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Physiological traits reveal potential for identification of drought tolerant mungbean [*Vigna radiata* (L.) Wilczek] genotypes under moderate soil-moisture deficit

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Abstract

Canopy temperature is an important physiological trait used for screening drought tolerance in several crop plants. Mungbean being often exposed to post-flowering drought, we evaluated a set of 48 genotypes for variability in post-flowering canopy temperature and its association with root traits and other physiological parameters contributing to drought tolerance under soil-moisture deficit stress conditions. Overall, canopy temperature depression revealed significant association with seed yield. Root traits like number of lateral branches and dry root weight exhibited significant negative correlation with canopy temperature. Leaf SPAD readings were positively associated with grain yield and most of the high SPAD genotypes maintained hot canopies under drought. Some genotypes with contrasting variation in SPAD levels (DMG-1050 and SML-1628) maintained their photosystem PSII health at par. Moreover, cool canopy was no guarantee for better PSII health or *vice versa*. This study identified some cool canopy genotypes (VC-6173-C, IC-325770 and ML-2082) and a genotype (DMG-1050) with novel trait combinations like high SPAD and better PSII health despite high canopy temperature which can be used as donors in mungbean breeding programs. Present study explores genetic variation in these adaptation traits contributing to plant performance under soil-moisture deficit stress conditions and potential of physiological breeding approaches for genetic enhancement of this legume crop.

Key words: Mungbeans, drought, canopy temperature, SPAD, chlorophyll fluorescence

Introduction

With the climate change threatening global food security, water availability will be one among the limiting constraints affecting food productivity which emphasizes improving water use efficiency in agriculture. In this backdrop, development of drought tolerant crop cultivars by conventional breeding/genetic engineering will be among the key strategies to meet global food demands. Traditional plant breeding programs have relied heavily on measurements of yield and yield components (branches per plant, pod number per plant, pod length, seeds per pod and others) in varying environments. However, yield is quantitative trait characterised by low heritability and high genotype x environment (G x E) interaction (Jackson et al. 1996). Therefore, breeding drought tolerant crop cultivars make it is imperative to identify the specific physiological traits that improve the crop adaptation to water limited environments (Subbarao et al. 1995). Several physiological selection techniques have been evaluated for their potential role to complement the empirical breeding procedures, although lack of efficient screening techniques while handling large number of genotypes continues to be a bottleneck. Canopy temperature is one among the important physiological traits associated with water uptake by roots and is a better indicator of genetic variation in transpiration rate, leaf porosity and stomatal conductance in crops (Jones et al. 2002, 2009; Rebetzke et al. 2013). Canopy temperature has been reported to have significant correlation with yield in wheat because genotypes with cooler canopy

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temperatures have better access to water due to presence of deeper root systems (Lopes and Reynolds 2010). In rice, canopy temperature correlated negatively with spikelet fertility and grain yield under drought stress (Prince et al. 2015). Canopy temperature has also been associated with grain yields of chickpea under drought (Purushothaman et al. 2015).

Chlorophyll content is another trait which reflects resilience of plants to abiotic stress (Chaves et al. 2002). Genetic dissection of wheat populations revealed yield QTL under heat and drought stress to be collocated with QTL for chlorophyll, demonstrating its contribution to yield (Reynolds and Langridge 2016). Chlorophyll fluorescence has been used to study stress responses of wheat to unfavourable conditions like heat stress (Shefazadeh et al. 2012).

Interestingly, there are still many important traits which are inaccessible to remote sensing techniques and need destructive harvests for their quantification (Reynolds and Langridge 2016). Root traits constitute an important component in this category and are the first to perceive the drought stress in several agriculturally important crop species. Although primary root remains unaffected by drought stress, lateral root growth is significantly reduced (Basu et al. 2016). Moreover, QTLs common to heat and drought stress tolerance have been linked to adaptive root responses (Pinto and Reynolds 2015).

Mungbean [*Vigna radiata* (L.) Wilczek] is an important warm season legume with a diploid chromosome number of 2n=2x=22 and has an estimated genome size of 579 Mb (1.2 pg per 2C) (Kang et al. 2014). Almost 90% of world's mungbean production comes from Asia, and India alone contributes over 50% (~6 million tons) to the global annual production (Raina et al. 2016). Mungbean is grown on about 4.2 million hectares in India with an annual average production of 1.3 million tons as in 2008 (Keatinge et al. 2011). Mungbean is an important source of protein in South and Southeast Asia (Somta et al. 2014) and is consumed as a soup of split seeds and spices called Dal or Dhal. Mungbean sprouts are also consumed as a vegetable rich in vitamins and minerals in various countries (Nair et al. 2012). Considering the importance of mungbeans in Indian vegetarian diet, present exercise was aimed at characterization of mungbean germplasm for genetic variability in physiological traits like canopy temperature, SPAD chlorophyll content, chlorophyll fluorescence and root traits and evaluate their potential in contributing to drought tolerance under deficit soil moisture conditions. Most of the earlier studies used vegetative or mid-reproductive stage canopy temperature as a physiological trait for screening drought tolerance. However, mungbeans being often exposed to terminal drought under Indian conditions (Singh et al. 2016), we explored the suitability of post-flowering canopy temperature as a selection criteria for drought screening. Dynamics of canopy temperature was also investigated as drought progressed.

