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Canopy temperature depression (CTD) and canopy greenness associated with variation in seed yield of soybean genotypes grown in semi-arid environment

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ABSTRACT

Method to measure canopy temperature depression (CTD) by employing thermal imaging system for crops like soybean, which is sensitive to low soil moisture has not been standardised/optimised. Hence, the present study was conducted to optimise the thermal imaging method and evaluated the CTD along with canopy greenness-based physiological traits in screening/selecting soybean genotypes suitable for semi-arid environment. The CTD and canopy greenness were measured six to eight times during different growth phases/stages using infrared (IR) and visible cameras mounted on a semi-automatic trolley that allowed rapid acquisition of high quality thermal and visible images, respectively. The CTD measured at the reproductive stage explained a major proportion of the variation in grain yield under both well-watered and water-stressed conditions. This could be attributed to close association between plant's capacity to keep its canopy cooler (low canopy temperature) and canopy greenness (higher chlorophyll content) as indicated by efficient photosynthesis which leads to grain yield. These results indicated that in addition to assess stay green features, CTD along with canopy greenness can also be used as a key trait of leaves in the selection of soybean genotypes for higher adaptability to low soil moisture stress conditions, a common feature exists under semi-arid regions.

1. Introduction

Soybean is a vital source of vegetable protein for both human and livestock (Fenta et al., 2014). It has emerged as a third major oilseed crop of India(Rai et al., 2016). Soil moisture stress as impacted by frequent drought limits soybean production particularly in semi-arid region. Its impact on seed yield of soybean can be very high if it occurs at the reproductive stage of the crop growth (Liu et al., 2003). Genetic improvement for soil moisture stress adaptation can be a long-lasting and less-expensive solution for drought management over many other agronomic options. Hence, cultivation of genotypes with stable yield under drought is highly crucial for future food security. Yield stability across varying environment is determined by multiple mechanisms involved in plant growth during low soil water supply condition (Tuberosa and Salvi, 2006). Advances in molecular biology allow us to generate new genotypes rapidly if used in combination with conventional breeding. This integrated approach can be successful, if we establish a perfect association between molecular markers/genes and their corresponding physiological trait implicated in drought tolerance. However, the easy and low cost phenotyping methods to assess physiological responses of plant to stress are not as efficient as molecular methods and platforms employed for understanding expression of genome to phenome.

Several physiological traits have been found to be associated with stress like drought (Salekdeh et al., 2009). The major determinant of plant health in vapour deficit environment is the leaf/plant water status, which is not easy to measure when a large number of genotypes are to be evaluated. Alternatively, since transpiration is the major cause of changes in leaf water status and leaf temperature, leaf or canopy temperature can be used as a better indicator to assess genetic variation in transpiration rate, leaf porosity and stomatal conductance in crop genotypes (Jones et al., 2002, 2009; Rebetzke et al., 2013). Stomatal closures for a considerable period of time are known to increase the leaf temperature (Kashiwagi et al., 2008) and maintenance of a cool canopy during grain filling period is a key physiological response of cropsfor high

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temperature stress tolerance as shown in wheat (Reynolds et al., 1998; Munjal and Rana, 2003); rice & potato (Fukuoka, 2005); maize (Masuka et al., 2012) and grain legumes (Farooq et al., 2016).

Canopy temperature is a promising trait for identifying drought tolerance and canopy temperature depression (CTD) has been shown to correlate well with the transpiration status in crops like rice, wheat and sugar beet (Fukuoka, 2005; Kumar et al., 2015). Preferability of this trait among the different other complex trait, has its own implications in the application of high-throughput phenotyping systems involving IR thermal imaging & analysis for the characterization of crop genotypes (Jones et al., 2009). Canopy temperature depression (CTD), defined as deviation of plant canopies temperature from the ambient temperature, has been recognized as the key trait for assessing/comparison of genotypes response to low water use, high temperature and other environmental stresses, (Balota et al., 2007). The CTD is expected to be influenced by several factors like the capacity of the crop plant to extract water, transpirational differences and crop phenological stages of growth. Ries et al. (2012) observed no difference in CT at varying VPD among slow, intermediate, and fast-wilting soybean. However, the issues relating to determining the optimal CTDdepend on sampling time and obtaining the maximum genetic discrimination was seldom addressed (Purushothaman et al., 2015). The CTD has been recognized as an indicator of overall plant water status. (Sivakumar, 1986; Penuelas et al., 1992; Balota et al., 2008). In addition, the other components of physiological processes that can keep optimum leaf temperature are crucial for plant growth and development under stress environment. For example in the absence of sufficient soil moisture, photosynthesis apparatus becomes more vulnerable to photo-inhibition.

