolerance of the mungbean plant at various growing stages. Morphophysiological traits can also be used as indicators in screening mungbean for drought tolerance.

Key words: *DREB*, drought stress, gene expression, plant available water, relative reduction.

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39 Material preparation, data collection and analysis were performed by Thi Thuy Hang Vu,
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41 Thi Thuy Hang Vu and all authors commented on previous versions of the manuscript.
42 All authors read and approved the final manuscript.

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44 Pham; Formal analysis and investigation: Thi Thuy Hang Vu and Thi Tuyet Cham Le;
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59 INTRODUCTION

60 Drought is a common abiotic stress that is severe threats to crop growth and food
61 production worldwide and can lead to more than 50% yield loss (Singh et al. 2015;
62 Nadeem et al. 2019). Drought stress affects the plants at different plant development
63 stages and at various levels of their organization from phenological, morphological,
64 physiological to molecular levels (Ahmad and Prasad 2012; Kaur and Asthir 2017; Nair
65 et al. 2019). Low germination rate, early flowering, flower abscission and young pod
66 abortion are observed throughout plant development stages under water deficit
67 (Ranawake et al. 2012; Bangar et al. 2019; Nadeem et al. 2019). Various
68 morphophysiological indicators such as growth and yield parameters (*viz.* plant height,
69 leaf number and area, number of pods per plant, number of pods per cluster, number of
70 clusters per plant, number of seeds per pod) and root and shoot characters (*viz.* root and
71 shoot length, number of roots, root diameter, fresh and dry weight of root and shoot)
72 have been used for screening drought tolerance in mungbean (Prakash et al. 2017;
73 Bangar et al. 2019).

74 At molecular level, the signaling network and transcriptional regulatory
75 pathways are important to abiotic stress responses (Atkinson and Urwin 2012;
76 Casassola et al. 2013; Yoshida et al. 2014). Various transcription factors (TFs) and their
77 binding sites, the so-called cis-acting elements have been identified as molecular
78 switches of stress-responsive gene expression. Five TF families [bZIP (mainly
79 *AREB/ABF*), *DREB (AP2/EREBP)*, *MYB/MYC*, *NAC* and *WRKY*] have been shown to
80 be associated with drought tolerance (Joshi et al. 2016). Among those TF families,
81 plant-specific *DREB* (dehydration-responsive element binding protein) originally
82 isolated in *Arabidopsis* (Liu et al. 1998) has been identified, isolated and characterized
83 in different plant species, such as common wheat, rice, maize and soybean (Chen et al.
84 2007; Joshi et al. 2016). The *DREB* transcription factor was also reported to be induced
85 by drought (Nakashima et al. 2000; Sakuma et al. 2006; Qin et al. 2007). The roles of
86 the *DREB2A* gene from *Arabidopsis* in abiotic stress tolerance have been well
87 characterized (Sakuma et al. 2006) but very limited in legume, especially in mungbean.
88 In cowpea, *VuDREB2A* was induced by drought and salt stresses, and heterologous
89 expression of *VuDREB2A* resulted in significant drought stress tolerance in *Arabidopsis*
90 (Sadhukhan et al. 2014). In soybean, two *GmDREB2A* homologs (*GmDREB2A;1* and
91 *GmDREB2A;2*) were identified (Mizoi et al. 2013). Although the peptide sequences are

very similar to each other, the induction of *GmDREB2A;2* was stronger than that of *GmDREB2A;1* and improved stress tolerance in *Arabidopsis* (Mizoi et al. 2013). The *DREB2A* genes from different species, such as common wheat (Egawa et al. 2006), pearl millet (Agarwal et al. 2007) and chrysanthemum (Liu et al. 2008) that caused physiological variations, were also overexpressed in *Arabidopsis thaliana* and improve stress tolerance under drought, salt and freezing stresses in transgenic plants. However, similar studies are very limited in mungbean, and the molecular mechanisms controlling plant tolerance to abiotic stress such as drought remains largely unknown.

