

## Original Research Article

# Effect of Photosynthetic Cessation on the Traits Associated with Waterlogging Tolerance in Pigeonpea

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## ABSTRACT

### Keywords

Seedlings, Dry Weight per Seedling, Seedling vigour, CRD, Waterlogging

Aim of the study is to evaluate the pigeonpea genotypes for waterlogging tolerance by assessment of characters like Survival percent, Dry Weight per Seedling and Seedling Vigour. Study design: Completely Randomized Design (CRD). Place and Duration of Study: The research trial was conducted at Pulse Research farm, Model bhitti and Department of Plant Breeding and Genetics Bihar Agriculture University, Sabour, Bhagalpur (Bihar). Which lies between 25°15'40" N latitude to 87°2'42" E longitude and 46 meters above sea level and the sowing was done on 21<sup>st</sup>July, 2018. 20 pigeonpea genotypes which have shown variability for waterlogging tolerance were taken for study. They were sown in pots and were flooded for waterlogging treatment (for 5 days). Observations were recorded before and after 8 days of de-submergence, and data analysis was done using Completely Randomized Design. The genotypes evaluated on the basis of three important characters viz. Survival percent, Dry Weight per Seedling (DWPS) and Seedling Vigour (SV). Survival percent for the genotypes varied from 0.0% to 60.0%. Genotypes ICP-5028, ICPL-20126, LRG-30 and MAL-9 were genotypes with most plants survived contrary to the genotypes ICP-7035, Manak, Pusa-991 and ICPL-20126 were with least survival percent. Genotypes like ICP-7035, Manak, ICPL-20125 and Paras exhibited drastic reductions in their dry weight per seedling i.e. 100%, 67%, 62.5% and 57% respectively as compared to genotypes ICP-5028, ICPL-20092, ICPL-20120 show almost same dry weight when compared with untreated control. Seedling vigour after treatment was found to be maximum with least reduction in genotypes ICP-5028(28%), LRG-30(48%), Mal-9(46%), Mal-15(58%) while at the lower end with maximum reduction compared to control genotypes ICP-7035(100%), Manak (97%), ICPL-20238 (89%), Paras(89%), ICPL-20120 (83%), Pusa 991(89%) were observed. Significant variability among the genotypes was found for waterlogging tolerance. Identified tolerant genotypes can be recommended for cultivation as well as for using them as parent for breeding programs for developing waterlogging tolerant cultivars.

## Introduction

Being a third world crop, pigeonpea has not got the kind of inquisitiveness other crops had. Changing climate and unstable natural

ecosystem has led to drastic changes in weather cycle especially in sub-tropical country like India. Monsoon rain has become a matter of concern in the current scenario, and we have faced either very less downpour

than required or heavy downpour causing flood like situations. Indian agriculture, which majorly depends upon the south-west monsoon, is adversely affected by this. It is well known that the pulse crop especially pigeonpea is much sensitive to waterlogging in its early to late vegetative stages. Despite being the largest producer, India is also the largest importer and consumer (23-24 million tonnes) of pulses in the world. Among pulses, pigeonpea hold second position after the chickpea in terms of area and production. Around 85% of total world's pigeonpea is being produced by India only, occupying area of around 46.5 lakh hectare giving 30.27 lakh tonnes of produce. In Bihar, it is cultivated on area of around 0.27 lakh hectare giving production of 0.39 lakh tonnes. The productivity of Bihar (1739 Kg/ha) is comparatively higher as compare to the national productivity (937 kg/ha).

Geographical presence of the river Ganga and its tributaries like kosi, gandak etc. cause this crop highly vulnerable to excessive hydrological stress every now and then in the state of Bihar. The sowing time of pigeonpea is around Mid-June to Mid-July in India, which makes the early stages of the crop to coincide with monsoonal downpours in the country. Being a kharif season crop, pigeonpea receives high rainfall. The presence of excessive moisture in the field or root zone enhances the incidence of *Phytophthora* and *Alternaria* blight.

Not only pods but also its other vegetative part is used as a feed, fodder, fuel and fertilizer by the rural section in Bihar. Survival percent is direct measure to assess the survival ability of plants under waterlogging stress. In addition, the reduction of dry matter is good indicator of plants internal well-being in terms of biosynthetic pathways and carbon assimilation under scarce conditions. Photosynthetic cessation is major setback, which plants start to

experience during anoxic stress. Aquaporin is also an important membrane protein that helps in passage of water into the cell and is damaged due to reduced hydrolic conduction and water-uptake during waterlogging (Tournaire-Roux *et al.*, 2003). The accumulation of fresh and dry mass is reduced significantly during the waterlogging stress (Shabala *et al.*, 2014). There is dire need to be addressed to develop or identify waterlogging tolerant genotypes which can thrive in low-lying gangetic plains of Bihar. In the present study, we have evaluated pigeonpea genotypes for its waterlogging tolerance on the basis of different physiological parameters.