Resilience of plants to abiotic stresses can also be indicated by chlorophyll content and its retention power of the absorbed solar radiation (Chaves et al., 2002).The contents of chlorophyll (Chl) and other photosynthetic pigments are directly associated with photosynthetic potential and primary production (Curran et al., 1990). High chlorophyll content is a desirable characteristic because it indicates a low degree of photo-inhibition in the photosynthetic apparatus. Hence, water deficit induced leaf senescence as indicated by loss of chlorophyll content is considered as another promising physiological trait which can be rapidly phenotyped for assessment of drought tolerance in crops (Vesali et al., 2015). Recently, image analyses for colour pixels based non-destructive techniques are emerging as new quantitative tools in agriculture research to estimate chlorophyll content of leaves (Li et al., 2014; Fahlgren et al., 2015). Methods to measure CTD by employing thermal imaging system for crops like soybean, which is sensitive to low soil moisture have not been standardised/optimised previously. Hence, thepresent study was focused on optimization of canopy imaging and analysis with IR and visible wavelengthsin soybean genotypes toassess variation in CTD and chlorophyll content (canopy greenness) respectively. Image analysis-based field phenotyping for CTD and canopy greenness presented in this study would be helpful to differentiate water-stress tolerance insoybean genotypes tolerance andto select superior soybean genotypeswith stable yield suitable for growing under semiarid environment.

2. Materials and methods

2.1. Plant materials and growing conditions

Field experiments were conducted at ICAR-NIASM research farm,Baramati, Maharashtra located in southern/central part of India (18°9 ′N, 74°28 ′E) during two growing seasons, 2014 and 2015. We used 32 advanced genotypes obtained from ICAR-Indian Institute of Soybean Research, Indore, Madhya Pradesh (India).In this study, a few genotypes *viz.* JS335, NRC37 and NRC 7 adapted to local conditions were used as checks for identifying superior genotypes. Planting was carried out manually with a plant to plant distance of 15 cm and row to row distance 45 cm. Experimental plots were applied with basal dose of fertilizer before planting (20 Kg Nitrogen, 40 Kg P_2O_5 and 20 Kg K_2O $_2O$ ha^{-1}). The genotypes were evaluated in irrigated and imposed low soil moisture stress environments, each year, for two years resulting in a total of four environments. In each environment, genotypes were planted in plots arranged in a lattice block design with four replications in plots of 1.8 m × 3.5 m (4rows 0.45 m apart).The crop was planted on 13 July 2014 and 11th July 2015 on an experimental farm featured by black soil with 30% silt and 40% clay and 60–70 cm deep.Weather parameters were recorded by automatic weather station installed at the institute research farm. Rains received were 214 mm and 175 mm for the year 2015 and 2016of experimental period respectively. Maximum and minimum ambienttemperatures remained close to 30 °C and 31 °C during both the crop seasons at the time of CTD and other physiological observation (10:00 to 14:00 h).

2.2. Soil moisture measurements

Post-anthesis water stress was imposed by withholding irrigation at anthesis (around 35 DAS) in water-stress plots. Soil samples from each experimental unit were collected in aluminium boxes with secure lids every week at two soil depths (15 and 30 cm) using augers. The samples were weighed immediately and then oven dried at 105 °C for 72 h for determining soil moisture content by gravimetric method. Soybean crop plot was exposed to 12–15 days moisture stress after 50% flowering was noticed in each plots (Fig. 1). The trend of soil moisture depletion was consistent across the years.

2.3. Grouping of genotype

Days to 50% flowering, yield and yield components/traits of soybean genotypes were used for clustering in to different groups. Data on days to 1st flowering, 50% flowering recorded daily from 22 DAS.Genotypes were clustered by Hierarchical clustering using Ward's minimum variance method (Ward, 1963) with standardised transformation of data recorded from the cultivar using traits matrix.

2.4. Canopy temperature

Thermal images for measuring canopy temperature were acquired between 10 h and 14 h on sunny, cloudless days by focusing lenses at the centre of a leaf canopy in each plot. To minimize the confounding effects of changes in environmental conditions on genotypic performance over this 4 h period, images of all the genotypes within a replication were acquired in about 20 min time period. All thermal images were obtained with a thermal imager (Vario CAM hr. inspect 575, Jenoptic, Germany) that operates in the wavelengths of 8–14 μm, with a thermal resolution of 0.01 °C, it was having a capacity to produce high quality thermal images with spatial resolution of 768 × 576 pixels. The thermal imaging system was mounted on a hand operated trolley that could move on an iron track installed in the experimental field. A thermal camera was mounted on a trolley in such a way that distance (about 1 m) as well asangle between the camera and crop canopy during all the measurements were ensured constant. Dry and wet filter papers were used as reference to mimic leaves of fully closed (full turgid) and fully opened (partial turgid) stomata, respectively (Jones et al., 2002) and to avoid extreme conditions during image capturing processes.

Observations were recorded during different reproductive stages. Image was captured from each plot five to six times during R1 through R8 growth stage which largely represent reproductive phase of the crop. Reproductive stages were indicated as R1: Beginning Bloom; R2: Full Flowering; R3: Beginning Pod Development; R4: Full Pod; R5–6: Full Seed; R7: Beginning Maturity; and R8: Full Maturity (Fehr and Caviness, 1977).IR thermal images were captured at 3–5 days interval from 42 DAS to 80 DAS, starting from the day of flowering. Emissivity for measurements of leaves and plant canopies was set at 0.96 (Jones, 2004). The captured radiometric images were stored and analysed