Materials and methods

Growing conditions and plant materials

Replicated field experiments were conducted during the mid-April month of year 2014 and 2015 at NIASM experimental farm, Baramati, occupying Southern part of India (18°92 N, 74°282 E) in a black 60-70 cm deep silty clay soil (around 40 % clay) over native basaltic soil. Field was prepared by making ridges and furrows 30 cm apart and sowing done in paired row manner on both sides of a ridge so as to maintain constant row-row spacing. Forty-eight genotypes (Table 1) with three replications were sown with 30 and 10 cm row-to-row and plant-to-plant spacing, respectively. In 2014, sowing was done on 05th April while in 2015, sowing was done on 11th April. During the crop season, mean temperature during growing season of 2014 was around 29.0°C, with an average maximum temperature of 36.7°C and average minimum temperature of 21.3°C. During growing season of 2015, mean temperature was around 29.4°C, with an average maximum temperature of 37.3°C and average minimum temperature of 21.5°C (Fig. 1). The genotypes were planted in four 2.5 m rows at a plant-plant spacing of 10 cm. Plot size consisted of 4 rows 0.3 m apart (2.5 m x 0.9 m). Seeds of all the genotypes used were treated with *Rhizobium* spp. before sowing. Plots were fertilized before planting with 20 kg Nitrogen, 40 Kg P_20_5 and 20 Kg K_2O ha^{-1}. Weeds were controlled manually as and when required. Immediately after sowing, 35 mm sprinkler irrigation was applied to ensure uniform emergence. Subsequently, the irrigated crop was irrigated at 21 DAS and 35 DAS although the drought treatment was irrigated only at 21 DAS and subjected to drought. However, there was a mild rainfall of 15 mm during the night of 20 May 2014 that increased the moisture levels of drought treated field. Three irrigations were applied to well-watered crop, each one supplying ~50 mm water. The crop also received a total rainfall of

Table 1. List of mungbean genotypes used

Genotype	IC Number	Status/pedigree	Origin
BM 2002-1	IC-0512342	Released variety	VNMKV, Parbani, Maharashtra
BM 2003-2	IC571727	Released variety	VNMKV, Parbani, Maharashtra
BM-7	-	-	-
BPMR-145	IC-0623699	Released variety	VNMKV, Parbani, Maharashtra
DMG-1050	-	Germplasm collection	-
DMG-1058	-	Germplasm collection	-
EC-48	-	Germplasm collection	-
H-705	-	Germplasm collection	-
IC-16033	IC-16033	Germplasm collection	-
IC-311397	IC-311397	Germplasm collection	Ahmedabad, Gujarat
IC-325770	IC-325770	Germplasm collection	Pali, Rajasthan
IC-325787	IC-325787	Germplasm collection	Pali, Rajasthan
IC-325833	IC-325833	Germplasm collection	Pali, Rajasthan
IC-370426	IC-370426	Germplasm collection	-
ML-1464	IC-0598295	Registered germplasm/Genetic stock	PAU, Ludhiana,Punjab
ML-1628	NA	ML 613 x UPM 98	PAU, Ludhiana,Punjab
ML-1721	NA	ML613 x ML1020	PAU, Ludhiana,Punjab
ML-1741	NA	ML613 x ML1020	PAU, Ludhiana,Punjab
ML-1907	NA	ML613 x ML1020	PAU, Ludhiana,Punjab
ML-1934	NA	ML818 x ML1165	PAU, Ludhiana,Punjab
ML-2037	NA	ML818 x VC-6372-45-8-1	PAU, Ludhiana,Punjab
ML-2056	IC-0619218	Released variety	PAU, Ludhiana,Punjab
ML-2081	NA	Pusa9971 x ML613	PAU, Ludhiana,Punjab
ML-2082	NA	Pusa9971 x ML818	PAU, Ludhiana,Punjab
ML-2083	NA	Pusa9971 x ML1260	PAU, Ludhiana,Punjab
ML-613		Released variety	PAU, Ludhiana,Punjab
SML-1012	NA	ML613 x BDYR1	PAU, Ludhiana,Punjab
SML-1018	NA	ML613 x BDYR1	PAU, Ludhiana,Punjab
SML-1019	NA	PS16 x SML504	PAU, Ludhiana,Punjab
SML-1023	NA	PS16 x SML504	PAU, Ludhiana,Punjab
SML-1115	IC-0617121	Released variety	PAU, Ludhiana,Punjab
SML-1124	NA	SML668 x SML713	PAU, Ludhiana,Punjab
SML-1150	NA	SML668 x ML1177	PAU, Ludhiana,Punjab
SML-1151	NA	PS16 x ML1137	PAU, Ludhiana,Punjab
SML-1168	NA	SML134 xV-6308	PAU, Ludhiana,Punjab
SML-1309	NA	SML470 x SML357	PAU, Ludhiana,Punjab
SML-668	IC-0623821	Released variety	PAU, Ludhiana,Punjab
SML-832	IC-0584165	Released variety	PAU, Ludhiana,Punjab
SML-859	NA	SML613 xML1184	PAU, Ludhiana,Punjab
SML-931	NA	SML134 x BDYR1	PAU, Ludhiana,Punjab
SML-970	NA	SML357 x Pusa Bold2	PAU, Ludhiana,Punjab
VAIBHAV	NA	Released variety (KDM1 x TARAM 18)	MPKV, Rahuri, Maharashtra
VC 3960-88	EC-0592172	landrace	Taiwan
VC 6173-B-10	EC-0592175	landrace	Taiwan
VC 6173-C	EC-0398949	landrace	Thailand
VC 6369-53-97	EC-0592178	landrace	Taiwan
VC 6370-30-65	EC-0592179	landrace	Taiwan
VC 6372-45-8-1	EC-0592180	landrace	Taiwan

