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EVALUATION OF DROUGHT TOLERANCE IN PULSES BY ASSESSING PS-II EFFICIENCY AND LEAF GREENNESS UNDER MOISTURE DEFICIT STRESS

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ABSTRACT

Pulses, vital to global food security, are susceptible to drought stress that occur at critical stages of crop growth, significantly impacting their growth and yield. This study evaluated the physiological responses of four major pulse crops such as greengram, blackgram, chickpea, and cowpea under depleting soil moisture to determine thresholds of tolerance for photosystem II (PSII) efficiency and leaf greenness (NDVI). Results revealed significant genetic variation among genotypes, with specific genotypes exhibiting higher tolerance to reduced soil moisture levels. NDVI and PSII efficiency metrics effectively differentiated responses under stress, suggesting their potential as selection criteria for drought tolerance in pulse improvement programs. These findings provide valuable insights for breeding strategies.

Key words : Pulses, Drought tolerance, PS-II, NDVI, Greengram.

Introduction

Pulses, members of the Fabaceae family, encompass about 700 genera and 18,000 species. These are categorized into cool-season legumes, including broad bean, lentil, chickpea and warm-season legumes, such as pigeonpea, cowpea and greengram, based on their climatic needs (Miller *et al.*, 2002; Toker and Yadav, 2010). Pulses are vital to global food security, representing 9-10% of the total food grain supply. They are a rich source of plant-based protein (20-25%), vitamins and minerals, often surpassing wheat or rice in protein content. Pulses play a key role in combating malnutrition, obesity, and diabetes. The nutritional content of various pulse crops is outlined in Table 1. In 2016, the United Nations acknowledged their significance by declaring the "International Year of Pulses" (Andrews and Hodge, 2010). In rainfed agricultural systems, pulses improve soil fertility by fixing atmospheric nitrogen, with contributions ranging from 72 to 350 kg of nitrogen per hectare annually. Around 80% of pulses are grown in rainfed conditions, while 20% are irrigated. In India, chickpea (*Cicer arietinum*) is the dominant pulse crop, with 65% of the area remain unirrigated (Pulses Revolution, 2018).

By 2020, global pulse production reached 89.8 million tons, with India being the largest producer, consumer, and importer, contributing 25% of global production and 27% of consumption. Pulses account for 7-10% of India's total food grain production, with over 60% of output from the Rabi season (Kaushal, 2021). However, pulses face challenges of climate change, including temperature rise, drought and salinity, which can reduce yields by over 50% (Suzuki *et al.*, 2014; Farooq *et al.*, 2008). Water scarcity impacts up to 45% of agricultural land, leading to yield losses. To meet growing demand, India must increase pulse production to 32 million tons by 2030 (IIPR, 2011).

In India, the absence of the summer monsoon specifically resulted in a significant loss in pulse yields (42% for pigeon pea, 71% for green gram and 74% for black gram) (Kulkarni *et al.*, 2016). In Chickpea the drought stress caused yield losses up to 50% (Sabaghpour

Table 1 : Different species and their genotypes used for the experiment.

Greengram	Blackgram	Chickpea	Cowpea
CO6	CO7	JG16	CG147
CO8	VBN11	GNG1581	CG251

et al., 2006). The impact of the drought worsens under semi-arid tropical conditions due to inconsistent and unexpected rainfall and high ambient temperatures at critical crop growth stages and soil conditions, which hamper plant responses to drought stress (Rosenzweig and Colls 2015). Pulses, grown in rainfed regions, are highly susceptible to drought, impacting growth and yield through physiological changes such as reduced germination, photosynthesis, and nitrogen fixation (Bita and Gerats, 2013; Iba, 2002; Farooq *et al.*, 2008). Drought stress affects different growth stages, with severe effects on plant size, root development, and biomass, particularly in species like greengram, blackgram, chickpea and cowpea (Sadasivam *et al.*, 1988; M Jincy *et al.*, 2019; Cayalvizhi Sai and Chidambaranathan, 2019; Bangar *et al*, 2019).

