

Chlorophyll Fluorescence to Evaluate Pigeonpea Breeding Lines and Mungbean for Drought Tolerance

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Abstract

Crops with increased biomass production and yield under suboptimal water availability are vital for sustainable production under abiotic stress. We identified drought tolerance in pigeonpea and mungbean cultivars by measuring chlorophyll fluorescence, an indicator to evaluate the efficiency of PSII photochemistry. Significant variation ($p < 0.05$) observed among cultivars and crops with high quantum yields (Fv/Fm) in Mungbean (Texas sprout) and Pigeonpea (W1, W3, G1) and were highly drought tolerant. Pigeonpea (Fv/Fm = 0.7933) and mungbean (Fv/Fm = 0.7993) were equally potential and significantly ($p < 0.05$) more tolerant to water stress (50 % field capacity) during early vegetative growth stage compared to soybean (Fv/Fm = 0.7870). The quantum yields were high (0.79-0.81) during early growth stage indicating high water use efficiency and low (0.71, pigeonpea; 0.77, mungbean) during podding and flowering stages respectively indicating distribution of photosynthetic assimilates. Among all the growth stages evaluated, the podding and maturity stages were with less water use efficiency as indicated by relatively less values of Fv/Fm in both crops requiring relatively less amount of water compared to active vegetative growth stage. The cultivars with shortest plant height and tan seed coat were observed with drought tolerance in pigeonpea. Among the six food legumes studied for mean comparisons, the chickpea, pigeonpea and mungbean were highly tolerant followed by soybean, mothbean and tepary bean while fababeen was less tolerant to drought stress.

Keywords: quantum yield, Fv/Fm, *Cajanus cajan*, *Vigna radiata*, fabaceae

1. Introduction

Drought stress, the most devastating abiotic stress, impacts economical yield losses when crops exposed to stress during early seedling growth stage due to lack of water availability (Ranjan et al., 2011). Crop loses its net photosynthetic leaf area due to rolling up of leaves, besides dried pollen, reduction in plant height and root system due to severe drought and heat negatively influencing yield. The United States of America (USA) had experienced severe losses in corn and soybean yields due to drought in 2012 (<http://www.drought.gov>). Both terminal and intermittent drought stress impact bean yield and quality and their effects will be amplified by their interaction with high temperature, disease and soil type (Urrea et al., 2007). The crop productivity is highly influenced by climate change which varies with crop and region with an estimated -40 to +15 percent change in US soybean yield (Adams et al., 1998). At the University of Wisconsin during summer 2012, drought effected soybean was observed with reduced root growth rate (0.9-1.2 cm/day), shortened internodes, reduced nitrogen fixation, abortion of flowers to set seeds and plant death. Therefore breeding drought tolerant new crop cultivars with high seed and quantum yields coupled with high water use efficiency during major crop growth stages under resource free crop situations will be helpful besides advanced agronomic practices.

Extensive research in the past revealed the role of new food legumes to restore soil fertility and in sustainable agriculture (Das & Ghosh, 2012). Pigeonpea is high yielding (of 650 kg/ha) drought tolerant pulse crop with excellent quality seed protein source (170-200 kg/ha) for tropics and subtropics (Saxena et al., 2002; Singh & Oswalt, 1992) and can fix up to 40 kg of N / ha (Saxena et al., 2010). Pigeonpea genotype, JSA 59, was identified as drought tolerant under rainout shelter (without irrigation) with less reduction in yield, high drought tolerance index, low drought susceptibility index, low membrane injury index, high harvest index and dry matter (Deshmukh & Mate, 2013). White and black cultivars were more tolerant than red cultivars with significant reduction in

germination rate, seedling fresh and dry biomass, seedling vigor index and germination index under drought stress in ten cultivated pigeonpea cultivars (Kumar et al., 2011). Improved growth, chlorophyll content, photosynthetic rate under drought stress was well documented with beneficial effects of arbuscular micorrhizae fungi in pigeonpea (Qiao et al., 2011). Mungbean is also a drought tolerant food legume with potentially high seed and protein yields in tropics and subtropics (Kabir & Sarkar, 2008) and was in investigation since 1997 in Virginia (Bhardwaj et al., 1999). Genotypes AUM-9, 25 and 38 were tolerant to drought at 50% field capacity (Aslam et al., 2013). At Queensland University of Technology, the studies on root development were in progress to develop cultivars with deep and widely spread root system for drought tolerance in Australia (Phy. sci. news, <http://www.veooz.com/news/1HDfjx2.html>).