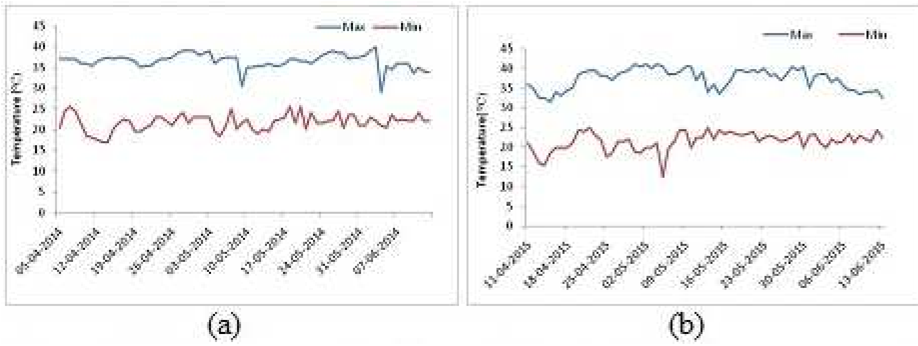

Fig. 1. Air temperature (maximum and minimum) during crop seasons of (a) 2014 and (b) 2015

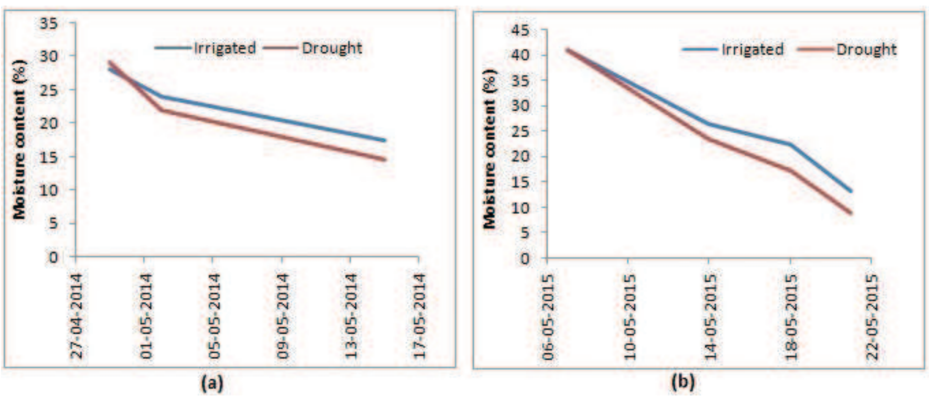

Fig. 2. Variation in soil moisture content of experimental field (irrigated and drought) of mungbeans for the crop seasons of (a) 2014 and (b) 2015

approximately 120.9 mm, while flood irrigation provided 150 mm additionally although total water requirement of the summer crop is 550-600 mm. However, drought plots received only two irrigations and rainfall. Soil moisture in drought treatment during reproductive stage (40-43 DAS) was around 9-15% (Fig. 2).

Agronomic and physiological measurements

Soil moisture was measured in weekly interval at two depths of 30 and 45 cm by gravimetric method. Soil samples were oven dried at 105°C and moisture content (%) was determined.

Canopy temperature was recorded by thermal imager (Vario Cam®hr inspect 575, Jenoptic, Germany) that operates in the wavebands 8-14 µm with a thermal resolution of 0.01°C. Thermal images with spatial resolution of 768 X 576 pixels were captured seven times covering crop growth stages just before and after anthesis. All thermal images were taken with the thermal imager on a tripod perpendicular to the area being imaged. Dry and wet references were used to mimic leaves with fully closed and fully open stomata, respectively (Jones et al., 2002) and to avoid extreme conditions while capturing images. Emissivity for all the measurements were set at 0.96 (Jones, 2004). IRBIS® software (Jenoptic, Germany) was used to analyse images. A total of four circular areas of interest in each image for analysis in the imager's software were outlined manually, by comparing thermal and normal digital images to exclude noise from ground area. Minimum canopy temperature in each of the sections in each image was considered to get a value that represents cooling capacity of cultivar. The measurements were done pre-flowering (25-27 DAS) and post flowering i.e., 43-48 DAS. Canopy temperature depression (CTD) was calculated as:

CTD (^{o}C) = Ambient temperature (^{o}C) - Canopy temperature (^{o}C).

