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Variations in ethylene sensitivity among mungbean [*Vigna radiata* (L.) Wilczek] genotypes exposed to drought and waterlogging stresses

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Abstract: Ethylene is an important phytohormone that regulates several aspects of plant development including those crucial for drought tolerance. The main aim of the present work was to investigate variability in ethylene sensitivity of mungbeans to seed germination, waterlogging, and leaf senescence, and the underlying transcriptional regulation of ethylene biosynthetic/responsive genes. Significant variation was observed for silver-nitrate–mediated germination inhibition and flooding stress tolerance among the mungbean genotypes. Genotypes with differential ethylene sensitivity (IC-325817 and IC-325756) revealed variable leaf senescence rates upon exposure to exogenously supplied ethephon. Drought induced significant upregulation of *VrACS7* and *VrERF* genes in the more sensitive genotype, implicating the role of plant moisture status in influencing ethylene biosynthesis/perception. Drought-mediated upregulation of *VrACS7* transcripts was accompanied by reduced lateral root formation in the more sensitive genotype (IC-325756), although the same did not follow in the less sensitive genotype (IC-325817), which had reduced ethylene responsiveness. These studies emphasize the variability in ethylene sensitivity of mungbeans and reveal a genotype- dependent transcriptional regulation of ethylene biosynthetic/responsive genes upon drought in this waterlogging-sensitive legume crop.

Key words: Mungbean genotypes, germination, chlorophyll fluorescence, ethephon, gene expression

1. Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is an important pulse crop in India, contributing to the protein requirement of the vegetarian population. It is rich in digestible protein (approximately 25%–28%) and enhances soil fertility through N_2 fixation. The crop fits well into multicropping systems because of its rapid growth and early maturity. However, its large-scale adoption is constrained by yield losses in areas exposed to various biotic and abiotic factors. Abiotic stress factors like heat, cold, drought, and flooding adversely affect crop productivity worldwide (Raina et al., 2018). Being sessile, plants have developed enormous diversity in their intrinsic factors which facilitate survival in a wide range of environments. Among the intrinsic factors, hormones are important chemical messengers which modulate plant growth in response to environmental cues. Although response to a particular stress condition is governed by a specific hormone, the overall response is in part regulated by extensive crosstalk among various hormones (Verma et al., 2016). Moreover, it is not only the variability in endogenous levels of a hormone but also the proportions thereof which contribute to stress adaptation. Genetic diversity in sensitivity to phytohormones also plays an important role in differential stress responses (Lee et al., 2018).

Although ABA is considered to be the principal hormone that regulates plant responses to abiotic stress, several studies have revealed the indirect role of other hormones in plant functions through extensive crosstalk. Ethylene is another plant hormone which has been implicated in several abiotic stress responses. It is a simple gaseous phytohormone whose endogenous concentration is normally low in tissues; however, its synthesis can be dramatically induced during seed germination, fruit ripening, and leaf and flower senescence, as well as in response to biotic and abiotic stresses (Kende, 1993; Yoon et al., 1997). Stress-induced ethylene production, for example under heat, flooding, air pollution, soil compaction, and drought can also directly influence yield, notably through reduced grain-filling rates and/or increased embryo and grain abortion (Hays et al., 2007; Wilkinson and Davies, 2010). There is also evidence that

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stress ethylene may directly reduce photosynthesis (Rajal and Peltonen-Sainio, 2001). Ethylene-insensitive mutants of tomato exhibit increased root biomass (Gallie, 2010). In maize, downregulation of ethylene biosynthesis improved the grain yield under abiotic stress conditions (Liu and Zhang, 2004; Wang et al., 2004; Habben et al., 2014;). However, an exogenous supply of ethephon increased stomata conductance and photosynthesis in mustard (Iqbal et al., 2011), signifying its role in enhancing transpiration. Contrary to this, ethylene caused leaf abscission in soybean under drought, resulting in reduced water loss (Arraes et al., 2015). Ethylene is also reported to play a role in regulating stomata closure which constitutes an important drought adaptation strategy of plants. Environmentally-induced ethylene accumulation has been reported to induce stomata closure in lettuce (Vysotskaya et al., 2011). Interestingly, ethylene has also been reported to antagonize drought- and ABA-induced stomata closure in Arabidopsis (Tanaka et al., 2005). Ethylene-mediated inhibition of stomata closure under moderate drought appears beneficial for carbon fixation and the leaf-cooling trait associated with improved yields in hot environments (Wilkinson et al., 2012).

Biosynthesis of ethylene in higher plants is mediated by enzymes aminocyclopropane-1-carboxylic acid synthase (ACS) and aminocyclopropane-1-carboxylic acid oxidase (ACO). The *ACS* gene controlling the rate-limiting step of ethylene biosynthesis belongs to a multigene family. In citrus, water-stress–mediated ABA accumulation promotes synthesis and accumulation of ACC through stimulation of ACC synthase activity in roots (Tudela and Primo-Millo, 1992). The *ACS* gene was upregulated in drought-tolerant soybean genotypes when the plants were exposed to water stress (Arraes et al., 2015). However, maize *Zmacs6* mutants with reduced ethylene biosynthesis exhibited reduced leaf senescence and higher CO_2 assimilation in expanding leaves under drought conditions (Young et al., 2004).

