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Competitive Research Grant

Sub-Project Completion Report on

Selection of Salt Tolerant Sunflower and Mustard Genotypes Based on Physiological and Biochemical Traits

Project Duration
May 2016 to September 2018

Central Laboratory, Oilseed Research Centre
Bangladesh Agricultural Research Institute
Gazipur-1701

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Acronyms

BARC	Bangladesh Agricultural Research Council
BARI	Bangladesh Agricultural Research Institute
CRG	Competitive Research Grant
NATP-2	National Agricultural Technology Program-Phase II Project
PCR	Project Completion Report
PIU	Project Implementation Unit
PI	Principal Investigator
RWC	Relative water contents
FW	Fresh weight
DW	Dry weight
TW	Turgor weight
MDA	Melondialdehyde / lipid per oxidation
H_2O_2	Hydrogen per oxide
$O_2^{\bullet-}$	Super oxide generation
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
APX	Ascorbate peroxidase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione *S*-transferase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Na^+	Sodium ion
K^+	Potassium ion
K-P buffer	Potassium-phosphate buffer
CDNB	1-Chloro-2, 4-dinitrobenzene
NBT	Nitro-bluetetrazolium
DAB	3, 3-diaminobenzidine
P_N	Photosynthesis
E	Transpiration
gs	Stomatal conductance
Ci	CO_2-assimilation rate

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Executive Summary

Salinity is one of the important abiotic stresses that affect in growth, photosynthetic parameters, ion accumulation, superoxide and hydrogen peroxide and antioxidant defense systems in oilseed crops. The goal of the project is develop salt tolerant sunflower and mustard genotypes or varieties to raise area and production in the coastal region. The present study investigates the identification of salt tolerant sunflower and mustard genotypes and better understanding of salinity tolerance mechanism and also to develop oil quantity and quality protocol of sunflower and mustard for awaring farmers and consumers. Two sunflower and three mustard promising genotypes have been selected against salinity after screening of forty sunflower and sixty five mustard genotypes. After screening, seven days old selected sunflower (GP-4030, BARI Surjamukhi-2) and mustard (Jun-0536, BARI Sarisha-16 and BARI Sarisha-11) seedlings were imposed to 0, 8, and 12 dSm^{-1} salinity for 10 days. Leaf relative water content (RWC), chlorophyll (Chl) content, photosynthetic parameters, K^+/Na^+ ratio, reactive oxygen species (ROS), proline (Pro) and lipid peroxidation (MDA) content, enzymatic antioxidants were investigated in fully expanded leaves. Salt stresses significantly reduced RWC, chl content, K^+ and K^+/Na^+ ratio, photosynthesis rate, transpiration rate, stomatal conductance, sub-stomatalCO_2 concentration rate, K^+ and as well as increased in levels of Pro and MDA content, Na^+,and superoxide and hydrogen peroxide (H_2O_2) in sunflower and mustard genotypes.The activities of superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione peroxidase, monodehydroascorbatereductase, dehydroascorbatereductase, and glutathione reductase increased in both genotypes, but the magnitude was higher in tolerant genotypes than in salt sensitive genotypes. Higher accumulation of Pro and MDA with salt stress improved physiological and biochemical parameters, and reduced oxidative damage by up-regulating their antioxidant defense system, better cellular protection from execs accumulation of ROS in selected genotypes as a result GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16 and BARI Sarisha-11showed better performance against salt stress. Oil quantity and quality parameters protocol has been developed for assessing oil content, quality, and awaring farmers and consumer in recognizing pure oils.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: **Selection of Salt Tolerant Sunflower and Mustard Genotypes Based on Physiological and Biochemical Traits.**
2. Implementing organization: **Bangladesh Agricultural Research Institute, Gazipur**
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s)**

Principal Investigator: **Dr. Md. Shakhawat Hossain**
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4. **Sub-project budget (Tk):**
   - 4.1 Total : **17,97,290/-**
   - 4.2 Revised: **17,34,290/-**

5. **Duration of the sub-project:**
   - 5.1 Start date (based on LoA signed): 1st April, 2017
   - 5.2 End date : 30 September 2018

6. **Justification of undertaking the sub-project:**

The acute shortage (71-74%) of edible oil is prevailing in Bangladesh for last several decades (Mallik, 2013). This shortage inherited from the past is met through imports, using a huge amount of foreign exchange every year. Bangladesh is produced 0.358 million tons of edible oil against the annual demand of 1.6 million tons, while the remaining 1.242 million tons of the country's domestic requirement is met through imports (Hossain, 2014). Bangladesh only 4% lands are produced in all oilseed crops. The area under oilseed crops cultivation is decreasing day by day due to competition of various cash crops, economic and technical reasons (Mia et al. 2014). Out of 2.83 million hectares of the coastal and offshore areas of Bangladesh about 0.88 million hectares are affected by different degrees of salinity (0.38 m ha in Khulna, 0.22 m ha in Pautuakhali, 0.15 m ha in Chittagong and 0.13 m ha in Barisal and Noakhali regions) (Amin et al. 2011). This land is more than 30% of the total cultivable lands. This vast land remains mostly uncultivated except some selected areas farmers grow mostly low yielding traditional rice. Moreover, it can fill up the gap by expanding the area of sunflower and mustard production. On the other hand, this situation is more crucial when there is no salt

tolerant variety. Importantly, many of the cultivable crops have been trialing to introduce in those areas without considering their saline tolerant potentiality. Therefore, potentiality (tolerance capacity) must be considered before introducing a crop in such problematic soil. Thus, there is a great scope to enhance crop production in this saline area by adapting salt tolerant high value crops such as sunflower and mustard with their technologies.

Sunflower (*Helianthus annuus* L.) is one of the most important crops grown for oil in both rabi and kharif seasons. Seeds of sunflower contain about 40-45% oil and protein about 22-24%. Sunflower oil is highly nutritious containing antioxidant, vitamins, α-tocopherol, minerals and high amount of linoleic (*ω*-6) and oleic (*ω*-9) fatty acids. In Bangladesh, sunflower is comparatively new but its oil is very much popular and high value edible oil in the world. So, as a high value oil crops it could be tried in the coastal area. Now-a-days, utilizing the coastal areas of Bangladesh sunflower production is increasing due to farmer awareness on nutritional aspect. However, till now, no such variety has been developed for the areas due to complexity of salinity tolerance trait.

Mustard (*Brassica sp*) is another important oilseed crop in Bangladesh which can be grown in the coastal area. Salinity may affect mustard plants at any stage of growth. Many studies have shown that seed germination, seedling growth (Wu, 2015), plant height, growth index, leaf area and fresh and dry weights of the shoot and root system (Ashraf and Ali, 2008) are affected by changes in salinity concentration. Under saline conditions, high accumulation of toxic ions such as Na^+ and Cl^- takes place in the chloroplast (Munns et al., 2006). High K^+/Na^+ ratio in plants under saline conditions has been suggested as an important selection criterion for salt tolerance (Ashraf, 2004). In addition, *Brassica* has some potential to cope with the toxicity of salts (Francis, 1984). However, until recent, no mustard varieties/genotypes have been developed tolerant to salinity.

Therefore, it is essential to develop a salt tolerant variety with understanding of the tolerance mechanism. Besides, short duration, existing comparatively salt tolerant mustard varieties and modern technologies has shown potential to reduce the existing gap between production and consumption of edible oil. Presently, we are investigating the biochemical and oxidative stress tolerance mechanism under salinity in mustard in Central Lab of ORC, BARI. With this view, the proposed project will be helpful in developing salt tolerant sunflower and mustard genotypes and will be evaluated some key biochemical and physiological parameters in both genotypes. Further this will provide an insight into the mechanism of salt tolerant in sunflower and mustard under different levels of salt loading.

7. Sub-project goal:

The goal of the project is develop salt tolerant sunflower and mustard genotypes or varieties to raise area and production in the coastal region.

8. Sub-project objective (s):

- To select salt tolerant genotypes of sunflower and mustard for the coastal area of Bangladesh.
- To determine the physiological mechanism of salt tolerance sunflower and mustard genotypes.
- To study the biochemical characterization of sunflower and mustard genotypes and to develop oil quantity/content and quality protocol of sunflower and mustard for awaring farmers and customers.

9. Implementing location (s):

Central Laboratory, Oilseed Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur

10. Methodology in brief:

Approach and Methodology

Plant Materials

This research work was implemented in Central laboratory, net house, and green house of Oilseed Research Centre (ORC), BARI, Gazipur. Firstly, forty sunflower and sixty five mustard genotypes were collected from ORC and Plant Genetic Resources Centre (PGRC), BARI, Gazipur. Notable screen work was started second week of October, 2017 for sunflower and last week of October, 2017 for mustard genotypes.

