SHORT COMMUNICATION

EFFECT OF FLOODING ON GROWTH AND BIOCHEMICAL PARAMETERS IN GARDEN PEA (*PISUM SATIVUM* L.)

SEEMA BEDI, PARDEEP KAUR AND P.S. BRAR*

Department of Botany, *Department of Vegetable Crops, Punjab Agricultural University, Ludhiana-141004

Received on 12th Nov., 2010; Revised and accepted on 23rd July, 2011

In the present study, field grown five pea cultivars *viz.*, MA-6, Arkel, Pb-87, Pb-88 and Pb-89 were exposed to flooding stress at vegetative (15 DAS) and reproductive (45DAS) stages for 24 and 48 h durations. Flooding stress adversely affected the growth parameters such as plant height, shoot and root dry weight in all the five cultivars. Flooding led to a reduction in chlorophyll content, total soluble sugars in leaves and roots and total soluble proteins in roots. Alcohol dehydrogenase (ADH) and cellulase activity were found to enhance after flooding stress. Reproductive stage was noted more sensitive to flooding damage compared with vegetative stage. Longer flooding durations were more detrimental to plant growth and development. Among the cultivars, Pb-88 and Pb-89 were least affected, while Arkel showed maximum effect of flooding stress.

***Key words*: ADH, cellulase activity, flooding, pea, plant growth**

Pea (*Pisum sativum* L.) is an important legume crop of the world. In north India, peas are widely cultivated in winter season for their fresh green seeds, tender pods, dried seeds and foliage. It requires irrigation one month after sowing and subsequently at flowering and pod filling stages. However, pea is very sensitive to flooding and due to untimely rains and excessive irrigation crop yields go down and sometimes even the crop fails. The present study was planned with an objective to analyse the effect of flooding at various growth stages on growth and to evaluate physiological and biochemical variability in common cultivars and to understand the mechanism of short term tolerance of hypoxia.

Five pea (*Pisum sativum* L. var Hortense) cultivars *viz.*, MA-6, Arkel, Pb-87, Pb-88 and Pb-89, were grown in the field area of the department of Vegetable Crops, PAU, Ludhiana as per recommended practices (Anon 2007). Crop was raised in a plot size of 3×3 m and row to row distance of 60 cm in randomized block design. Flooding stress was imposed at vegetative (15 days after sowing) and reproductive (45 days after sowing) stages for 24 and 48 h durations. Flooding stress was administered by applying heavy irrigation 15 days after sowing (DAS) for 24 h flooding stress and both 15 DAS and 16 DAS for 48 h flooding stress. The procedure was likewise repeated at 45 DAS. Control plants were grown under normal irrigation. Data was analyzed using two-way ANOVA and CD between the cultivars and between the treatments was calculated. All the observations were recorded five days after each flooding treatment.

Plant height was measured from the base of the shoot near the soil surface to the base of the topmost leaflet (modified tendril) using a centimeter scale. For measuring the length of the tap root, plant was uprooted and length was measured from its base to the tip. From

*Corresponding author, email: sbedipau@yahoo.com

the uprooted plant, at maturity stage, root and shoot were separated and were oven dried at 60°C. The dry weight was recorded when the samples reached constant weight (3 days). Total chlorophyll content (Hiscox and Israelstam 1979) and total soluble sugars in the leaves (Dubois *et al.* 1956) were estimated. Total soluble sugars (Dubois *et al.* 1956), total soluble proteins (Lowry *et al.* 1951), cellulase activity (Mendels *et al.* 1974) and alcohol dehydrogenase activity (Crawford 1966) were estimated in roots.