Genetic variability of stress-related ethylene generation has been reported in wheat (Balota et al., 2004). Wide genotypic differences were observed for ethylene biosynthetic ability in *Dianthus caryophyllus* (Olsen et al., 2015). Moreover, enhanced flower life was associated with low expression levels of *DcACS1*, *DcACS2*, and *DcACO1* genes in *Dianthus caryophyllus* plants (Olsen et al., 2015). Variations in ethylene sensitivity have been associated with losses in foliage and grain yield under stress, senescence, and growth of plants (Balota et al., 2004; Hays et al., 2007). Screening for variation in ethylene sensitivity can also facilitate the identification of genotypes with low stress ethylene production, which can serve as genetic stocks for studying the mechanisms regulating the aspects of plant development in the context of stress tolerance. It was also intriguing to investigate the molecular and physiological basis of differential ethylene responsiveness. Hence, this study was conducted to determine the genetic variations in ethylene responsiveness, its interaction with drought, and underlying molecular responses in mungbeans.

2. Materials and methods

2.1. Plant materials and growth conditions

The 10 mungbean genotypes used in this study (Supplementary Table) were obtained from ICAR-National Bureau of Plant Genetic Resources, New Delhi, and multiplied at NIASM farm for future use. For the treatments, plants were raised in plastic pots during April–June as described earlier (Raina et al., 2016) with slight modifications. Briefly, pots (28 cm × 22.5 cm) were filled with equal amount of sand, silt, and farmyard manure in equal proportions, and fertilizers (1.9 g single superphosphate and 0.42 g muriate of potash per 1 kg of soil) were uniformly added to the soil before filling the pots. Each genotype was planted in 14 pots, out of which 7 pots were used as controls and 7 subjected to drought treatment. Three seeds were sown per pot; after germination, only 1 plant was allowed per pot. Drought was applied by withholding watering at 21 days after sowing until the moisture content of the medium was reduced to 50% of field capacity (FC), while moisture content of control pots was maintained at FC. To calculate water requirement at FC, 7 pots were filled with equal amount of dry soil and weighed. These pots were watered uniformly until the excess water started to drain from the bottom. Once the draining of water from the bottoms of the pots stopped, the pots were again weighed and the average difference in the weight before and after the irrigation was designated as the field capacity (FC). FC was achieved by adding water equal to calculated FC to the dry pots. Pots were periodically weighed to maintain the water level at FC, while 50% FC was maintained by adding water equal to half the calculated FC.

Grain yield was measured as described previously (Raina et al., 2016). Briefly, pods were harvested at 54, 60, and 66 days after emergence, and yield was determined by summing up the 3 harvests.

For evaluating root parameters under controlled conditions, half-strength MS with 0.5% sucrose (pH 5.7, 1.2% agar) was used as control. Osmotic stress was induced by applying 10% polyethylene glycol to the growth media (Delong and Fricke, 2017). One seed was placed in each of the glass tubes with PEG-infused 1/2MS media under aseptic conditions. The seeds of the 2 genotypes were germinated for 4 days under dark conditions and then transferred to light conditions. The temperature during the growth was 25 ± 2 °C with a 16 h photoperiod and light intensity maintained at 1000 lux. Twenty days

after planting the seeds, the lateral roots were scored by manually counting the roots which were >0.3 cm, using 20 seedlings each from the control and the osmotic stress treatment groups. Roots were dried at 72 °C for 48 h and the dry weight was measured with a balance.

2.2. Seed germination

The seeds were washed several times with sterile Milli-Q water, and 10 seeds were placed in each of the petri dishes lined with filter papers moistened with distilled water or 100 µM silver nitrate solution; they were then germinated in an incubator for 5 days at 23 ± 1 °C and 85 ± 5% humidity in the dark. There were 5 replicates for each genotype under control (distilled water) as well as treatment (100 µM silver nitrate) conditions; the experiment was laid in a completely randomized design (CRD). The seeds were sprayed with distilled water every 6 h and with the treatments at 18 h and 36 h (that is, treated twice). Seeds in which the radicles emerged (1–2 mm) through the seed coat were considered to have germinated. The number of seeds that germinated at 3 days after planting were counted, and germination percentage calculated as follows:

Germination % age = (No. of seeds with radical emerged/total number of seeds plated) × 100.

2.3. Relative leaf water content (RLWC)

The RLWC of young fully expanded leaves (third leaf from the top) was measured as described previously (Sengupta et al., 2013). The LRWC was calculated as: LRWC (%) = [(Lfw – Ldw) / (Ltw – Ldw)] × 100, where Lfw is leaf fresh weight, Ltw is leaf turgid weight (obtained after keeping leaf samples in distilled water for 24 h), and Ldw is leaf dry weight after oven drying at 105 °C until constant weight was obtained.

2.4. Flooding treatment

Since plant survival under waterlogging/submergence stress is largely regulated by ethylene, in order to correlate waterlogging tolerance with ethylene responsiveness, we subjected the potted plants to flooding for a period of 7 days as described earlier (Islam et al., 2008). Briefly, 21-day-old plants were flooded with 2.5 cm of standing water while control plants were maintained at optimal soil moisture level. Treatments were arranged in a randomized block design with 9 single plant replicates of each genotype per treatment. The experiment was conducted in natural conditions with precautions taken to avoid rainfall during the stress induction period. Seven days after the flooding treatment ended, mortality was recorded and expressed as percentage of total plants exposed to treatment.