Mungbean (*Vigna radiata* L. Wilczek) is the third most important pulse crop after chickpea and pigeon pea and predominantly grown across Asia (Nair et al. 2013, 2019). The mungbean, a short growth duration crop, is grown widely under rainfed conditions and an ideal legume for catch cropping, intercropping, relay cropping and soil nutrient improvement but still considered as an underutilized crop. Due to uneven temporal and spatial distribution of rainfall, the crop often suffers from water stress at any growth stages in its growing cycle leading to reduced growth and productivity. In addition, being grown on marginal lands and under hot climate conditions (Nair et al. 2013; van Zonneveld et al. 2020), mungbean is largely considered as a moderately drought tolerant, and this distinctive characteristic makes it a valuable tropical legume for studying the molecular tolerance mechanisms of various abiotic stresses, particularly drought (Kim et al. 2004, 2015). Recently, the *VrDREB2A* gene with conserved AP2 domains and transactivation ability was isolated from mungbean by Chen et al. (2016). The expression of the *VrDREB2A* gene is induced by drought, high salt concentrations and abscisic acid treatment at seedling stage. Moreover, the overexpression of *VrDREB2A* in transgenic *Arabidopsis* activates the expression of downstream genes, resulting in enhanced tolerance to drought and high-salt stresses and no growth retardation (Chen et al. 2016). This indicates that *VrDREB2A* functions as an important transcriptional activator and may help increase the abiotic stress tolerance of the mungbean plant. However, it has not been known yet how the expression of *VrDREB2A* would affect mungbean under water deficit at different growth stages, viz. vegetative and flowering stages.

Therefore, a better understanding of the responses of mungbean under different drought stress conditions in aspects of physiological traits and gene expression is required for mungbean improvement. Within this aim, the growth and the associated

125 expression of *VrDREB2A* gene in response to drought stress at the vegetative and
126 flowering stages in mungbean cultivars of Vietnam were studied.

127 **MATERIALS AND METHODS**

128 **Plant materials and drought imposition**

129 Plant materials included two mungbean cultivars, DX208 (improved cultivar,
130 G1) and Tam Thanh Hoa (landrace, G2), obtained from the Plant Resources Center of
131 Vietnam Academy of Agricultural Sciences. These two cultivars are suitable to dry
132 and sandy soils so that they are somewhat moderately to highly drought tolerance
133 (Nguyen et al. 2017).

134 The mungbean cultivars were planted in greenhouse of the Faculty of
135 Agronomy, Vietnam National University of Agriculture from May to September, 2019
136 (summer season). The seeds were sown in 30 x 40 cm in diameter and height pots, each
137 pot was left with two plants. The pots were filled with substrate containing 6.5 kg
138 alluvial soil mixed with fine sand (1:1, v/v) and 0.5 kg Song Gianh microbial-organic
139 fertilizer containing 0.45 g phosphorus and kept in greenhouse under temperature
140 condition 32 °C with regular watering every day before water was withheld in drought
141 treated plants. The experiment was arranged in a randomized complete block with two
142 replications with four pots each.

143 For drought imposition at vegetative stage, the plants were fully watered until 20
144 days after emergence (2 true-leaf stage) and then water was withheld for 8, 12, 15 and 20
145 days. For drought imposition at flowering stage, the plants were fully watered for 33
146 days after emergence (first flower bud appearance) and the drought was imposed for
147 duration times similar to those at vegetative stage. The control plants were fully watered
148 throughout the experiment.

149 The recovery experiment was set up separately with the same cultural preparation
150 and drought imposed at vegetative and flowering stages, and with two replications (4
151 plants per replication). After 20 days without water, all pots were simultaneously
152 watered to evaluate the recovery.

153 **Evaluation of plant growth characteristics**

154 At the setup of the experiment, the pots were saturated with water and weighted.
155 All pots were weighted weekly to measure the water used by plants. After 8, 12, 15 or
156 20 days without water, all pots were also weighted to measure the water used. The

characteristics measured at 8, 12, 15 and 20 days post drought exposure included plant height, leaf number, the fresh and dry weight of the above-ground mass and roots.

The recovery measurements included plant height, leaf number, yield components and individual yield (g plant⁻¹). The recovery was scored using 1 – 4 scale: (1) plant death plant; (2) less than 30% wilting leaf recovered; (3) more than 60% wilting leaf recovered; (4) completely recovery with more than > 90% wilting leaf recovered.

RNA extraction, synthesis of cDNA and qRT-PCR reaction

The leaf and root samples were harvested from at 8, 12, 15 and 20 days post drought exposure. The samples were cleaned, wrapped in aluminium foil and immediately immersed in liquid nitrogen and stored at -80°C freezer before RNA extraction.