Methodology and Stress Treatments

(A) Sowing of seeds for screening of sunflower and mustard genotypes under saline condition

The research work was conducted during second week of October, 2017 (sunflower) and last week of October 2017 (mustard) at net house and green house of Central Laboratory, ORC, BARI, Gazipur. Forty sunflower and sixty five mustard genotypes were screened under different levels of salinity. Before sowing, seeds were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with distilled water (DW). The plastic pots (35 cm diameters at top and 30 cm at bottom) were filled with sandy loam soil. Full-strength Hoagland's nutrient solution was added on alternate days to each pot. The pH of the solution was adjusted to 6-6.5. After 15-16 days of germination, seedlings were thinned to maintain six (sunflower) or nine (mustard) plants in each experimental unit/pot. After 18-20 days acclimatization period, solutions were adjusted to the

desired salinities (0, 8, and 12 dSm^{-1} NaCl) and plants were simultaneously treated with an aqueous solution of Hoagland's nutrient solution. Salinity levels were measured by an EC meter (Spectrum direct soil EC meter, USA). Control treatments were maintained by distilled water. Fifteen days after treatment, all treated plants were evaluated and selected salt tolerant genotypes.

(B) Performance of selected sunflower and mustard genotypes under salinity stress in pot culture

Forty sunflower and sixty five mustard genotypes were screened against different levels of salinity (8, 10 and 12 dSm^{-1} salinity). Among them genotypes two sunflower (GP-4030 and variety BARI Surjamukhi 2) and three mustard (Jun-0536, BARI Sarisha 16 and BARI Sarisha 11) were survived whole life in all salinity levels and thus, they used consider as tolerant genotypes. On the other hand, sunflower genotype PS-2 and BARI Sarisha 14 variety were found as most salt sensitive genotype.

In this study, we included a previously reported a salt tolerant sunflower genotype Hysan 33 (Anwar-ul-Haq et al. 2013, this tolerant genotype compared with our selected genotypes), and another tolerant BARI Sarisha 11 (Mondol et al. 2003). Selected all genotypes were grown (1^{st} week of December, sunflower and 1^{st} week of November, mustard) in pots under green house of ORC, BARI, Gazipur. The plastic pots (35 cm diameters at top and 30 cm at bottom) were filled sandy loam soil. Full-strength Hoagland's nutrient solution was added on alternate days to each pot. The pots were arranged in complete randomized design with three replication and all experiments were repeated three times. Seedlings were grown under control conditions (relative humidity 60-70%, temperature 25 ± 2^{o}C, light 655 µmole $m^{-1}s^{-1}$ and photoperiod from 12 to 14 h) in green house. After 10 days of germination, seedlings were thinned to maintain three plants in each experimental unit. The 0, 8, and 12 dSm^{-1} NaCl was applied with Hoagland's nutrient solution after 10 days of germination. The NaCl concentration was increased step-wise in aliquots of 8 dSm^{-1} up to 12 dSm^{-1} finally attained. Salinity levels were measured by an EC meter. Four weeks after the exposure to salt stress, three plants per replicate were harvested and separated into shoots and roots. After washing well with distilled water, fresh masses of shoots and roots were recorded. The samples were then oven-dried at 80^{O}C for 1 week so as to record dry masses. Control treatments were also maintained under same condition. The remaining plants were used for recording data on different parameters in fully expanded leaves of 10 days stressed seedlings for the following variables.

(C) ***Morphological, physiological, and biochemical responses of selected sunflower and mustard genotypes under pots culture at salt loading***

Plant harvest:

Plants were harvested at vegetative or maturity stage and data were recorded. After 40-50 days of salinization, the samples were harvested for analysis. After recording fresh weights of shoots and

roots, the samples were dried at 65^{o}C up to three days. For most of the physiological and biochemical parameter data, plants were grown up to 55-60 days after sowing (DAS).

Relative water contents (RWC)

Relative water content was determined in fresh leaves (second and third). Samples were fresh weighed (FW) quickly and immediately floated on double distilled water in petridishes to saturate them with water for next 12 h, in dark. The excessive water was blotted and turgor weight (TW) was taken. The leaves were dried at 80°C for 24 h and the dry weight (DW) was recorded. The RWC was calculated using following formula (Smartand Bingham, 1974):

$$RWC\ (\%) = (FW\text{-}DW/TW\text{-}DW) \times 100$$

Measurement of chlorophyll content

The chlorophyll contents were determined following Arnon (1949). Fresh leaves (1g) were obtained from each replicate, chopped it into very fine pieces, placed in test tube containing 10 ml of 80% acetone for overnight. On the coming day, chlorophyll sap was filtered using a filter paper (Whatman No.1). Absorbance of the samples were read at 645, 652 and 663 nm with a Hitachi spectrophotometer (Hitachi, Model U2001, Tokyo, Japan). Chl. a and Chl. b was calculated using following formula:

"Chl. a (mg g^{-1} F.Wt.) = [12.7 (OD_{663})-2.69(OD_{645}) ×V/1000 x W]"

"Chl. b (mg g^{-1} F.Wt.) = [22.9 (OD_{645})-4.68(OD_{663}) ×V/1000×W]"

Total chlorophyll = Chl. (a) +Chl. (b)

Where,

W = weight of fresh leaf tissue (g), V = volume of the leaf extract (ml)

Measurement of photosynthetic parameters

Gas exchange parameters were determined using a LiCOR 6400 open system portable infrared red gas analyzer (IRGA) (Lincoln, USA). These parameters were determined in noon and cloudless clear days when light intensity was fully expanded leaves (at 9 a.m. to 2.00 p.m.). A young fully expanded leaf (first and third) was used for estimating photosynthetic rate, transpiration rate, intracellular CO_2 concentration and stomatal conductance. Before taking the measurement, the IRGA was calibrated and adjusted approximately every 30 min during the measurement. Leaf was enclosed in a 1-litre gas exchange chamber for 60 sec. The conditions used for the equipment/leaf chamber were as follows: ambient pressure 99. 2 kPa , atmospheric CO_2 concentration (Cref) 400 µmol mol^{-1}, leaf surface area 6 cm^2, PAR (Qleaf) was maximum up to 900-1000 µmol m^{-2} s^{-1} and the chamber water vapor pressure varied from 4.0 to 5.8 mbar (Ali et al., 2008).

Inorganic Ions (Na^+ and K^+) measurements:

Inorganic ions were determined following Ashraf et al. (2001). For the determination of Na^+ and K^+ contents, 10–100 mg of well-ground dried material of the third fully expanded young leaf from top was digested in 8.0 ml concentrated HNO_3, and Na^+ and K^+ ions were determined with a flame photometer (Jenway PFP7).

Determination of Proline

Proline colorimetric determination was preceded according to Bates et al. (1973) based on proline's reaction with ninhydrin. Fresh leaf tissue (0.5g) was homogenized in 10 ml of 3% sulfosalicylic acid in ice. The homogenate was centrifuged at 11,500 × g for 15 min. Two ml of the filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. After incubation at 100^0C for 1 hr it was cooled and 4 ml of toluene was added. The optical density of the chromophore containing toluene was read spectrophotometrically at 520 nm using toluene as a blank. The amount of proline was determined by comparison with a standard curve.

Measurement of Lipid Peroxidation

The level of lipid peroxidation was measured by estimating melondialdehyde (MDA), a decomposition product of the per-oxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid (TBA) as the reactive material following Heath and Packer (1968). The leaf samples (0.5g) were homogenized in 3 ml 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 11,500×g for 10 min. One ml supernatant was mixed with 4 ml of TBA reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95^0C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged at 11,500×*g* for 15 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for nonspecific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} and was expressed as nanomole of MDA g^{-1} FW.

Measurement of H_2O_2

H_2O_2 was assayed according to Yu et al. (2003). The extracted was prepared by homogenizing 0.5 g of leaf tissue with 3 ml of 50 mM K-P buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (3 ml) was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 11,500×*g* for 15 min at room temperature. The absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content (ϵ= 0.28 $\mu M^{-1}cm^{-1}$) and expressed as micromole g^{-1} FW.

Measurement of the $O_2^{\bullet-}$ generation Rate

Superoxide radical was determined according to Elstner and Heupel (1976) with slight modifications. Leaves (0.3 g) was homogenized in 3 ml of 65 mM (K-P) buffer (pH 7.8) on an ice bath and then centrifuged at 4°C for 10 min at 5,000×*g*. The supernatants (0.75 ml) was mixed with 0.675 ml of 65 mM K-P buffer (pH 7.8) and 0.07 ml of 10 mM hydroxylamine chlorhydrate and the reaction was incubated at 25°C. After 20 min, 0.375 ml of 17 mM sulfanilamide and 0.375 ml of 7 mM α-naphthylamine was added, and the mixture was incubated at 25°C for another 20 min before it was mixed with 2.25 ml of diethyl ether. The absorbance was measured at 530 nm and the $O_2^{\bullet-}$ concentration was calculated from a standard curve of $NaNO_2$.