For evaluating root parameters under controlled conditions, 1/2 MS with 0.5% sucrose (pH 5.7, 1.2 % agar) was used as medium. One seed was placed in each of the glass tube having 1/2MS media under a asceptic conditions. The seeds were allowed to germinate and grown for 20 days. After 20 days, the lateral roots were scored by manually counting roots (>0.3 cm) in 5 seedlings of each of the genotype. Root fresh weight was measured after cleaning the samples of all the media traces.

The Minolta SPAD chlorophyll meter (SPAD-502, Minolta, Japan) was used to measure the leaf relative chlorophyll content of the plants. The mean of three observations from the portable chlorophyll meter was obtained from individual fully emerged 3rd-trifoliate leaves.

The quantum efficiency of photosystem II was measured 37-40 DAS as described previously (Govindasamy et al. 2017). Chlorophyll fluorescence was determined from third leaf from the top using a dark-acclimated Handy Plant Efficiency Analyzer chlorophyll fluorometer (FMS2, Hansatech Instruments, King's Lynn, Norfolk, UK). Briefly, leaves were previously adapted to the dark for 30 min and F0 obtained using low-intensity light (less than 0.1 mol/m^2/s^1), which did not induce any effect in the fluorescence variable. The Fm was obtained using continuous light excitation (at 2500 mol/m^2/s^1) by an array of LEDs focused on the leaf surface to provide homogeneous irradiation over a 4-mm (0.16 in)-diameter leaf surface. The fluorescence variable (Fv) was calculated from the difference between Fm and F0. The Fv/Fm ratio indicated the quantum efficiency of PSII.

Mature pods were harvested and allowed to dry before thrashing them to get the seeds. Yield per plant was obtained by dividing the plot yield by number of plants per plot.

Statistical analysis

Experiment was laid in alpha lattice design with three replications. Traits like canopy temperature, SPAD and yield per plant were studied for two calendar years while chlorophyll fluorescence and root traits were studied during one calendar year only (2015). Broad-sense heritability (H^2), defined as the proportion of total phenotypic variation (σ^2P) attributable to genotypic variation (σ^2G) was calculated as described previously (Sharma et al. 2016). Simple statistics were computed to differentiate the treatment effects by SPSS 16 (SPSS Inc., Chicago, IL, USA).

Results

Canopy temperature variability and its association with other traits

Significant variability (p<0.05) was observed for post-flowering canopy temperature (CT) among the genotypes (Table 2) during 2014 field trial. The CT among the genotypes varied from 30.27 to 37.8°C under drought conditions (Fig. 3a). Some genotypes like ML-2082, IC-325770, SML-931, VC-6173-C were significantly (p<0.01) cooler than the local check Vaibhav with ML-2082 maintaining its canopy approx. 3°C cooler than Vaibhav. The post-flowering canopy temperature depression (CTD) among the genotypes ranged from 6.23°C to –1.29°C. Genotypes with a 5°C or higher CTD were ML-2082, IC-325770, IC-325733, SML-931, VC-6173-C and ML-2037. To further understand the dynamics of CT, we introduced a term delta CTD (ΔCTD).

Table 2. Means square values, F-value and p- values for various traits

Trait	N	Mean square	F-value	Significance
CT_irrigated_2014	48	2.062	9.305	0.000
CT_drought_2014	48	9.066	5.274	0.000
CT_irrigated_2015	48	3.712	2.742	0.000
CT_drought_2015	48	14.822	5.036	0.000
SPAD_drought_2014	25	39.073	7.865	0.000
SPAD_drought_2015	25	23.802	2.926	0.001
Root branches_2015	25	318.22	5.363	0.000
Root fresh weight_2015	25	12562.022	7.724	0.000
Yield_drought_2014	48	2.884	1.939	0.003
Yield_drought_2015	48	1.759	1.915	0.004

$$\Delta CTD = CTD_{pre\text{-}flowering} - CTD_{post\text{-}flowering}$$

The pre-flowering (25 DAS) CT which defined CT of drought plot under unstressed conditions, varied from 27.76°C in DMG-1058 to 32.48°C in SML-1023 with an average of 30.1°C. The ΔCTD was calculated for all the genotypes and those with a low ΔCTD value (≤2.5) included VC-6173-C, SML-1012, ML-1907, IC-325770, VC-6370-30-65 and ML-2037 implying least impact of drought on CT in these genotypes. Genotypes with a high ΔCTD value (≥8) included VC-6173-B-10, VC-6369-53-97, SML-832 and SML-1019, signifying huge variation in their CTD from pre to post-flowering stage. In 2014 field trial, significant variation for grain yield was observed among the genotypes (Table 2). Yield per plant varied from 1.92-5.94 gm per plant with maximum grain yield recorded in IC-325770 followed closely by ML-613, Vaibhav and VC-6173-C. Under drought conditions, post-flowering CTD revealed a significant (p<0.05) positive correlation with yield per plant (Fig. 3b).

