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Comparative Analysis of Pigeon Pea Genotypes for Physiological Traits Under Water Logging Conditions Using Hydroponics and Pot Culture

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ABSTRACT

Pigeon pea (*Cajanus cajan* L. Millspaugh) is more susceptible to water logging stress than other legumes and is known to be one of the major factor for limiting growth and production of pigeon pea across the globe. The timing and duration of water logging vary with soil type, frequency of heavy rainfall and risk of flooding. Therefore, the best method for screening pigeon pea genotypes under water logging stress is important. However, there is currently no useful and efficient selection method of screening for this trait, which is an obstacle in breeding programmes. Hydroponics is the simplest and effective method used to study the water logging tolerance in different crops. A study of pigeon pea genotypes under hydroponics condition revealed that after 4 days of stress condition (anaerobic) decrease in chlorophyll content was observed and was correlated with relative index of root length, shoot length and shoot fresh weight under water logging stress and control condition while in pot condition the variation was studied using various physiological and morphological parameters like plant stand, leaf senescence, chlorophyll content, relative water content, membrane stability index. Under hydroponics medium Pusa 992 and Pusa 2002 was found to be highly sensitive and tolerant genotypes while under pot condition Pusa 992 and Pusa 2002 as moderately sensitive and tolerant genotypes respectively. The study in response to water logging stress in two pigeon pea genotypes under two different medium was found to be not correlated significantly and further evaluation of this trait under field condition will help to understand their potential utility in crop improvement plan.

Key words *Pigeonpea; Water logging; Hydroponics; Chlorosis*

Pigeon pea (*Cajanus cajan* L. Millspaugh) is cultivated in tropical and sub-tropical areas between 300N and 300S latitude. It is an important grain legume of Asia (especially, the Indian subcontinent), Latin America and Eastern and Southern Africa. Globally, it is grown on ~5 million hectares (mha) in about 82 countries of the world. Pigeon pea has a unique place in Indian farming and accounts for about 90 % of the global production. It is the second most important pulse crop next to chickpea, covering an area of around 4.42 m ha (occupying about 14.5 % of area under pulses) and production of 2.86 mt (contributing to 16 % of total pulse production) and productivity of about 707 kg/ha. It is an excellent source of protein (20-22 %), supplementing energy rich cereal diets in a mainly vegetarian population (Saxena et al. 2010). However, the productivity of this crop is hampered by various biotic and abiotic stresses among which water-logging stress is of major importance. In India, pigeon pea crop is generally grown in monsoon or rainy (kharif) season and is exposed to water-logging stress conditions caused by erratic and prolonged rains. Moreover, pigeon pea is primarily grown in deep vertisols and in the areas with mean annual rainfall of 600-1,500 mm, hence water-logging becomes a serious problem (Chaudhary et al. 2011). In India, about 8.5 m ha of arable land is prone to this problem. A recent comparative analysis of pigeon pea growing regions revealed that almost all the states that grow pigeon pea in India are affected by water-logging. It is estimated that around 1.1 mha of the total area under pigeon pea is affected by excess soil moisture, causing an annual loss of 25-30 % in production (Chaudhary et al. 2011).

Water-logging occurs when the water table attains a level at which the soil pores in the root zone of the plants are fully saturated and restrict normal air circulation. Consequently, oxygen level in the soil declines and carbon dioxide concentration increases, which adversely affects the growth and development of plant roots. Drastic reduction in oxygen level and increase in carbon dioxide concentrations are the primary stresses to which the plants are exposed under water-logging conditions (Vartapetian & Jackson, 1997). The germination and vegetative growth stages of pigeon pea crop are more sensitive to water-logging stress in comparison to flowering stage. Wilting, senescence, chlorosis, and abscission of lower leaves are few symptoms begin within two days of imposing stress condition which ultimately decides the crop stand and productivity (Else et al., 1996; Takele et al., 1995; Cho et al., 2006; Kumutha et al., 2008; Singh et al., 1986). The water-logging stress result significant decline in RWC (Min and Barhthalomew, 2005). Water logging have been reported to induce considerable membrane damage in different crop plants such as forty times increase in solute leakage results from four days waterlogged pea plants (Oberson et al., 1999; Rawyler et al., 2002; Jackson et al., 1982). Thus, it is immediate to develop pigeon pea genotypes which are high yielding as well as are tolerant to water-logging.

