EFFECT OF FLOODING STRESS ON SEEDLING GROWTH AND PROTEIN PROFILE OF ROOTS IN MAIZE

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SUMMARY

Seedlings of three maize (*Zea mays* L.) cultivars (Parbhat, Paras and Navjot) were exposed to flooding under laboratory conditions for 24h at 7, 14 and 21 days after emergence. Excess moisture stress adversely affected growth parameters such as root length, shoot length, leaf chlorophyll content, seedling fresh and dry weight. Electrophoretic studies of the root proteins indicated repression of some proteins due to flooding. Pre-treatment of seedlings with $CoCl_2$ (10 and 15mg ml^{-1}) and KCl (1%) ameliorated the adverse effects of flooding and restored protein pattern to some extent.

***Key words*: Flooding, maize, protein profile, seedling growth.**

INTRODUCTION

Maize is a major cereal crop of the world. Predominantly, it is grown in summer/rainy season in India and other tropical and subtropical countries. Flooding and submergence, caused by heavy rainfall waterlogging and poorly drained soil or high water table is one of the most common constraints for maize production (Zaidi *et al*. 2005). Flooding inhibits supply of oxygen to plant roots leading to hypoxia followed by anoxia. In the present investigation, an attempt has been made to study the effect of flooding stress on seedlings of three cultivars of maize (*Zea mays* L.), viz. Parbhat, Paras and Navjot. $CoCl_2$ is an inhibitor of ethylene biosynthesis (Lau and Yang 1976) and role of K is well known in osmoregulation of plants. Therefore, to overcome the effects of flooding, the seedlings were pre-treated (sprayed) with a solution of $CoCl_2$ and KCl and thereafter, observations were recorded on various growth parameters and dynamics of protein profile of the roots.

MATERIAL AND METHODS

The seeds of maize (*Zea mays* L) cultivars, Parbhat, Paras and Navjot were obtained from the department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.The seeds were surface sterilized by dipping in a solution of $HgCl_2$ (0.1%) for 1 min followed by rinsing in distilled water and sown in disposable plastic cups perforated at the base and filled with sand (Zaidi *et al.* 2005). The cups were placed in plastic trays holding water to a depth of 1.0 cm. The sand in the cups got fully saturated with water entering from the hole present at the base of the cups through capillary action. Two seeds per cup were sown, at a depth of 2.0 cm in the fully saturated sand and there were six replicates for each cultivar. Cultivars were randomized among different trays. Trays along with cups were kept at 25°C in a seed germinator. The experiments were replicated three times. The cups were observed daily and remoistened as and when required. A seed was considered to have emerged when its plumule exceeded 1.0 cm.

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Flooding treatment: The cups were flooded with water (1.0 cm above the sand surface) 7, 14 and 21 days after emergence. In order to maintain the level of flooding in the cups, these were placed in plastic tray containing 3 cm of water. Flooding water was drained off after 24h and the seedlings were uprooted 48h after draining off. Biometric and physiological estimations were recorded in the uprooted seedlings.

Pre-treatments: Prior to flooding, the seedlings were sprayed with a solution of cobalt chloride ($CoCl_2$) (10µg ml^{-1} and 15µg ml^{-1}) and potassium chloride (KCl) (1%). The flooding water was drained off after 24h and the seedlings were again sprayed with solutions of $CoCl_2$ and KCl. The observations were recorded 24h after the second treatments (i.e. 48h after draining off water). Observations were recorded on shoot and root length, shoot and root weight, number of adventitious roots, leaf chlorophyll content (Hiscox and Israelstam 1979) and changes in the protein profile in the roots of seedlings.

Protein profile: Fresh root tissue (2.5 g) was taken in 2ml of Tris – HCl buffer (pH 6.8), and ground in pestle and mortar. The homogenized tissue was centrifuged in a refrigerated centrifuge at 4°C for 20 min at 10,000 rpm. Supernatant was collected as crude protein sample and stored at 4°C. The protein sample was mixed with sample buffer in the ratio of 1:1 and heated for 2 minutes. Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out at a constant current of 1.5 mA per cm, until tracking dye reached near the bottom of the slab gel (Walker 1996).