2.5. Ethephon treatment

For ethephon treatment, third (from top) fully expanded leaves of healthy potted plants of all 10 genotypes were detached and placed on a wet paper towel containing 3 mM MES buffer (pH 5.8) plus 30 mM of ethephon (Sigma-Aldrich, St. Louis, MO, USA), and kept at 28 °C/16 h and 23 °C/8 h cycle in the dark. For the control group, leaves were placed on a wet paper towel containing 30 mM 2-(N-morpholino) ethanesulphonic acid (MES) buffer pH 5.8, and kept at 28 °C/16 h light and 23 °C/8 h dark. Samples were collected individually at 0, 12, 24, 48, and 72 h after treatment. Samples from control and ethephon treatments were analyzed for photosynthetic efficiency (Chen et al., 2012).

2.6. Chlorophyll fluorescence

Photosynthetic efficiency of the control (MES buffer treatment only) and ethephon (30 mM) treated leaves was recorded as ratio of variable to maximum fluorescence (Fv/Fm) in excised leaves (third fully expanded leaf from the top) by capturing the leaf images at given time points with the aid of a chlorophyll fluorescence measuring system (PSI, Drasov, Czech Republic), and the data were analyzed using Fluorochrom7 software.

2.7. Quantitative PCR analysis (qPCR)

For gene expression analysis, total RNA was extracted from the leaves (third fully expanded leaf from the top) using an RNeasy Mini kit (Qiagen, Valencia, CA, USA). The quality of RNA was determined using a Shimadzu spectrophotometer; first strand cDNA was synthesized from 1 µg of total RNA using Revert-AID H-minus cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). RT-PCR reaction was performed in a 40-cycle program (95 °C for 10 s, 58 °C for 30 s) on a CFX 1000 instrument (Biorad, Hercules, CA, USA), using IQ SYBR GREEN master mix (Biorad, Hercules, CA, USA). The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to normalize and calibrate transcript values relative to the endogenous mungbean actin gene. Three biological replicates were used for the gene expression analysis. The primer sets used for amplifying different target genes are shown in Table.

2.8. Statistical analysis

The data were subjected to analysis of variance according to the model for completely randomized design using the SPSS program (SPSS Inc, Chicago, IL, USA). Student's t- test was used to determine the significance of the differences between mean values of control and treated plants. Differences among means of several treatments were evaluated by the Duncan multiple range test at 0.05 probability level.

3. Results

3.1. Seed germination

To investigate the role of ethylene in the germination of mungbeans, seeds of 10 mungbean genotypes were treated with silver nitrate solution. Silver ions produced from silver nitrate act as ethylene perception inhibitors and inhibit the

Table. Details of primers used in the present study.

Primer name	Sequence	Amplicon size (bp)
VrActinrtF	CCCGAAGTTCTGTTCCAGCCAT	240
VrActinrtR	GTATTTCCTCTCTGGTGGTGCG	
VrEREF	TCTGCTAGACCTCACGCTCA	175
VrERER	CGCTCGAATTTGAGTCACTG	
VrACS7F	AGAAGTGCTCTGTTCAACGG	120
VrACS7R	CACGCATAGACTAAGACCAGG	
VrACS6F	CTGGTTTCGGATGTGCTTTG	93
VrACS6R	TCGTGATTTGACTCTTGGACG	

downstream ethylene responses. Under control conditions, seed germination ranged from 66.67% to 100% with an overall average of 82.1%. However, there was no significant variation for seed germination among the genotypes under control conditions. With the application of silver nitrate, germination among the genotypes ranged from 3.33%–43.33% with an overall average of 20.93%, indicating germination inhibition. ANOVA revealed significant ($P < 0.05$) genotypic variation for seed germination upon silver nitrate treatment (Figure 1a). However, genotypes varied in rate of germination inhibition, with IC-325817 and IC-325770 being the least affected (<50% reduction), while IC-324036 and IC-325788, IC-324012, IC-370498, IC-325753, IC-325787, and IC-325833 were moderately affected (50 to <80% reduction). IC-325756 had the maximum (96%) germination inhibition due to silver treatment. Mean germination values of IC-325817 and IC-325756 were significantly different from each other ($P < 0.05$).

3.2. Tolerance to waterlogging

The tolerance of the mungbean genotypes to flooding stress was evaluated. Among the genotypes studied, IC-325756 was highly susceptible with ~96% mortality, followed by IC-370498 with a mortality rate of ~63% (Figure 1b). IC-325817 emerged as a tolerant genotype with the lowest mortality (~4% mortality).

Based on the silver-nitrate–mediated germination inhibition studies, which were further supplemented by the flooding stress experiment, genotypes IC-325817 and IC-325756 with differential phenotypic responses were identified for further characterization.

3.3. Leaf senescence

For leaf senescence studies, genotypes IC-325817 and IC-325756 were selected as they exhibited maximum (and statistically significant) variation for silver-nitrate–mediated germination inhibition. Moreover, these 2 genotypes also revealed maximum variation for plant mortality under flooding stress. The differential ethylene responsiveness of these 2 genotypes was characterized by exposing their excised leaves to 30 mM ethephon treatment; leaf senescence was monitored by change in chlorophyll fluorescence (Fv/Fm) over a period of time. Leaves of both genotypes treated with buffer only maintained similar levels of chlorophyll fluorescence; no significant reduction in the efficiency of the PSII system was noted even up to 72 h posttreatment (Figure 2a). When exposed to ethephon treatment, the leaves of IC-325756 had a significant ($P < 0.01$) reduction in chlorophyll fluorescence 12 h after treatment, while IC-325817 had chlorophyll fluorescence comparable to the control up to 24 h posttreatment (Figure 2a). However, at 48 h posttreatment, both genotypes had a significant reduction in chlorophyll fluorescence compared to the control (Figure 2b). Based on these results, IC-325756 was considered more sensitive to ethylene, while IC-325817 was considered a less sensitive genotype. We also tested efficacy of low (5 and 15 mM) ethephon concentrations and found these to be less effective in influencing PSII efficiency (Supplementary Figure 1).