Plant height was adversely affected by flooding stress applied at both vegetative and reproductive stages (Table 1). However, plants for which the stress was applied at reproductive stage (45 DAS) were more affected than the plants which were flooded at vegetative stage (15 DAS) for 48 h. Cultivar MA-6 showed least plant height in control while under flooded conditions, cultivar Pb-88 and Pb-89 showed least plant height. Maximum reduction in plant height over control was observed in cultivar Arkel (18.1%) after flooding at 45 DAS for 48 h while minimum reduction was recorded in the cultivar MA-6 (7.6%). Likewise there was a progressive decline in shoot and root dry weight with increase in flooding duration. Flooding at reproductive stage (45 DAS) was more detrimental (Table 1). Maximum reduction in root dry weight was observed in cultivar MA-6 (21%) and Arkel (17%) and minimum in Pb-88 (11.7%). For shoot dry weight this decrease was non-significant for all the cultivars.

Waterlogging stress severely affects plant growth and development. Genetic variability with regard to seedling growth under fully saturated soil conditions, as observed in the present study, has been reported in maize (Zaidi *et al.* 2003). Excess soil moisture stress leads to decline in root and shoot length and decrease in dry matter production (Jiang and Wang 2006). Flooding changes almost every aspect of root and shoot growth, however, root growth and development is affected more than shoot growth (Zaidi *et al.* 2003).The reduction in dry weight might be attributed to decline in water uptake and to suppression of photosynthetic output and carbohydrate synthesis.

Chlorophyll content was recorded at vegetative and reproductive stage (Fig. 1). Flooding at 15 DAS led to a reduction in leaf chlorophyll content over control. The longer flooding duration led to further reduction in chlorophyll content. For the cultivar MA-6, reduction in chlorophyll content was non-significant. Plants flooded at reproductive stage (45 DAS) also showed the similar trend of reduced chlorophyll content in plants subjected to longer duration of stress (48 h). The decrease in chlorophyll content was non-significant for cultivar Pb-88. A reduction in chlorophyll accumulation has been reported under flooded conditions. Leaf senescence is a common symptom of flooding injury and is characterized by changes in chlorophyll content (Jamei *et al.* 2008). Root hypoxia leads to changes in biochemical composition, functional activity and structure in pea chloroplasts (Ladygin 2003) leading to leaf chlorosis. In maize it has been shown that reduction of chlorophyll content in hypoxia stress is probably due to slow synthesis and fast destruction of chlorophyll pigment and further chlorophyll destruction and membrane deterioration are related to degree and speed of flooding injury (Yan *et al.* 1996).

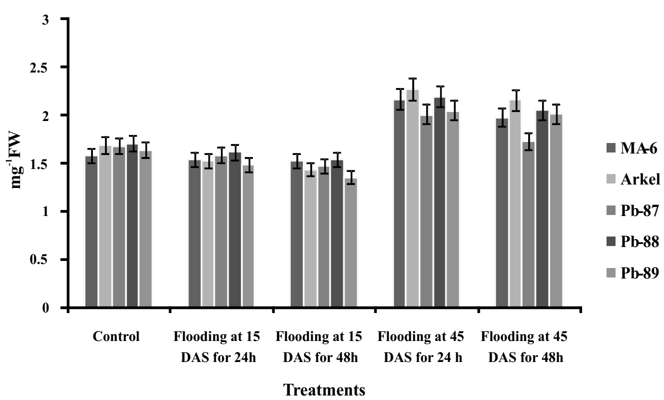

Fig. 1. Effect of flooding stress imposed on total chlorophyll content of leaves (mg g^{-1} FW) at vegetative stage (20 DAS) reproductive stage (50 DAS) in five pea cultivars

Total soluble sugars showed decrease with flooding stress (Table 2). At vegetative stage plants flooded at 15 DAS for 48 h had lower sugar level than plants flooded for 24 h. The cultivar Arkel had maximum amount of sugars while cultivars MA-6 and Pb-89 had least amount of sugars under both control and flooded conditions. At reproductive stage also, sugar level decreased with progressive increase in flooding stress duration. However, sugar levels were higher than those

Table 1. Effect of flooding stress imposed at vegetative (15 DAS) and reproductive stage (45 DAS) on height (PHt), shoot dry weight (SDW) and root dry weight (RDW) at maturity stage in five pea cultivars.