### **Materials and Methods**

Freshly harvested seeds of 20 pigeonpea genotypes were evaluated for waterlogging tolerance in a short period of time using a simple screening method using pots (Table 1). Pots were filled with 8 kg of soil and were mixed with soil: FYM in ratio 50:1(w/w) and Fertilizer (nitrogen, phosphorus and potassium, NPK) was also applied as basal doses. Ten seeds/pot were sown, for each genotype three pots were sown i.e. 2 treated and one as a control. To avoid the incidence of fungal infection, seed treatment was done with contact fungicide Thiram (dithiocarbamate) dust @ 2.5g per kg of the seeds before sowing. For each genotype, three pots were prepared (two pots for imposing stress treatment and one kept as a control, i.e. no treatment). Before application of waterlogging stress treatment, the number of plants in each pot was counted. The stress treatment imposed pots were kept in trench having depth slightly more than the height of pots to create anaerobic waterlogged conditions. Observations involving survival percent, dry weight per seedling and seedling vigour were recorded after 8 days of de-submergence.

### **Survival percentage**

It was recorded by counting the number of plants before and after waterlogging treatment and then taking their ratio followed by multiplication of 100 to get it in percent.

### **Dry Weight per Seedling (DWPS)**

Whole plants were first sundried in natural condition then inside incubator at 40°C till stable weight was attained. Then the plants were weighed and recorded in grams for treated and control plants.

### **Seedling vigour**

It is a composite character, which depends upon the data of survival percent and Dry Weight per Seedling (DWPS). Seedling Vigour tells about the tolerability of plants towards waterlogging stress, which is the outcome of various adjustments in biosynthetic pathways to cope up with the adversity.

SEEDLING VIGOUR(SV) = SURVIVAL PERCENT X DRY WEIGHT PER SEEDLING (DWPS)

Analysis of generated data was done in Completely Randomized Design (CRD) in two replications over the control pots. Analysis of variance among genotypes for the character survival percent, dry weight per seedling and seedling vigour was done using Indostat software. Phenotypic Correlation was determined among characters using Indostat software. Tocher's clustering was also done using the same software.

## **Results and Discussion**

### **Survival percentage**

Critical reduction was observed in the survival percentage of genotypes after 8<sup>th</sup> day

of waterlogging stress. The genotypes ICP-5028 (60%) along with ICPL-20126 (55%) and MAL-9 (50%) were least affected or least mortile due the waterlogging stress while genotypes ICP-7035 (0%), Manak (5%) and Pusa-991 (10%) were the most affected ones. Average performers include genotypes Maruti (19%), Asha (25%), Paras (27.5%), ICPL-20237 (30.5%), Mal-15 (40%) and ICPL-332 (40%) etc. At brief exposure of excessive moisture genotypes does show variation in their survival (Chauhan *et al.*, 1987).

Various studies on environment conditions of southeast Asian countries like India and Thailand unfolds that reduced diffusion of gases specially O<sub>2</sub> and along with this low irradiance sometimes are important towards plant mortality (Setter *et al.*, 1997). When a plant maintains constant level of growth as compared to normal conditions even in waterlogged ones then this is to be known as tolerance towards waterlogging (Setter and Waters, 2003). Kumutha *et al.*, (2009) suggested that waterlogging caused the loss as much as 96% in susceptible genotypes of Pigeonpea. Roots are most affected part due to shortage of oxygen which is further conducted to shoots (Yordanova *et al.*, 2001). This coupled with physiological absence of water to the plant tissues causes wilting and finally mortality of the plant. When tissues are hypoxic or anoxic, the oxygen-dependent pathways, especially the energy-generating systems, are suppressed, the functional relationship between roots and shoots are disturbed, and both carbon assimilation and photosynthetic utilisation are suppressed (Vartapetian and Jackson, 1997).

### **Dry Weight per Seedling (DWPS)**

Genotypes like ICP-7035, Manak, ICPL-20125 and Paras exhibited drastic reductions in their dry weight per seedling i.e. 100%, 67%, 62.5% and 57% respectively as

compared to genotypes ICP-5028, ICPL-20092, ICPL-20120 show almost same dry weight when compared with untreated control.