Due to several production constraints, pigeonpea and mungbean yields were reduced in highly cultivated regions of the world during the past decade which opened the avenues for exploring its potential for cultivation in non-traditional areas in the USA (Phatak et al., 1993; Bhardwaj et al., 1997) by introducing food legumes as alternative crops to commercial crops like tobacco in southern USA. Mungbean and pigeonpea yield were high when planted in May-June (Bhardwaj et al., 1997, 1999) in Virginia. Seed quality traits of pigeonpea, produced during two different planting times (Narina et al., 2012), tepary bean (Narina et al., 2014a, 2014b), two cultivars of mungbean and chickpea (Xu et al., 2012, 2013) identified as useful high yielding cultivars of new food legumes for production in Virginia (Narina et al., 2014c). Since the temperatures in summer is high in USA, it is essential to develop new food legume cultivars with short growth duration, high yield and tolerance to high temperature and water stress. No studies were conducted to date to evaluate the drought tolerance of the breeding lines and developed cultivars in pigeonpea, mungbean and other new food legume germplasm available in chickpea, faba bean, mothbean, and lupin for production in southern USA or drought prone areas of USA (Bhardwaj et al., 1999, 2002).

Chlorophyll fluorescence analysis is unique and powerful tool to characterize the germplasm for drought tolerance (Burke, 2007) and was widely used by plant breeders, physiologists and eco-physiologists (Maxwell & Johnson, 2000). Chlorophyll fluorescence will measure the energy that is converted to chemical energy to drive the photosynthesis and energy that is emitted as heat (non photochemical quenching). Fluorescence parameters F_0 is the minimal fluorescence, F_m is the maximal fluorescence, F_v is the variable fluorescence measured as the difference between F_0 and F_m . PSII photochemical efficiency (F_v/F_m) will measure the imbalance between absorbed light energy by chlorophyll and the utilized energy for photosynthesis or carbon fixation which indirectly measures the photo-oxidative damage or tolerance to water or heat stress. Thus fluorescence was used to measure the efficiency of PSII photochemistry to evaluate the stress in a leaf and associated transpiration efficiency (Xin et al., 2008) and photochemical efficiency was a useful indicator for tolerance to drought and varietal screening (Favaretto et al., 2011; Narina et al., 2014).

Drought tolerant genotypes (MCC392, MCC877) showed higher PSII photochemical efficiency (F_v/F_m) compared to sensitive genotypes (MCC68, MCC448) (Rahabarian et al., 2011), high proline content with reduced chlorophyll content with high yields under water stress (Mafakeri et al., 2010) in chickpea and mungbean (Ocampo & Robles, 2000) and pigeonpea (Kumar et al., 2011). The numbers of leaves, leaf area, stem length, amount of dry matter accumulated and yield were reduced due to water stress during six weeks after planting in mungbean (Ranawake et al., 2011) and teparybean (Narina et al., 2014).

Forty three breeding lines (PP1 to PP43) and four cultivars (G1, G2, W1 and W3) of pigeonpea and two mungbean cultivars developed from new crop germplasm available at Agricultural Research Station (ARS) of Virginia State University (VSU) were investigated for drought stress tolerance based on quantum photosynthetic yield in the leaf during four major growth stages (early vegetative, flowering, podding and maturity) in greenhouse, and the data obtained was used to compare the drought tolerance capabilities of these two crops with other food legumes to identify the highly drought tolerant accessions during early vegetative growth stage. We compared various growth stages for highly drought tolerant legumes to know the stages that were highly susceptible to water stress and needs irrigation. We included available food legume crops (fababean, mothbean, chickpea, soybean and teparybean) which help us to identify the potential food legume cultivars useful for crop production during summer after wheat crop in Virginia and south eastern USA as alternatives for tobacco and to improve farm income from domestic and international markets. The knowledge gained through fluorescence studies will be used for further analysis of the morphological traits for water stress in these two crops by relating to the genomic information available in the public data base for pigeonpea, teparybean and soybean which will help to improve the economically important traits for drought tolerance specific to legume crops in short time through advanced breeding techniques.