Genetic analysis of tolerance to any abiotic stress can be successfully accomplished if a repeatable, precise and quick screening technique is available. Screening for water logging tolerance under field conditions, that too in

Table 1. Composition and concentration of Hoagland stock solution used under hydroponics study

Compounds	Molecular Weight (g/mol)	Concentration of stock solution (mM)	Concentration of stock solution (g/L)	Volume of stock solution per litre of final solution (ml)
A)$Ca(No_3)_2$	164.088	3.64	364.0	1.25
KNO_3	101.1032	2.213	221.3	1.25
KH_2PO_4	136.086	0.621	62.1	1.25
$MgSO_4$	120.366	2.176	217.6	1.25
B)$CuSO_4.7H2O$	285.7156	0.0035	0.35	
$MnSO_4$	151.001	0.0609	6.09	
$ZnSO_4$	161.47	0.0097	0.97	0.125
H_3BO_3	61.83	0.1269	12.69	
H_2MoO_4	161.973	0.04	4.0	
C) Tartaric acid + $FeSO_4$	150.087+ 151.048	0.04+0.05	4+5	0.6

the rainy season involves complications such as uncontrolled rainfall and percolation of water as well as occurrence of diseases like phytophthora blight in pigeon pea. In addition, genotypes interact differently with the varying environment, and it is difficult to screen genotypes precisely and to define the testing environment. Moreover, the results are not repeatable in natural field conditions due to these unpredictable and uncontrolled test conditions. By looking to these complications, it is necessary to standardize the screening technique for water-logging under controlled condition which will be precise, repeatable and free from uncertainties of natural environment, so as to identify tolerant genotypes and for designing genetics of the tolerance. Moreover, it has also been reported that screening of pigeon pea genotypes in seedling stage for water logging tolerance is suitable to predict water logging tolerance at later stage (Singh et al. 1986, Kumutha et al. 2008 and Li et al 2008).

However, in view of the complexity of water logging tolerance and its great variation at intra-specific and inter-specific levels, it is difficult to identify single criteria, which could be used as effective selection targets. Rather it is most meaningful if morphological and physiological indicators for individual species are determined rather than generic indicators. It is also important to find out association of morphological and physiological parameters with results of screening at the seedling stage. So far there are only few scientific reports available on screening of water logging tolerance in pigeon pea under pot and field conditions, but there has been no scientific efforts made to standardize the screening methods using hydroponics in pigeon pea. Thus

Table 2. Paired t-test for comparison of mean performance for morphological parameters (RL, SL, TL, NL, RFW, SFW, LFW, TFW, RDW, SDW, LDW and TDW) of two genotypes under control and Water Logging (WL) stress conditions in hydroponics

Traits	Pusa 992			Pusa 2002		
	Control	WL stress	T test	Control	WL stress	T test
RL (cm)	24.3	14.1	0.63	27.7	21.3	3.80
SL (cm)	22.3	12.9	2.44	19.2	17.9	0.88
TL (cm)	46.7	27	4.22	46.8	39.2	3.12
NL	11.0	4	8.009*	10.0	7.7	3.99
RFW (g)	0.32	0.11	6.92*	0.35	0.14	2.05
SFW (g)	0.48	0.19	4.95*	0.51	0.3	2.53
LFW (g)	0.75	0.15	7.72*	0.79	0.4	2.16
TFW (g)	1.55	0.44	6.95*	1.65	0.85	2.18
RDW (g)	0.12	0.07	0.15	0.11	0.05	2.44
SDW (g)	0.07	0.08	0.06	0.14	0.03	20.24**
LDW (g)	0.18	0.35	2.48	0.16	0.06	3.35
TDW (g)	0.37	0.48	0.09	0.41	0.13	2.86

**Significant at 1%, *Significant at 5%

Table 3. Relative index (RI) of SL, RL and SFW of two genotypes under control and WL stress conditions in hydroponics

Traits	Pusa 992			Pusa 2002		
	Control	Stress	RI (%)	Control	Stress	RI (%)
SL	22.3	12.9	57.8	19.2	17.9	93.2
RL	24.3	14.1	58	27.7	21.3	76.9
SFW	0.48	0.19	39.6	0.51	0.3	58.8

in the present study an effort has been made to standardize screening technique using hydroponics under controlled environment to have more precision and repeatability of the results. Therefore, by keeping the above into consideration the present study involves comparative study of pigeon pea genotypes for water logging tolerance using both hydroponics and pot culture to find correlation between the traits observed under hydroponics with biochemical and physiological parameters estimated in pot culture under waterlogged condition.