Histo-chemical detections of $O_2^{\bullet-}$ and H_2O_2

Superoxide ($O_2^{\bullet-}$) and H_2O_2 were visualized in leaves according to Wang et al. (2011) with modifications. Briefly, the second leaves were stained in 0.1% nitro-bluetetrazolium (NBT) or 1% 3, 3-diaminobenzidine (DAB) solution for 8 h under dark and light, respectively. Incubated leaves were then decolorized by immersing in boiling ethanol which allowed visualization of blue insoluble formazan (for $O_2^{\bullet-}$) or deep brown polymerization product (for H_2O_2). After cooling, glycerol was used to open the leaves and photographs were taken by placing the leaves between two glass plates.

Extraction of soluble protein and enzyme assay

Using a pre-cooled mortar and pestle, 0.5g of fresh leaves of sunflower and mustard seedlings were homogenized in 0.0090 g Ascorbic acid, 10 ml of 500 mM ice-cold K-P buffer (pH 7.0), 5 ml KCl in 1 M solution, 5 mM 25 µl β-marcapto ethanol and volume up to 50ml. The homogenates was centrifuged at 11,500×g for 15 min at 4°C, and the supernatant was used for enzyme assay. The protein concentration in the leaf extracts were determined according to Bradford (1976) using BSA as a protein standard.

Assay of enzymatic activities

Superoxide dismutase (SOD) (EC: 1.15.1.1): To determine SOD activity of whole cell homogenate, the reaction was prepared on ice in 50 mM potassium phosphate buffer (pH 7.8, with 1.34 mM EDTA) and an indirect competitive inhibition assay was used for the measurement (Spitz and Oberley, 1989). This assay is based on the competition between SOD and an indicator molecule nitro-bluetetrazolium (NBT) of 2.24 mM, for superoxide production from 2.36 mM xanthine and xanthine oxidase. One unit of activity was defined as the amount of protein required to inhibit NBT reduction by 50%. SOD activity was expressed as units as $min^{-1}mg^{-1}$ protein.

***Peroxidase* (POD)** (EC: 1.11.1.7): POD activity was estimated according to Hemeda and Klein (1990). The reaction mixture contained 25 mM K-P buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and the protein solution. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation for 1 min using extinction coefficient of 26.6 mM^{-1} cm^{-1}.

***Catalase* (CAT)** (EC: 1.11.1.6): CAT activity was measured according to Rohman et al. (2016a) by monitoring the decrease of absorbance at 240 nm for 1 min caused by the decomposition (or degradation) of H_2O_2. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 0.7 ml. The reaction was initiated with the addition of enzyme extract, and the activity was calculated using the extinction coefficient of 39.4 M^{-1} cm^{-1}.

***Ascorbate peroxidase* (APX)** (EC: 1.11.1.11): APX activity was assayed following of Nakano and Asada (1981). The reaction solution contained 50 mM K-P buffer (pH 7.0), 0.50 mM ASA, 0.10 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final volume of 0.7 ml. The reaction was started by the addition of H_2O_2, and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

***Glutathione peroxidase* (GPX)** (EC: 1.11.1.9): GPX activity was measured as described by Elia et al. (2003) using H_2O_2 as a substrate. The reaction mixture consisted of 100 mM sodium-phosphate buffer (pH 7.5), 1 mM EDTA, 1 mM NaN_3, 0.12 mM NADPH, 2 mM GSH, 1 unit GR, 0.6 mM H_2O_2, and 20 µl of sample solution. The reaction was stated by the addition of H_2O_2. The oxidation of NADPH was recorded at 340 nm for 1 min, and the activity was calculated using the extinction coefficient of 6.62 mM^{-1} cm^{-1}.

***Glutathione reductase* (GR)** (EC: 1.6.4.2): GR activity was measured following of Hossain et al. (2010). The reaction mixture contained 0.1 M K-P buffer (pH 7.8), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme solution in a final volume of 1 ml. The reaction was initiated with GSSG, and the decrease in absorbance at 340 nm due to NADPH oxidation was recorded for 1 min. The activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

***GlutathioneS-transferase* (GST)** (EC: 2.5.1.18): GST activity was determined spectro-photometrically according to Rohman et al. (2016a). The reaction mixture contained 100 mM Tris-HCl buffer (pH 6.5), mM GSH, 1 mM 1-Chloro-2, 4-dinitrobenzene (CDNB), and enzyme solution in a final volume of 0.7 ml. The enzyme reaction was initiated by the addition of CDNB, and the increase in absorbance was measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Determination of oil extraction

Oil content was determined following method (Soxlhet) described by Pena et al. (1992) with some modifications. For solvent extraction, mustard and sunflower seeds were crashed in grinder machine and were dried in an oven at 60^0C for 30 min. Two-three gram of ground/powder sample was placed into cellulose paper and extracted using 20-25 ml petroleum ether (40-60^0C)/n-hexane in a 2-1 Soxlhet extractor for 15-45-10 min. The oil was recovered by evaporating of the solvent using rotary evaporator (Heidolph model, Germany) and residual solvent was removed drying in an oven at 60^0C for 1h. The n-hexane/petroleum ether brown oil extracts obtained was weighed and stored in the refrigerator until it is required for further analysis. All experiments were repeated three times.

Determination of Fatty acid composition

Fatty acid composition in seeds under study was carried out according to the procedure with some modifications as described by Were et al. (2006). Ten-twelve mustard seeds were crashed and were taken in a 15 ml screw capped pyrex glass tubes having 15 cm length and 1 cm internal diameter. Then 5 ml of ethylate reagent (sodium hydroxide, ethanol and petroleum ether mixed) was added and was vortex the sample tube 1 min. The glass vials were put in 10-12 h overnight and cooled. Then five milliliter of salt solution (sodium hydrogen sulphate and sodium chloride mixed) was added in each tube and shake after 1 min. After then the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile.

Statistical analysis

All data was analyzed by SAS (SAS Institute Inc. Cary, NC, USA, Version 9.3) and R statistical packages following complete randomized design (CRD) and the mean differences was compared by Tukey's tests. Differences at the P≤0.05 level were used as a test of significance.

11. Results and Discussion:

(a) Screening of sunflower genotypes under saline condition:

Forty sunflower genotypes were screened under net house condition. After screening, two sunflower genotypes**,** BD-4030, and BARI Surjamukhi-2 genotypes have been selected against salinity (12 dSm^{-1}). When the plants were at the stage of 2-3 true leaves, 12dSm^{-1} NaCl solution was added to the pots gradually by every alternate day until the desired concentration was achieved.

Screening and data collection of sunflower genotypes under saline condition

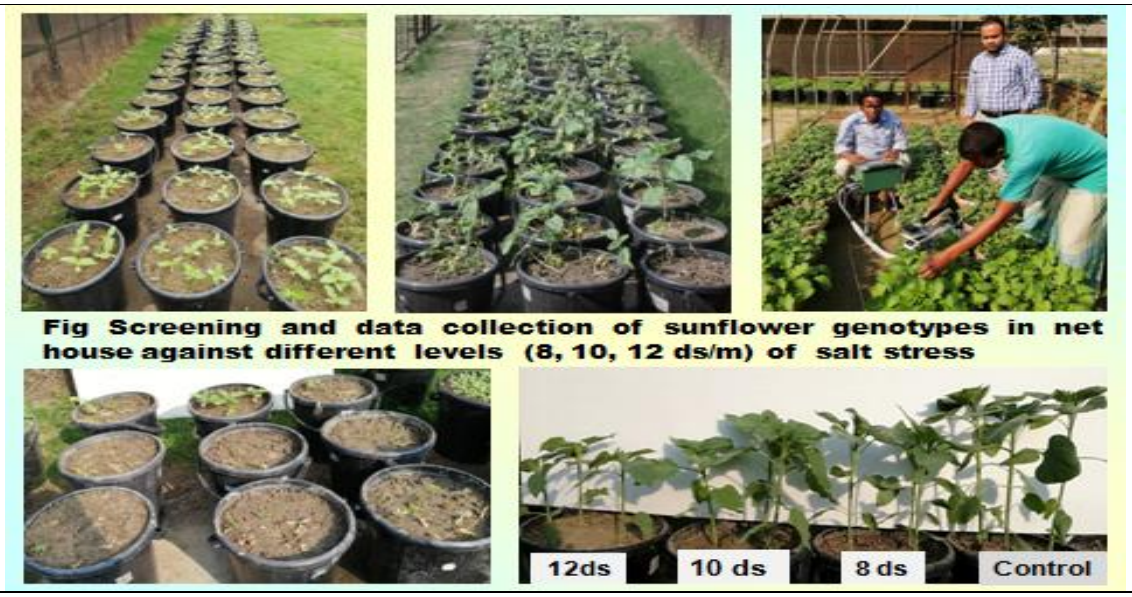


Fig Screening and data collection of sunflower genotypes in net house against different levels (8, 10, 12 ds/m) of salt stress

(b) Sowing of seeds for screening of mustard genotypes under saline condition:

Sixty five mustard genotypes were screened under green house condition and three mustard genotypes, Jun-0536, BARI Sarisha-16, and BARI Sarisha-11 genotypes were selected against salinity (12 dSm^{-1}). When the plants were at the stage of 25-30 days, 12 dSm^{-1} NaCl solution was added to the pots gradually by every alternate day until the desired concentration was achieved.