Treatments \ Cultivars	M A-6			Arkel			Pb-87			Pb-88			Pb-89			C.D (5%)		
	PHt (cm)	SDW (g)	RDW (g)	PHt (cm)	SDW (g)	RDW (g)	PHt (cm)	SDW (g)	RDW (g)	PHt (cm)	SDW (g)	RDW (g)	PHt (cm)	SDW (g)	RDW (g)	PHt	SDW	RDW
Control	98.0	25.63	2.16	110.0	24.60	2.23	105.0	24.50	2.12	100.0	23.80	1.95	98.3	23.9	2.10	1.82	NS	0.12
Flooding at 15 DAS for 24 h	97.6	24.20	1.92	103.0	23.80	2.17	101.0	24.30	2.03	97.0	21.80	1.89	96.4	22.20	2.01	1.66	5.07	0.16
Flooding at 15 DAS for 48 h	96.0	23.3	1.87	97.0	23.20	2.07	99.0	24.10	1.95	93.2	20.10	1.85	93.1	21.90	1.95	1.42	4.22	0.09
Flooding at 45 DAS for 24 h	94.0	21.30	1.73	95.6	22.30	1.92	94.0	23.60	1.86	90.6	19.50	1.79	90.0	20.20	1.90	1.61	5.20	0.10
Flooding at 45 DAS for 48 h	90.6	20.60	1.69	90.0	20.30	1.85	90.1	22.20	1.79	88.0	18.70	1.72	88.0	19.60	1.86	1.78	4.52	0.10
C.D (5%)	1.054	NS	0.03	1.069	NS	0.04	1.032	NS	0.03	0.851	NS	0.02	0.813	NS	0.02			

Table 2. Effect of flooding stress on total soluble sugars in leaves and roots (mg g^{-1} FW) at vegetative (20 DAS) and reproductive stage in five pea cultivars.

Cultivars	MA-6		Arkel		Pb-87		Pb-88		Pb-89		C.D (5%)	
Treatments	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
VEGETATIVE STAGE (20 DAS)												
Control	53.1	51.8	64.9	55.7	52.9	49.1	54.5	50.4	52.5	47.8	1.43	0.934
Flooding at 15 DAS for 24 h	51.8	51.1	61.3	54.4	51.8	47.6	52.6	49.8	51.0	47.0	1.07	1.33
Flooding at 15 DAS for 48 h	49.1	50.2	59.1	53.1	50.4	46.0	51.1	49.1	49.3	44.7	1.27	1.85
C.D (5%)	2.11	0.933	1.99	NS	0.398	0.993	1.64	0.800	1.86	1.29		
REPRODUCTIVE STAGE (50 DAS)												
Control	188.5	59.0	204.5	73.8	175.7	64.9	187.5	68.8	170.4	63.7	1.81	1.21
Flooding at 45 DAS for 24 h	158.6	57.7	175.7	72.1	157.3	62.8	169.2	67.0	156.0	62.0	1.82	1.64
Flooding at 45 DAS for 48 h	153.4	55.7	163.9	70.6	142.9	60.5	154.8	65.5	142.9	60.9	1.85	1.65
C.D. (5%)	1.83	1.39	2.00	1.44	1.98	NS	1.99	1.48	1.38	1.72		

DAS- Days after sowing

at vegetative stage. When compared with control, plants flooded at 45 DAS for 48 h had minimum sugar content. Maximum sugars were observed in cultivar Arkel and minimum sugars were observed in cultivar Pb-89.

The amount of total soluble sugars was less in roots as compared with leaves. Sugar content was found to decrease with increase in flooding stress duration at both vegetative and reproductive stage. Plants flooded at 45 DAS for 48 h were among the most affected by flooding stress. Among the cultivars, Arkel had maximum sugar content at both the stages while Pb-89 and MA-6 had minimum sugar content at vegetative and reproductive stage respectively.