MATERIALS AND METHODS

Plant material and experiment

The plant material in the present study consists of two pigeon pea genotypes viz., Pusa 992 (water-logging sensitive) and Pusa 2002 (water-logging tolerant). These genotypes were evaluated for various morpho-physiological traits viz., RL (root length), SL (shoot length), TL (total length), NL (number of leaves), RFW (root fresh weight), SFW (shoot fresh weight), LFW (leaf fresh weight), TFW (total fresh weight), RDW (root dry weight), SDW (shoot dry weight), LDW (leaf dry weight) and TDW (total dry weight) under hydroponic condition and for plant stand, leaf senescence, total chlorophyll content, relative water content and membrane stability index under pot condition during the year 2013-2014 at the Division of Genetics, IARI, New Delhi and National Research Centre of Plant Biotechnology, Pusa Campus, IARI, New Delhi. The experiment was laid out in Complete Randomised Design (CRD) with two replications, and phenotyping was carried out for a number of traits. The standard cultivation practices were followed precisely.

a) Hydroponics screening

Plant growth conditions

Seed Germination

Seeds were germinated in a tray on the germinating paper and kept for continuous growing for 10 days then 3 uniform plants of each genotype were selected and transferred in good quality of plastic duplicate trays with size 20 x 15x 5 cm and the roots of the propagules were inserted through the holes of the tray slightly large sized plastic net trays of size 30 x 20x 5 cm having holes of 5 x 5 mm were placed above the plastic container containing working nutrient solution for each water logging (anaerobic) and control (aerobic) conditions for 2 days under continuous light and aeration by fitted aquarium air pump to produce bubbling so that continuous oxygen will be provided for respiration of the plants and solution were replaced every 5-6 days to maintain the proper nutrient level and concentration in the solution. The pH of the nutrient solution was measured daily. Then first sensitive symptom (time/days after treatment) was noted among the genotypes and root as well as shoot were harvested separately by blotting to eliminate the entrained moisture.

Composition and Preparation of Nutrient Solution

Modified Hoagland solution Epsteim (1972) prepared as described by Taiz and Zeiger (2002) were used for hydroponic study as shown in Table 1. The stock solution of each nutrient was prepared separately and appropriate volume of each was mixed together to make up the final volume and concentration of the nutrient solution. The pH of the solution was adjusted to 6.8 using 0.1 N HCl or NaOH.

Sampling, drying and weighing methods

Sampling

Samples of water logging stress and control were collected at 8th day after creating anaerobic condition and 38 days of growth. At each interval, plants were harvested from each treatment, washed thoroughly in distilled water and blotted to dryness. Morphological parameters such as root/shoot length, leaf area and relative index were recorded. For biochemical analyses, root, stem and leaves were sampled. A minimum of 6 plants of each treatment were separately cut into pieces, randomized and sampled in duplicates for each analysis.

Table 4. Variation between two pigeonpea genotypes for morphological and physiological parameters under pot experiment for Control (C) and Water logging stress (WL)

Genotype	RWC (%)		MSI (%)		Chlorophyll content (SPAD)		Plant Stand		Shoot Length (cm)	
	C	WL	C	WL	C	WL	C	WL	C	WL
Pusa 992	29.5	59.9	75.4	45.3	38.0	33.6	2	1	15	14
Pusa 2002	13.4	54.5	99.5	51.8	51.3	48.75	5	4	11	11

Table 5. Paired t-test for comparison of mean performance for physiological and biochemical parameters (RWC, MSI and Chlorophyll content) of two genotypes under control and Water Logging (WL) stress conditions in hydroponics

Genotype	RWC (%)			MSI (%)			Chlorophyll content (SPAD)		
	C	WL	T-test	C	WL	T-test	C	WL	T-test
Pusa 992	29.5	59.9	469.38**	75.4	45.3	340.96**	38.0	33.6	8.91*
Pusa 2002	13.4	54.5	202.9**	99.5	51.8	119.87**	51.3	48.75	0.272

**Significant at 1%, *Significant at 5%

Morphological measurement

Growth of plants was assessed in terms of root length, stem length, and leaf area.

Root and stem length

The sampled plant seedlings were washed in distilled water, and length of root, stem and number of leaves were measured manually, using a graduated scale. Measurements of not less than six plant seedlings were recorded each time.

Dry weight

Samples of root stem and leaves collected as described earlier were weighed in pre-weighed containers using electronic balance. Fresh weight obtained was recorded and the weighed samples were then placed in hot air oven at 60° C for one week. Dry weight of each sample was taken on the 8^{th} day of drying and weighing was repeated until values become constant.

Relative Index percentage

Relative Index percentage was calculated according to the method of Turner (1994)

$$RI = \frac{\text{Observed value of a trait in solution under stress} \times 100}{\text{Observed value of a trait in solution under control}}$$

b) Pot screening

Seed germination

Seeds were germinated in the pots (size 12 x 8 cm) containing 1kg of soil with 34° C temperature at day and 25° C at night with relative humidity approximately 68 % and a photoperiod of 16/8 h. Four seeds were sown per pot. The plants were irrigated every alternate day with normal tap water. After 35 days from sowing, water logging stress was induced by putting the pots into the plastic trays filled with stagnant water and making the water level up to 5cm above the soil upto 8 days. The intensity of the water added to the pots was calculated periodically to maintain the water level of the pots. After 8 days of water logging stress screening based on physiological and morphological parameters was also studied before and after water logging.

