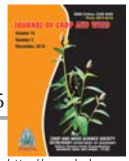
Journal of Crop and Weed, 15(3): 145-149 (2019)

ISSN- O : 2349 9400 ; P : 0974 6315



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Comparative physiology of salinity, drought and heavy metal stress during seed germination in ricebean [*Vigna umbellata*(Thunb.) Ohwi and Ohashi]

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Received : 11.11.2019 ; Revised : 10.12.2019 ; Accepted : 20.12.2019

DOI: https://dx.doi.org/10.22271/09746315.2019.v15.i3.1251

ABSTRACT

Abiotic stresses, likely salinity, drought, heavy metals, temperatures (high or low), and other environmental extremes are the primary factors that causes of low crop yields and reduced plant growth in the biome. Such type of abiotic stress may cause metabolic impairment, nutrient imbalance, reduced synthesis of photosynthetic pigments which are closely related with biomass production in plant, thus, causing serious loss in crop productivity. The present experiment was undertaken to study the biochemical and physiological effects of salinity, drought and heavy metal (copper and lead) stress on seed germination in ricebean variety Bidhan 1. For studying the effect of iso-osmotic potential of salinity and drought stress solutions of NaCl and PEG 6000 with -0.2, -0.4 and -0.8 MPa osmotic potential were used whereas the solutions of 50, 100 and 200 µM Cu and Pb supplemented in the form of $CuSO_4.5H_2O$ and $Pb(NO_3)_2$ were used to study the effects of equimolar concentrations of copper and lead. Drought was found to produce more adverse effects on speed of germination, reserve mobilization by germinating seeds as well as radicle and plumule growth in the seeds of ricebean. The proline content in cotyledon increased under all the treatments of salinity, drought stress as well as metal stress. The highest intensity of salinity stress was found to produce more adverse effects than drought in respect of lipid peroxidation in germinating seeds of ricebean in the present experiment. The presence of lead in the germinating medium produced more detrimental effects than equimolar concentrations of copper.

Keywords: Drought stress, heavy metal, lipid peroxidation, proline, salinity stress, SOD

Salinity stress can affect plants initially by creating an osmotic stress then it induces ion toxicity that lead to cyto-toxicity, metabolic impairment, nutrient imbalance and finally death of the plant. Initially, the presence of salts in high concentration makes difficult for plants to withdraw water from soil due to very low osmotic potential. In effect, the plants suffer from a sort of osmotic stress which restricts growth of plant causing yield reduction. In the later stages of stress, due to the absorption of sodium and chloride ions in high concentration plants suffer from cyto-toxicity which result in reduction of growth, leaf burn and plant death. The presence of high concentration of Na^+ and Cl^- ion also reduces the availability of other ions like K^+, Ca^{2+}, Mg^{2+}, thus, causing nutritional disorders.

Being a meteorological term drought reffered as the period without significant rainfall. Drought stress normally occurs under depleting soil moisture and the intensity of drought increases under atmospheric conditions conducive to increased water loss by transpiration and/ or evaporation. Crop growth and yield unfavourably affected by water deficit, which is one of the major abiotic stress. The effects are mainly related to uptake and translocation of ion, carbohydrate biosynthesis, altered metabolic functions, reduced synthesis of photosynthetic pigments, nutrient metabolism and synthesis of growth promoters. The reduction in fresh and dry biomass (Farooq *et al*., 2009) is a major adverse effect of water stress on different crops.

Physiological and metabolic alteration occurs when plants are exposed to toxic levels of heavy metals. Copper is an essential element for plants (Gang *et al*., 2013). It plays important role in assimilation of carbon as well as ATP synthesis. Excess amount of copper in soil causes cytotoxicity and induces injury to plants. This, in turn, leads to retardation of plant growth and leaf chlorosis (Lewis *et al*., 2001). Copper toxicity also causes excess generation of ROS and oxidative stress (Stadtman and Oliver, 1991), which ultimately leads to disturbance of metabolic pathways and damage to macromolecules (He-gedus *et al*., 2001). Lead is considered as one of the most abundant and ubiquitously distributed toxic elements present in the soil. Morphology, photosynthetic processes and growth of plants are adversely affected by lead (Yadav, 2010).Important enzymes with interference of lead induces seed germination under lead toxicity. The present experiment has been designed to study the comparative effects of different levels of salinity, drought, lead and copper stress on some physiological and biochemical parameters of ricebean at seed germination stage.