2. Materials and Methods

The legume cultivars selected for each crop were grown at Randolph farm of VSU during 2011-12 and were hand harvested to raise seedlings in the greenhouse for drought tolerance studies during 2012-2013. The experiment was conducted twice in replicated block design with six crops (chickpea, mungbean, mothbean, pigeonpea, fababean, tepary bean and control soybean) and with two treatments (irrigated and water stress). We selected high yielding cultivars from six legume crops including soybean to test drought tolerance initially to confirm the tolerance of mungbean and pigeonpea.

Six seeds were planted in pots filled with regular growing mix with all the nutrients required for normal plant growth under 10-12 hr photoperiod, 60%-85% relative humidity with 28 °C/18 °C day and night temperatures on an average respectively. The radiation received during the midday in the greenhouse ranged from 27 °C. We followed the recommended fertilizer, insecticide and fungicide dose as necessary in treatment and control pots. The moisture content of water stress pots was maintained at 0.26 m³/m³ and control pots was maintained at 0.45 m³/m³ which are 50 and 100 percent field capacity respectively. The drought treatments in three replications in the treatment pots were induced by withholding watering for one week during growth stage. The pigeonpea and mungbean were evaluated for water stress four times at vegetative, flowering, podding and maturity stages with two weeks interval on an average between each stage.

2.1 Measurement of Chlorophyll Fluorescence

Leaf discs of 100 mm size were collected twice in triplicates from control and treatments in 24 well plates (CELLTREAT scientific products, LLC, Shirley, MA01464) in liquid water and transported to research lab in ARS at VSU. Measurement was performed with modulated chlorophyll fluorometer (Model: OS1-FL, <http://www.optisci.com>) and the leaf discs were read using light adapted test (test type 2) for yield measurements of quantum efficiency under photosynthetic conditions immediately after sample collection and after exposing to heat by incubating the leaf discs at 42 °C in a hot air oven for 20 minutes. The incubated samples were allowed to cool for 20 minutes and were used for fluorescence measurements to know the efficiency of PSII and the energy lost due to quenching and to know the amount of damage caused due to stress. The variations in chlorophyll fluorescence readings obtained before and after incubations were used to estimate the drought tolerance due to non-photochemical quenching and impairment in the PS II efficiency respectively in each individual cultivar to identify the potential cultivars. The heat stress induced artificially, not in field conditions as designed to conduct greenhouse evaluation initially to reduce the number of lines for field screening. The number of highly drought tolerant lines will be screened in the greenhouse and in field conditions for drought tolerance by measuring the yield (Fv/Fm) values and measuring the photosynthetic gas exchange rates in near future.

Comparisons for potential drought tolerance were done by taking the average quantum yield (Fv/Fm) values from the high yielding cultivars in tepary bean, pigeonpea, chickpea, soybean, mothbean and mungbean during water stress at early vegetative growth stage (Table 2). The seed yield values mentioned in the Table 2 were from field experimentations done in Virginia during previous years (Bhardwaj et al., 1997, 1999). The high yielding cultivars in each crop used in the greenhouse experiment, were compared for seed yield values to their quantum yield values. The results of drought stress during early growth stage (Figure 2) were also compared with those during other stages of growth in soybean (control), mungbean and pigeonpea (Table 1, Figure 1), to understand the tolerance by these two selected crops during various growth stages.