Physiological Parameters

Physiological parameters like relative water content, membrane stability index, total chlorophyll content and plant stand were calculated by Weatherley (1950), Sairam et al. 1997 and SPAD meter respectively.

Statistical analysis

t-test for paired samples was carried out as per the method given by Panse and Sukhatme (1989). The results have been presented in Table 2 and Table 5.

RESULTS AND DISCUSSION

As we know that water logging induces adverse effect on several morphological and physiological parameters of plants by creating deficiency in essential nutrients like Ca, Mg, K and N_2 which lead to the formation of adventitious roots, lenticels and aerenchyma formation to adapt under oxygen deficient environment (Ashraf et al. 2012). Water logging stops the influx of CO_2, which leads to decrease in photosynthetic parameters observed in many crops like maize, barley, mung bean, and tomato respectively (Yan et al., 1996; Yordanova et al., 2001; Else et al., 1996; Cho et al., 2006). Chlorophyll content is directly influenced by reduction in photosynthesis and with increase in the duration of stress (Bansal and Srivastava 2015) as it is important part of light harvesting system in photosynthetic apparatus. Pezeshki (2001) reported that increase in the chlorophyllase activity under water logging stress condition is responsible for reduction in chlorophyll content in the plants. Reduction in chlorophyll content during water logging stress was also reported in plants like wheat (Collaku and Harrision 2002) and soybean (Sorte et al., 1996).

Decrease in energy supply and altered redox state of the cells are responsible for increase in ROS production under water logging stress condition causing oxidative injury which may leads to reduction in membrane stability due to lipid peroxidation and it is continued to increase with increase in the duration of the stress (Yan et al., 1996) reported in many plants like corn, winter rape and barley. Fig 1a shows the effect of water logging stress on two genotypes in which Pusa 2002 was shown to be the tolerant and Pusa 992 as a susceptible genotype during hydroponics screening. The first symptom of chlorosis and wilting of susceptible genotype was noticed after 4 days of starting of anaerobic condition while the tolerant genotype was remained as it is even after 21 days of continuation of stress. This is could be due to the adaptation of tolerant genotype by formation of lateral roots at the junction of root and shoots internode as shown in Fig 1a. The screening of genotypes in soil/pot was also done simultaneously to compare the result in the same environment under two