Waterlogging has shown significant reduction in photosynthetic efficiency and biological yield in maize (Zaidi *et al.*, 2003; Dhillon *et al.*, 1998; Ashraf and Rehman 1999; Scholowing and Teching, 1997), tomato (Else *et al.*, 2009), soybean (Cho *et al.*, 2006) and barley (Yordanova and Popova, 2001; Yan *et al.*, 1996). Short term exposure of 6 days waterlogging reduced dry weight of genotypes significantly in case of maize (Liu *et al.*, 2010). According to study on plants *Hyparrhenia rufa* and *Andropogon gayanus* by Filho and Lopes (2011) it has been found that the reduction in dry matter was more in waterlogged plants when compared with those grown at field capacity.

Singh *et al.*, (2017) suggested that waterlogging caused reduction in dry weight of seedlings with variations among genotypes. Dry weight enables to determine the biomass content of genotypes produced as result of photosynthetic assimilations after deducting the respiratory losses. Short duration exposure of waterlogging resulted in reduction of biomass by reducing the leaf area, which is mainly responsible of photosynthesis (Takele and McDavid, 1995). According to (Araki *et al.*, 2012), wheat roots and shoots were lighter when compared to control ones due to excessive moisture treatment pots.

The response of photosynthesis to soil flooding resembled that produced by other stresses (osmotic shock and drought). The common events of this response are the slowed rate of carbon assimilation, inhibition of RuBPC activity, and changes in photorespiratory carbon metabolism (Kicheva *et al.*, 1994, Popova *et al.*, 1996, Tsonev *et*

*al.*, 1998). All this contributes towards reduce carbon assimilation thus affecting the Dry weight per seedling.

### **Seedling Vigour (SV)**

Seedling vigor was obtained by multiplying dry weight per seedling with Survival Percent. Results here correspond with that of DWPS and survival percent. Seedling vigour gives overall acceptability of plants for being used as waterlogging tolerance genotype.

Seedling vigour after treatment was found to be maximum with least reduction in genotypes ICP-5028(28%), LRG-30(48%), Mal-9(46%), Mal-15(58%) while at the lower end with maximum reduction compared to control genotypes ICP-7035(100%), Manak (97%), ICPL-20238 (89%), Paras (89%), ICPL-20120 (83%), Pusa 991(89%) were observed. Seedling vigour is directly correlated to survival percent.

Significant positive correlation of Seedling Vigour with Survival percent and Dry Weight per Seedling was found i.e.  $r=0.85^{**}$  and  $0.62^{**}$  respectively.

Shortage or insufficient oxygen supply attributes to reduced vigour of seedlings. 16.6 to 58.3 % reduction was found in pigeonpea genotypes after 8 days of waterlogging treatment (Lal M., 2014).

As oxygen is final electron acceptor in electron transport chain so the final sink for electron becomes unavailable thus affecting the whole respiration process thus hampering Adenosine Triphosphate (ATP) production, which results in decreased vigour and poor germination. This is also in lieu with reports of Johnson *et al.*, (1989). These results also had similarity with results of Wang *et al.*, (2012).

**Clustering of genotypes using tocher's method**

The character used for Tocher's Method clustering (using Indostat software) was Survival percent. As Survival percent is easily deducible and direct measure for waterlogging tolerance among pigeonpea

genotypes. The results obtained were concurrent with classification on the basis of absolute values of Survival Percent. Details of various clusters include viz. Highly Susceptible (Cluster 6), Susceptible (Cluster 2), Moderately Tolerant (Cluster 4), Moderately Susceptible (Cluster 1), Tolerant (Cluster 3) and Highly Tolerant (Cluster 5).