RESULTS AND DISCUSSION

There was a reduction in length of longest root in response to flooding in the seedlings from all the three cultivars (Tables 1,2,3). Under prolonged excess moisture condition, plant roots suffer from hypoxia followed by anoxia. Root growth is inhibited under depleted oxygen supply as roots tips are highly susceptible to anaerobic stress and may die within few hours of anoxic stress (Roberts *et al*. 1984, (Fausey *et al*. 1985). The number of adventitious roots increased over control in all the three cultivars under the flooding treatments (Tables 1,2,3), although, this increase was non-significant, probably because the present study was confined to very young seedlings. In flooded plants, the original root system is often replaced by adventitious roots (Visser and Voesenek 1996, Zaidi and Singh 2002). In maize, nodal roots are initiated within 1 to 2 days of flooding both from below and above ground nodes and were found to be closely related to final grain yield (Zaidi and Singh 2001). In addition, hypertrophic growth near the base of the stem was observed in flooded seedlings (Fig. 1). This type of growth that appears as a swelling of the mesocotyl near the base of the stem and roots is important because of the transitional role of mesocotyl in carrying oxygen from shoots to roots. This growth is due to radial cell division and expansion and is often accompanied by cell collapse and aerenchyma formation (Kawase 1981).

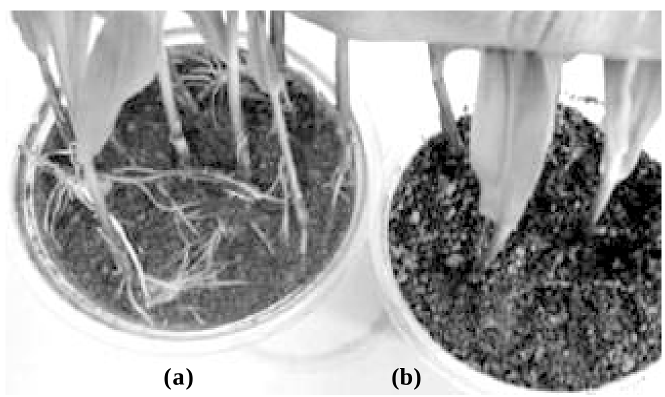

Fig. 1. (a) showing hypertrophic growth in flooded seedlings, (b) control seedling without hypertrophic growth

There was reduction in shoot length in the seedlings from all the three cultivars (Parbhat, Paras and Navjot) exposed to flooding after differential days of emergence (Tables 1,2,3). This trend was reflected in fresh and dry weight of the seedlings. Inhibition of shoot growth is almost immediate following flooding. Though shoots are not directly exposed to excess moisture conditions, however they respond to metabolic status of the roots. It has been shown that maize crop is more susceptible to waterlogging during early seedling to tasseling stage (Zaidi and Singh 2002). Leaf growth and stem elongation are severely restricted by root anoxia in the short term either as a

Table 1. Effect of flooding and treatments with $CoCl_2$ and KCl on various growth parameters in 10 d old seedlings of maize (*Zea mays* L.) cultivars