Table 1. Comparison of quantum yield (Fv/Fm) values during various growth stages of mungbean and pigeonpea for drought tolerance

Crops/growth stage	Pigeonpea	Mungbean	Soyabean (Carter)
Vegetative_T	0.7933	0.7993	0.7870
Vegetative_C	0.7945	0.7968	0.7802
Flowering_T	0.7676	0.7795	0.7950
Flowering_C	0.7550	0.7967	0.7782
Podding_T	0.7143	0.7914	0.7777
Podding_C	0.7078	0.8011	0.7882
Pod maturity_T	0.7715	0.7983	0.7883
Pod maturity_C	0.7356	0.7980	0.7912

The values were means of all the cultivars from the data obtained from two experiments and the differences among crops and stages were significant at 5 per cent. The standard deviation was ranging from 0.00 to 0.10; Column 1: T indicates treatment drought (water stress) and C indicate Control (irrigated) for various stages.

Table 2. Comparison of drought tolerance during early vegetative growth stage (six weeks) of food legumes adapted to Virginia

Crops	Quantum Yield (Fv/Fm)	Seed Yield (Kg/ha)
Pigeonpea	0.7933	1236
Mungbean	0.7993	1416
Chickpea	0.8044	719 (kabuli)-1153 (Desi)
Teparybean	0.7290	1816
Mothbean	0.7703	*
Fababean	0.6570	*
Soybean	0.7403	3010.8

Mean seed yield data was retrieved from previous publications of Bhardwaj et al 1997, 1999, 2002 (pigeonpea, mungbean, chickpea, tepary bean) and Virginia Department of Agriculture and Consumer Services (soybean). * No data available on seed yield in Virginia or southern USA.

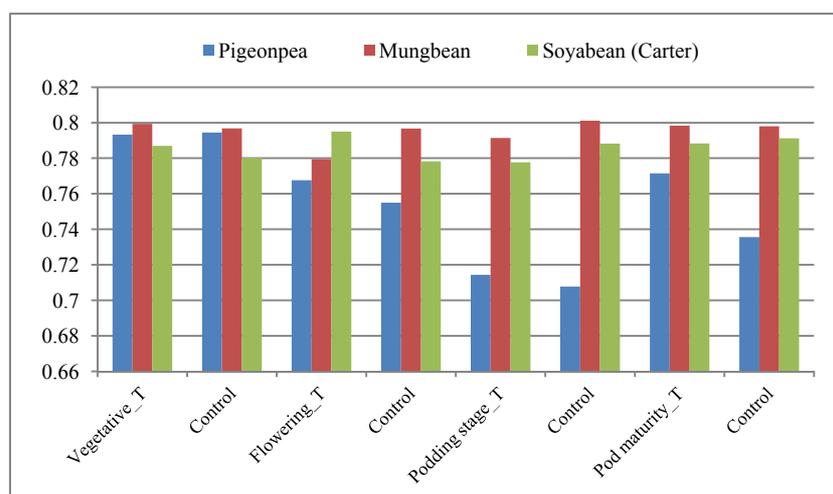


Figure 1. Quantum efficiency of photosystem II (Fv/Fm) during different growth stages in irrigated (Control) and water stress (T) treatments in pigeonpea, mungbean and soybean

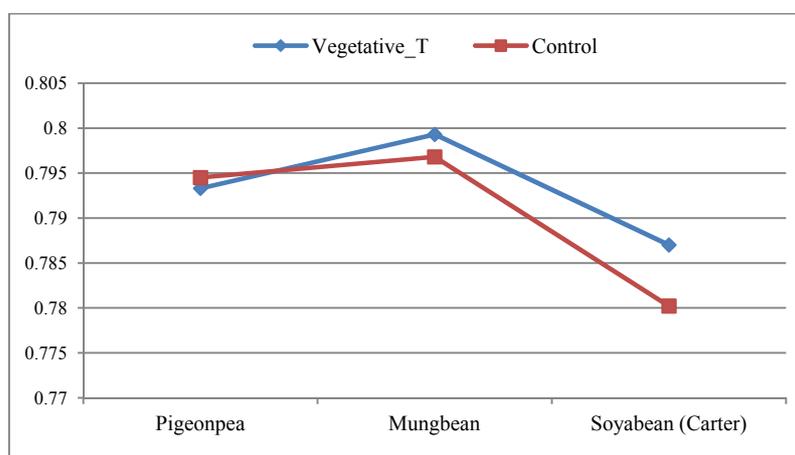


Figure 2. Quantum efficiency of pigeonpea, mungbean and soybean under water stress (T) and irrigated (control) conditions during early growth stage (at six weeks age)