3.4. Genotypic variation in response to drought

The relative leaf water content (RLWC) of the more sensitive and less sensitive genotypes was also determined under drought stress condition. The RLWC of IC-325817 and IC-325756 under well-watered conditions were 81.27% and 80.68%, respectively (Figure 3a). However, drought caused a significant ($P < 0.05$) reduction in the RLWC in both IC-325817 (58.85%) and IC-325756 (63.86%) (Figure 3a).

Grain yield of both genotypes was also significantly ($P < 0.05$) reduced when the plants were exposed to drought (Figure 3b). In IC-325817, grain yield/plant under drought was reduced by ~47% while in IC-325756, a reduction of 63.25% was recorded compared to well-watered conditions. Hence, 2 genotypes (IC-325817 and IC-325756) were evaluated for root traits like the number of lateral roots and root dry weight under osmotic stress. Compared to the control, a significant reduction in the

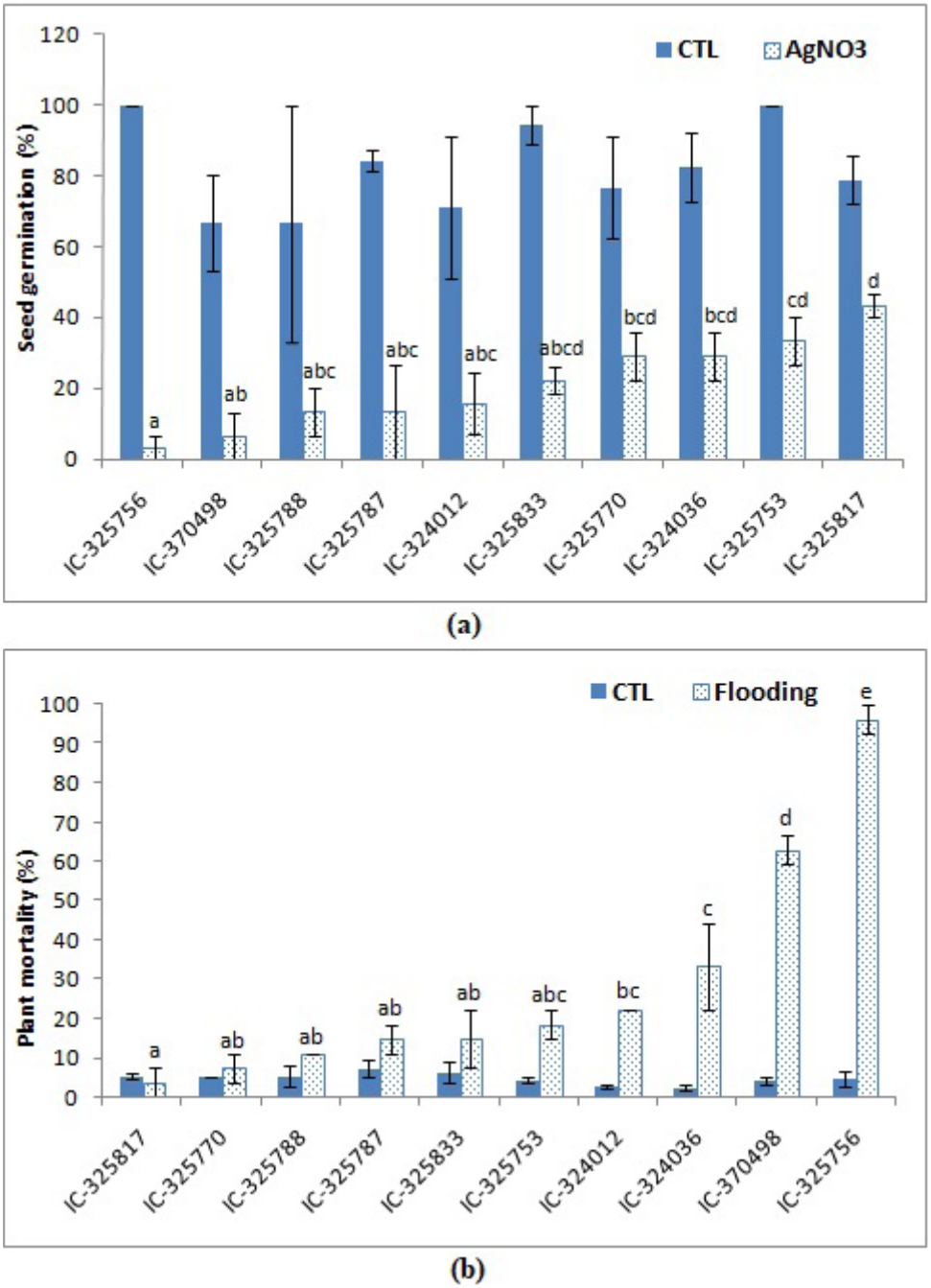

Figure 1. Evaluation of mungbean genotypes for various physiological processes. (a) Variation for silver nitrate mediated inhibition of seed germination among mungbean genotypes. From each biological replicate, an equal number of seeds from each genotype was exposed to water or 100 μM silver nitrate solution; the number of seeds producing radicle within 3 days from start of experiment were considered germinated. The values shown here represent means of 4 independent experiments with error bars representing SE. (b) Genetic variation for plant mortality as measured by percentage of plants which died due to flooding. Plants were exposed to flooding for 7 days and percentage mortality was recorded 7 days after stopping the flooding treatment. Error bar represents SE, n = 21. Significant differences in each graph are marked by different letters at the 0.05 level.