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MATERIALS AND METHODS

Seeds of ricebean [*Vigna umbellata* (Thunb) Ohwi and Ohashi] variety Bidhan-1 were used in the experiment. The seeds were collected from AICRP on Forage Crops, Kalyani Centre. For germination studies, the seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes followed by thorough washing in distilled water. After that, the seeds were set to germinate in petridish, diameter of 9 cm with Whatman No.1 filter paper at a temperature of 28±1°C and relative humidity of 80±1% and moistened with 5 ml each of NaCl and PEG 6000 solution with -0.2, -0.4 and -0.8 MPa osmotic potential as well as solutions of 50, 100 and 200 µM Cu and Pb supplemented in the form of $CuSO_{4.}$ $5H_2O$ and $Pb(NO_3)_2$, respectively. A control set containing distilled water was used for comparison.

On the 4th day of germination the final germination count data was taken in the experiment. The speed of germination was also calculated as per Czabator (1962). The fresh and dry weight of radicle and plumule were recorded under each treatment. Proline content was measured as per Mohanty and Sridhar (1982) while lipid peroxidation activity was calculated as per Heath and Packer (1968). Antioxidative enzymes like SOD and catalase was also calculated as per Giannopolitis and Ries (1977) and Cakmak *et al*. (1993). Stress response index (SRI) for individual character was calculated as as per Chen *et al*. (2007).

The mean data in all the cases were subjected to statistical analysis following completely randomised design using INDOSTAT version 7.1 software.

RESULTS AND DISCUSSION

The treatments varied significantly among themselves in case of germination parameters and all the growth parameters under study. The mean values for germination percentage for all the treatments were same except at 12 and 18% PEG treatment where it was 96 and 92 % respectively. In the present experiment, the variety Bidhan 1 recorded 34.95 and 44.27 % reduction in speed of germination in 200 mM NaCl and 18 % PEG solution producing an osmotic potential of -0.8 MPa, respectively, over that of control. The corresponding values for copper and lead at the highest concentration were 2.09 and 6.96 %, respectively.

The data indicated that fresh and dry weights of radicle and plumule registered more counter effects of drought stress in comparison with salinity stress at iso-osmotic potentials. In the present experiment, the variety Bidhan 1 recorded 22.38 and 70 % reduction for radicle fresh weight, 46.51 and 53.48 % for radicle dry weight under highest intensity of salinity and drought stress. The corresponding reduction were 14.89 and 80.10 % for plumule fresh weight and 43.84 and 69.23 % for plumule dry weight in 200 mM NaCl and 18 % PEG solution producing an osmotic potential of -0.8 MPa, respectively, with respect of control. The corresponding values of radicle dry weight was 46.51 and 53.48 % and incase of plumule dry wight was 36.15 and 74.61 % respectively, for copper and lead at the highest concentration.

Drought stress at the lowest osmotic potential was found to produce more adverse effects on reserve mobilization during seed germination stage of ricebean in the present experiment as compared to iso-osmotic potential of salinity. The PEG treatments showed linear decrease in reserve depletion during the seed germination with the extent of decrease being more as the osmotic potential of the medium decreased more. Earlier the adverse effects of drought stress (Macar, 2008, Adele *et al*., 2014) and salinity stress (Gamze 2005, Petrovic *et al*., 2016) on reserve mobilization and seed germination in legumes were reported by different workers. The effects of lead was found to be more drastic than copper at equimolar concentrations. These comparative effects of stresses on reserve mobilization were well reflected in the speed of germination.