Screening of mustard genotypes under saline condition

Fig : Project activity & screening of sixty five mustard genotypes in green and net house against different levels (0, 8 and 12 ds/m) of salt stress

Morphological, physiological and Biochemical parameters:

Sowing of selected sunflower and mustard genotypes in pots under salinity condition

Two selected sunflower genotypes, BD-4030, and BARI Surjamukhi-2, one salt susceptible line, PS-2 and one tolerant variety Hysan-33 were grown in pots to collect physiological and biochemical data. On the other hand, three selected mustard genotypes, Jun-0536, BARI Sarisha-16, and BARI Sarisha-11 and one salt sensitive variety BARI Sarisha-14 were grown in pots under net house for study on physiological mechanism under salt loading. The pots were arranged in complete randomized design with three replication and all experiments were repeated three times.

Growth attributes:

Salt stress sharply decreased the shoot and root length, and fresh and dry weights of all sunflower and mustard genotypes when compared with the control (0 dSm^{-1}). Data showed that newly selected sunflower (GP-4030 and BARI surjamukhi-2) and mustard (Jun-0536, BARI Sarisha-16) as well as Hysan-33 and BARI Sarisha-11 had greater shoot and root length, fresh and dry biomass than the sensitive genotype PS-2 (sunflower) and BARI Sarisha-14 (mustard) under saline conditions table 1a and 1b. However, all the parameters were significantly higher in all the tolerant genotypes than the sensitive genotype, PS-2 and BARI Sarisha-14 at 12 dSm^{-1} salinity.

Salt stress caused a substantial decrease in the growth of all sunflower and mustard genotypes table 1a and 1b. Salt-induced reduction in different growth attributes are parallel to previous report in different crops such as sunflower (Akram et al. 2009; Akram and Ashraf 2011), maize (Nawaz and Ashraf 2010), wheat (Khan et al. 2006), tomato (Zribi *et al.,* 2009), eggplant (Abbas et al. 2010), strawberry (Keutgenand Pawelzik, 2009), mulberry (Ahmad and Sharma 2010), okra (Saleem et al. 2011), *Populousalba* (Imadaand Tamai, 2009) and proso millet (Sabir *et al.,* 2011). Differences in growth of sunflower and mustard genotypes in response to salt stress observed in the present study might have been due to variation in a number of biochemical or physiological traits associated with the processes related to mechanism of salt tolerance table 1a and 1b. Generally, salt stress causes reduction in cell division as well as cell elongation (Pitann et al. 2009) mainly due to salt-induced perturbate in uptake of nutrients, high accumulation of ROS (Ashraf 2009), cytoplasmic enzyme inhibition, turgor loss (Pitann et al. 2009) and hormonal imbalance (Ashraf et al. 2010; Iqbaland and Ashraf 2011) which in turn impairs plant growth in terms of yield or biomass production.

Table 1a: Mean values of shoot length and root length of sunflower and mustard genotypes under different level of salinity

Cultivar /genotypes	**Shoot length (cm)**				**Root length (cm)**		
	0 ds/m	**8 ds/m**	**12 ds/m**		**0 ds/m**	**8 ds/m**	**12 ds/m**
Sunflower							
GP-4030	65.89	50.21	37.44		33.93	24.22	17.88

BARI Surjamukhi-2	68.51	51.53	38.16		37.05	26.51	19.44
Hysan-33 (tolerant)	40.81	33.54	28.22		34.33	25.37	18,21
PS-2 (salt sensitive)	38.53	28.22	21.31		29.07	18.44	11.74
Mustard							
Jun-0536	44.01	37.07	22.23		25.33	18.31	12.91
BARI Sarisha-16	55.41	44.71	39.41		23.11	19.61	14.91
BARI Sarisha-11 (tolerant)	45.41	34.81	29.04		22.42	15.09	11.55
BARI Sarisha-14(salt sensitive)	49.33	37.42	31.11		20.11	14.34	10.88

Table 1b: Mean values of fresh weight and dry weight of sunflower and mustard genotypes under different level of salinity

Cultivar/ genotypes	**Fresh weight (g/plant)**				**Dry weight (g/plant)**		
	0 ds/m	**8 ds/m**	**12 ds/m**		**0 ds/m**	**8 ds/m**	**12 ds/m**
Sunflower							
GP-4030	58.87	45.94	30.93		8.91	7.11	5.76
BARI Surjamukhi-2	52.07	37.05	33.93		9.47	7.55	3.54
Hysan-33 (tolerant)	49.21	33.82	29.26		8.06	6.92	5.08
PS-2 (salt sensitive)	44.84	27.75	23.36		7.83	5.74	4.44
Mustard							
Jun-0536	34.87	25.94	19.93		7.91	4.91	3.96
BARI Sarisha-16	36.21	27.05	20.93		8.43	5.35	3.94
BARI Sarisha-11 (tolerant)	31.84	21.75	14.36		6.43	4.74	4.14
BARI Sarisha-14(salt sensitive)	33.21	23.82	17.26		8.26	5.92	3.08

Relative water content (RWC)

Results pertaining to relative water content revealed significant reduction in test materials as compared to control under salt stress. Maximum reduction of RWC was observed salt sensitive sunflower (PS-2) and mustard (BARI Sarisha-14) genotypes at 12 dSm^{-1} salinity and minimum reduction was observed in tolerant variety Hysan-33 and BARI Sarisha-11 as well as selected genotypes, GP-4030, BARI Surjamukhi-2, and Jun-0536, BARI Sarisha-16 in NaCl stress (Fig. 1a and 1b). The RWC in the selected genotypes was almost similar to Hysan-33 and BARI Sarisha-11.

Regulation of water balance measured in terms of leaf RWC is considered as one of the most important adaptations under salinity stress (Ashraf 2004; Noreen et al. 2010). In the present study, RWC decreased in all sunflower and mustard genotypes compared to the control (Fig. 1a and 1b) whereas the selected tolerant genotypes GP-4030, BARI Surjamukhi-2, and Jun-0536, BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 maintained higher RWC compared to salt sensitive genotypes PS-2 and BARI Sarisha-14 (1a and 1b). Similar decrease in RWC due to salt stress was reported earlier in a salt sensitive genotypes rice (Hasanuzzaman et al. 2014), pea (Ahmad and Jhon 2005), *Populuscathayana* (Yang et al., 2009), olive (Boussadia et al., 2008), turnip (Noreen et al., 2010) and mulberry (Ahmad and Sharma 2010). Increases concentration of soil water under salinity

hampers the uptake of water and nutrients inducing osmotic effects and ion toxicity (Duan et al. 2005). The tolerant genotypes also differed significantly in RWC compared to the salt sensitive genotype at high salt (12 dSm^{-1}) which might be due to genotypic variation.