As flooding stress reduces photosynthetic rates, it therefore leads to a decrease in total carbohydrate content. In the present study, in field grown crop at vegetative stage, maximum sugar content in leaves was observed in control plants that also recorded maximum chlorophyll content. Higher leaf sugar content as compared to root sugar content may be attributed to reduced phloem transport under stress conditions (Wample and Davies 1983). Maintaining adequate levels of fermentable sugars in flooded roots is important for long term survival of plants under flooding. Saglio *et al.* (1988) suggested that under anoxia, high energy charge and metabolic activity can be maintained through anoxic fermentation pathway. The anoxic fermentation in roots flows through glycolysis and fermentation rate in roots is controlled by amount of available sugars. Further Zhang and Greenway (1994) found that exogenous sugar prevent depletion of sugar supplies in beetroots and enhance ethanolic fermentation.

The amount of total soluble proteins decreases as duration of flooding stress increases (Fig. 2). At vegetative stage plants flooded at 15 DAS for 48 h showed maximum decrease. Among the cultivars, Pb-89 showed non-significant decrease. At reproductive stage also, plants flooded at 45 DAS for 48 h showed greater reduction in root proteins. All cultivars showed significant reduction in proteins except MA-6.

Under flooding stress, nitrogen uptake and assimilation is severely affected. Nitrogen is the main constituent of proteins. Roots which are surrounded by excess water are not able to absorb nitrogen (in form

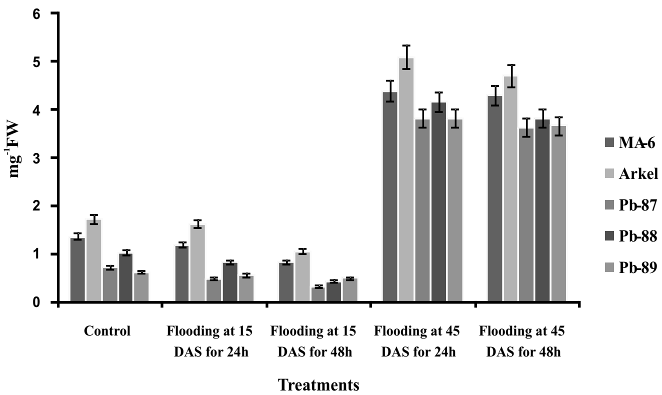

Fig. 2. Effect of flooding stress on total soluble proteins in roots (mg g^{-1} FW) at vegetative stage and reproductive stage in five pea cultivars.

of nitrate ions) from the rhizosphere. Nitrate reductase and glutamine synthetase, the two key enzymes in nitrate reduction and ammonia assimilation influencing the total nitrogen balance, are affected by flooding (Buwalda *et al.* 1988). Further nitrogen fixation in legumes is also inhibited under flooded conditions (Trung *et al.* 1985). Waterlogging interferes with nitrogen fixing capacity through the degradation of leghaemoglobin due to decrease in oxygen supply to root system. As a result, the composition and quantity of proteins and amino acids is affected. Zhen and Zhen (2006) have reported decrease in total protein content in wheat, in response to soil waterlogging.

The alcohol dehydrogense (ADH) enzyme was assayed from roots of field grown pea plants flooded at vegetative and reproductive stage (Table 3). The enzyme activity was found to increase with increase in flooding duration. At vegetative stage, plants flooded at 15 DAS for 48 h showed higher enzyme activity than plants flooded for 24 h. Similar trend was observed at reproductive stage. Among the cultivars when subjected to flooding at 15 DAS for 48 h, Pb-89 showed maximum increase (5.7%) while Arkel showed minimum increase (3.8%) in ADH activity over control. Likewise at reproductive stage also, maximum increase (19.7%) in ADH activity was seen in Pb-89 and minimum in Arkel (5.9%).

Under root anoxia, cells inevitably undergo anaerobic fermentation. Thus, Krebs cycle, the major source of ATP production is absent and ADH is responsible for recycling NAD^+ needed for glycolysis pathway to continue and the end product is ethanol instead of carbon dioxide and water. Zaidi *et al.* (2003) observed that

Table 3. Effect of flooding stress on ADH activity (µ moles of NAD reduced min^{-1}) and cellulase activity in roots (µg of reducing sugars liberated min^{-1}) at vegetative and reproductive in five pea cultivar.