number of lateral roots and root dry weight was recorded in the 2 genotypes under osmotic stress (Figures 3c and 3d). Compared to control conditions, there was a reduction in lateral roots of 27.78% and 19.73% in IC-325817 and IC-325756 respectively when they were exposed to osmotic stress. Similarly, dry root weight under osmotic stress was

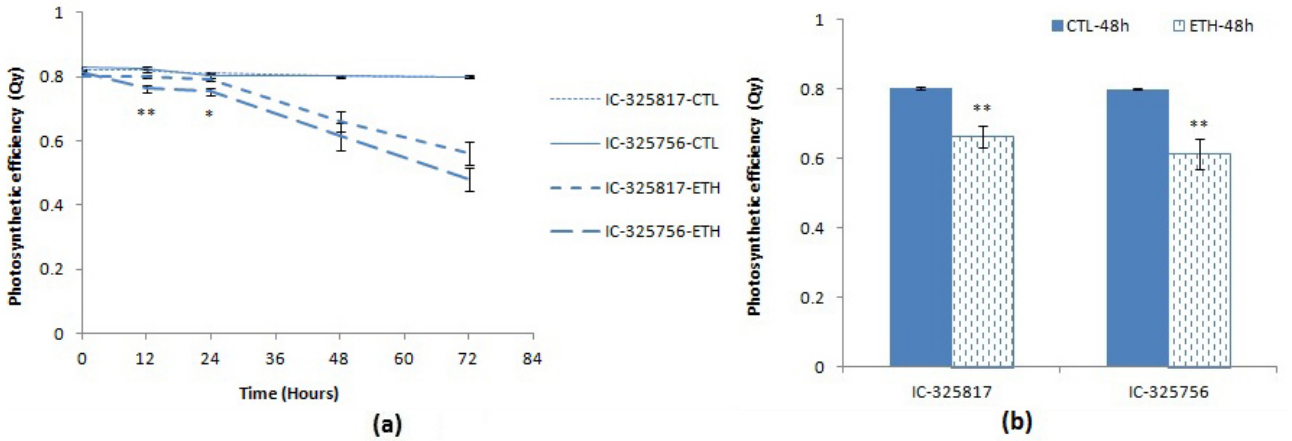

Figure 2. Evaluation of leaf senescence in detached leaves of mungbean genotypes upon ethephon treatment. (a) Graph depicting change in chlorophyll fluorescence of excised leaves of mungbean genotypes IC-325817 and IC-325756 exposed to buffer alone (CTL) or ethephon (ETH) over a period of indicated time points. (b) Bar graph indicating change in chlorophyll fluorescence in excised leaves of 2 genotypes 48 h after ethephon treatment. Ethephon-mediated leaf senescence was measured by change in chlorophyll fluorescence (Fv/Fm or Qy) of the PSII system over a period of 72 h at 12/24-h intervals. Detached leaves of the control plants were exposed to buffer only and fluorescence measured regularly. Significant differences ($P < 0.05$) are marked with a single asterisk, while highly significant differences ($P < 0.01$) are marked with a double asterisk.

reduced by 28.48% in IC-325817, while in IC-325756, it was reduced by 15.9% when compared to the control.

3.5. Differential transcript accumulation in mungbean genotypes

Genetic variability in ethylene-mediated physiological processes prompted us to analyze the expression levels of 1-aminocyclopropane-1-carboxylate synthase (ACS), the rate-limiting enzyme that controls the synthesis of ethylene. The present study has revealed that ethephon induced leaf senescence in mungbean (Figure 2). Hence, we analyzed the expression levels of ethylene biosynthetic genes (*ACS6* and *ACS7* genes) in mungbean plants exposed to drought. The expression level of ethylene response factors (ERFs), which is also important in mediating ethylene responsiveness, was also analyzed. This gene revealed sequence identity with *ERF4* of *C. arietinum*. The transcripts of *VrACS6* did not reveal any significant changes in the 2 genotypes when the plants were exposed to drought (Figure 4a). However, *VrACS7* transcript was 2.37-fold in IC-325756 under drought conditions, while in IC-325817 it was 2-fold compared with their respective controls (Figure 4b). The expression of *VrERF* increased under drought conditions by 3.2- and 4.5-fold in IC-325756 and IC-325817, respectively (Figure 4c).

