Title: Salinity tolerance dynamics in different crop varieties under controlled condition

PI: Professor Md. Shariful Islam, PhD; Department of Agricultural Chemistry, PSTU

Co-PI: Professor Md. Nizam Uddin, PhD; Department of Agricultural Chemistry, PSTU

Abstract

Salinity is one of the major problems in agriculture all over the world, including Bangladesh. Seawater intrusion leads to elevated soil- and water salinity levels in the coastal regions of Bangladesh during the dry season. It decreases soil productivity and increases food insecurity. The present research will be conducted to find out the salinity tolerance dynamics in different crop varieties to combat the present and future salinity threat to sustainable agricultural production due to climate change and food security in salinity prone areas. Seeds of crop varieties which are nutritionally and economically importance in Bangladesh such as from high-valued vegetable, fibrous, pulse and fodder crop species will be taken for germination and pot experiments under different levels of salinity ranges from 0 to 16 dS m^{-1}. Different agronomical and physiochemical parameters will be measured to determine the salt tolerance dynamics within the tested varieties. Finally, salt tolerant crop varieties will be identified and recommended for pilot field experiments subjecting these to different levels of soil salinity. This way, a potentially huge amount of saline soil can be brought under cultivation in the salinity prone coastal regions of Bangladesh.

Problem statement

Soil salinization is one of the major factors contributing to the loss of productivity of cultivated soils. This problem is increasing rapidly throughout the world. Researchers have estimated that more than 50% of the Earth's arable land could be salinized by 2050 (Jamil *et al*., 2011; Menason *et al*., 2015). According to an estimate of the FAO, over 6% of the world's land is affected by salinity and about 2000 ha of irrigated land per day is degraded by salts (Parihar *et al*., 2015; Qadir *et al*., 2014). Both magnitude and extent of soil salinity in Bangladesh are also increasing with time, being 0.83 Mha (million hectare) in 1973, 1.02 Mha in 2002 and 1.06 Mha in 2009 (SRDI, 2010), posing a serious threat to sustainable agricultural production. As a result, the coastal agricultural production will decrease and problems associated with future food security will become ever present, which creates a vast socio-economic impact upon the coastal populations. Therefore, suitable salt tolerant crop cultivars should be identified analyzing their agronomical, physiological and biochemical responses to different salinity level to combat the future salinity threat to sustainable agricultural production. By selecting and cultivating these crop varieties, currently unused large areas of saline soil can be brought under cultivation and existing agricultural land will be more productive, which will ensure sustainable future crop production and food security for the coastal inhabitants living in salinity prone areas.

Objectives and research questions

The current research has been designed with the following research questions –

a) What are the salinity tolerance levels of different crop varieties at germination stage?
b) Which varieties are most suitable to grow under different salinity stress levels and for what reasons?

By answering the above questions, we set the following objectives-

a. To screen different crop varieties for different degrees of salt tolerance at germination stage;
b. To examine the suitability of the selected crop species under salt stress at controlled field condition based on agronomical, physiological and biochemical traits.

Methodology

The following methodologies will be followed to achieve the above objectives.

Salinity tolerance at germination stage: Seeds of crop varieties which are nutritionally and economically importance in Bangladesh namely, Spinach (*Basella alba*), River spinach var. BARI Gimakalmi-1 (*Ipomoea reptans* Poir), Snake bean (*Vigna unguiculata* subsp. *sesquipedalis*), Cucumber (*Cucumis sativus* L.) from vegetable species; Mung bean/green gram (*Vigna radiata* L. R. Wilczek), Grass pea (*Lathyrus sativus* L.), Lentil (*Lens culinaris* Medicus) from pulse crop species; Jute (*Corchorus olitorius* L. var. BJRI Tosha Pat-8 (MG-1) and *Corchorus capsularis* L. var. BJRI Deshi Pat-9 (BJC-5003)), kenaf (*Hibiscus cannabinus* L. var. BJRI Kenaf-3/HC-3) from fibrous species and Triticale (*Triticum aestivum*), Millet (*Pennisetum glaucum*) and Sorghum (*Sorghum bicolor*) from fodder crop species will be placed in petri plates and allowed to germinate on a moist cotton bed with different levels of salinity treatments: 0, 4, 8 and 12 d*S* m^{-1} added as a NaCl solution. The total number of petri plates will be 224 (14 varieties x 4 salinity treatments x 4 replications).

Salinity tolerant dynamics at controlled field conditions: A pot culture experiment will be conducted by sowing and/or transplanting the new seeds or seedlings (varieties that have passed the germination test) in the soil of different salinity levels (0, 4, 8, 12 and 16 d*S* m^{-1} from NaCl salt added with half Hoagland nutrient solution) in a net house. Sandy or sandy loam soil will be collected from non-saline areas and physico-chemical analysis will be done in the laboratory to describe the soil type and composition. About 5-10 kg of air dried and clean soil per pot will be filled in a series of plastic pot. Depending on the germination percentage (>60% depends on varieties and salinity level) of the selected crop species, the numbers of pots will be varied and minimum four replications will be maintained. Soil salinity in petri plates and pots will be developed by slowly increasing the salt concentration to avoid a large osmotic shock to seedlings in the higher salinity treatment groups. Proper precautionary measures will be taken to protect the pot from heavy rain. Intercultural operations necessary for proper crop development, such as application of recommended doses of fertilizers, weeding, earthing, irrigating, and training of plants on bower and stakes for vine crops will be done as and when necessary. Soil moisture and salinity in the pots will be regularly monitored by using soil moisture meter (TDR 350, FieldScout, USA) and direct soil EC meter (2265FS, FieldScout, USA), respectively. The electrical conductivity (EC) and quantity of irrigation water provided will also be recorded.