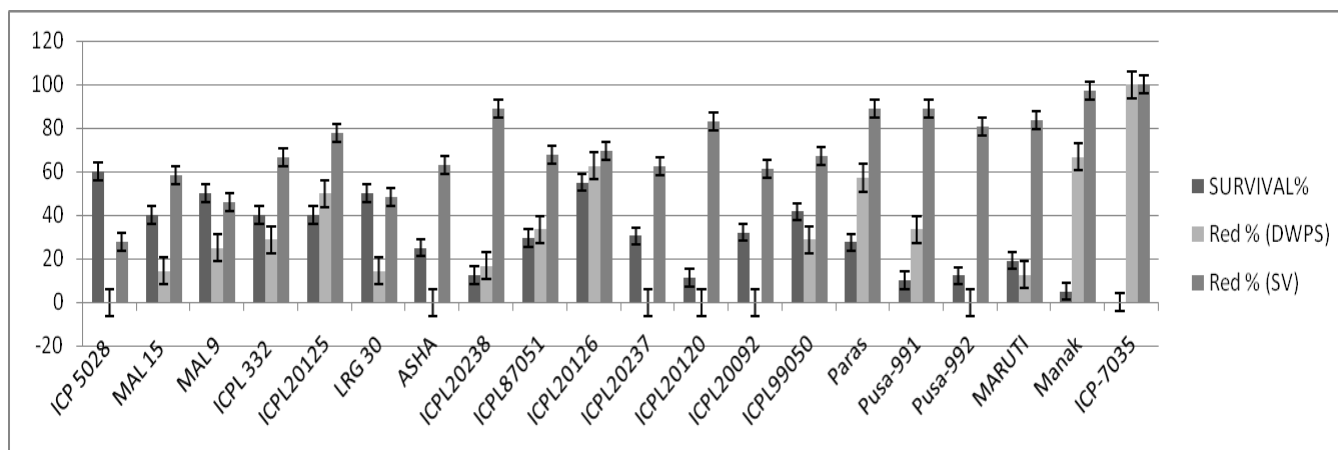
**Table.1** List of genotypes and mean performances for the traits recorded

S.NO.	GENOTYPE	SURVIVAL%	DWPS (WL)	DWPS (N)	SV (WL)	SV (N)
1	ICP 5028	60	0.07	0.06	3.8	5.27
2	MAL 15	40	0.06	0.07	2.57	6.14
3	MAL 9	50	0.06	0.08	2.83	5.22
4	ICPL 332	40	0.05	0.07	2.07	6.21
5	ICPL20125	40	0.03	0.06	1.09	4.91
6	LRG 30	50	0.06	0.07	2.84	5.49
7	ASHA	25	0.07	0.07	1.79	4.83
8	ICPL20238	12.5	0.05	0.06	0.59	5.26
9	ICPL87051	29.5	0.04	0.06	1.16	3.59
10	ICPL20126	55	0.03	0.08	1.64	5.38
11	ICPL20237	30.5	0.08	0.07	2.25	5.97
12	ICPL20120	11.3	0.06	0.05	0.59	3.51
13	ICPL20092	32	0.07	0.07	2.05	5.28
14	ICPL99050	41.5	0.05	0.07	2.17	6.61
15	Paras	27.5	0.03	0.07	0.62	5.62
16	Pusa-991	10	0.04	0.06	0.61	5.42
17	Pusa-992	12.1	0.07	0.07	0.85	4.4
18	MARUTI	19	0.07	0.08	1.24	7.57
19	Manak	5	0.02	0.06	0.13	4.48
20	ICP-7035	0	0	0.06	0	5.34
<b>Mean</b>		<b>29.5</b>	<b>0.05</b>	<b>0.07</b>	<b>1.54</b>	<b>5.32</b>
<b>C.V.</b>		<b>14.46</b>	<b>15.97</b>	<b>6.1</b>	<b>18.79</b>	<b>10.04</b>
<b>C.D. 5 %</b>		<b>5.34</b>	<b>0.02</b>	<b>0.01</b>	<b>0.6</b>	<b>1.12</b>
<b>Ranges</b>		<b>0.00-60.00</b>	<b>0.00-0.08</b>	<b>0.050-0.08</b>	<b>0.00-3.80</b>	<b>3.51-7.57</b>