The proline content in the cotyledon significantly increased under all the treatments of salinity and drought stress in comparison with unstressed control. The increase in proline under osmotic and salinity stress were observed earlier by Martinez *et al*., (2005), Chen *et al*., (2009); Ibrahim (2013) and Maimaiti *et al*. (2014).In salinity treatments the proline content increased linearly along with simultaneous increase in the concentration of NaCl solution, whereas in case of drought it decreased linearly as the osmotic potential of the growing medium decreased more. Thus, proline content registered more adverse effects under drought stress in comparison with salinity stress at iso-osmotic potentials. Likewise, the cotyledon proline content mostly increased under both the metal (Pb, Cu) treatments with the effects being less as the molar concentration increased. From the above data it might be noted further that under Pb 200 µM concentration the proline content reduced as compared to unstressed control, thus, showing more detrimental effect of lead in comparison with copper for this variety of ricebean.

In the present experiment the extent of membrane damage was measured by determining the level of lipid peroxidation. The ricebean variety recorded 154.42 and 144.11 % increase in lipid peroxidation for both 200 mM NaCl and 18 % PEG solution generating an osmotic potential of -0.8 MPa, respectively, over that of control. This increase in lipid peroxidation, in the present experiment, indicated membrane damage leading to more leakiness. Earlier the adverse effects of salinity stress

Table 1: Effect of salinity, drought and heavy metal stress on seed germination , speed of germination and reserve depletion in cotyledons of germinating seeds of ricebean cv. Bidhan 1

Treatments	Germination (%)	Germination speed	Res depletion (%)
Control	100.00	23.83	28.70
NaCl 50 mM	100.00	23.67	19.13
NaCl 100 mM	100.00	19.17	22.61
NaCl 200 mM	100.00	15.50	20.87
PEG 10%	100.00	20.50	25.22
PEG 12%	96.00	18.00	24.35
PEG 18%	92.00	13.28	16.52
Cu 50 µM	100.00	23.83	26.96
Cu 100 µM	100.00	23.67	24.35
Cu 200 µM	100.00	23.33	20.00
Pb 50 µM	100.00	23.17	23.48
Pb 100 µM	100.00	22.83	17.39
Pb 200 µM	100.00	22.17	13.91
SEm(±)	**0.36**	**0.55**	**1.32**
LSD (0.05)	**1.04**	**1.13**	**3.84**

Table 2: Effect of salinity, drought and heavy metal stress on fresh and dry weight of radicle and plumule from germinating seeds of ricebean cv. Bidhan 1

Treatments	Radicle FW(mg)	Radicle DW(mg)	Plumule FW(mg)	Plumule DW (mg)
Control	0.210	0.043	0.920	0.130
NaCl 50 mM	0.323	0.033	0.903	0.103
NaCl 100 mM	0.244	0.033	0.813	0.090
NaCl 200 mM	0.163	0.023	0.783	0.073
PEG 10%	0.180	0.037	0.750	0.070
PEG 12%	0.160	0.023	0.480	0.063
PEG 18%	0.063	0.020	0.183	0.040
Cu 50 µM	0.233	0.043	0.920	0.110
Cu 100 µM	0.140	0.033	0.613	0.090
Cu 200 µM	0.113	0.023	0.250	0.083
Pb 50 µM	0.104	0.023	0.520	0.103
Pb 100 µM	0.93	0.023	0.210	0.063
Pb 200 µM	0.053	0.020	0.107	0.033
SEm(±)	**0.0042**	**0.0041**	**0.0051**	**0.0046**
LSD (0.05)	**0.0123**	**0.0120**	**0.0147**	**0.0134**

and drought stress were reported by Dar *et al.* (2007), Verma *et al.* (2012) and Pratap and Sharma (2010) on lipid peroxidation in legumes. Thus, the highest level of salinity stress led to more membrane damage than iso-osmotic drought stress. Likewise, the lipid peroxidation also increased under both the metal (Pb, Cu) treatments with the effects being more as the molar concentration increased. The corresponding increase in lipid peroxidation under copper and lead treatments at the highest concentration were 48.54 and 79.42 % respectively, over control.