Drought stress reduces the electron transport rate (ETR) and photosystem efficiency (Fv/Fm), impairing PSII activity critical for photosynthesis (Ahmed *et al.*, 2002; Stirbet *et al.*, 2018). In mungbean, drought stress disrupts energy transfer pathways and affects the D1 protein in thylakoid membranes, reducing PSII activity (Batra *et al.*, 2014; Bano *et al.*, 2021). Chickpea under drought stress shows a significant decline in Fv/Fm during seedling, flowering and podding stages (Rahbarian *et al.*, 2011). In cowpea, drought stress reduces Fv/Fm and increases intrinsic water-use efficiency, highlighting stomatal regulation as a key limitation to photosynthesis (Singh and Reddy, 2011).

Normalized difference vegetation index (NDVI) is widely used to assess vegetation greenness, ground cover, and crop canopy photosynthetic capacity, utilizing data from the ground to satellite heights (Pietragalla and Vega, 2012). It strongly correlates with grain yield and is recognized as a proxy for drought-adaptive traits. NDVI also helps evaluate growth rate, seedling vigor and senescence in wheat (Pietragalla and Vega, 2012; Ramya *et al.*, 2016). In maize, cultivars like DK9901, CP301, and S7328 grown under water deficit showed NDVI reductions of 18.7%, 23.3%, and 19.4%, respectively, demonstrating its effectiveness in identifying stress impacts (Pipatsitee *et al.*, 2022). Most of the studies were done on point measurements ignoring soil moisture levels, limit the ability to interpret drought tolerance thresholds in genotypes (Raina *et al.*, 2019). To address this, the physiological responses of the pulse crops selected for the present study were thoroughly examined to better understand their adaptability under depleting soil moisture conditions.

Materials and Methods

Seed Material

The seeds of greengram and blackgram were collected from TNAU Pulse Unit, and chickpea and cowpea varieties were collected from ICAR - NIASM. In order to differentiate the genotypes from different species of having similar name, it is prefixed with 'Gr', 'Bl', 'Ch' and 'Co' for greengram, Blackgram, chickpea, and cowpea respectively in the illustration. The genotypes were selected based on the performance under water deficit in previous studies, in order to assess the threshold tolerance of PSII and leaf greenness (NDVI) in response to depleting soil moisture conditions.

Plant Growth conditions

The experiment was conducted from 2021-2022 at the National Plant Phenomics facility of ICAR National Institute of Abiotic Stress Management (NIASM), Malegaon (Baramati). The seeds were sown in the plastic pots (Nisarga 302) filled with 3.8 kg clay loam soil outside a greenhouse in open air (natural) conditions. The soil has the following physicochemical properties: pH 8.4, EC 0.24 dSm^{-1}, organic carbon 6.3 g kg^{-1}, 170 kg nitrogen, 17 kg phosphorous and 140 kg potash ha^{-1}, 72 per cent clay, 24.4 per cent sand and 4 per cent silt. In each container, ten seeds were sown, and only three uniform seedlings were kept subsequently after germination. The pots were watered manually using a weighing balance. The field capacity was measured using gravimetric method. For well-watered conditions, 60% field capacity was maintained. And for water stressed plants water was with held.

Chlorophyll Fluorescence

The analysis of chlorophyll fluorescence reveals the health of the PSII, which powers photosynthesis in plants and is often employed in assessing the plant responses to abiotic stress (Stirbet *et al.*, 2018). At around 09:00 h, a sampling of fully formed third leaves at the top of each replication was obtained, and samples were then brought to a dark room and acclimated and stabilized for one hour in the dark. Samples that had been stabilized and acclimated to the dark were collected, and chlorophyll fluorescence was measured on each sample using an image fluorometer (Handy FluorCam, P.S.I., Brno, Czech Republic; Nedbal *et al.*, 2000). Fluorescence was detected using a high-sensitivity charge-coupled device camera operated by the FluroCam software (FluorCamversion 1.2.5.3). First, images of the dark-adapted fluorescence level (Fo) were captured using non-actinic measuring flashes generated by exceptionally bright light- emitting diodes (LEDs). The maximum fluorescence (Fm) level was then determined using a halogen lamp with 800-ms long pulse of saturating light radiation (2,500 mol photons m^{-2} s^{-1}). Variable fluorescence (Fv), which represents the distinction between Fm and Fo, was used to calculate the maximum photochemical efficiency of PSII (Fv/Fm).

Normalized difference Vegetation Index (NDVI)

NDVI (Greenseeker) was employed to detect and quantify the live green fraction of plants based on absorption and reflection of specific bands of light in the electromagnetic spectrum. The absorption and reflection values of red and NIR (near-infrared) light are considered to calculate the NDVI by using following formula.