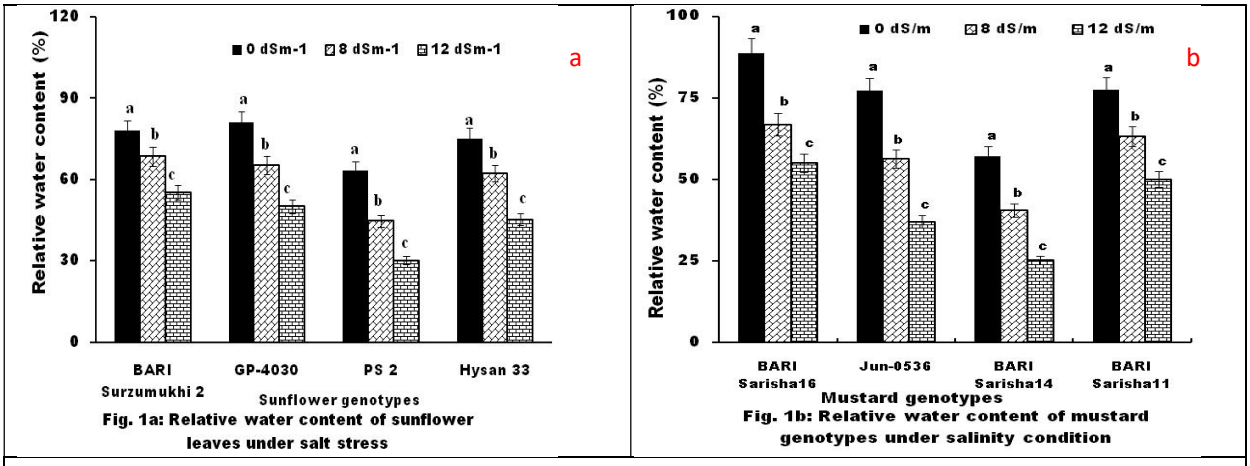


Fig 1a, b: Effect of salinity on relative water content of sunflower and mustard genotypes

Total chlorophyll (chl a and chl b) pigments:

Chlorophyll (Chl) content of sunflower and mustard leaves were decreased significantly under salinity as compared to control and the magnitude of loss was higher in salt sensitive genotype sunflower (PS-2) and mustard (BARI Sarisha-14) in all the salt levels (Fig. 2a and 2b). However, Hysan-33 and BARI Sarisha-11 maintained comparatively higher Chl content at 12 dSm^{-1} than the selected sunflower and mustard genotypes but it was statistically similar.

Photosynthetic pigments have been reported to play a key role in maintaining photosynthetic capacity of most plants (Dubey 2005). This study shows that photosynthetic pigments of leaves were reduced by salinity in all genotypes (Fig. 2a and 2b). Decrease in chlorophyll concentration in salinized plant could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy and Vora 1986) or due to the disruption of fine structure of chloroplast and instability of pigment protein complexes by ions. These results are in agrement with Sakr et al. (2012). It has been reported that chlorophyll content decreased in plants such as wheat (Arfan et al. 2007), rice (Hasanuzzaman et al. 2014) radish (Jamil et al. 2007), and pea (Hamada and El-Enany 1994) but has been increased in some plants sugar beet and cabbage (Jamil et al. 2007) under saline condition. However, the tolerant genotypes maintained higher Chl as compared to salt sensitive genotypes suggesting tolerant genotypes can continue photosyntheic rate to sustain in saline condition.

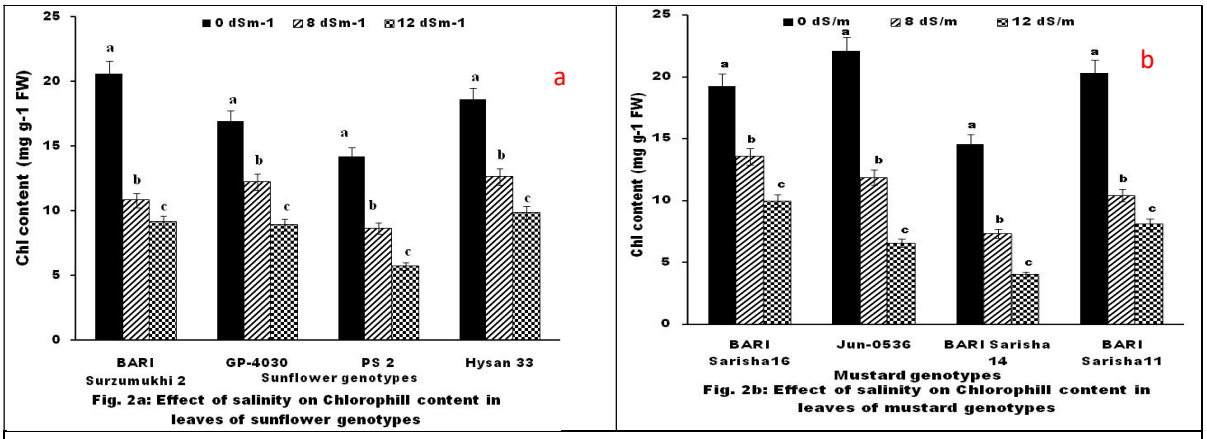


Fig. 2a: Effect of salinity on Chlorophill content in leaves of sunflower genotypes

Fig. 2b: Effect of salinity on Chlorophill content in leaves of mustard genotypes

Fig 2a, b: Chlorophyll content of sunflower and mustard genotypes under different levels of salinity

Gas exchange characteristics:

Gas exchange attributes were significantly reduced by salt stress. A substantial reduction in photosynthetic rate (*pN*), transpiration rate (*E*), stomatal conductance (*gs*), and intercellular CO_2 concentration rate (*Ci*) were observed in salt stress (Fig. 3a-h). Importantly, *pN, E* and *gs* were higher in all tolerant genotypes compared to susceptible genotypes PS-2 and BARI Sarisha-14 (Fig. 3a-f). However, the *Ci* was higher in Hysan-33 and BARI Sarisha-11 as compared to BARI Surjamukhi-2 and BARI Sarisha-11. On the other hand, in GP-4030 and BARI Sarisha-11, the *Ci* content was significantly higher than both Hysan-33 and Jun-0536. Moreover, salt stress reduces g_S and *Ci* in all sunflower genotypes; this ultimately reduces biomass production (Fig. 3g-h).

Saline stress considerably decreased *pN*, *gs*, *Ci* and *E* in all sunflower and mustard genotypes compared to control (Fig. 3a-h). However, a sharply reduction was observed in salt sensitive genotype PS-2 and BARI Sarisha-14 compared to salt tolerant genotype Hysan-33, BARI Sarisha-11 as well as GP-4030, BARI surjamukhi-2, Jun-0536, BARI Sarisha-16 in NaCl stress. It is well known that stomatal closure due to salt-induced abscisic acid accumulation is one of the vital factors which cause retardation in vital photosynthetic processes (Noreen et al. 2010; Akram and Ashraf 2011; Saleem et al. 2011). On the other hand, *pN* was markedly decreased by salt stress and this was accompanied by decrease *gs* (Fig. 3a, b, e, f). Photosynthesis has a well-established role in plant growth and dry matter production (Baker 1996). Salinity tolerance is related to the maintenance of *pN* and g_S (Khan et al. 2007). A positive significant relation between *pN* and *gs* may suggest that the reductions in *pN* were largely associated with stomatal closure, and therefore stomatal effects could be the most important to justify photosynthesis depression. From these observations, it is clear that inter-genotypic variation in the studied sunflower and mustard genotypes for salt tolerance were only due to genetic variation in photosynthetic rate which could be used as effective selection criteria for salt tolerance in different crops. Besides, *E* and *Ci* rate also decreased considerably with increasing salt concentration (Fig. 3c, d, g, h), resulting in reducing biomass production. The present research, selected tolerant sunflower and mustard genotypes had better

growth compared salt sensitive genotype under saline conditions due to higher *pN* as well as biomass production. This result correlates with earlier findings on different crops e.g. sunflower (Noreen and Asraf 2008), safflower (Siddiqi et al. 2009), wheat (Arfan et al. 2007), okra (Saleem et al., 2011), wheat (James et al., 2002), maize (Crosbie and Pearce, 1982), asparagus (Faville et al., 1999), cotton (Pettigrew and Meredith, 1994) and *Cynodondactylon* (Akram et al., 2007).