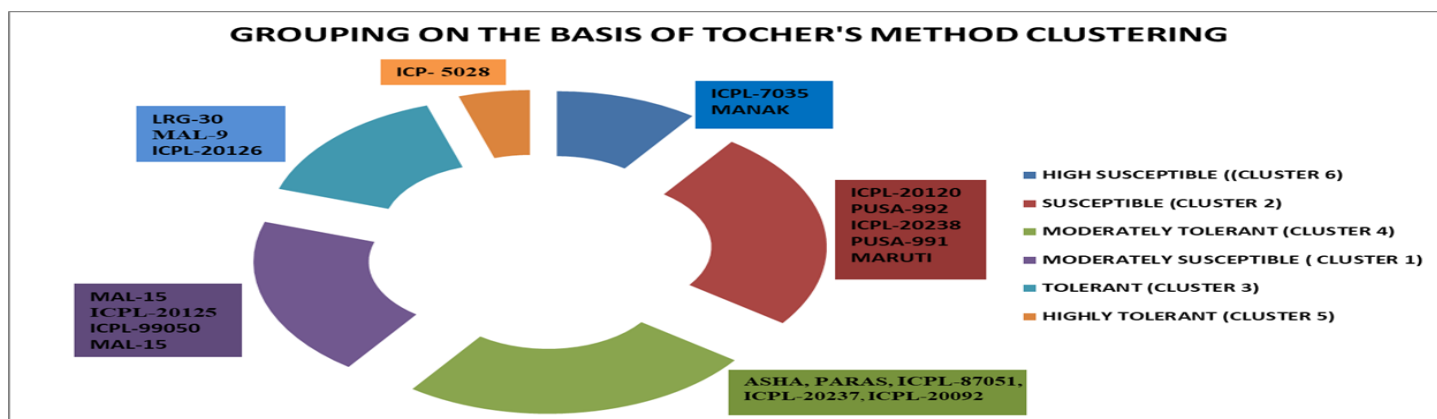
Note: DWPS= Dry Weight per Seedling, SV= Seedling Vigor, WL- Waterlogging, N= Normal/Control



**Fig.1** Comparison of survival percent and Reduction in Dry matter Per Seedling (Red DWPS) and Seedling Vigour (Red SV)



**Fig.2** Classification of genotypes on the basis of Tocher's Method clustering by Survival % and cluster distances



	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	0.09	19.34	3.8	4.16	10.37	37.37
Cluster 2	19.34	0.47	39.66	6.01	57.77	3.33
Cluster 3	3.8	39.66	0.52	15.45	1.91	64.21
Cluster 4	4.16	6.01	15.45	0.38	27.28	17.16
Cluster 5	10.37	57.77	1.91	27.28	0	86.87
Cluster 6	37.37	3.33	64.21	17.16	86.87	0.72

When compared with cluster 6 the distances were in order: Cluster 2 (3.3) < Cluster 4 (17.16) < Cluster 1 (37.27) < Cluster 3 (64.21) < Cluster 5 (86.87). It is very clear from the distance table that cluster 6 (highly susceptible pool) is at maximum distance from cluster 5 (highly tolerant pool).

Cluster 5 which show highly tolerant group contains single genotypes ICP-5028, cluster 3 containing tolerant genotypes has genotypes LRG-30, Mal-9 and ICPL-20126. Cluster 1 is moderately susceptible group having genotypes Mal-15, ICPL-20125 and ICPL-99050 likewise other genotypes are

grouped into remaining clusters shown in fig 2.

In conclusion, significant genotypic variability was observed among the pigeonpea genotypes screened for waterlogging tolerance; hence there is ample scope for selection of promising lines for waterlogging tolerance. The promising waterlogging tolerant lines may be utilized either directly or through introgression breeding to transfer waterlogging tolerance trait in high yielding cultivars of pigeonpea.

The photosynthetic capability and water uptake capability of plants are the two major factors which play key role in deciding the tolerability towards waterlogging. Therefore, the traits like survival percentage, DWPS and seedling vigour would help in evaluation, and thus genetic improvement through selection for these traits would be rewarding.