During 2015 summer, significant variability (p<0.05) was observed for post-flowering CT among the genotypes (Table 2) and CT under drought conditions ranged from 29.94-38.78°C (Fig. 4a). Some genotypes like ML-1464, IC-325770, VC-6173-C, H-705, SML-859, ML-2082 and ML-1741 were significantly (p<0.01) cooler than the local check Vaibhav. All these genotypes were at least 5°C cooler than the local check, Vaibhav under drought conditions. The post-flowering canopy temperature depression (CTD) among

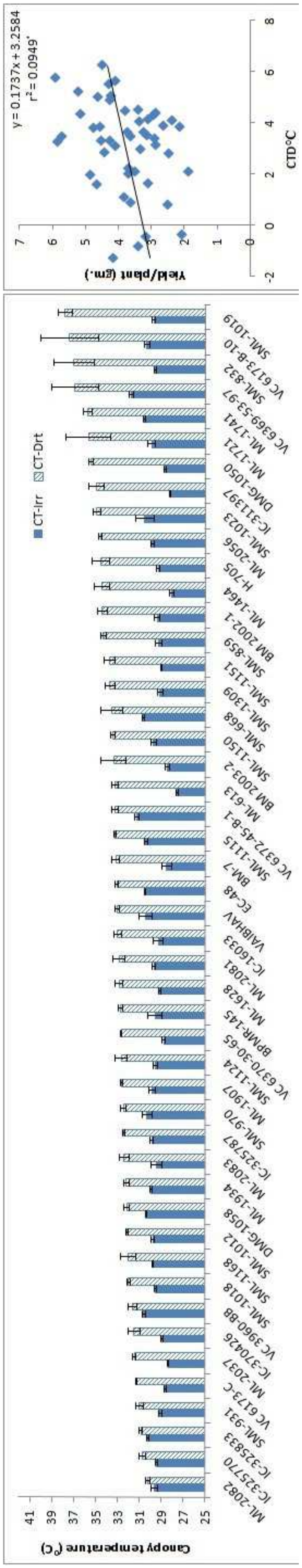

Fig. 3. Variability for canopy temperature (CT) in mungbeans during the crop season 2014. (a) CT of 48 mungbean genotypes under irrigated and drought conditions. (b) Relationship of canopy temperature depression (CTD) and the grain yield for 48 mungbean genotypes under drought stressed environment. Solid bars indicate CT under irrigated condition (CT-irr) while light bars with lines indicate CT under drought conditions (CT-drt). Error bars indicate standard deviation with n = 12

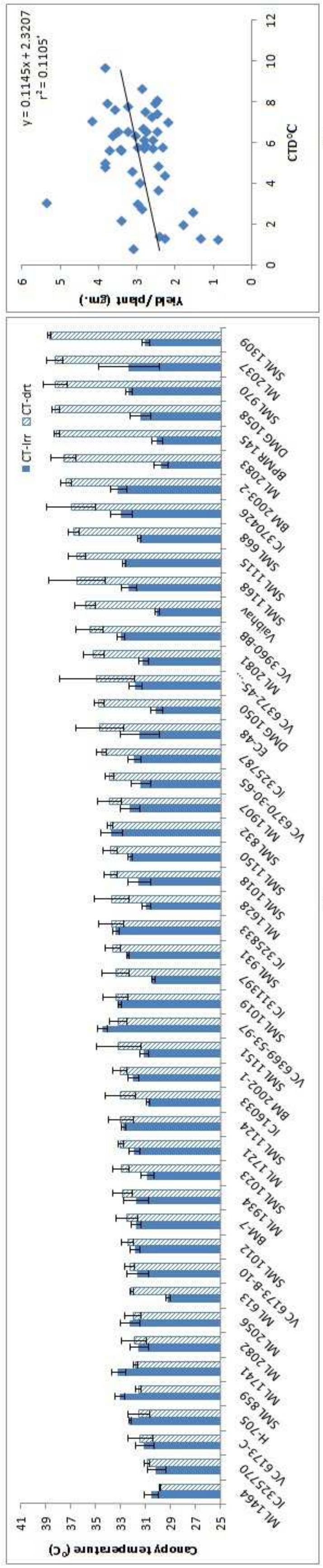

Fig. 4. Variability for CT in mungbeans during the crop season 2015. (a) CT of 48 mungbean genotypes under irrigated and drought conditions. (b) Relationship of canopy temperature depression (CTD) and the grain yield for 48 mungbean genotypes under drought stressed environment. Solid bars indicate CT under irrigated condition (CT-irr) while light bars with lines indicate CT under drought conditions (CT-drt). Error bars indicate standard deviation with n = 12

the genotypes ranged from 9.56°C to 0.73°C. Genotypes with CTD of 7.5°C or above included ML-1464, IC-325770, VC-6173-C, H-705, SML-859, ML-1741 and ML-2082. The pre-flowering CT (27 DAS) varied from 28.1°C in IC-325787 to 30.6°C in VC-6173-8-10 (S3) with an average of 29.3°C. Genotypes with a low ΔCTD value (≤2.5) were ML-1464, VC-6173-B-10, ML-1741, H-705, SML-859, ML-2082, IC-325770, MB-7, ML-613, VC-6173-C and ML-2056 while those with a high ΔCTD value (≥8) included SML-1309, DMG-1058, SML-970 and ML-2083.Significant variation (p<0.05) was observed for yield under drought among the mungbean genotypes during 2015 (Table 2). The yield under drought conditions varied from 0.9-5.36 gm per plant with maximum yield recorded in SML-1168 followed by SML-1012 and IC-325787. Under drought conditions, post-flowering CTD revealed a significant (p<0.05) positive correlation with yield per plant (Fig. 4b).