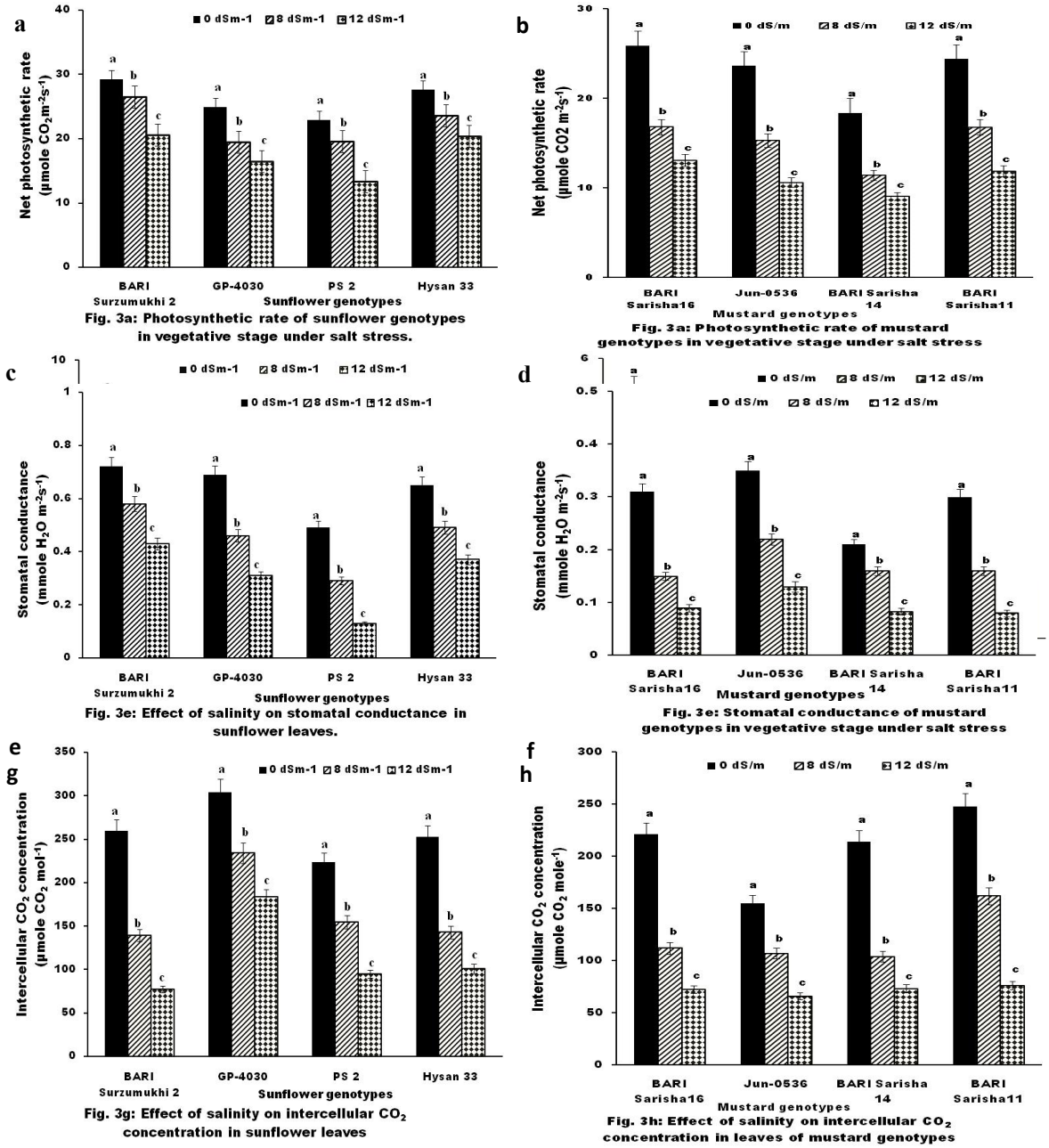


Fig. 3a: Photosynthetic rate of sunflower genotypes in vegetative stage under salt stress.

Fig. 3a: Photosynthetic rate of mustard genotypes in vegetative stage under salt stress

Fig. 3e: Effect of salinity on stomatal conductance in sunflower leaves.

Fig. 3e: Stomatal conductance of mustard genotypes in vegetative stage under salt stress

Fig. 3g: Effect of salinity on intercellular CO_2 concentration in sunflower leaves

Fig. 3h: Effect of salinity on intercellular CO_2 concentration in leaves of mustard genotypes

Fig 3a-h: Photosynthesis, stomatal conductance, CO_2-assimilation rate and transpiration rate of sunflower and mustard leaves under different levels of salinity

Effect of salinity on Na^+ and K^+ concentration and their ratio in sunflower and mustard leaves:

As compared to control, sodium ion content was significantly increased with salt levels in all sunflower and mustard genotypes (Fig. 4a-f). It was remarkable that the content Na^+ at 12 dSm^{-1} was significantly higher in PS-2 and BARI Sarisha-14 compared to all tolerant (GP-4030, BARI Surjamukhi-2, and Jun-0536, BARI Sarisha-16) genotypes. However, the content Na^+ in BARI Surjamukhi-2, GP-4030, and Jun-0536, BARI Sarisha-16 genotypes were almost similar to Hysan-33 and BARI Sarisha-11. Unlike Na^+, K^+ decreased with salt stress in all the genotypes. All though K^+ was comparative lower in PS-2 and BARI Sarisha-14, the level was all most similar to the tolerant genotypes. Data of clearly indicated that ionic balance in all the tolerant genotypes was better than salt sensitive genotypes (Fig. 4a-f).

Plant survival under saline conditions primarily depends on the ion homeostasis. Ion flux regulation is important for ensuring ionic balance where the concentration of essential ions is greater and the toxic ions below the range. Salinity can create an ionic imbalance (Chen et al. 2005). The increase in Na^+ ion content and decrease in K^+ ion uptake disturbs ionic imbalance as observed in most species exposed to salt stress.

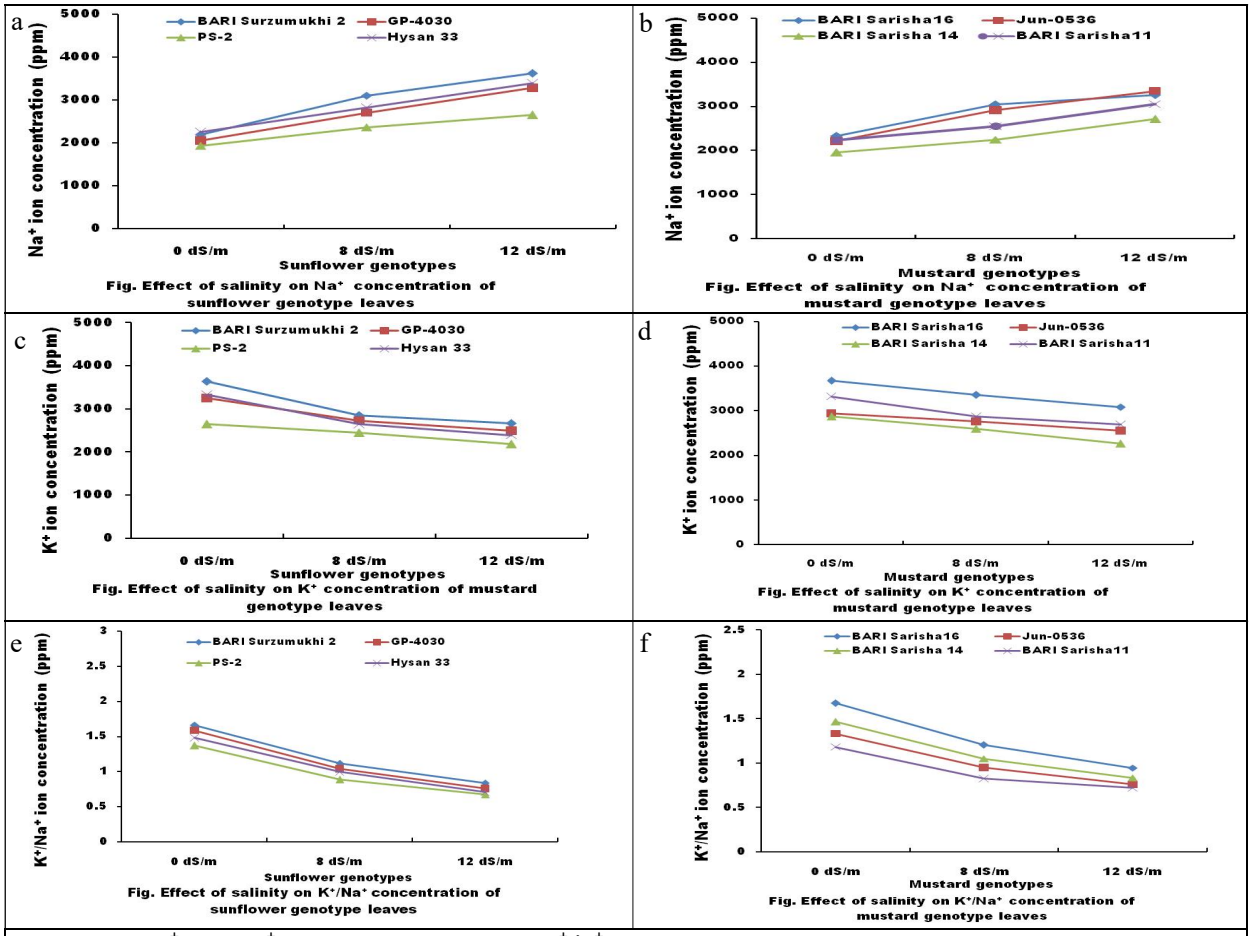


Fig 4a-f: Na^+ and K^+ concentration and Na^+/K^+ ratio of sunflower and mustard genotypes under different levels of salinity