4. Discussion

Ethylene is a phytohormone which plays a fundamental role in germination, leaf senescence, flower senescence, fruit ripening, and plants' responses to biotic and abiotic stress (Cheng et al., 2009). Evaluation of selected mungbean genotypes for variability in ethylene-mediated phenomena like seed germination, waterlogging stress, and leaf senescence was carried out in the present study. Involvement of ethylene in seed germination has already been established in several species (Kępczyński and Karssen, 1985; Lalonde and Saini, 1992; Petruzzelli et al., 1995; Kępczyński et al., 2003; Gianinetti et al., 2007). Ethylene produced during seed germination promotes radicle cell elongation (Kucera et al., 2005). Being potent inhibitors of ethylene signaling, silver ions have been frequently used to study the role of ethylene in physiological plant processes. Silver nitrate (a source of silver) has been reported to inhibit germination in mungbeans and *B. nigra* (Chaudhuri and Kar, 2008; Amooaghaie et al., 2015), suggesting the role of ethylene in promoting germination. In the present study, genetic variation was observed for silver-nitrate–mediated germination inhibition in mungbeans. Silver ions (produced from silver nitrate or other sources) are thought to disturb ethylene binding sites (Rodriguez et al., 1999). Therefore, variation in ethylene receptors across the genotypes could account for the differences observed. Genotype IC-325817, which had less silver-nitrate–induced germination inhibition, probably had receptors/receptor modifications which deter binding of silver ions or allowed binding of fewer silver ions compared to those which had higher rates of germination inhibition. It was reported that *Arabidopsis thaliana* has 5 structurally and functionally diverse ethylene receptors differing in their sequences and domains (Gallie, 2015). Seed coat permeability to silver ions across the genotypes is another factor that needs to be investigated (Salanenka and Taylor, 2011; Subhash et al., 2017).

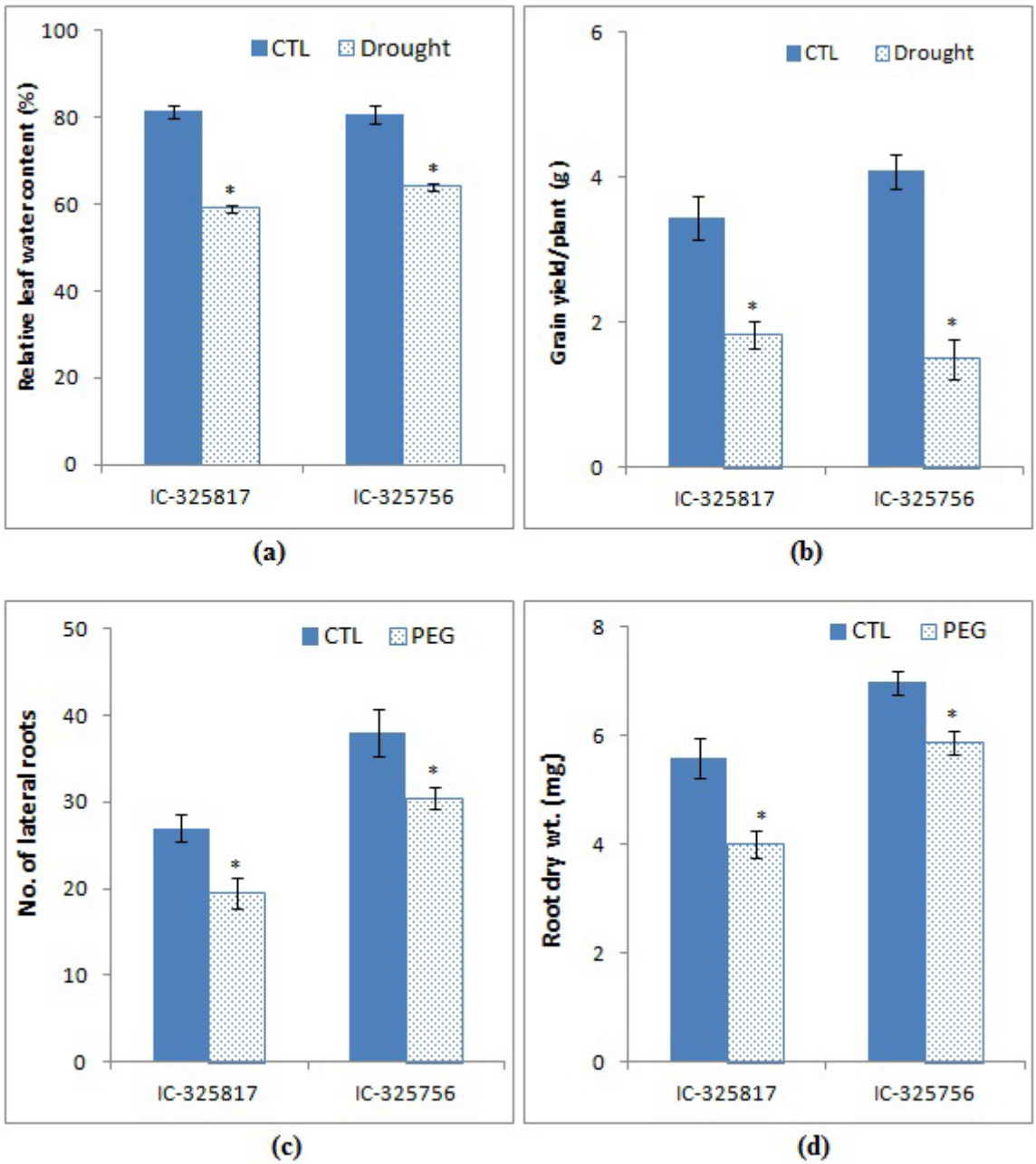

Figure 3. Evaluation of mungbean genotypes IC-325817 and IC-325756 for drought responses. (a) Relative leaf water content and (b) grain yield under control and drought conditions. (c) Lateral branches and (d) root dry weight in control (1/2 MS alone) and osmotic stress (1/2 MS supplemented with 10% PEG 8000). Treatments with significant differences ($P < 0.05$) are marked with an asterisk.