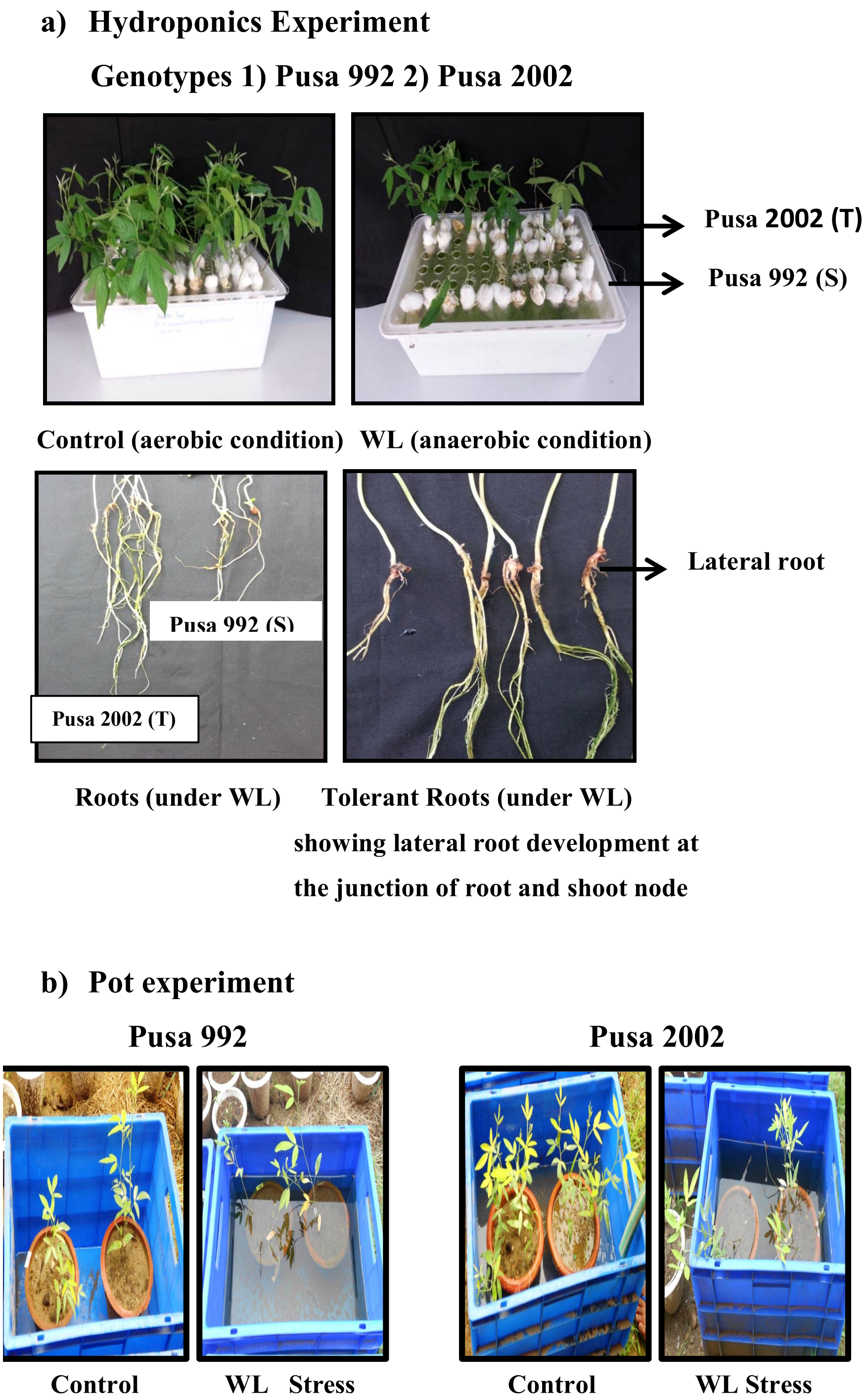

Fig. 1. Screening of pigeon pea genotypes in control and waterlogging stress condition under hydroponics and pot experiment

different methods. The comparative morphological analysis viz., chlorosis and wilting of leaves of the two genotypes under both the screening methods was found to be similar as shown in Fig 1a and 1b. In the present study analysis of paired t-test (Table 2) shows that the mean under control and waterlogging stress were significantly differed for the traits RL, SL, TL, NL, RFW, SFW, LFW, TFW, RDW, SDW, LDW and TDW. The mean values for various traits (RL, SL, TL, NL, RFW, SFW, LFW, TFW, RDW, SDW, LDW and TDW) recorded under control and WL stress conditions for the two varieties Pusa 992 and Pusa 2002 in hydroponics presented in Table 2. The mean values were compared by 't' test as per Panse and Sukhatme (1989). The results indicated that the genotype Pusa 992 had significantly lower performance under waterlogging stress for NL, RFW, SFW, LFW and TFW than that under control. The second genotype Pusa 2002 had non- significant reduction in the performance for almost all the traits except for SDW for which this genotype showed significant reduction thus, it could be concluded that the genotype Pusa 2002 showed relative tolerance as compared to Pusa 992 to waterlogging stress under hydroponic conditions. These results are

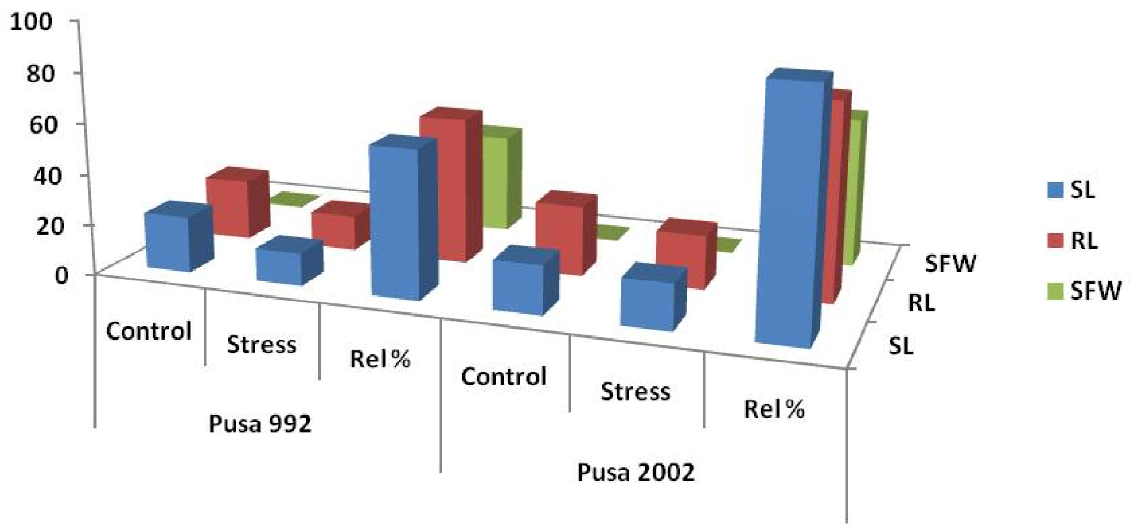

Fig. 2. Graph showing relative tolerance index of SL, RL and SFW traits in two pigeon pea genotypes under control and stress condition

parallel to the results obtained under pot screening experiment. Thus, it revealed that hydroponics screening procedure may be a good substitute for pot screening test. However, the screening under hydroponic conditions should be further verified by screening large number of pigeon pea genotypes, including tolerant and sensitive to waterlogging stress, under hydroponic condition in order to fine tune the protocol of screening for waterlogging