Agronomical and physiological data collection: Percentage of germination, root and shoot length, plant height, leaf number, yield, chlorophyll and proline content will be determined from all crop varieties.

Chemical analysis: EC (both saturated paste extract and 1:5 extract), pH, soil organic carbon, translocation factor (TF), bio-concentration factor (BCF), sodium adsorption ration (SAR), soluble sodium percentage (SSP), exchangeable sodium percentage (ESP), plant essential macro- and microelements like N, P, K, Ca, Mg, S, Na and Cl will be analyzed as described below. After harvesting, plant samples will also be analyzed for different nutritional elements such as P, K, Ca, S, Na and protein in addition to crop growth rate, photopigments and yield.

pH and EC: pH and EC of soil (1:2.5= soil:water for pH, and 1:5= soil:water and saturated paste extract for EC) and irrigation water samples will be determined with a glass electrode pH meter and conductivity meter (EC Meter), respectively (Jackson 1962; Tandon, 1995).

Chlorophyll: Leaves will be treated using 80% chilled acetone in order to determine chlorophyll concentrations with the help of UV-VIS spectrophotometer using the following equation given by Lichtenthaler and Wellburn (1983).

Chlorophyll a = $12.21A_{663} – 2.81A_{646}$ ………. (i)

Chlorophyll b = $20.13A_{646} – 5.03A_{663}$ ………. (ii)

Proline: Proline will be determined in fresh leaf samples after homogenization with sulfosalicylic acid and color development with acid-ninhydrin and toluene reagent. The absorbance of color will be determined at 520 nm using UV-VIS spectrophotometer (Bates *et al*., 1973)

Elemental analysis: After wet oxidation of plants and necessary extraction of soil samples, organic carbon, Na, K, Ca, Mg, P, S, N and Cl will be determined. Organic carbon will be measured using the Walkley Black chromic acid wet oxidation method

(Walkley and Black, 1934), Na and K by the flame emission spectrophotometric method; Ca and Mg by the atomic absorption spectrophotometric method, chloride by the argentometric method of titration; S and P by the UV-VIS spectrophotometric method after necessary color development; and protein and nitrogen (N) by the Kjeldahl method of analysis.

Sodium adsorption ratio (SAR), soluble sodium percentage (SSP), exchangeable sodium percentage (ESP), translocation factor (TF) and bio-concentration or bio-accumulation factor (BCF or BAF) of Na will be determined from the collected samples using the following formulas.

SAR = $\frac{[Na^{+}]}{\sqrt{([Ca^{2+}] + [Mg^{2+}])/2}}$ ……… (iii)

Where, $[Na^+]$, $[Ca^{2+}]$, and $[Mg^{2+}]$ are the concentrations in meq/L

SSP=[soluble sodium concentration (meq/L)/total cation concentration (meq/L)] ×100 .…(iv)

ESP = (Na^+/CEC) × 100 ……….(v)

Where: Na^+ = Measured exchangeable Na (cmol/kg); CEC = Cation exchange capacity (cmol/kg); [numerically centimoles/kg is equal to milli equivalents per 100 g of soil].

BCF: The ability of the plant to accumulate or resist the salt with respect to the salt concentration in the substrate.

BCF= (Concentration of salt or Na^+ in plant tissue) / (Initial concentration of salt or Na^+ in external soil) ………….(vi)

TF: The relative translocation of salts or Na^+ from soil to other parts (root and shoot) of the plant species. The most salt accumulating part of plant (root/shoot/fruits) will be identified using the TF.

TF = (Concentration of salts or Na^+ in plant tissue (parts)) / (Concentration of salts or Na^+ in corresponding soil or root) …………(vii)

Statistical analysis: Data will be analyzed using a standard statistical program, such as SPSS or R.

Work plan:

Activities	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Seeds and materials collection and processing for Petri plate culture	■											
Petri plate culture		■										
Agronomical and chemical analysis			■									
Soil collection and processing				■								
Chemical analysis of soil					■							
Pot experimentation						■	■	■	■			
Post harvest soil and plant sampling and processing							■		■	■		
Chemical analysis of soil and plant samples							■	■		■	■	
Data collection		■	■			■	■	■	■			
Data analysis and report writing												■

Budgetary items: Total budget will be 13000 Euro (One Thousand Three Hundred Euro)

Sl. No.	Name of Items	Unit Price (Euro)	Numbers	Total cost (Euro)
1	Direct Soil EC meter (2265FS, FieldScout, USA)	900	1	900
2	Vacuum filtration apparatus	1000	1	1000
3	Laboratory glass wares	Various	Various	960
4	Petri plate	4	224 Pcs.	896
5	Plastic pot	5	120 Pcs.	600
6	Filter paper	120	5 pac	600
7	Chemicals and reagents	Various	Various	4054
8	Vertical rack	240	1	240
9	Labour for soil collection, processing and pot experimentation	6	100	600
10	Stationery	Various	Various	50
11	Contingency	Various	Various	100
12	Student scholarship (MS/PhD)	250 per month	12 months	3000
Total: Thirteen Thousand Euro Only				**13000**

Outcome: High salt tolerant crop varieties will be identified and recommended for pilot field experiments in salinity prone coastal regions of Bangladesh.

Outreach to local farmers and other stakeholders: Farmers from salinity prone areas will benefit using high-value salt tolerant crop varieties identified, which will ensure more sustainable crop production and food security. Students will experience about how crop varieties respond to abiotic stresses and identify suitable or resilient crop varieties to minimize the climate change impact of salination.

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