Our results also showed that the Na^+ content in salt-treated sunflower significantly increased with the increasing salinity level concentrate with reversal of K^+. Salinity caused higher Na^+ accumulation which in K^+ concentrations in PS-2 and BARI Sarisha-14 as compared Hysan-33 and BARI Sarisha-11 as well as GP-4030, BARI Surjamukhi-2, and Jun-0536, BARI Sarisha-16 (Fig 4a-f). The increase of Na^+ in plants is generally associated with a decrease in K^+. Lower K^+/Na^+ ratio is also indicated Na^+ mediated damage. Maintenance of low ratios of K^+/Na^+ is suitable for the metabolic processes occurring within the plants and essential for the plants to survive salt stress and K^+/Na^+ may be used as a possible criterion for selecting salt tolerant genotypes (Chen et al. 2005). However, the preservation of the favorable K^+/Na^+ ratio in the cytoplasm under salt stress may be due to an effective partitioning of both ions (Munns et al. 2006). High K^+/Na^+ in plants have been suggested to be an important selection criterion of salt tolerance for the species (Greenway and Munns 1980; Ashraf 2002; Wenxue et al. 2003). Our results are in support of the above data, as well as of the findings of Chen et al. (2007), showing higher K^+/Na^+ ratio in shoot of selected sunflower and mustard genotypes under all saline treatments (Fig. 4a-f).

Proline content:

Variation in proline accumulation among the genotypes was found under salt stress, although several folds of proline were recovered in 12 dSm^{-1} salinity in all the genotypes (Fig. 5a-b). Genotypes, GP-4030, BARI Surjamukhi-2, and Jun-0536, BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 had significantly proline accumulation than salt sensitive genotype PS-2 and BARI Sarisha-14 (Fig. 5a-b). However, the proline content was similar among the tolerant genotypes.

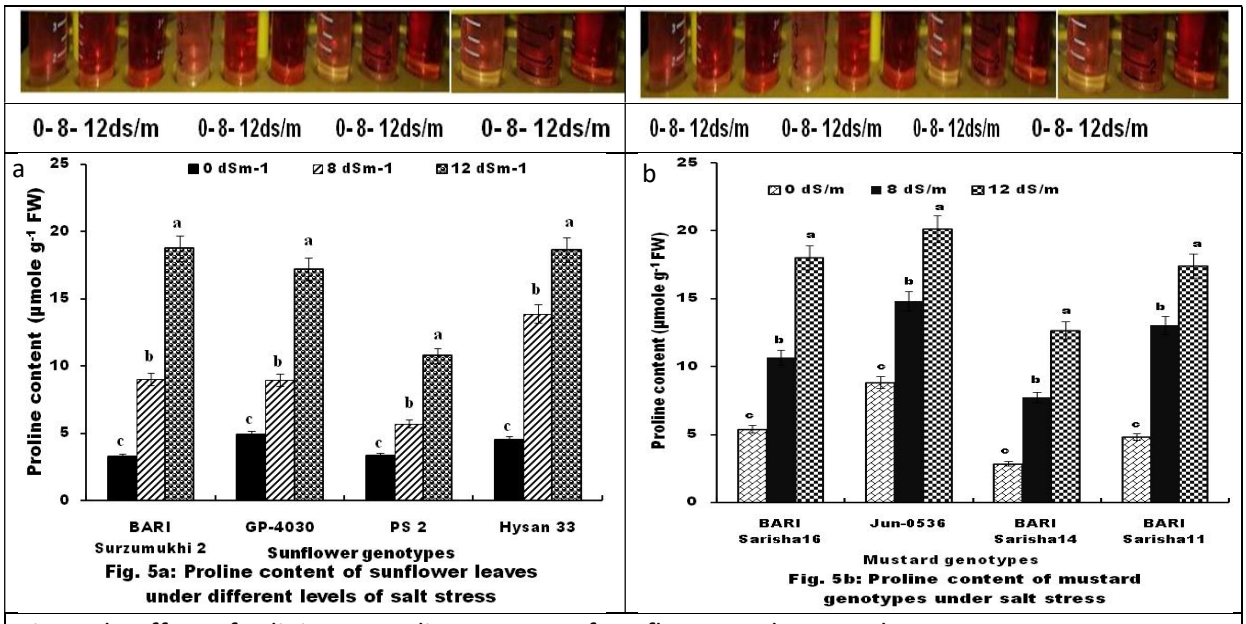


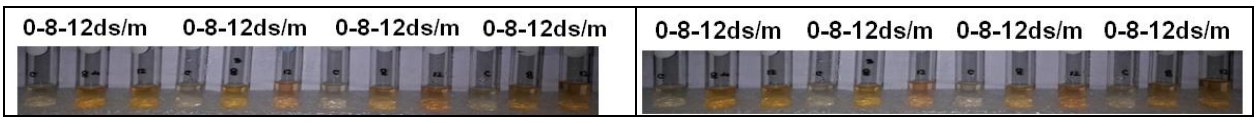
Fig 5a, b: Effect of salinity on proline content of sunflower and mustard genotypes

Proline is known to function in cellular osmotic adjustment, protection of cellular damage, detoxification of ROS, and protection of membrane integrity and stabilization of enzymes, storage of nitrogen and scavenging of free radicals and hence, it is often suggested as a selection criteria under stress environment (Ashraf and Foolad 2007; Hayat et al. 2012). In our experiment, both selected and referred tolerant genotypes accumulated higher proline as compared to salt sensitive genotype under any level of salt stress (Fig. 5a-b). Proline accumulation as a non-toxic protective osmolyte under salinity has been reported in maize (Rohman et al. 2016a) and canola (Bandeh-hagh et al. 2008). Besides, relatively high accumulation of proline in salt tolerant genotypes has been reported in pea (Noreen and Ashraf 2009), *B. juncea* (Hayat et al. 2011), proso millet (Sabir *et al.,* 2011) and sugarcane (Chaum and Kirdmanee 2009).

Lipid peroxidation and accumulation of Malondialdehyde (MDA)

Malondialdehyde (MDA) content (indicator of lipid peroxidation) gradually increased as salinity level increased at 12 dSm^{-1} salt stress in all sunflower and mustard genotypes (Fig. 6a, b). Data also revealed higher MDA production in salt sensitive genotypes PS-2 and BARI Sarisha-14 compared to Hysan-33 and BARI Sarisha-11 and selected genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16. Important that selected genotype GP-4030 had comparatively lower MDA at 12 dSm^{-1} than Hysan-33 and BARI Sarisha-11.

Lipid peroxidation is a well-known index for determining is extent of oxidative stress because increased MDA content has been found to be highly correlated with oxidative damages induced by various abiotic stresses including salinity (Garg and Manchanda 2009). As oxidation product, MDA formed when ROS such as $OH^{\bullet-}$ and $^1O_2$ attack poly unsaturated fatty acid (PUFA) of cell wall (Gill and Tuteja 2010). Thus lower level of MDA content in maize tested indicated better protection against oxidative damage under saline stress (Rohman et al. 2016a). In our study, lower MDA concentration was observed in the tolerant genotypes under salinity, suggesting lower oxidative damage to sustain under salinity. Such a pattern of MDA accumulation has already been observed in sesame (Koca et al. 2007), maize (Azooz et al. 2009), okra (Saleem *et al.*, 2011), mulberry (Ahmad *et al.,* 2010a) and wheat (Ashraf et al. 2010).