Cultivars / Treatments	MA-6		Arkel		Pb-87		Pb-88		Pb-89		C.D (5%)	
	ADH	Cellulase	ADH	Cellulase	ADH	Cellulase	ADH	Cellulase	ADH	Cellulase	ADH	Cellulase
VEGETATIVE STAGE (20 DAS)												
Control	0.253	0.121	0.397	0.141	0.210	0.098	0.260	0.083	0.132	0.103	0.008	0.003
Flooding at 15 DAS for 24 h	0.260	0.139	0.409	0.163	0.217	0.112	0.267	0.102	0.138	0.134	0.082	0.004
Flooding at 15 DAS for 48 h	0.267	0.152	0.413	0.172	0.221	0.120	0.271	0.113	0.140	0.149	0.014	0.005
C.D (5%)	0.001	0.002	0.003	0.003	0.002	0.005	0.003	0.004	0.002	0.006		
REPRODUCTIVE STAGE (50 DAS)												
Control	0.245	0.256	0.368	0.283	0.179	0.213	0.231	0.198	0.102	0.243	0.009	0.006
Flooding at 45 DAS for 24 h	0.254	0.339	0.384	0.352	0.193	0.249	0.243	0.219	0.113	0.312	0.007	0.007
Flooding at 45 DAS for 48 h	0.282	0.382	0.391	0.399	0.204	0.278	0.252	0.231	0.127	0.368	0.008	0.008
C.D (5%)	0.007	0.005	0.013	0.002	0.014	0.004	0.003	0.003	0.009	0.008		

under excessive moisture condition, NAD^+-alcohol dehydrogenase activity increased exponentially in flood tolerant maize genotypes. On the other hand, in highly sensitive genotypes increase in ADH-activity was nominal. This may be one of the most important features for their high susceptibility to excess moisture stress.

The cellulase enzyme was assayed from the roots of pea plants at vegetative (15 DAS) and reproductive (45 DAS) stage (Table 3). The enzyme activity was found to increase with increase in flooding duration. 45 d old plants flooded for 48 h showed maximum enzyme activity while minimum enzyme activity was found in control plants. Cellulase activity showed a trend similar to ADH activity. At vegetative stage maximum increase in enzyme activity over control was observed in Pb-89 (23.1% and 30.0% respectively) after flooding at 15 DAS for 24 h and 48 h respectively. At reproductive stage, however no particular trend in cellulase activity was seen. Cellulase plays an important role in cell wall degradation leading to formation of aerenchyma which is an adaptive response of plants to flooding stress (Peschke and Sachs 1993).

Under hypoxic conditions such structural changes are believed to enhance internal transfer of atmospheric or photosynthetic oxygen from shoot to submerged tissues. Increased oxygen transport may sustain aerobic respiration and growth (Armstrong and Webb 1985). According to studies conducted on pea, hypoxic stress caused by high water content or low oxygen levels induce cell lysis in central vascular cylinder of pea primary roots which result in formation of long vascular cavities (Gladish and Niki 2000).

The activities of enzymes ADH and cellulase are important determinants of flooding tolerance. In the cultivar Pb-89 has highest activities of these enzymes which explain the highest yield in terms of number of pods in this cultivar. Flooding inhibits most of the metabolic pathways like photosynthesis and nitrogen assimilation. The direct consequence of reduced metabolism is in reduction of metabolites such as carbohydrates, proteins and lipids. The cultivar Pb-88 has maximum amount of leaf chlorophyll at the reproductive stage which explains higher amount of metabolites which are translocated to pod and contributes to higher pod weight. Seeds are the foremost storage regions for the metabolites. If there is less accumulation of storage metabolites, the yield of the plant will be ultimately affected. Thus flooding has a direct adverse affect on growth, biochemical constituents and seed productivity.