RNA was isolated from leaf and root samples using Easy-spin™ IIP Plant RNA Extraction Kit (Intron Bio-Korea). cDNA was amplified using reverse transcription PCR (RT-PCR) with MLV Reverse Transcriptase (Promega, Tokyo, Japan). The primers of *VrDREB2A* gene and the reference gene *VrActin* (Chen et al. 2016) were used for qRT-PCR reaction (Table 1). The Master mix for qRT-PCR was accompanied by the kit GoTaq(R) qPCR Master Mix (Promega, A6001) containing 0.1 µg/µl master mix, 1.6×10⁻³ µg/µl primer of each kind, 0.08 µg/µl cDNA and water.

The leaf and root samples of the control plants (fully watered) of the cultivar DX208 were included in each qRT-PCR reaction as reference to enable the calculation of the relative gene expression under drought stress.

Table 1. Primer sequences used for qRT-PCR reaction

Gene	Primer	Sequence
<i>VrDREB2A</i>	<i>VrDREB2A-F</i>	5'-CTGCTCTTGCTTATGATGAA-3'
	<i>VrDREB2A-R</i>	5'-ATGTAGTGGTAGTAGTAGTAGTG-3'
<i>VrActin</i>	<i>VrActin-F</i>	5'-TCCACGAGACAACATATAACT-3'
	<i>VrActin-R</i>	5'-TCCTTGCTCATCCTATCAG-3'

The thermal RT-PCR cycle was run as follows: 95°C for 1 min, 40 cycles 95 °C in 15 seconds, 60 °C for 1 min, the curve was noted from 55-95° C, 72 °C in 18

seconds. After the cycle terminated, the process was transferred to 72 °C for 7 min and the samples were kept at 12 °C. The qRT-PCR was done with two technical replications for each sample to ensure the reliability and homogeneity. A reference sample was selected arbitrary from any RNA sample in each qRT-PCR.

Statistical analysis

Plant available water and expression of physiological traits

The plant available water (PAW) was deterimed as follows:

$$\text{PAW (\%)} = \frac{W_t - W_i}{W_s - W_i} \times 100$$

Where: W_t is the pot weight at 8, 12, 15 and 20 days after drought exposure

W_s is the weight of pot saturated with water

W_i is the weight of pot initially filled with substrate

The relative differences (%) of evaluated traits between the treatment (imposed drought at vegetative and flowering stages) and control were used for analysis:

$$\text{Relative difference (\%)} = \frac{P_t - P_c}{P_c} \times 100$$

Where: P_t and P_c are the trait in drought and control treatment respectively;

The drought resistance index (DRI) of mungbean cultivars was calculated by formula presented by Fischer and Maurer (1978):

$$\text{DRI} = \frac{Y_t/Y_c}{\bar{Y}_t/\bar{Y}_c}$$

Where: Y_t and Y_c are the grain yield in drought and control treatment respectively;

$\bar{Y}_t$ and $\bar{Y}_c$ are the average values of all examined genotypes of grain yield in drought and control treatment respectively.

ANOVA analysis was used to evaluate the differences in physiological traits between varieties exposed to drought at different stages (vegetative and flowering) and at different times (8, 12, 15 and 20 days after withholding water). The mean values were calculated from replicates.

Relative expression of VrDREB2A

The relative expression of *VrDREB2A* was presented by $R = 2^{-\Delta\Delta C_t}$ based on housekeeping gene and calibrator as described by Livak and Schmittgen (2001). $2^{-\Delta\Delta C_t}$ is

the relative gene expression of the test sample in comparison with the control. In this study, the relative gene expression was determined based on the expression of *VrDREB2A* in leaves and roots to the reference gene *VrActin* and to the calibrator (leaf sample of G1 under full watering condition) using the following equation:

$$R = 2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct_T - \Delta Ct_C)}$$

$$= 2^{-[(Ct_{T-VrDREB2A} - Ct_{T-VrActin}) - (Ct_{C-VrDREB2A} - Ct_{C-VrActin})]}$$

Where:

$Ct_{T-VrDREB2A}$ is the threshold cycle of *VrDREB2A* in drought sample

$Ct_{T-VrActin}$ is the threshold cycle of reference gene *VrActin* in drought sample

$Ct_{C-VrDREB2A}$ is the threshold cycle of *VrDREB2A* in the calibrator sample

$Ct_{C-VrActin}$ is the threshold cycle of reference gene *VrActin* in the calibrator sample

The R value determined the the expression of *VrDREB2A* in drought treatment at 8, 12, 15 days 20 post stress exposure. In biological terms, $R \leq 0.5$ or ≥ 2.0 is considered as change in gene expression.