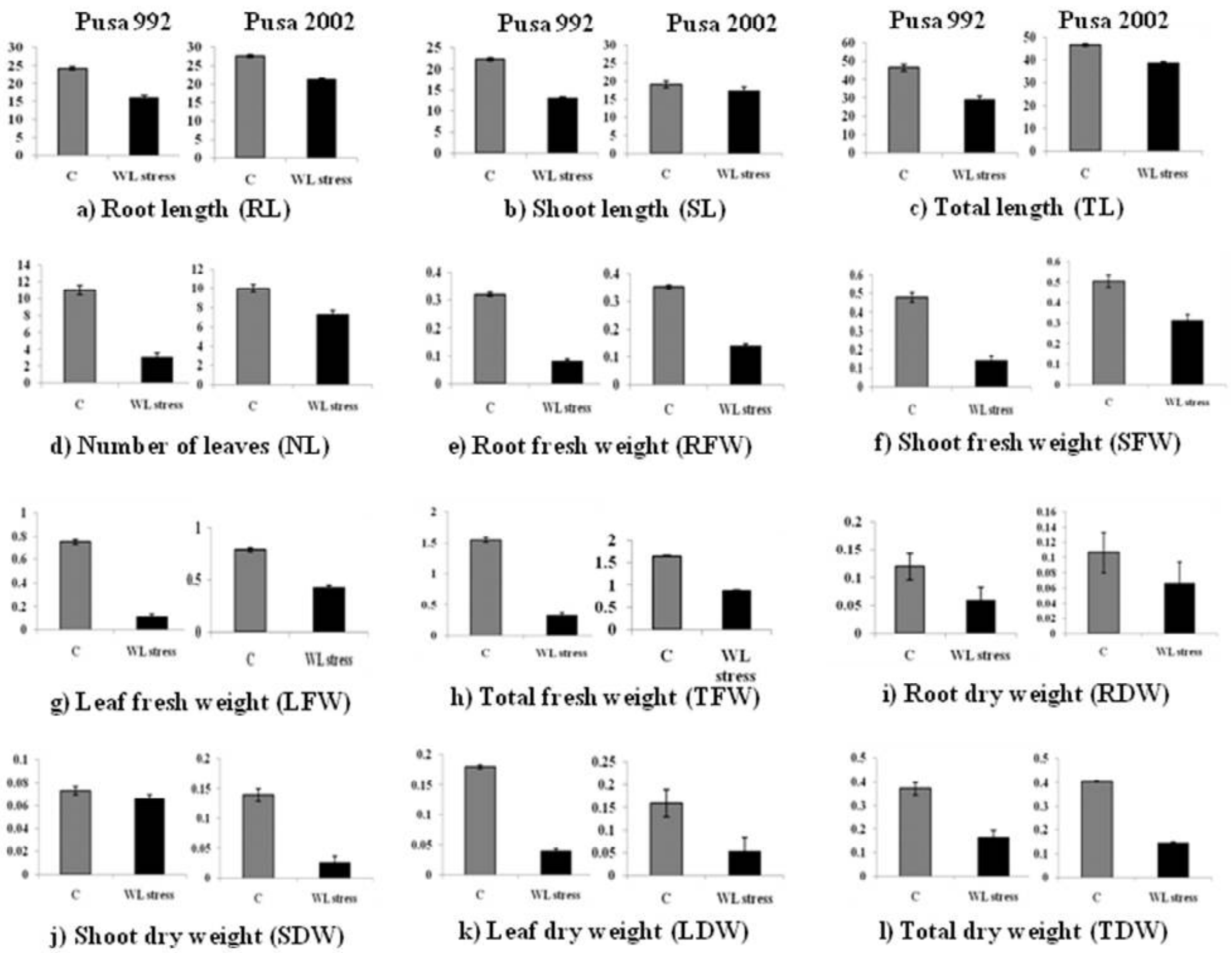

Fig. 3. Graph showing comparison of mean performance for morphological and physiological parameters of two genotypes under control (C) and Water Logging (WL) stress conditions in hydroponics a) RL b) SL c) TL d) NL e) RFW f) SFW g) LFW h) TFW i) RDW j) SDW k) LDW and l) TDW. Data shown here is the mean ± S.E.