REFERENCES

Anon. (2007). Package of practices for Vegetable Crops of Punjab (Rabi) PAU, Ludhiana, pp. 33-35,

Armstrong, W. and Webb, P. (1985). A critical oxygen pressure for root extension in rice. *J. Exp. Bot.* **36:** 1573-1583.

Buwalda, F., Barrett–Lennard, E.G., Greenway, H. and Davies, B.A. (1988). Effects of growing wheat in hypoxic nutrient solutions and of subsequent transfer to aerated solutions. II. Concentrations and uptake of nutrients and sodium in shoots and roots. *Aust. J. Plant Physiol.* **15:** 599-612.

Crawford, R.M.M. (1966). Alcohol dehydrogenase activity in relation to flooding tolerance in roots. *J. Exp. Bot.* **18:** 458-464.

Dubois, M., Giles, K.A., Hamilton, J.K., Roberts, P.A. and Smith, F. (1956). Colorimetric methods for determination of sugars and related substances. *J. Anal. Chem.* **28:** 350.

Gladish, D.K. and Niki, T. (2000). Factors inducing cavity formation in the vascular cylinders of pea roots (*Pisum sativum* L., cv. Alaska). *Env. Exp. Bot.* **43:** 1-9.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for extraction of chlorophyll from leaf tissues with maceration. *Can. J. Bot.* **51:** 1332-1334.

Jamei, R., Heidari, R., Khara, J. and Zare, S. (2008).The interaction effects of floording and kinetin or growth criteria, chlorophyll content and 5 Aminolevulinic acid dehydratase activity in corn seedlings. *TUBITAK.* **32:** 253-257.

Jiang, Y. and Wang, K. (2006). Physiological and anatomical response of creeping bentgrass cultivars to different depths of waterlogging. *Crop Sci.* **46:** 2420-2426.

Ladygin, V.G. (2003). Effect of iron shortage and root hypoxia on photochemical activity, spectral characterstics and structure of pea (*Pisum sativum* L.) chloroplasts. *Biophysics.* **50:** 80-93.

Lowry, U., Rosenbrough, N.K., Farr, A.L. and Randell, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Biochem.* **193:** 256-275.

Mendels, M., Hontz, I. and Nystrom, C. (1974). Enzymatic hydrolysis of waste cellulose. *Biotectnol. Bioeng.* **16:** 1471-1493.

Peschke, V.M. and Sacks, M.M. (1993). Multiple pyruvate decarboxylase genes in maize are induced by hypoxia. *Mol. Gen. Genet.* **240:** 206-212.

Saglio, P., Drew, M.C. and Pradet, A. (1988). Metabolic adaptation to anoxia induced by low oxygen pre-treatment in root tips of *Zea mays*. *Plant Physiol.* **86:** 61-66.

Trung, B.H., Yoshida, S. and Kobayashi, Y. (1985). Influence of excess soil moisture on the nitrogen nutrition and grain productivity of mungbean. *Japan J. Crop Sci.* **54:** 79-83.

Wample, R.L. and Davies, R.W. (1983). Effect of flooding on starch accumulation in chloroplasts of sunflower (*Helianthus annuus* L.) *Plant Physiol.* **73:** 195-198.

Yan, B., Dai, Q. and Liu, X. (1996) Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil* **179:** 261-68.

Zaidi, P.H., Rafique, S. and Singh, N.N. (2003). Response of maize (*Zea mays* L.*)* genotypes to excess moisture stress; morpho-physiological effects and basis of tolerance. *Eur. J. Agron.* **19:** 383-399.

Zhang, Q. and Greenway, H. (1994). Anoxia tolerance and anaerobic catabolism of aged but root storage tissues. *J. Exp. Bot.* **45:** 567-75.

Zhen-Zhu, X.U. and Zhen-Wen, Y.U. (2006). Nitrogen metabolism in flag leaf and grain of wheat in response to irrigation regimes. *J. Pl. Nut. Soil Sci.* **169:** 118-126.