		Treatments					
Growth Parameters	**Cultivars**	**Control**	**Flooded**	**$CoCl_2$ (10µg ml^{-1})**	**$CoCl_2$ (15µg ml^{-1})**	**KCl (1%)**	**C.D. (p=0.05)**
Root length (cm)	Parbhat	10.7	9.8	11.5	11.6	12.7	0.01
	Paras	12.6	10.0	12.5	12.9	13.0	0.09
	Navjot	12.5	11.1	12.0	12.3	12.3	0.10
Shoot length (cm)	Parbhat	12.5	12.2	13.5	13.7	13.7	0.05
	Paras	12.5	11.4	12.6	12.3	12.5	0.08
	Navjot	12.3	10.3	13.1	14.0	13.2	0.91
Number of adventitious roots	Parbhat	8	10	9	10	10	NS
	Paras	10	13	11	10	12	NS
	Navjot	9	10	11	11	11	NS
Seedling fresh weight (g)	Parbhat	0.30	0.25	0.22	0.31	0.34	0.04
	Paras	0.33	0.23	0.27	0.29	0.30	0.03
	Navjot	0.30	0.20	0.21	0.22	0.25	0.05
Seedling dry weight (g)	Parbhat	0.18	0.12	0.15	0.16	0.17	0.05
	Paras	0.18	0.10	0.19	0.17	0.17	0.04
	Navjot	0.18	0.11	0.19	0.16	0.17	0.04
Chlorophyll content (mg g^{-1} fw)	Parbhat	0.794	0.756	0.984	0.954	0.959	0.01
	Paras	1.137	0.907	1.209	1.225	1.226	0.06
	Navjot	0.794	0.709	0.740	0.790	0.758	0.01

consequence of lack of nitrogen or other major nutrients, or through inhibition by ethylene. In the long term, slow growth rates, may persist because of the accumulation of metabolic toxins, or the lack of water and nutrients. Net assimilation rates and photosynthetic rates decline in plants experiencing root anaerobiosis due to stomatal closure or biochemical modifications (Jackson and Drew 1984).

Chlorophyll content was measured in the 3rd leaf of the seedlings exposed to flooding conditions. The amount of both chlorophyll a and b in the leaves of flood treated plants was lower than the control. It has been suggested that reduction in chlorophyll content in hypoxia stress is probably due to the slow synthesis or fast destruction of chlorophyll pigments (Ashraf 2003). The rate of chlorophyll destruction and membrane deterioration are related to the degree and speed of flooding injury (Yan *et al*. 1996). Leaf senescence is a common symptom of flooding injury and is characterized by changes in chlorophyll content (Jamei *et al*. 2008). In tobacco, flooding accelerated senescence in leaves and was associated with chlorophyll degradation which was due to phenolic-peroxidase-H_2O_2 systems (Hurng and Kao 1994). Yordanava and Papova (2001) showed that flooding in barley for 72 h resulted in a remarkable decrease in photosynthesis and dry matter accumulation.

Effect of pre-treatments with $CoCl_2$ and KCl on alleviation of flooding effects in maize: A set of plants were sprayed with solution of $CoCl_2$ (10µg ml^{-1} and 15µg ml^{-1}) and KCl (1%) one day prior to flooding and followed by 1 day after flooding**.** Thereafter, growth parameters were recorded 10, 17 and 24 days after emergence (DAE), i.e. 2 days after the spray treatment. In all the three cultivars, the root length was significantly reduced after 10, 17 and 24 days of flooding (Tables 1, 2, 3). Treatment with $CoCl_2$ and KCl alleviated the adverse effects of flooding in all the three cultivars at all the three stages of flooding to varying extent. In the present study

Table 2. Effect of flooding and treatments with $CoCl_2$ and KCl on various growth parameters in 17d old seedlings of maize (*Zea mays* L.) cultivars