Significant outcome of the two trials was that genotypes like VC-6173-C, IC-325770 and ML-2082 emerged as cooler canopy genotypes during both the trials. Moreover, genotypes with low values of ΔCTD across both the trials included ML-2082, IC-325770, VC-6173-C,

Table 3. Mean values, range, genotypic variance and heritability for the CT of 48 mungbean genotypes estimated during crop season of 2014 and 2015

CT during	Trial mean	Range of means	Genotypic variance	Heritability
2014	33.36324	30.27-37.80	2.449	0.587571977
2015	34.24069	29.94-38.78	3.959	0.573643

SML-1151 and SML-1012 while those with high values of △CTD were Vaibhav, DMG-1058, BM-2003-2, SML-668 and DMG-1050. Moreover, post-flowering CTD revealed a significant positive association with yield under drought. The statistical parameters of post-flowering CT during the two trials are listed below (Table 3). During both the trials, a medium heritability (broad sense) of 0.57-0.59 was recorded for post-flowering CTD under water stress conditions.

In several crops like wheat and chickpea, canopy temperature has been associated with root traits. To explore this association in mungbeans, we studied the root traits of a subset of 25 genotypes under *in vitro* conditions. These genotypes were evaluated for traits like primary root length, lateral root branches and fresh root weight (Table 4). Primary root length among the genotypes ranged from 9.75 cm (IC-325833) to 13.5 cm (ML-2056) with an average of 11.75 cm. The number of root branches ranged from 17.75 (Vaibhav) to 50.75 (ML-2056) with an average of 32.31. The fresh root weight ranged from 114.305 mg (ML-1934) to 279.895 mg (SML-859) with an average of 187.1442 mg. A highly significant (p<0.01) negative association of the canopy temperature with lateral root branches was observed while a significant (p < 0.05) negative association of canopy temperature with fresh root weight was recorded.

Table 4. Range, mean and standard deviation of various root traits measured in 25 mungbean genotypes under *in vitro* conditions

Trait	Range	Mean	Standard deviation
Primary root length	9.75-13.5 cm	11.755	1.1339
Lateral branches	17.75-50.75	32.31	8.262
Root fresh weight	114.305-279.895mg	187.14	54.978

Variability for leaf chlorophyll content and chlorophyll fluorescence

The negative correlation observed between CT and lateral roots was further characterized by evaluating these genotypes for their SPAD chlorophyll content. Significant variability (p<0.05) for SPAD values was observed during 2014 and 2015 under drought stress (Table 2). During 2014, average SPAD values under drought conditions ranged from 42.37 (ML-1907) to 54.13 (SML-832) while average SPAD values ranged from 43.26 (ML-2081) to 54.17 (ML-1907) during 2015. The genotypes with statistically distinct (p<0.05) SPAD values (high SPAD and low SPAD genotypes) are listed in table below (Table 5). During 2014 trial, high SPAD genotypes like ML-2056, SML-1023, DMG-1050 and VC-6369-53-97 maintained significantly hot canopy temperature compared to cool canopy genotypes i.e., ML-2082, IC-325770 and VC-6173-C (Fig. 5a). However, DMG-1050 and Vaibhav only maintained hot canopies during 2015 when compared to cool canopy genotypes when exposed to drought (Fig. 5). These results identify DMG-1050 as a genotype capable of maintaining high leaf SPAD despite a significantly hot canopy during both the trials. SPAD revealed a significant (p<0.05) positive association with the yield under drought conditions during 2015 trial.

Table 5. List of high and low SPAD mungbean genotypes identified on the basis of performance in 2014 and 2015

High SPAD genotypes	Low SPAD genotypes
Vaibhav, VC-6369-53-97, ML-2056, DMG-1050 and SML-1023	ML-2081, EC-48, IC-311397, ML-1907, SML-1124, VC-6173-B-10, SML-1018, ML-1628 and IC-325787

Genotypes with consistently high and low SPAD values respectively identified during the two growing seasons (Table 5) were evaluated for their chlorophyll fluorescence. The cool canopy genotypes identified during the two successive seasons (VC-6173-C, IC-325770 and ML-2082) were also included in this study. The reduction in chlorophyll fluorescence among the genotypes upon exposure to drought stress was recorded and two categories could be identified. DMG-1050 (high SPAD), ML-1628 (low SPAD) and ML-1907 (low SPAD) revealed no significant reduction in their fluorescence under drought stress (Fig. 6). All other genotypes exhibited significant reduction in their