NDVI = (NIR-Red)/ (NIR+Red)

NDVI values range from -1 to +1. A higher or more positive value indicates greater plant vigour and general health. Thus, in mathematical terms, comparing the red and near- infrared light signals can help differentiate between healthy and non-healthy plants (Govaerts and Verhulst, 2010).

Results

Threshold of tolerance of key physiological processes

Photosystem II sensitivity and Leaf greenness in response to depleting soil moisture were the key physiological parameters selected for the study and measured based on the chlorophyll fluorescence kinetics and NDVI, respectively. The results of experiments on threshold of tolerance are given in the following section.

Photosystem II sensitivity

It was observed that as the PS-II efficiency, as depicted by QY_max, decreased by more than 50% relative to initial values before imposing stress in blackgram. There were no differences among the genotypes at initial stage; however, the severe depletion of soil moisture could differentiate the responses of genotypes. As evident from the Fig. 1, the genotype VBN11 could retain its 75% of PSII activity at 42% FC of soil whereas the genotype CO7 shown 75% of efficiency even under 38% FC of soil indicating distinct level of threshold of tolerance to soil moisture depletion. In chickpea, the genotype GNG1581 could retain 75 % of PSII efficiency only up to 45% FC of soil whereas; the genotype JG16 could retain 75% efficiency even under 39% FC of soil (Fig. 2). In contrast, there was no significant difference between the genotypes of cowpea chosen for this experiment (Fig. 3). However, these genotypes could retain 75 % of PSII efficiency even at 38% FC soil. In greengram, the genotype CO6 exhibited retention of 75% efficiency at 42 % moisture, whereas the genotype CO8 had 75% of activity even under the 38% soil moisture indicating existence of genotypic variation in this trait (Fig. 4).

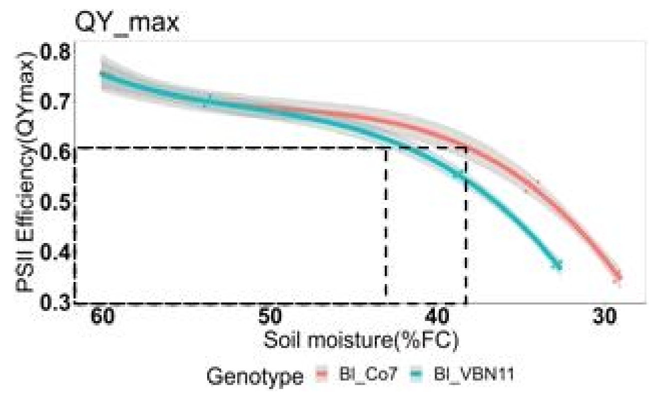

Fig. 1 : PS II efficiency as indicated by QY_max under depleting soil moisture in blackgram.

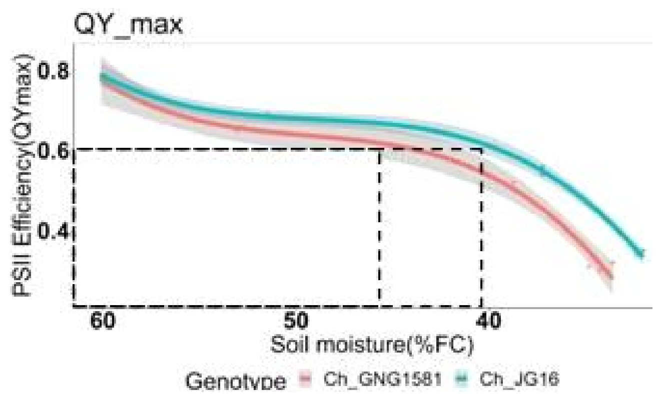

Fig. 2 : PS II efficiency as indicated by QY_max under depleting soil moisture in chickpea.