RESULTS

Plant available water under water stress at vegetative and flowering stages

The plant available water (PAW) differed apparently among time duration exposed to drought and mungbean cultivars (Table 2). Eight days post drought exposure PAW quickly reduced to 63.3 and 36.8% for G1 and 55.0 and 31.5% for G2 at vegetative and flowering stage, respectively. After 12 and 15 days when water was withheld, PAW continued to decrease since the plants were still able to uptake water to maintain growth. Twenty days post-exposure to drought the water was completely used up by the plants with PWA $\leq 1.0\%$. Reduction of PAW per day varied around 4 – 5%. Table 2. The PAW (%) of mung bean varieieies at vegetative and flowering stages at 8, 12, 15 and 20 days post drought exposure

Drought stage	Cultivar	PAW (%) post drought exposure				Reduction of PAW (%/day)
		8 days	12 days	15 days	20 days	
Vegetative	G1	63.3	45.4	29.0	1.0	5.03

stage	G2	55.0	38.8	17.1	0.5	5.06
Flowering stage	G1	36.8	10.4	3.8	0.3	4.35
	G2	31.5	8.3	3.6	0.3	4.15

238 **Effect of drought on the growth and development of mungbean**

239 The analysis of variance for drought imposition stage and duration time showed
 240 that drought significantly affected plant growth, such as plant height and leaf number as
 241 compared with the control. Increased drought duration time adversely reduced plant
 242 growth (Table 3; Fig. 1). When imposing drought at vegetative stages for 8 – 20 days,
 243 the relative reduction in plant height increased from 14.6 to 43.4% for G1 and from 5.3
 244 to 45.2% for G2. Similar pattern was also observed for the relative reduction in plant
 245 height caused by drought at flowering stage (Table 3a). The relative reduction in the
 246 number of leaves compared to the control at 8-20 days varied from 5.1 to 38.5% and
 247 from 3.9 to 25.0% for drought imposed at vegetative and flowering stages respectively
 248 (Table 3b). In general, relative reduction in plant growth was somewhat higher in G1.

249 Drought stress at vegetative as well as flowering stages significantly affected the
 250 accumulation of plant biomass (Fig. 1). The fresh and dry plant weight, and fresh root
 251 weight significantly reduced when exposed to drought at both vegetative and flowering
 252 stage (Table 3 c-e). As soon as water was withheld, the plant and root fresh weights
 253 dramatically reduced as compared to the control, with the rate of 5.3% to 16.1% at 8
 254 days. The reduction was proportional with the time of exposure to drought with high
 255 relative reduction, even up to 80% as observed for fresh root weight at 20 days.

256 Table 3. Relative reduction (%)^a of measured mungbean characters as influenced by
 257 drought in relation to fully watered plants

Measured characters	Cultivar	Duration of drought exposure at vegetative stage				Duration of drought exposure at flowering stage			
		8 days	12 days	15 days	20 days	8 days	12 days	15 days	20 days
a. Plant height	G1	-14.6	-24.2	-33.6	-43.4	-17.9	-32.6	-35.6	-35.5
	G2	-5.3	-29.3	-34.7	-45.2	-8.7	-13.1	-13.2	-17.1
b. Leaf number	G1	-8.6	-18.8	-33.3	-38.5	-13.2	-15.6	-19.4	-25.0
	G2	-5.1	-14.3	-16.7	-28.6	-3.9	-5.3	-7.7	-7.7
c. Fresh plant weight	G1	-32.8	-45.5	-65.3	-75.8	-14.6	-36.7	-47.5	-67.1
	G2	-28.6	-46.4	-56.1	-79.5	-5.3	-8.0	-43.8	-66.3
d. Fresh root weight	G1	-57.9	-67.7	-74.6	-84.8	-16.1	-27.3	-39.0	-80.8
	G2	-41.7	-62.5	-73.9	-83.8	-12.0	-27.3	-38.9	-63.3

e. Dry plant weight	G1	-22.9	-37.6	-65.4	-66.0	-7.9	-39.9	-40.3	-42.9
	G2	-24.0	-42.3	-43.0	-76.8	-3.2	-9.1	-37.9	-42.0