Growth Parameters	Cultivars	Treatments					
		Control	Flooded	$CoCl_2$ (10µg ml^{-1})	$CoCl_2$ (15µg ml^{-1})	KCl (1%)	C.D. (p=0.05)
Root length (cm)	Parbhat	14.6	13.9	15.6	15.7	15.9	0.07
	Paras	15.6	15.0	15.2	15.5	16.8	0.10
	Navjot	15.9	14.4	15.6	15.8	16.2	0.09
Shoot length (cm)	Parbhat	15.7	13.1	15.0	13.5	14.7	0.06
	Paras	16.5	15.1	16.5	17.0	17.7	0.90
	Navjot	18.8	16.6	20.1	22.1	21.3	0.09
Number of adventitious roots	Parbhat	13	15	13	12	14	NS
	Paras	14	18	18	16	17	NS
	Navjot	11	14	15	16	14	NS
Seedling fresh weight (g)	Parbhat	0.45	0.40	0.44	0.42	0.43	0.02
	Paras	0.46	0.43	0.45	0.44	0.45	0.01
	Navjot	0.41	0.38	0.37	0.38	0.38	0.01
Seedling dry weight (g)	Parbhat	0.24	0.19	0.20	0.22	0.21	0.02
	Paras	0.26	0.22	0.20	0.20	0.21	0.03
	Navjot	0.23	0.17	0.19	0.20	0.20	0.03
Chlorophyll content (mg g^{-1} fw)	Parbhat	1.255	1.201	1.350	1.320	1.332	0.02
	Paras	1.350	1.235	1.328	1.354	1.365	0.01
	Navjot	1.227	1.104	1.203	1.304	1.202	0.02

$CoCl_2$ and KCl did not significantly affect the number of adventitious roots (Tables 1, 2, 3). $CoCl_2$ is an inhibitor of ethylene biosynthesis (Lau and Yang 1976). Therefore, its application negates deleterious effects of ethylene under flooded conditions. KCl enhances general growth and metabolism. Nitrogen (N) and potassium (K) content of various plant parts have also been reported to decrease under waterlogging (Srivastava *et al.* 2007). Further role of potassium is well known in osmoregulation of plants and higher potassium content suggests better osmoregulation in waterlogged plants. Likewise, there was a significant reduction in shoot length due to flooding which was enhanced by treatment with $CoCl_2$ and KCl (Tables 1, 2, 3).

Flooding significantly reduced seedling fresh and dry weight, in all the three cultivars (Tables 1, 2, 3). A decrease in dry weight accumulation due to waterlogging has been reported in creeping bent grass (Jiang and Wang 2006) and winter oats (Cannel *et al.* 1985). A decline in the growth parameters of maize seedlings in response to flooding have been attributed to imbalance in the quantity of internal phytohormones (increased ABA content, decreased auxin, gibberellins and cytokinin content) (Zhang and Younis 1991). Pre-treatments with $CoCl_2$ and KCl enhanced seedling fresh and dry weight over untreated flooded. Spray with $CoCl_2$ and KCl significantly enhanced chlorophyll content over flooded seedlings in all the three cultivars under flooding (Tables 1, 2, 3). For all the parameters, $CoCl_2$ (15µg ml^{-1}) was more effective than 10µg ml^{-1} $CoCl_2$ and KCl (1%) in amelioration of flooding affects.

Changes in root protein profile: There was an alteration in root protein profile. SDS-PAGE showed 4 prominent bands with molecular weight ranging between 14 to 24 kDa (lane 1, 5, 9) (Fig. 2). In Navjot (Lane 9), there is absence of 20 kDa polypeptide. This

Table 3. Effect of flooding and treatment with $CoCl_2$ and KCl on various growth parameters in 24d old seedlings of maize (*Zea mays* L.) cultivars

Growth Parameters	Cultivars	Control	Flooded	$CoCl_2$ (10µg ml^{-1})	$CoCl_2$ (15µg ml^{-1})	KCl (1%)	C.D. p=0.05
Root length (cm)	Parbhat	19.5	17.5	17.0	17.7	17.9	0.03
	Paras	20.7	18.7	19.7	20.1	21.7	0.06
	Navjot	22.7	20.3	20.7	21.8	22.0	0.04
Shoot length (cm)	Parbhat	22.5	21.5	18.5	18.0	18.7	0.09
	Paras	22.7	19.6	19.9	21.0	20.2	0.10
	Navjot	21.7	19.7	21.1	21.5	21.5	0.09
Number of adventitious roots	Parbhat	14	16	17	17	18	NS
	Paras	13	17	18	19	17	NS
	Navjot	14	16	16	15	14	NS
Seedling fresh weight (g)	Parbhat	0.55	0.49	0.47	0.48	0.48	0.02
	Paras	0.56	0.46	0.45	0.46	0.43	0.02
	Navjot	0.57	0.52	0.50	0.49	0.49	0.03
Seedling dry weight (g)	Parbhat	0.35	0.30	0.26	0.27	0.27	0.04
	Paras	0.37	0.33	0.31	0.31	0.32	0.02
	Navjot	0.35	0.31	0.30	0.31	0.30	0.02
Chlorophyll content (mg g^{-1} fw)	Parbhat	1.357	1.249	1.351	1.395	1.295	0.02
	Paras	1.337	1.244	1.347	1.351	1.305	0.02
	Navjot	1.092	1.258	1.214	1.209	1.305	0.01