2.2 Statistical Analysis

Analysis of data was done following the statistical procedures for Analysis of Variance and Correlation using tools on MS Excel 2010. The differences were considered significant with p values more than or equal to 0.05. The R^2 and standard deviation were ranging from 0.90-0.98 and 0.00 to 0.10 respectively.

3. Results and Discussion

Significant differences ($p < 0.05$) between the treatment means were observed for all the stages and in all the cultivars and between the crops (Tables 1 and 2). Pigeonpea samples PP 9, 11, 28, 43 were missing due to either non-germination and/or due to lack of sample on the plant during data collection time (Table 3). Relatively high quantum yields (Fv/Fm) in leaf disc samples were observed from vegetative to flowering stage in pigeonpea indicated high water use efficiency of cultivars during these stages. The quantum yield values were slightly reduced towards podding stage which might be due to partitioning of assimilates for pod filling. The values were normal at maturity stage which indicates reduced or no water requirement during pod maturity (Tables 1, 3 and Figure 1). The reduction in Fv/Fm values indicate less tolerance to drought or photo-oxidative stress (Faveretto et al., 2011) and low water use efficiency which is due to high evapotranspiration losses and reduced efficiency of PSII system due to reduced electron transport for carbon fixation. Therefore high water use efficiency indicated by high values of Fv/Fm under full sunlight and reveals high tolerance to drought conditions and high efficiency of PSII photochemistry (Narina et al., 2014; Kao et al., 2011).

Table 3. Quantum photosynthetic yield (Fv/Fm) under water stress during various growth stages in mungbean cultivars, pigeonpea breeding lines and cultivars

Breeding line	Flower Color	Seed coat Color	Plant Height	Quantum Photosynthetic yield (Fv/Fm)			
				Vegetative	Flowering	Podding	Maturity
PP1	Y	T	Tall	0.7753	0.7697	0.626	0.736
PP2	Y	Light T	Tall	0.7691	0.778	0.695	0.808
PP3	Y	T	Medium	0.7763	0.7597	0.638	0.773
PP4	Y	T	Short	0.771	0.7707	0.576	0.771
PP5	Y	T	Medium	0.771	0.769	0.746	0.775
PP6	M	T	shortest	0.774	0.7493	0.696	0.77
PP7	Y	T	Tall	0.7808	0.7567	0.7	0.755
PP8	Y	T	Medium	0.775	0.7723	0.684	0.767
PP10	Y	T	Short	0.7666	0.775	0.693	0.775
PP12	Y	T	Tall	0.7743	0.7587	0.698	0.782
PP13	R	T	Tall	0.744	0.7615	0.723	0.77
PP14	Y	T	Tall	0.7643	0.7763	0.73	0.776