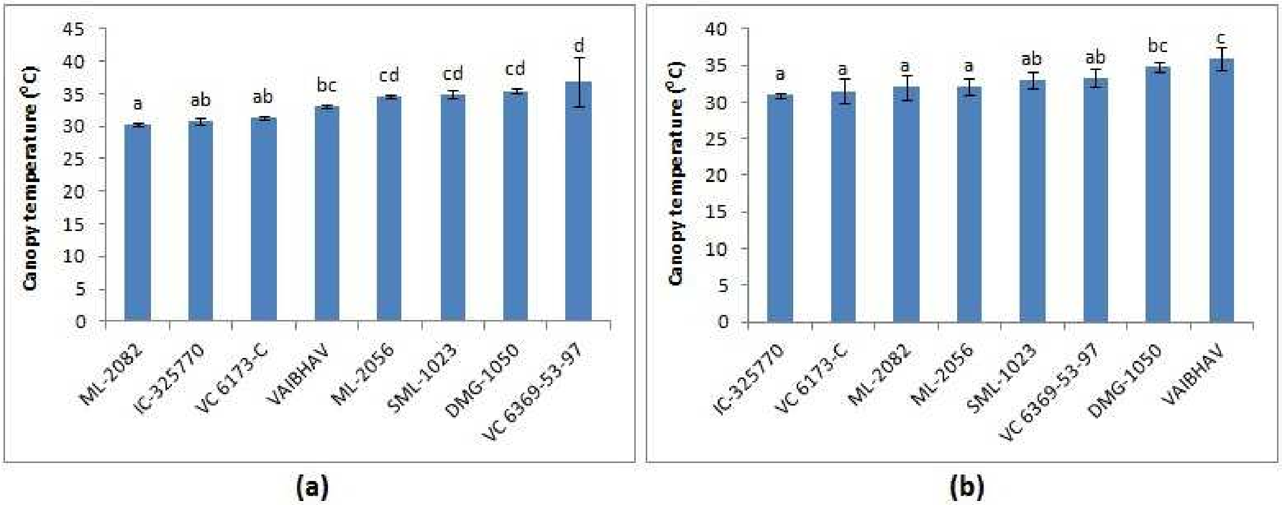

Fig. 5. CT comparison of selected mungbean genotypes. (a) Comparison of CT of cool canopy genotypes with high SPAD genotypes during crop season of 2014 and (b) 2015. Error bars indicate standard deviation with n= 15. Means with same letters are not significantly different at $p < 0.05$

fluorescence when exposed to drought stress (Fig. 6). Even the cool canopy genotypes *viz*., VC-6173-C, IC-325770 and ML-2082 exhibited significant reduction in their fluorescence under drought.

Discussion

Conventional plant breeding over the time has shifted from morphological to physiological selection criteria since they are time consuming and rely on present genetic variability. Physiological traits on the other hand can be selected for in early generation progeny to increase favourable gene frequencies. The physiologically inferior lines can be discarded in early generation, thereby reducing the cost of yield testing (Reynolds et al. 2012). Physiological traits like photosynthetic rate and canopy temperatures have been used as screening tools for evaluation of heat tolerant lines in wheat (Reynolds et al. 1998). Present study was undertaken to evaluate feasibility of post-flowering CT as a screening methodology for drought stress in mungbeans. VC-6173-C, IC-325770 and ML-2082 were identified as the coolest among all the genotypes in both the trials. Interestingly, two of them recorded low ΔCTD value in both the trials indicating