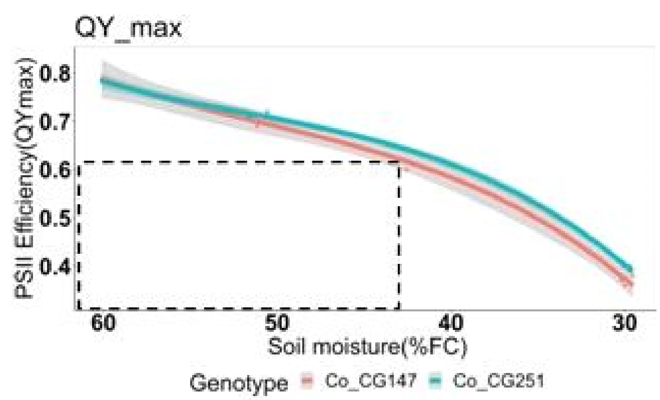

Fig. 3 : PS II efficiency as indicated by QY_max under depleting soil moisture in cowpea.

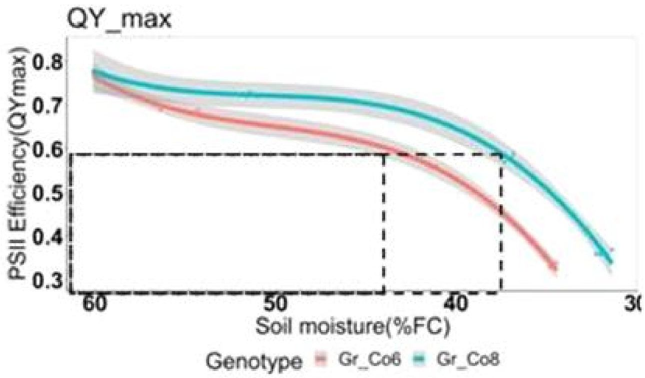

Fig. 4 : PS II efficiency as indicated by QY_max under depleting soil moisture in greengram.

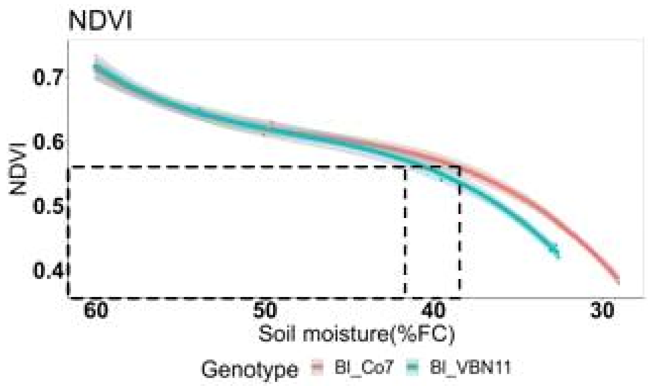

Fig. 5 : NDVI under depleting soil moisture in blackgram genotypes.

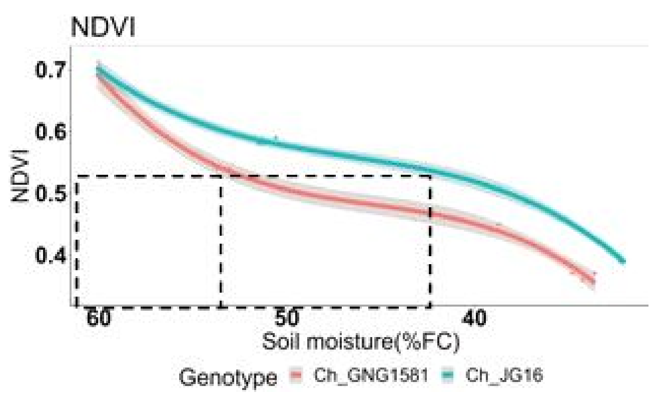

Fig. 6 : NDVI under depleting soil moisture in Chickpea genotypes.

Leaf Greenness

It was observed that the depletion in soil moisture can drastically affect plant health and chlorophyll content as indicated by NDVI. There was gradual decrease in NDVI with the depletion of soil moisture up to 40% FC of soil, however, then onwards there was sharp decline in NDVI in all the pulse species. In blackgram, the genotype CO7 had shown early decline in NDVI as compared to the genotype VBN11(Fig. 5). In chickpea the genotype GNG1581 shown early decline in NDVI as compared to the genotype JG16 (Fig. 6). Cowpea

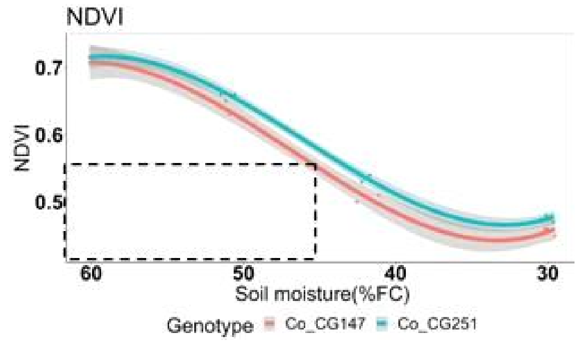

Fig. 7 : NDVI under depleting soil moisture in cowpea genotypes.