Interestingly, in some legumes like chickpeas, induction of thermodormancy at higher temperatures is associated with reduced ethylene production (Gallardo et al., 1991). The same has been observed in other crops like sunflower (Corbineau et al., 1988) and lettuce (Prusinski and Khan, 1990). With this background, mungbean genotypes like IC-325817 and IC-325770 (with reduced ethylene dependency for seed germination) are expected to have a selective advantage over other genotypes like IC-325756 under unfavorable growing conditions.

There are conflicting reports regarding the effects of ethylene on photosynthesis. Khan et al. (2000) have reported an increase in photosynthesis by ethylene, while others (Rajala and Peltonen-Sainio, 2001) have reported its inhibitory effect. Ethephon treatment reduced the maximum quantum yield of the PSII system by 27.5% in

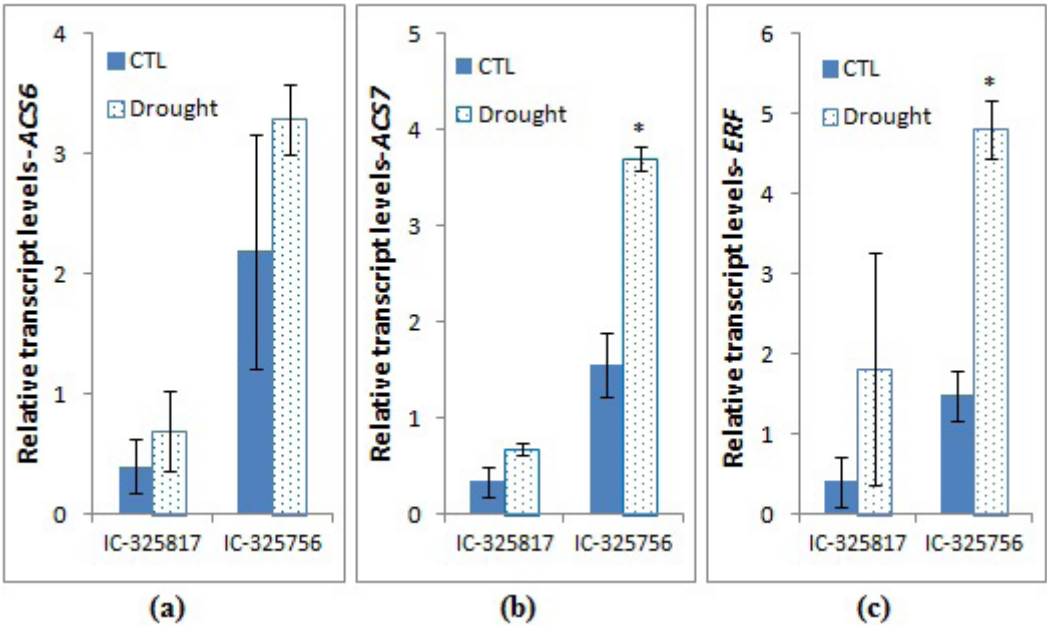

Figure 4. Changes in gene expression in mungbean genotypes IC-325817 and IC-325756 exposed to drought. Relative transcript abundance of (a) *VrACS6*, (b) *VrACS7*, and (c) *VrERF* genes in the 2 genotypes under control and drought conditions. The expressions were normalized to that of *VrActin*, and expression in the control was set to 1.0. The error bars indicate SE of means of 3 independent experiments. Significant differences ($P < 0.05$) are marked with an asterisk.

detached leaf segments of quick-leaf–senescence maize, while stay-green maintained its PSII efficiency with up to 3 days of treatment (Zhang et al., 2012). Interestingly, variation was observed in this trait, and the more sensitive genotype IC-325756 exhibited decreased PSII efficiency within 12 h of exposure to ethephon. However, IC-325817 was less sensitive to ethylene treatment, maintaining its chlorophyll fluorescence up to 24 h posttreatment. Earlier studies also reported that ethylene/ethephon treatment reduced PSII photosynthetic activities in several crop plants (Choe and Whang, 1986; Chen et al., 2010). This observation indicates differential ethylene sensitivity in IC-325817 and IC-325756. Ethylene is an important component of signal transduction underlying O_3-injury in plants that reduces the PSII efficiency and net photosynthesis (Kobayakawa and Imai, 2015). Mungbeans exposed to elevated O_3 levels showed a reduction in photosynthetic rates and photochemical efficiency (Mishra and Agrawal, 2015).

Increase in ethylene production is associated with various stresses including drought (Yang et al., 2009). Ethylene is reported to induce stomata closure (Wilkinson et al., 2012), an important feature contributing to moisture conservation under drought condition. Substantial crosstalk between ABA and ethylene signaling pathways has been observed in plants under drought conditions (Hopper et al., 2016). However, in biological processes that influence yield under stress conditions, ABA and ethylene have been reported to act antagonistically (Wilkinson et al., 2012). Based on these reports, we evaluated the drought responses of the genotypes with differential ethylene sensitivity. Interestingly, no significant variation in drought responses was observed between the 2 genotypes, although altered ethylene levels cannot be ruled out in the 2 genotypes since no ethylene measurements were conducted.