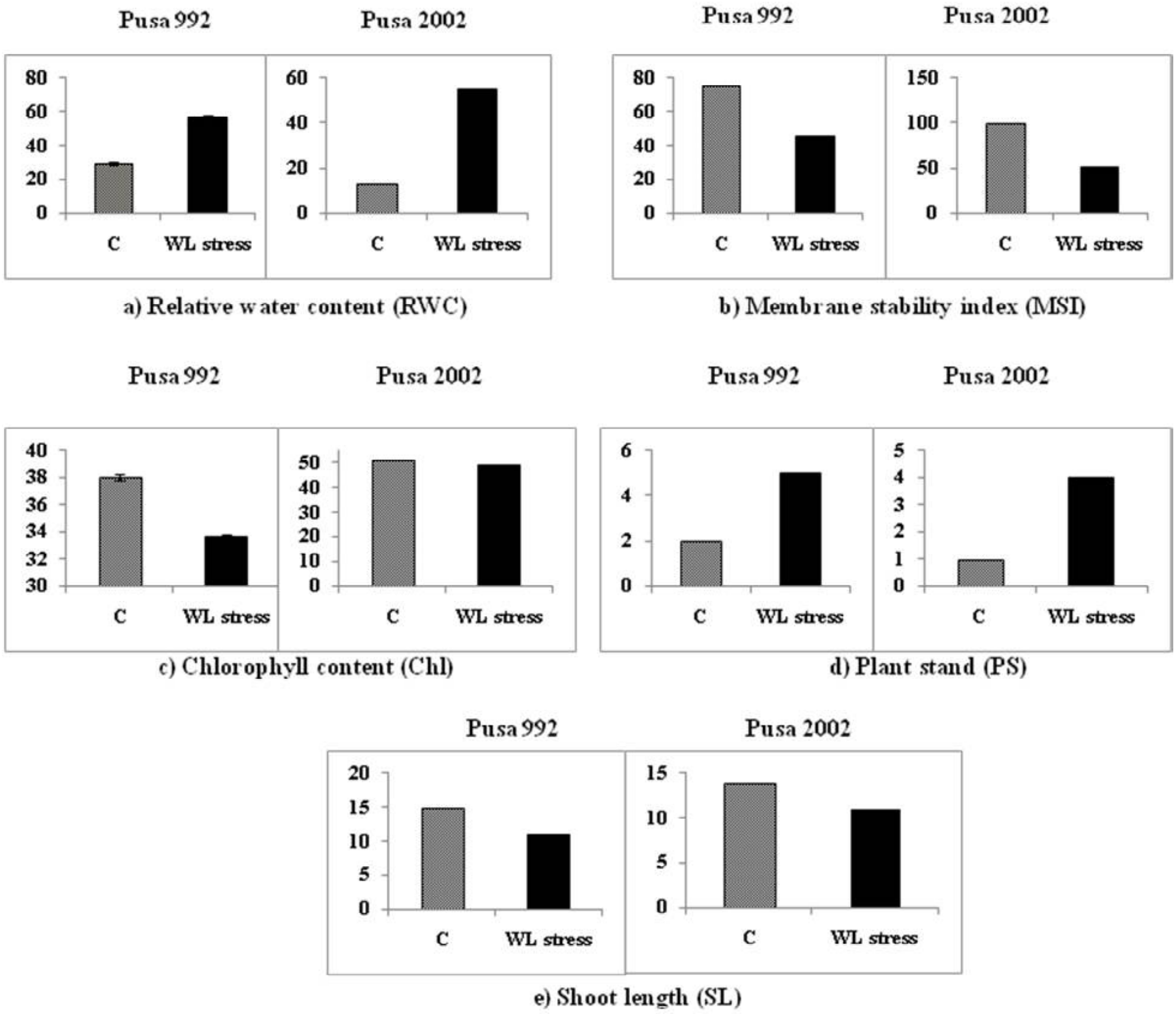

Fig. 4. Graph showing variation between two pigeon pea genotypes for morphological and physiological parameters under pot experiment for Control (C) and Water logging stress (WL) a) Relative water content (RWC) b) Membrane stability index (MSI) c) Chlorophyll content (Chl) d) Plant stand (PS) and e) Shoot length (SL). Data shown here is the mean ± S.E.

stress under hydroponics. The relative index percentage data shows that SL, RL and SFW traits for screening water logging tolerance under hydroponics study shows the highest variation under control [SL (57.8), RL (58.0) and SFW (39.6)] and stress [SL (93.2), RL (76.9) and SFW (58.8)] conditions as shown in Table 2 and Fig 2. On the other hand during pot screening it was found that plant stand, relative water content, membrane stability content and total chlorophyll content decreases after stress in comparison to control condition as shown in Fig 3 and 4. Based on the physiological and morphological data Pusa 992 was found to be moderately sensitive while Pusa 2002 as tolerant genotype under water logging. A lot of study under hydroponics for screening water logging tolerance based on similar study during seedling stages was also studied in different crops such as maize, wheat and sorghum (Qui et al. 2007, Singh et al. 2003 and Jordan et al. 1979).