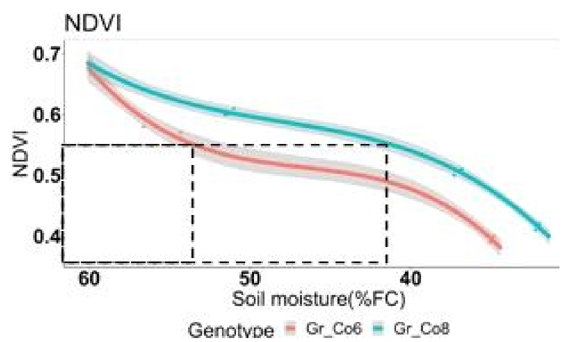

Fig. 8 : NDVI under depleting soil moisture in Greengram genotypes.

genotypes could retain 75% of the initial NDVI values only up to 42% Field capacity and there was no significant difference between the genotypes (Figure 7). In greengram, the CO8 could maintain the 75% NDVI even at 42% FC of soil in contrast to the genotype CO6 which could retain it's the same level of NDVI only up to 53% FC of soil thus indicating the existence of genetic variation (Fig. 8).

Discussion

Often physiological parameters are considered to assess the genotypic responses based on point measurement ignoring the level of soil moisture, which restricts the scope for interpreting threshold level of tolerance of genotype to drought or depleting soil moisture (Raina *et al*., 2019). To bridge this gap, the physiological responses of the pulse crops selected for present study was examined based on the threshold of tolerance of PSII and canopy greenness as indicated by response curve drawn against the soil moisture levels. As expected, the photosystem II efficiency drastically decreased with depletion of soil moisture. However, there were apparent differences in level of tolerance among the genotypes of each species as indicated by retention of certain level of original values. For example, the blackgram genotype, VBN11, could retain 75% PSII efficiency even at 38%soil moisture, however, CO7 genotype could retain same level only up to 42%of soil moisture. This tolerance level shown by the genotype VBN11 can be possibly explain less reduction in the biomass under water-stressed as compared to well-watered condition. In chickpea, the highly popular genotype of water scarce zone like JG16 could maintain efficiency even up to 39% FC of soil in contrast to the genotype GNG1581 which could retain the same level only up to 45% FC of soil clearly indicating the exploitable genetic variation for genetic improvement of the crop (Fig. 2). High level of soil moisture stress tolerance of the genotype JG16 could be one of the reasons for relatively higher biomass as compared to other genotypes even under water-stressed conditions. The similar results obtained in greengram clearly separating the genotypes CO6 and CO8 which were differing in their capacity to retain PSII efficiency. The reduction in the PSII efficiency was reported to be due to impact in energy transfer mechanisms by causing changes in the thylakoid membrane protein D1 (Batra *et al*., 2014). The existence of genetic variation in the chlorophyll fluorescence under water-stressed condition has been reported in greengram (Raina *et al*., 2019) just based on point measurements. The present experiments confirm earlier observations and clearly indicate possibility of using threshold of tolerance of PSII as selection criteria.

This study demonstrates the importance of using threshold tolerance to soil moisture, assessed through NDVI, for evaluating genotypic responses in pulses. The reduction in NDVI, primarily due to leaf senescence and biomass cover loss, is a key indicator of drought stress (Pipatsitee *et al*., 2022). While NDVI is widely employed to assess abiotic stress in crops like rice (Irsyad *et al*., 2022), wheat (Thapa *et al*., 2019) and maize (Pipatsitee *et al*., 2022), previous studies have not considered threshold levels.

This study highlights the significance of assessing genotypic responses to soil moisture stress using threshold tolerance levels of PSII efficiency and NDVI, providing a more comprehensive evaluation than point measurements. Incorporating soil moisture depletion thresholds can enhance genotype selection strategies for improving drought tolerance in pulse crops.

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