PP15	Y	W	Medium	0.7673	0.7765	0.76	0.774
PP16	Y	T,R	Medium	0.7605	0.771	0.75	0.777
PP17	Y	T	Med-Tall	0.7788	0.7733	0.771	0.775
PP18	Y	T	Short	0.7752	0.774	0.63	0.768
PP19	Y	W,T	Tall	0.772	0.7697	0.721	0.768
PP20	R	T	Tall	0.7797	0.7647	0.757	0.769
PP21	Y	T	Tall	0.765	0.7393	0.709	0.759
PP22	Y	T, Dark R	Tall	0.7493	0.7657	0.742	0.757
PP23	Y	T	Tall	0.7628	0.7757	0.721	0.771
PP24	Y	T,R,W	Medium	0.7688	0.7703	0.733	0.754
PP25	Y	T	Shortest	0.761	.	0.695	0.779
PP26	R	T	Medium	0.779	0.7743	0.764	0.76
PP27	R	T	Tall	0.7713	0.779	0.74	0.74
PP28	Y	T	Tall	.	0.7737	.	.
PP29	Y	T	Tall	0.744	0.768	0.747	0.772
PP30	Y	W	Medium	0.765	0.7657	0.727	0.776
PP31	Y	W	Short	0.7714	0.7747	0.725	0.775
PP32	M	W	Short	0.67	0.764	0.63	0.78
PP33	Y	T	Tall	0.7662	0.7723	0.75	0.779
PP34	R	W	Medium	0.773	0.7753	0.724	0.781
PP35	R	W	shortest	0.7667	0.751	0.706	0.785
PP36	Y	W	Tall	0.7598	0.77	0.719	0.774
PP37	Y	T,W	Tall	0.7698	0.778	0.756	0.783
PP38	Y	T	Tall	0.7753	0.7783	0.764	0.786
PP39	Y	T	Tall	0.7042	0.769	0.729	0.775
PP40	Y	T	Tall	0.7769	0.7593	0.732	0.765
PP41	Y	T	Tall	0.7638	0.7553	0.688	0.753
PP42	Y	T	Tall	0.7703	0.7543	0.755	0.789
G1	Y	T	Medium	0.7923	0.7763	0.72	0.777
G2	Y	W	Medium	0.787	0.755	0.772	0.779
W1	R	T	Tall	0.7978	0.762	0.776	0.801
W3	R	T,R	Tall	0.796	0.721	0.779	0.798
Berkem	Y	G	Short	0.7933	0.7801	0.7901	0.7944
Texas Sprout	Y	G	Short	0.8053	0.7789	0.7927	0.8022
Soya bean (Carter)	Cream	W	Medium	0.787	0.795	0.7777	0.7883

Flower color: Y-Yellow; R-Red, M- mixed yellow and red; Seed coat color: R = Red, T = Tan, W = White, G = Green; Plant Height: Tall = above 4inches; medium=2.5 - 4inches shortest =< 2.5 inches; the values were means of three observations and three replications from two experiments and the differences among cultivars were significant at 5 per cent. The standard deviation was ranging from 0.00 to 0.05. Numerical values in bold were selected lines with tolerance to water stress.PP1-PP42 refer to Pigeonpea breeding lines and G1,G2,W1 &W3 are Pigeonpea cultivars.

During flowering stage, mungbean cultivar, Berkem (0.7801), pigeonpea breeding lines, PP2 (0.7780), PP14 (0.7763), PP15 (0.7765), PP27 (0.7790), PP37 (0.7780), PP38 (0.7783) and cultivar G1 (0.7763) were superior with high values of Fv/Fm indicating drought tolerance. During Podding stage, mungbean cultivar, Texas Sprout (0.7927) and pigeonpea cultivars W1 (0.776) and W3 (0.779) were tolerant compared to the pigeonpea breeding lines and soybean control (Cv. Carter). At Pod maturity stage, mungbean cultivar, Texas Sprout (0.8022) and pigeonpea cultivar, W1 (0.8010) were superior compared to soybean Cv. Carter (0.7883). Some of the breeding lines (PP12, 14, 16, 25, 30, 32, 33, 34, 35, 37, 38, and 42) and the cultivars G1, G2, and W3 of pigeonpea were observed tolerant with high values of Fv/Fm during pod maturity due to distribution of photosynthetic assimilates and less requirement of water.