^a Negative values indicate the relative reduction (%) of the measured traits in drought treatment to the control

Fig. 1 Severe symptoms of plants and roots after 20 days exposed to drought at the flowering stage on two mung bean cultivars, DX208 (A) and Tam Thanh Hoa (B) (left: control, right: drought stress)

Effect of drought on plant recovery

At 20 days post-drought exposure, mung bean plants apparently showed wilting, leaf dropping and death. After rewatering, only those leaves that remained on the plant recovered, contributing to the regrowth of the plants. For drought imposed at the vegetative stage, plant height increased by 7.6 – 22.5% and leaf number increased by 7.5 – 31.3% (by 0.4 – 1.3 leaves) compared to the point prior re-watering (Table 4). This recovery trends were not observed for drought imposed at the flowering stage, with the slightly increase in plant height (6.2 – 8.0%) but reduction in leaf number (18.8 – 25.5%). However, in comparison with the control plants, the growth characteristics of stressed plants remained much lower regardless of stages exposed to drought, with ranges of 24.5 – 47.2% for plant height and of 10.5 – 37.6% for leaf number. The recovery scores of cultivar G2 were higher than those of cultivar G1.

Table 4. Recovery of mungbean plants 7 days after rewatering^b

Drought stage	Cultivar	In relation to control (%)		In comparison to point prior re-watering (%)		Plant height (cm)	Leaf number	Recovery score
		Plant height	Leaf number	Plant height	Leaf number			
Vegetative stage	G1	-47.2	-37.6	22.5	31.3	27.0	5.3	2.8
	G2	-36.5	-17.0	7.6	7.5	32.0	5.4	3.3
Flowering stage	G1	-37.5	-14.1	8.0	-18.8	34.3	4.9	2.0
	G2	-24.5	-10.5	6.2	-25.5	38.5	4.5	2.2

^b Negative values indicate the relative reduction (%) of the measured traits in drought treatment to either control or to the point prior re-watering; The positive indicates the relative increase (%) after 7 days of recovery compared to the point prior re-watering.

Water deficit at both plant growth stages significantly and differently affected

the components of yield, viz. number of pod clusters, number of pods per plant, pod weight and plant individual yield (Table 5). The relative reduction of yield components was rather high in comparison with the control, ranging from around 28 – 60%, especially the number of pods and individual yield, but lower in cultivar G2. The pod cluster and pod number per plant in both cultivars reduced by 28.6 – 55.2% and 30.0 – 58.1%, respectively, leading to reduce pod weight by 53.5 – 62.2% and plant individual yield by 49.6 – 56.6% in relation to the control. It appeared that cultivar G2 was better tolerant to drought than cultivar G1 as evidenced by higher plant yields (2.08 and 2.31 g plant⁻¹) and drought resistance indices (1.024 and 1.069) (Table 5).

Table 5. Effects of drought at vegetative and flowering stage on yield components, individual yield relative to control and drought resistance index (DRI)^a

Drought stage	Cultivar	Reduction (%) in relation to control				Plant yield (g plant ⁻¹) ± s.e	DRI
		Pod cluster number	Pod number	Pod weight	Plant yield		
Vegetative stage	G1	-55.2	-57.9	-56.3	-56.6	1.68 ± 0.27	0.972
	G2	-46.4	-30.0	-53.5	-53.3	2.08 ± 0.72	1.024
Flowering stage	G1	-37.1	-58.1	-62.2	-55.7	1.69 ± 0.47	0.980
	G2	-28.6	-47.1	-54.1	-49.6	2.31 ± 0.47	1.069

^a Negative values indicate the relative reduction (%) of the measured traits in drought treatment to the control

Relative expression level of *VrDREB2A* gene

The t-test indicated that there was difference in gene expression of *VrDREB2A* between normal and drought conditions at both vegetative and flowering stages (Fig. 2 & 3).

The response to drought set in differently in mungbean cultivars, time point and duration, and stage of plant growth when water was withheld. Generally, at 12 and 8 days onwards after drought exposure at the vegetative and flowering stages respectively, the relative gene expression levels in leaves and roots were higher than the control plants (Fig. 2). At vegetative state, the relative expression level of *VrDREB2A* in root started to increase significantly early at 8 days post-drought exposure earlier in cultivar G1 (R = 2.689) and at 20 days after withholding water (R = 2.041). Significant expression was also found leaves of cultivar G2 at 15 days (R = 3.137) and in roots (R = 2.925) at 20 days after withholding water.