indicates inherent genetic variability among the cultivars. Flooding caused disappearance of certain proteins in all the genotypes, i.e. a band in the range of (20 to 24 kDa) in the cultivars Parbhat (lane 3) and Paras (lane 7) and a band at 24 kDa in Navjot (lane 11). In Navjot (lane 11) band between 20 to 24 kDa, however, did not disappear under flooding. The difference in behavior of proteins is because of an inherent genetic variability with regard to waterlogging tolerance (Sachs *et al*. 1996). Treatments with $CoCl_2$ and KCl caused reappearance of these bands, though to a lesser extent. In the cultivar Navjot, only one very faint band reappeared in the 24 kDa range (lane 10, 12). Treatment with KCl led to very faint bands in the range of 20-24 kDa in the cultivar Parbhat (Lane 4). In the cultivat Paras, a prominent band between 20 and 24 kDa appeared due to KCl treatment (lane 8) while in cultivar Navjot, only one band between 20 and 24 kDa was prominently visible (lane 12). Anaerobic treatment of maize seedlings caused repression of pre-existing protein synthesis and

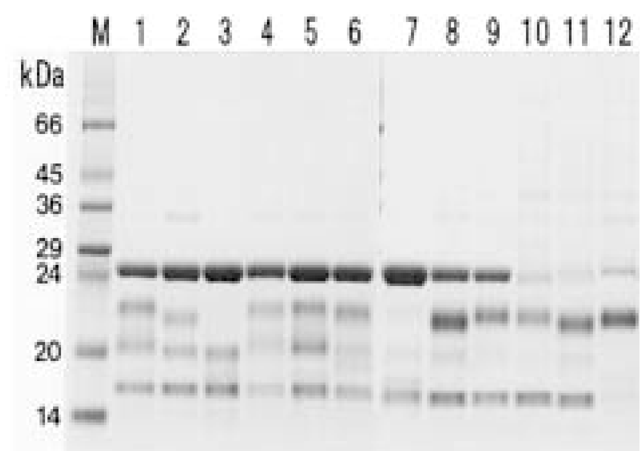

Fig. 2. SDS-PAGE profile of protein from roots of maize seedlings as affected by flooding and spray application with $CoCl_2$ (15µg ml^{-1}) and KCl (1%) in 24 d old seedlings. Lame M=Marker, Prabhat, lane 1=control (unflooded), lane 2=$CoCl_2$ (15µg ml^{-1}), lane 3= flooded, lane 4=KCl (1%), Paras, lane 5=control (unflooded), lane 6=$CoCl_2$ (15µg ml^{-1}), lane 7= flooded, lane 8=KCl (1%), Navjot, lane 9=control (unflooded), lane 10=$CoCl_2$ (15µg ml^{-1}), lane 11= flooded, lane 12=KCl (1%)

induced synthesis of about 20 anaerobic proteins (ANPs). Most hypoxia-induced proteins in the maize root tip cells are the enzymes involved in glycolysis and primary carbohydrate metabolism (Chang *et al*. 2000). An alteration in expression of proteins due to pre-treatments explains ameliorative effects of these pre-treatments on seedling growth.

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