High values of photosynthetic quantum yield (Fv/Fm) at maturity stage compared to vegetative and flowering

stage (Tables 1 and 2) was supported by Allahmoradi et al. (2011) indicating high water use efficiency under water stress during early stages of growth compared to podding and maturity stage. It was also indicated high water requirement during early stages influences productivity at later stages of growth and for better crop yields. The results were supported by Dutta and Bera (2008) and Ranawake et al. (2011) who stated that seed germination and early seedling growth were considered critical for successful agricultural crop production in arid and semiarid conditions. We observed similar results of non germination with teparybean seed in both field and greenhouse conditions during water stress (unpublished)

Among all the 42 pigeonpea breeding lines, the shortest cultivars were observed drought tolerant with more leaves per stem and with oval thick coated leaf surface to prevent transpiration losses during extreme climates. The four improved cultivars G1, G2, W1 and W3 were significantly highly tolerant compared to 42 breeding lines. Among the 42 breeding lines, PP1, 7, 17, 20, 26 were tolerant with mean Fv/Fm values of 0.77 to 78. Red flowered, W1 and W3 (Fv/Fm = 0.7969) cultivars were equally potential in drought tolerance to yellow flowered cultivars G1 and G2 (Fv/Fm = 0.7897) in pigeonpea (Table 3) with significant ($p < 0.05$) differences for chlorophyll fluorescence. The cultivars with tan seed coat were observed to be highly tolerant compared to red seed coat and were in line with the previous observations in pigeonpea by Kumar et al. (2011). Studying the morphological features of leaf from these pigeonpea cultivars and its inheritance will help us to utilize the trait in other food legumes like teparybean, favabeen which were adapted to climate in USA.

Significant variation ($p < 0.0001$) observed for photochemical efficiency during heat incubation experiment of leaf disc and the values were significantly ($p \leq 0.05$) low (Fv/Fm $\leq 12.5\%$) compared to water stress. The lines with drought tolerance were selected with high quantum yield values. The decrease in the quantum yield is due to impairment of the PSII system due to heat treatment for 20 min (results were not presented). These observations are in line with the previous observations in food legumes like chickpea (Rahabarian et al., 2011) due to decreased supply of water due to abiotic stress and increased evapotranspiration losses due to increased leaf temperature under water stress (Ghassemi-Golezani & Lotfi, 2012). The cultivars which were tolerant to water stress were significantly potential with heat stress tolerance as supported by Mafakheri et al. (2010) in chickpea.

Mungbean was highly tolerant during early growth stage compared to pigeonpea (Figure 2) and was revealed that mungbean is adaptable to the environments with 30-80 % moisture availability due to its morphological features of leaf and root to shoot ratio under water stress (Aslam et al., 2013). Among the four cultivars developed in pigeonpea, the cultivars W1 and W3 and among two cultivars of mungbean, the cultivar Texas Sprout were with high values for Fv/Fm with extreme tolerance to drought stress compared to soybean as supported by previous results in mungbean (Ocampo & Robles, 2000) and pigeonpea (Kumar et al., 2011). The lower Fm values in the breeding lines, cultivars and crops during water stress indicated impaired photosystem due to non - availability of electrons to water splitting complex which reduced the efficiency of the PSII influencing crop specific Fv/Fm ratio, the quantum yield (Table 1, Figure 2) and was supported (Kao et al., 2011).

4. Conclusion

Present study revealed preliminary data on quantum yield potential of food legume cultivars. Further studies on leaf samples for photosynthetic gas exchange during mid day conditions, for stability of seed and quantum yield due to heat induction under field condition will be helpful to identify the potential drought resistant lines in these food legume crops. Due to enormous capabilities of adaptability to wide range of climates and with observed highly significant drought tolerance values under water stress during the four stages of growth, pigeonpea and mungbean were identified as potential alternative crops to tobacco farmers in Virginia and ideal crops for sustained agriculture production under limited water availability in USA during summer after wheat. As the Fv/Fm values were greatly influenced by temperature and water stress (climate), evaluation of fluorescence for the selected lines (from each crop) in high tunnel (with and without irrigation) under field conditions and future studies directed towards plant height, leaf shape, size, thickness, foliage and flower color, pod color, seed coat color under water stress will help us to improve the potential cultivars in each crop for production in southern Virginia. Further, analysis of multiple parameters (agronomic, morphological, physiological, biochemical and molecular) will be more reliable for successful breeding for drought tolerance and crop production purposes as multiple factors and their interaction contribute for tolerance and will help understanding the osmotic adjustments under water stress in field conditions for better grain yield and quality.

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