308 **Fig. 2** Relative expression of *VrDREB2A* gene in leaves and roots of two mung bean
309 cultivars, DX208 (G1) and Tam Thanh Hoa (G2), exposed to drought at the vegetative
310 stage for (a) 8, (b) 12, (c) 15, (d) 20 days in comparison with the control

311 Unlike drought exposure at vegetative stage, the relative expression level of
312 *VrDREB2A* when plants exposed to drought at flowering stage was earlier and higher in
313 comparison to the control both in leaves and roots (Fig. 3). Significant expression levels
314 in leaves were observed for both G1 and G2 throughout 8 – 20 days after withholding
315 water (R varied from 0.901 to 4.780). Significant expression levels in roots for G1 and
316 G2 were identified on 8 days exposure to drought with R of 2.271 and 2.855
317 respectively.

318

319 **Fig. 3** Relative expression of *VrDREB2A* gene in leaves and roots of two mung bean
320 cultivars, DX208 (G1) and Tam Thanh Hoa (G2), exposed to drought at the flowering
321 stage for (a) 8, (b) 12, (c) 15, (d) 20 days in comparison with the control

322 **DISCUSSION & IMPLICATION**

323 The larger reduction of PAW at flowering stage can be attributable to larger
324 plant size (*viz.* higher plant height and leaf number) at the point of water withholding as
325 compared with that at vegetative stage (Table 2). As results, plants used up water more
326 quickly at the flowering stage than at the vegetative stage, with PAW reaching to $\leq 5\%$
327 by 15 days. Thus, mungbean can stand for drought up to 15 – 20 days upon the growth
328 stages that drought occurs. Some studies also applied this range of days for evaluation
329 of drought tolerance in mungbean, such as Iseki et al. (2018) (7 – 22 days) and Bangar
330 et al. (2019) (15 days), suggesting the maximum time mungbean can stand for without
331 irrigation.

332 Drought significantly affects plant growth regardless of growth stages. The
333 longer the exposure to drought the higher is the reduction of physiological traits, such as
334 plant height, leaf number, above-ground plant biomass and root weight (Table 3). The
335 relative reduction in those physiological traits can be varied largely from 4.0 – 85.0%,
336 which results in large relative reduction in yield component traits and individual yield
337 (mostly around 50 – 60%) (Table 5). Therefore, drought affects adversely plant yield as
338 seen by the reduced number of pods per plant, number of pod clusters per plant, pod
339 weight and individual yield at both the vegetative and flowering stages. Other traits such

340 as relative water content, membrane stability index, chlorophyll content, protein and
341 proline contents, and harvest index were also found to significantly reduced by 1.0 –
342 80% compared to the control (Bangar et al. 2019; Nadeem et al. 2019). Fathy et al.
343 (2018) and Nadeem et al. (2019) reported yield loss in the range of 31 – 60% at
344 flowering and 26% at post flowering/podding stages in mungbean due to drought stress.
345 The vegetative stage was more sensitive to drought stress than the flowering stage with
346 higher relative reduction in plant height and leaf number after 8 – 20 days post
347 withholding water and 7 days of recovery. This phenomenon was also observed in
348 previous studies (Allahmoradi et al. 2011; Ranawake et al. 2012; Ratnasekera and
349 Subhashi 2015; Bangar et al. 2019) and is probably due to the low water absorption
350 capacity during the vegetative stage.

351 The re-watering time point is critical for recovery of plants. In this experiment,
352 plants were rewatered at 20 days after exposure to drought, which is the common
353 duration of drought that plants can stand for. The common symptoms of plants exposed
354 to drought are leaf wilting, rolling and even dropping, and wilting growing tips (Vu et
355 al. 2015). Therefore, the more remnant leaves on the plants, the more ability for plants
356 to recover after re-watering. Plants exposed to drought at the vegetative stage were able
357 to increase their height and leaf number after re-watering. However, the leaf number
358 was reduced after recovery 7 days since dropping and death of lower leaves near the
359 ground (Table 4). In addition, in this experiment, cultivar G2 seemed to be more
360 drought tolerant than cultivar G1 with smaller relative reduction in physiological traits,
361 and higher individual yields, recovery scores and drought resistance indices.