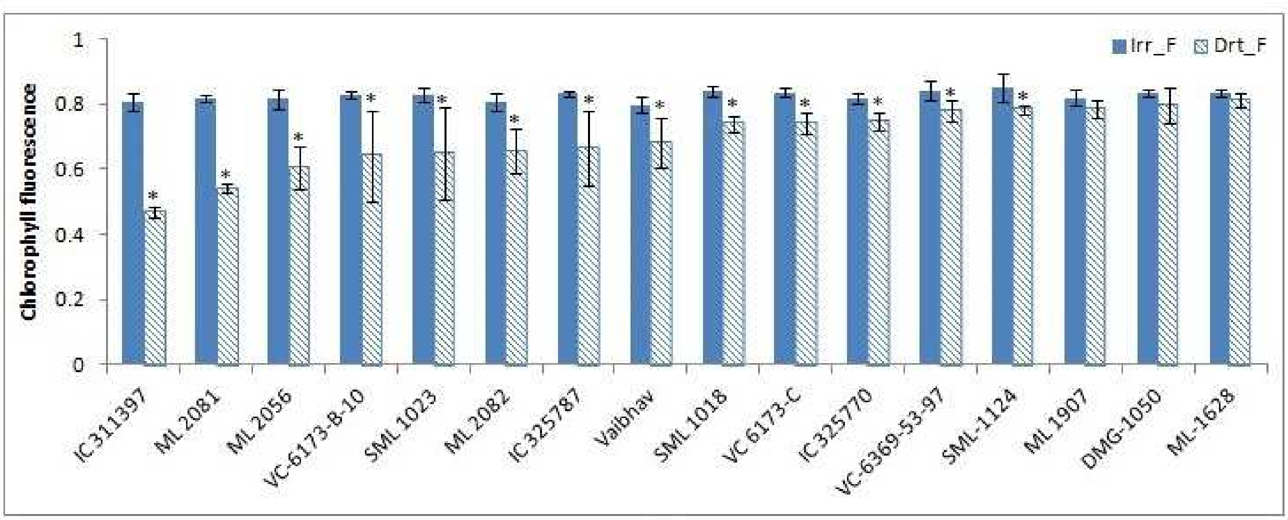

Fig. 6. Variation in chlorophyll fluorescence of selected mungbean genotypes under irrigated (Irr_F) and drought (Drt_F) conditions. Error bars indicate standard deviation with n = 15. Means with single asterisk (*) indicate significant reduction compared to irrigated fluorescence at $p < 0.05$

least sensitivity of their CT to water deficit. Some other genotypes like VC-6369-53-97, VC-3960-BB, VC-6372-45-8-1 and SML-832 maintained high canopy temperature under well-watered condition, signifying low evaporative cooling despite water availability. Thus, water conserved under well-watered conditions can be made available when plants are exposed to drought. Interestingly, most of these genotypes were able to maintain a relatively cooler canopy (compared to overall average canopy temperature of all the genotypes) under drought conditions. Hence, these genotypes provide useful trait combination suitable for cultivation under deficit soil moisture conditions. A medium heritability of CTD was recorded in mungbeans indicating moderate effectiveness of selection process in altering the trait mean.

The QTLs related to cooler canopy temperature have been associated with optimal root distribution in wheat which is important for water extraction from deeper layers under drought conditions (Pinto and Reynolds 2015). The finding that canopy temperature associates negatively with spikelet fertility and grain yield under drought (Prince et al. 2015) is in concurrence with our results. To supplement our observations, Babu et al. (2003), reported negative correlation of canopy temperature with biomass, an important yield component. In contrast to our findings, reduced lateral root branching density has been reported to confer drought tolerance in maize (Zhan et al. 2015). However, maize has an adventitious root system while mungbean has a well developed tap root system and these differences in basic root morphology need to be considered while drawing the analogy of the results across the species. It may be also be worth to mention here that no association of CT with primary root length was observed in our study. Interestingly, primary root is reported to remain unaffected by drought stress (Basu et al. 2016).

SPAD value is an indicator of relative chlorophyll content of canopy of the plants. As chlorophyll plays vital role in conversion of light energy into chemical energy of carbohydrates, the SPAD value assumes significance. In cotton, high yielding genotypes recorded higher SPAD values than those with low yields (Feng et al. 2016). The positive association between yield and SPAD under drought observed in mungbeans indicates that drought tolerant genotypes are well adapted to retain chlorophyll pigments under drought that contributes to their enhanced grain yields. DMG-1050 with a significantly high canopy temperature was able to maintain a high SPAD value across both the years. This indicates its capacity to retain high leaf chlorophyll content despite moisture stress and hence serves as useful trait for drought adaptation.

Chlorophyll fluorescence (Fv/Fm) of dark adapted leaves indicates relative health of PSII and is often used as an expression of photo-inhibition (Critchley 1998; Kitao et al. 2000; Krause et al. 1999; Schensker & van Rensen 1999). Drought stress is known to inhibit PSII activity and affect energy transfer processes in mungbeans (Batra et al. 2014). In light of this, DMG-1050 and ML-1628 seem resistant to photo-inhibition under drought stress. DMG-1050 has a capacity to maintain high chlorophyll fluorescence and high SPAD under drought stress and can be used as a donar parent for this trait in breeding programs and as well used as a genetic stock to investigate the mechanisms regulating this trait. The high fluorescence of ML-1628 despite a low SPAD can be explained by the observation that photo-inhibition is not necessarily accompanied by chlorophyll degradation and a reduction of A_{max} (Bunmann and Oesterhelt 1995; Critchley 1998; Demmiig-Adams et al. 1998). Cool canopy genotypes VC-6173-C, IC-325770 and ML-2082 revealed significant reduction in their fluorescence under drought while ML-1907, DMG-1050 and ML-1628 with better PSII health exhibited significantly high canopy temperature, indicating that cool canopy is not necessarily a guarantee for better PSII health.

Overall, these investigations revealed significant variability in physiological traits which is a pre-requisite for a holistic crop improvement strategy towards the development of drought tolerant mungbeans. Moreover, the genotypes with useful traits/trait combinations can serve as donors for crop improvement programs in mungbeans.

Authors' contribution

Conceptualization of research (SKR, JR); Designing of the experiments (SKR, JR); Contribution of experimental materials (SKR, JR, PSM); Execution of field/lab experiments and data collection (SKR, VG, MK, SCE); Analysis of data and interpretation (SKR, AKS, NR); Preparation of manuscript (SKR, JR, PSM).

Declaration

The authors declare no conflict of interest.

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