The rate-limiting step in biosynthesis of ethylene is the conversion of S-adenosyl methionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC) mediated by enzyme ACC–synthase (ACS). The *ACS7* gene has been associated with ethylene induction in plants exposed to stress/elicitation (Guan et al., 2015; Li et al., 2015). Moreover, *Arabidopsis thaliana* knockout mutants for *ACS7* gene lacking in *ACS7* transcript accumulation exhibited ethylene emissions approximately one-third those of the wild type (Dong et al., 2011). *ACS7* acts as a negative regulator of ABA sensitivity and its accumulation under abiotic stresses, which might act as a molecular link between ethylene biosynthesis and the ABA-mediated abiotic stress signal pathway (Dong et al., 2011). Significant upregulation of *VrACS7* and *VrERF* genes observed in more sensitive mungbean genotypes is in consonance with the upregulation of *ACS* and *ACO* genes observed in soybeans exposed to drought (Arraes et al., 2015). In *Citrus*, water-stress–induced leaf abscission was associated with *ACC* expression and concomitant increase in ethylene (Gomez-Cadenas et al., 1996). The upregulation of *VrERF*

in the sensitive genotype is in agreement with previous reports where 5 *StERF* genes were upregulated in response to drought and salt stress in potato (Wang et al., 2015).

Ethylene accumulation plays an important role in flooding-induced adventitious root formation (Visser et al., 1996); mungbean genotypes tolerant to waterlogging exhibited adventitious root proliferation (Kumar et al., 2013). Interestingly, roots contribute to plant tolerance in soil-moisture deficit conditions, and ethylene (ethephon) is known to inhibit lateral root production in mungbeans (Huang et al., 2013). Differential transcriptional regulation of the *VrACS7* gene which controls the rate-limiting step of ethylene biosynthesis (Guerra et al., 2015) was observed in mungbean genotypes exposed to drought. Nevertheless, the response of lateral roots to osmotic stress was uniform (significant reduction) in both, indicating potential variability in ethylene responsiveness/signaling. Moreover, in consonance with the studies of Young et al. (2004), drought-mediated upregulation of *VrACS7* expression may partly explain faster ethephon-mediated leaf senescence in a more sensitive genotype compared to that observed in a less sensitive one. These studies reveal genotype-dependent transcriptional regulation of ethylene biosynthetic/responsive genes in mungbeans exposed to drought, and the potential of *VrACS7* and *VrERF* as markers for ethylene responsiveness.

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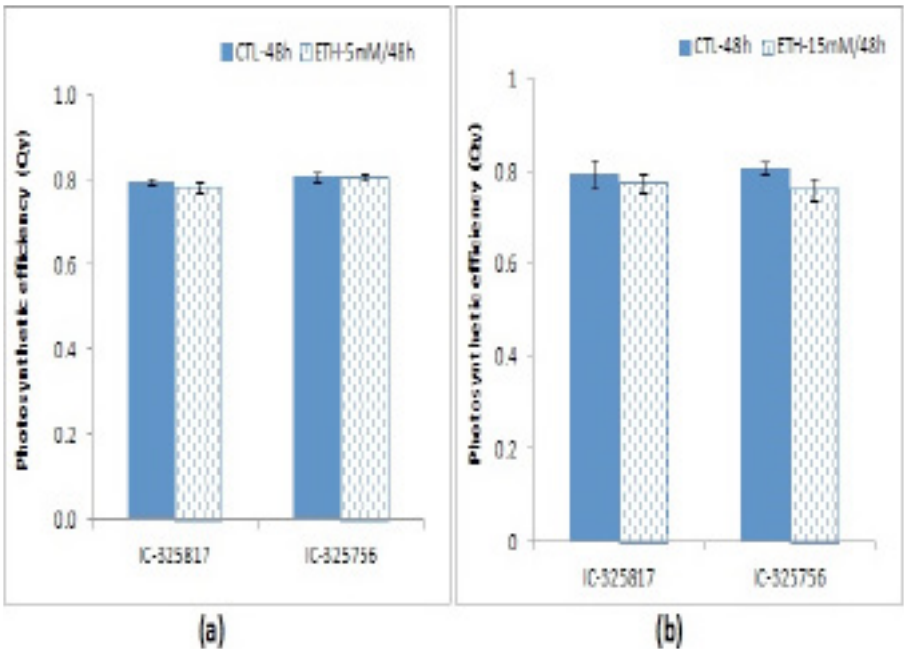
Supplementary Table. Basic information about the genotypes used in study.

Accession	Source	Pod length	100 seed wt	Days to 50% flowering
IC324012	ICAR-NBPGR	7.2	3.25	44.5
IC324036	ICAR-NBPGR	7.4	2.93	43.5
IC325756	ICAR-NBPGR	7.7	2.96	44
IC325770	ICAR-NBPGR	6.85	4.35	39
IC325787	ICAR-NBPGR	7.25	3	39.75
IC325788	ICAR-NBPGR	7.8	3.73	41.75
IC325817	ICAR-NBPGR	7.8	2.88	41.25
IC325833	ICAR-NBPGR	7.45	3.65	38.25
IC370498	ICAR-NBPGR	7.35	3.37	41.75
IC325753	ICAR-NBPGR	7.95	2.99	41.75



Supplementary Figure 1. Evaluation of different ethephon concentrations in influencing the maximal efficiency of PSII system. (a) Graph depicting change in chlorophyll fluorescence of excised leaves of mungbean genotypes IC-325817 and IC-325756 exposed to buffer alone (CTL) or 5 mM ethephon (ETH), and (b) 15 mM ethephon over a period of 48 h. Ethephon-mediated leaf senescence was measured by changes in chlorophyll fluorescence (Fv/Fm or Qy) of the PSII system. Control plants were exposed to buffer only and fluorescence measured regularly.