The equilibrium between generation of ROS and its scavenging is often perturbed by different biotic and abiotic factors. Such a disturbance may increase the intracellular levels of ROS .Under this situation different enzymatic and non-enzymatic mechanisms are activated in plant cell to combat against oxidative stress. Several antioxidant enzymes like catalase (CAT), superoxide

Table 3: Effect of salinity, drought and heavy metal stress on lipid peroxidation, proline content and activity of superoxide dismutase (SOD) and catalase (CAT) enzymes in cotyledons of germinating seeds of ricebean cv. Bidhan 1

Treatments	Lipid peroxidation[a]	Proline[b]	SOD[c]	CAT[d]
Control	17.16	628.19	14.96	1.43
NaCl 50 mM	29.02	725.27	19.11	1.02
NaCl 100 mM	33.31	745.70	27.85	1.66
NaCl 200 mM	43.66	861.94	20.79	0.73
PEG 10%	28.77	1072.70	17.29	1.59
PEG 12%	36.84	752.09	18.15	1.23
PEG 18%	41.89	706.11	16.09	0.85
Cu 50 µM	17.41	920.70	25.99	1.55
Cu 100 µM	19.66	642.24	21.24	1.19
Cu 200 µM	25.49	638.41	19.15	0.73
Pb 50 µM	27.51	960.30	16.38	1.25
Pb 100 µM	30.28	864.50	16.55	1.12
Pb 200 µM	30.79	587.32	14.74	0.88
SEm(±)	**1.32**	**7.07**	**1.32**	**0.04**
LSD (0.05)	**3.84**	**20.57**	**3.84**	**0.12**

Note : [a] Data expressed as µM of TBARS contentg^{-1} fresh weight
[b] Data expressed as µM g^{-1} fresh weight
[c] Data expressed as Unit min^{-1} g^{-1} fresh weight
[d] Data expressed as Unit min^{-1} g $^{-1}$ fresh weight

dismutase (SOD) contribute to plant defense and helping in scavenging of ROS. In case of drought and salinity stress the activity of SOD enzyme significantly increased over unstressed control. The activity showed sigmoidal pattern of changes. The activity increased along with increase in stress intensity from mild to medium followed by a significant decrease as the osmotic potential reached the minimum value under study. Earlier Verma *et al.* (2012) observed in salinity that enzymatic scavengers such as superoxide dismutase (SOD) resulted in the significant increase at 100 mM NaCl concentration. In the present experiment, the variety Bidhan-1 recorded 38.97 and 7.35 % increase in SOD activity for both 200 mM NaCl and 18 % PEG solution producing an osmotic potential of -0.8 MPa, respectively, against control. Thus, drought stress registered more adverse effects on SOD activity as compared to salinity stress in comparison with salinty stress at iso-osmotic potentials. The activity of SOD also decreased linearly under both the metal (Pb,Cu) treatments as the concentrations of the metal in germinating medium increased. Copper was found to be more detrimental for SOD content in comparison with lead. The 200 mM concentration of copper caused an increase in the enzyme activity by 28 % and lead 1.47 % decrease over control, respectively.

Catalase (CAT) activity significantly decreased under most of the salinity and drought treatments as compared to control. Only in case of 100 mM NaCl and 10% PEG solution the activity increased over that of control. Earlier the detrimental effects of salinity stress (Dar *et al.*, 2007) and drought stress (Pratap and Sharma 2010, Jiang-Jing Long *et al.*, 2013) on CAT activity in legumes were reported by different workers. Likewise, the cotyledon CAT activity also decreased under all the concentrations of lead treatments with the effects being more as the molar concentration increased. In contrast, the activity increased at 50 µM of copper followed by significant decrease under higher concentrations of the metal. However, copper was found to be more detrimental than lead for CAT activity.

Summarizing the results, it might be concluded that the drought stress was found to register more drastic effects on different biochemical and physiological parameters of germinating seed as compared to iso-osmotic potential of salinity stress, especially, at highest intensity of stress. Also the lead toxicity produced more adverse effects than copper toxicity at equimolar concentration in ricebean cultivar Bidhan 1 at germination stage.

ACKNOWLEDGEMENT

The authors acknowledge the assistance extended by AICRP on Forage Crops, Kalyani Centre, for supplying plant materials.

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