Youngsukying and Nakasathien (2008) studied the four clones of Eucalyptus to water logging stress condition under hydroponics based on physiological parameters like net photosynthesis, maximum quantum yield, chlorophyll content and biomass. Four two-month-old eucalyptus clones were grown in half-strength Hoagland's solution and subjected to water logging conditions for 16 days. Physiological parameters were monitored at days 0, 8 and 16. All the parameters were decreased after 16 days of water logging in comparisons to control. The physiological parameters of gas exchange and the Fv/Fm ratio were proven to be suitable indicators of water logging-tolerant traits.

Soffer and Burger (1988) studied the effect of dissolved oxygen concentrations in Aero-hydroponics on the formation and growth of adventitious roots of cuttings of woody (*Ficus*) and herbaceous (*Chrysan thymum*) in the range of 0-8 mg/L. The result showed that the lowering the dissolved oxygen concentration increased the time required to form adventitious roots, reduced rooting percentage, reduced number of roots formed per cutting, and reduced average root lengths.

Zhou et al. 2011 showed that WLT was mainly controlled by additive genes and had high heritability, and hence is suitable for screening in early generations but accurate phenotyping is crucial for identifying better QTL. Stress tolerance is obtained indirectly by testing, at best, advanced breeding lines in multi-environmental trials and finally by approval of cultivars with the highest mean grain yield in variety testing. A trait-based selection method, either for root growth or for chlorophyll fluorescence, to improve WLT would probably be more successful than just breeding for yield. The risk of discarding interesting genotypes on the way would be reduced and it would be possible to introgress special alleles for high WLT.

Pang et al. 2004 suggested the use of photosynthetic rate or total chlorophyll for selection of parental genotypes and chlorophyll fluorescence when screening large numbers of breeding lines. However, there are no reports on the heritability of these traits and their use in practical breeding. Li et al. 2008 used indices of leaf chlorosis, plant survival and plant biomass reduction to identify quantitative trait loci (QTL) associated with water logging in barley and found seven such QTL. Some of these QTL affected multiple adaptations to water logging-related traits, e.g. by reducing leaf chlorosis and increasing plant biomass under water logging stress.

Bertholdsson (2013) Studied the screening for Barley waterlogging tolerance in Nordic Barley cultivars (*Hordeum vulgare* L.) using chlorophyll fluorescence on hydroponically grown plants. In this study water logging can reduce crop yield by 20-50 % or more and lack of efficient selection methods is an obstacle in plant breeding. Methods based on root growth inhibition and on fluorescence on plants grown hydroponically were evaluated against data on biomass accumulation water logging soil. As both traits were correlated and it was easier to measure fluorescence, this method was further evaluated.

Hydroponics

In the present study analysis of paired t-test showed that the mean under control and water logging stress for the two varieties Pusa 992 and Pusa 2002 were significantly differed for the traits viz., relative water content (RWC), moisture susceptibility index (MSI) and total chlorophyll, but non-significant for shoot length (SL) of Pusa 2002 in hydroponic (Table 2). The mean values for the studied traits under control and WL stress conditions for varieties Pusa 992 and Pusa 2002 in hydroponics are presented in Table 2. The results indicated that the genotype Pusa 992 had significantly lower performance for all the studied traits under water logging stress relative to control (Fig. 1). The second genotype Pusa 2002 also showed significant reduction for RWC, MSI and total chlorophyll except SL under water logging condition relative to control, but reduction in performance was significantly lower compared to Pusa 992 (Fig. 2).

Pot experiment

The same traits viz., RWC, MSI, total chlorophyll and SL were also studied in pot culture under water logging and control conditions, and the results are in agreement with that of hydroponics. The paired t-test showed that mean performance of variety Pusa 992 differ significantly under water logging and control conditions for all the studied traits, whereas the Pusa 2002 showed significant difference in mean performance under two conditions for RWC and MSI but non-significant for the traits viz., total chlorophyll and SL.

On the basis of above study it is concluded that no efforts have been made so far towards standardization for screening techniques under hydroponics for identification of water logging tolerant genotypes. Thus, the standardized protocol in this study shall be useful towards the understanding of mechanism and genetics of water logging tolerance in pigeon pea. This shall enable Plant Breeders to screen Pigeonpea genotypes more rapidly and with more precision under controlled environment condition. In the present study Pusa 2002 was found to be the tolerant genotype as compared to Pusa 992 which is susceptible under water logging stress condition in hydroponics as well as pot condition. SL, RL and SFW were found to be the better traits for screening water logging tolerance under hydroponic condition as shown in Table 3. In the pot screening Pusa 2002 was found to be tolerant and Pusa 992 as moderately sensitive genotype as shown in Table 4. This is the preliminary study on standardization of water logging tolerance under hydroponics on which further improvement can be made on the basis of physiological, biochemical and anatomical parameters for precise screening of the genotypes.

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