## References

- Araki H, Hossain MA, Takahashi T. Waterlogging and hypoxia have permanent effects on wheat root growth and respiration. *Journal of Agronomy and Crop Science*. 2012 Aug; 198(4):264-75.
- Ashraf M. Interactive effects of nitrate and long-term waterlogging on growth, water relations, and gaseous exchange properties of maize (*Zea mays* L.). *Plant Science*. 1999 Jun 14; 144(1): 35-43.
- Chauhan, Y.S., Venkataratnam, N., Sheldrake, A.R. A perennial cropping system from pigeonpea grown in post-rainy season. *Indian Journal of Agricultural Sciences*, 1987, 57, 895-899.
- Dhillon BS, Thind HS, Malhi NS, Sharma RK. Effect of excess water stress on grain yield and other traits in maize hybrids. *CROP IMPROVEMENT-INDIA*-. 1998; 25(2): 209-14.
- Else MA, Janowiak F, Atkinson CJ, Jackson MB. Root signals and stomatal closure in relation to photosynthesis, chlorophyll a fluorescence and adventitious rooting of flooded tomato plants. *Annals of botany*. 2009 Jan 1; 103(2): 313-23.
- FilhoMbD, Lopes Md. Screening for tolerance to waterlogging in forage plants. In *Embrapa Amazônia Oriental-Artigoemanais de congresso (ALICE)* 2011. In *International Symposium on Forage Breeding*.
- Jin-Woong CHO, Ji HC, Yamakawa T. Comparison of photosynthetic response of two soybean cultivars to soil flooding. *J. Fac. Agr., Kyushu Univ*. 2006; 51(2): 227-32.
- Johnson BA, Shirokawa JM, Aswad DW. Deamidation of calmodulin at neutral and alkaline pH: quantitative relationships between ammonia loss and the susceptibility of calmodulin to modification by protein carboxyl methyltransferase. *Archives of biochemistry and biophysics*. 1989 Jan 1; 268(1):276-86.
- Kicheva MI, Tsonev TD, Popova IP. Stomatal and nonstomatal limitations to photosynthesis in two wheat cultivars subjected to water stress. 1994.
- Kumutha D, Ezhilmathi K, Sairam RK, Srivastava GC, Deshmukh PS, Meena RC. Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes. *Biologia Plantarum*. 2009 Mar 1; 53(1):75-84.
- Lal, M. *Physiological Evaluation of Pigeonpea [Cajanus cajan (L.)*

- Millsp.] Genotypes for Waterlogging and Salinity Tolerance, 2014 (Doctoral dissertation, Plant Physiology, CCSHAU, Hisar).
- Liu YZ, Bin T, Zheng YL, XU SZ, QIU FZ. Screening methods for waterlogging tolerance at maize (*Zea mays* L.) seedling stage. *Agricultural Sciences in China*. 2010 Mar 1; 9(3): 362-9.
- Metodiev MV, Tsonev TD, Popova LP. Effect of jasmonic acid on the stomatal and nonstomatal limitation of leaf photosynthesis in barley leaves. *Journal of plant growth regulation*. 1996 Apr 1;15(2):75.
- Scholowing T, Teching C. Effect of waterlogging on growth and yield of maize VII: Recovery response of maize plants after drainage. *Bulletin of the National Pingtung Polytechnique Institute*. 1997; 6:85-93.
- Setter T. L., Ellis M., Laureles E. V., Ella E. S., Senadhira D., Mishra S. B., Sarkarung S., Datta S., *Physiology and Genetics of Submergence Tolerance in Rice*, *Annals of Botany*, Volume 79, Issue suppl\_1, 1 January 1997, Pages 67–77
- Setter TL, Waters I. Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and soil*. 2003 Jun 1; 253(1): 1-34.
- Shabala S, Shabala L, Barcelo J, Poschenrieder C. Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding. *Plant Cell & Environment*. 2014 Oct; 37(10): 2216-33.
- Singh O, Singh S P , Bahadur R , Ram M , Singh P, Prajapati S. Physiological Basis of Water Logging Tolerance in Wheat at Vegetative Stage Under Sodic Soil. *International Journal of Current Microbiology and Applied Sciences*, 2017, 6(4): 1993-2004.
- Takele A, McDavid CR. The response of pigeonpea cultivars to short durations of waterlogging. *African Crop Science Journal*. 1995; 3(1).
- Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature*. 2003 Sep; 425 (6956): 393-7.
- Tsonev TD, Lazova GN, Stoinova ZG, Popova LP. A possible role for jasmonic acid in adaptation of barley seedlings to salinity stress. *Journal of Plant Growth Regulation*. 1998 Sep 1;17(3):153-9.
- Vartapetian BB, Jackson MB. Plant adaptations to anaerobic stress. *Annals of Botany*. 1997 Jan 1;79 (suppl\_1):3-20.
- Wang L, Zhang Y, Qi X, Li D, Wei W, Zhang X. Global gene expression responses to waterlogging in roots of sesame (*Sesamum indicum* L.). *Actaphysiologiaeplantarum*. 2012 Nov 1; 34(6): 2241-9.
- Yan B, Dai Q, Liu X, Huang S, Wang Z. Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant and soil*. 1996 Feb 1; 179(2): 261-8.
- Yordanova RY, Popova LP. Photosynthetic response of barley plants to soil flooding. *Photosynthetica*. 2001 Dec 1; 39(4): 515-20.
- Zaidi PH, Rafique S, Singh NN. Response of maize (*Zea mays* L.) genotypes to excess soil moisture stress: morpho-physiological effects and basis of tolerance. *European Journal of Agronomy*. 2003 Jul 1; 19(3): 383-99.