362 Drought causes fluctuations in relative expression levels of *VrDREB2A* gene in
363 leaves and roots. Increase in *VrDREB2A* expression generally occurred earlier at 8 days
364 compared with 12 days for drought imposed at the flowering and vegetative stages
365 respectively (Fig. 2 & 3). At the vegetative stage, *VrDREB2A* gene seemed to be
366 strongly and clearly express in the roots, especially close to the 20 days with the relative
367 expression levels about 5.9 – 27.2 times of the control (Fig. 2d). At the flowering stage,
368 the relative expressions of *VrDREB2A* were generally higher in both stressed leaves and
369 roots than in the control. Although the relative expression decreased with the duration
370 time of drought exposure, it remained at higher level with more than twice compared to
371 the control. The significant expression was more obvious in roots than in leaves and
372 lasted from 8 to 20 days (Fig. 3). At 1-week-old seedling stage, expression of

373 *VrDREB2A* in response to drought began to increase within 1 h (around 6-fold) and
 374 continued to increase after 3 h (around 13- fold) (Chen et al. 2016). Thus, these results
 375 were in line with study by Chen et al. (2016) that the activation of expression of
 376 *VrDREB2A* gene was induced by abiotic stresses, including drought. The earlier and
 377 longer activation of *VrDREB2A* gene at the flowering stage can explain the more
 378 tolerance of mungbean plants to drought than at the vegetative stage. However, the
 379 increase in relative levels of *VrDREB2A* expression in this study are not as high as in
 380 other studies on DREB when plants are exposed to abiotic stresses. This might be
 381 correlated to the time plant samples should be collected and analyzed for gene
 382 expression. For example, relative expression levels of *VrDREB2A* were 6 - 13-fold after
 383 1 - 3 h post drought treatment and after 1 - 24 h post salt treatment (Chen et al. 2016).
 384 *ThDREB* expression levels in root and stem of *Tamarix hispida* were around 369-fold of
 385 control under NaCl stress for 6 h and 6-fold of control under PEG stress for 12 h (Yang
 386 et al. 2017). The expression of other drought tolerant related gene in mungbean,
 387 *VrSnRK2.6c*, showed the highest level (12-fold) when 11-day-old mungbean plants
 388 exposed to 3 days withholding water (Fatima et al. 2020). The upregulation of *VrDREB*
 389 in drought tolerant mungbean was also observed during recovery from drought and
 390 higher in leaf than in petiole (Meena et al. 2021). In addition, the increase in gene
 391 expression in plants is not always the cases when plants are exposed to drought. The
 392 expression of drought tolerance gene might occur in other plant organs depending on
 393 plant species. For instance, the expression of *GmHK06* (encoded for protein
 394 homologous to CK12/AHK5 in *Arabidopsis*) in soybean reduced in roots but increased
 395 in shoots in plants exposed to water stress. In contrast, drought stress led to increased
 396 expression of *GmRR34* in both soybean roots and shoots (Hoang et al. 2014).

397 The results of the present study clearly show that water stress adversely
 398 influenced plant growth and yield of mungbean in different manners as reported in
 399 various studies (Blum 1996; Sanchez et al. 2012; Iseki et al. 2018). Under water stress
 400 the expression of *VrDREB2A* gene was activated to combat plant water deficit. The
 401 expression level increased significantly when water stress occurs at flowering stage and
 402 in leaves. In relation to the genotype, the drought resistant cultivar showed stronger
 403 *VrDREB2A* expression. Therefore, this knowledge of physiological responses and gene
 404 expression in mungbean under drought condition might contribute to ongoing studies of
 405 drought resistance in mungbean.

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Figures

A

B

Figure 1

Severe symptoms of plants and roots after 20 days exposed to drought at the flowering stage on two mung bean cultivars, DX208 (A) and Tam Thanh Hoa (B) (left: control, right: drought stress)

Figure 2

Relative expression of VrDREB2A gene in leaves and roots of two mung bean cultivars, DX208 (G1) and Tam Thanh Hoa (G2), exposed to drought at the vegetative stage for (a) 8, (b) 12, (c) 15, (d) 20 days in comparison with the control

Figure 3

Relative expression of VrDREB2A gene in leaves and roots of two mung bean cultivars, DX208 (G1) and Tam Thanh Hoa (G2), exposed to drought at the flowering stage for (a) 8, (b) 12, (c) 15, (d) 20 days in comparison with the control