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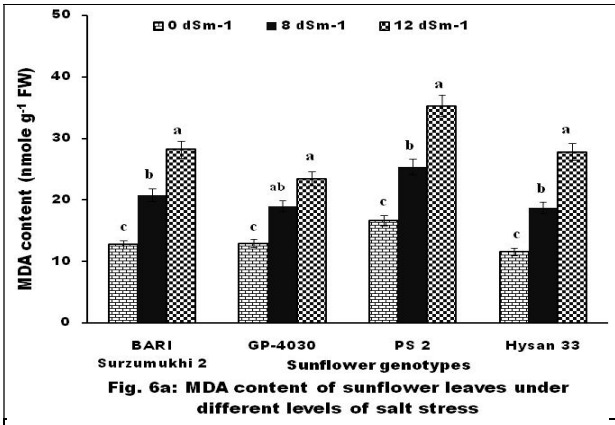


Fig. 6a: MDA content of sunflower leaves under different levels of salt stress

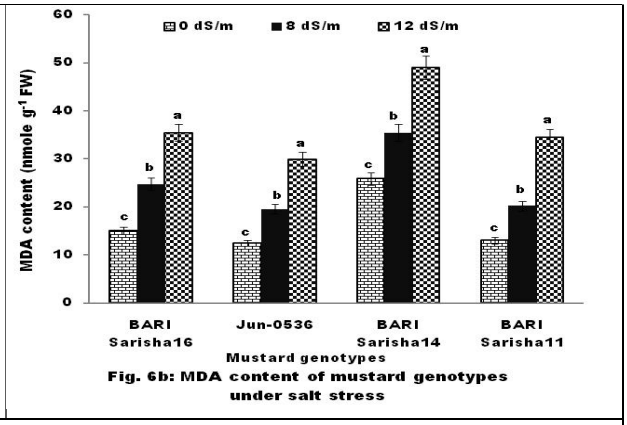


Fig. 6b: MDA content of mustard genotypes under salt stress

Fig. 6a, b: Effect of salinity on lipid peroxidation (MDA) content of sunflower and mustard genotypes

Histochemical detection of Hydrogen peroxide and superoxide generation content

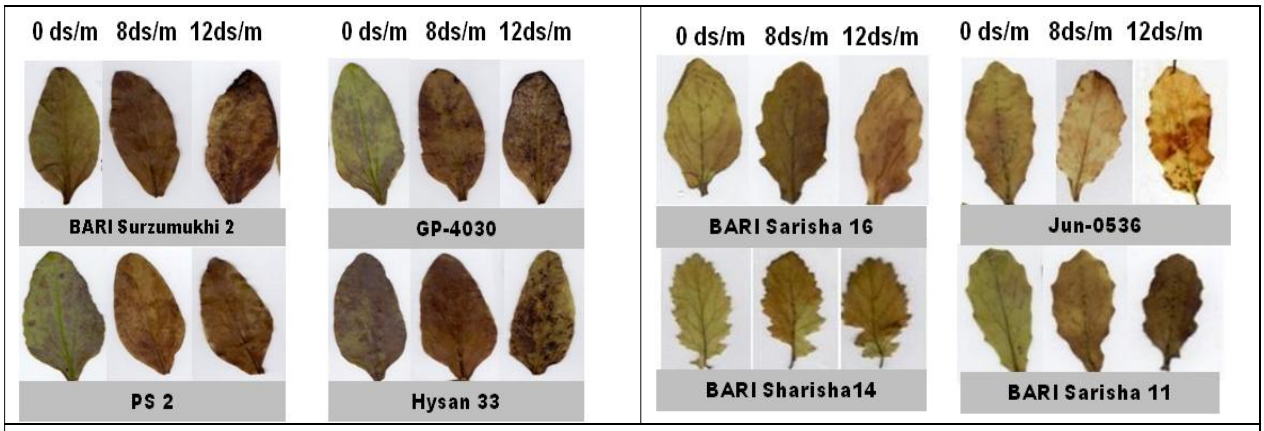
Histochemical staining also showed detected higher H_2O_2 and $O_2^{\bullet-}$ with dark blue insoluble formazan product or spots and deep brown polymerization product or spots localization, respectively, in salt loaded leaves of sunflower and mustard seedling. These results supported the results of H_2O_2 production and $O_2^{\bullet-}$ generation rate in Fig. 7a-d.

Hydrogen peroxide (H_2O_2) and $O_2^{\bullet-}$ generation content:

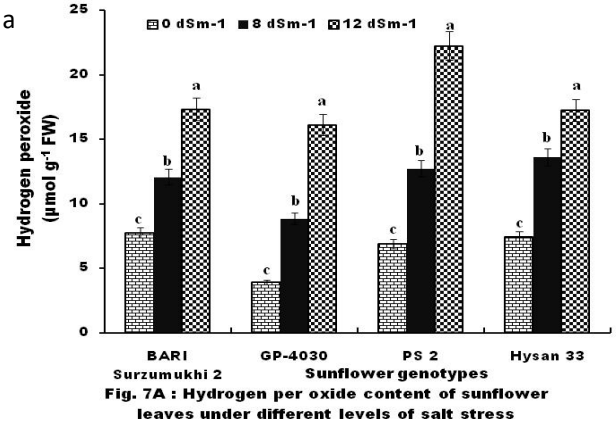
As salt level increased both H_2O_2 and $O_2^{\bullet-}$ increased sharply and significantly as compared to respective control (Fig. 7a-d). It was very clear that sensitive genotype showed significantly higher H_2O_2 concentration and $O_2^{\bullet-}$ production rate as compared to all sunflower and mustard tolerant genotypes at 12 dSm^{-1} salt level, although the values were almost similar among the tolerant genotypes.

Overproduction of ROS as compared to control remarkable increases in $O_2^{\bullet-}$ and H_2O_2 contents were observed in all sunflower and mustard genotypes under salinity, and the levels were significantly higher in salt sensitive PS-2 and BARI Sarisha-14 compared to Hysan-33 and BARI Sarisha-11 as well as selected genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16 (7a, d). Both $O_2^{\bullet-}$ and H_2O_2 are highly reactive and can modify protein, lipid and pigments to lead cell death (Gill and Tuteja 2010). Therefore, they must be kept under toxic level. With this view, a genotypes with lower level of $O_2^{\bullet-}$ and H_2O_2 have least possibility of oxidative damage. Therefore, salt sensitive PS-2 and BARI Sarisha-14 has more possibility of cell damage, because both higher $O_2^{\bullet-}$ and H_2O_2 as well MDA are highly risky for leakage of cell wall. Previously, tolerant genotypes under salinity have been reported with lower $O_2^{\bullet-}$ and H_2O_2 as well MDA (Rohman et al. 2016a; Hasanuzzaman et al. 2014). Therefore, lower ROS in the selected genotypes as well as Hysan-33 may

be important for their salt tolerance, which was in agreement with several previous reports (Azooz et al. 2009; Weisany et al. 2012).



Histo-chemical detection of H_2O_2 in sunflower and mustard leaves under different levels of salinity



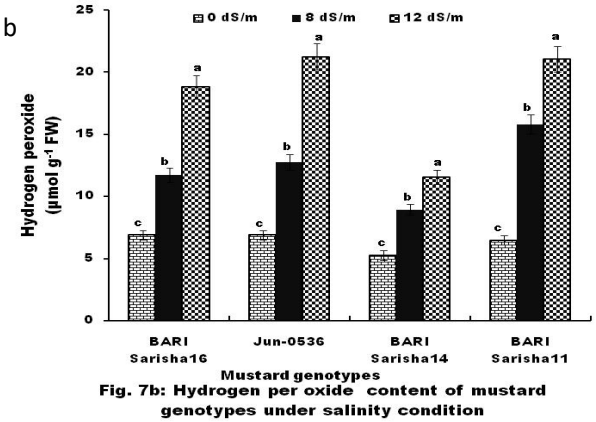
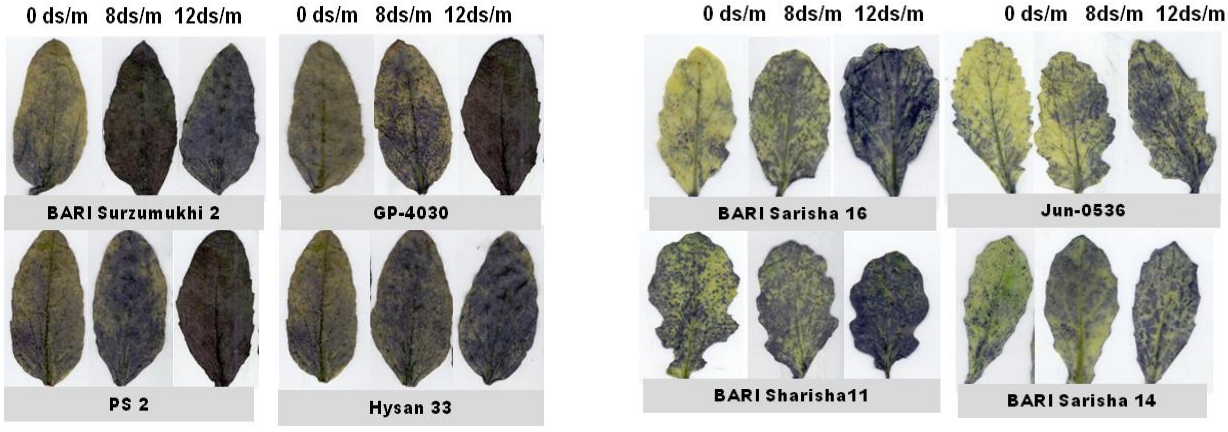


Fig 7 a, b: Hydrogen peroxide in sunflower and mustard genotypes leaves under different levels of salinity



Histo-chemical detection of $O_2^{\bullet-}$ in sunflower and mustard leaves under different levels of salinity

C d

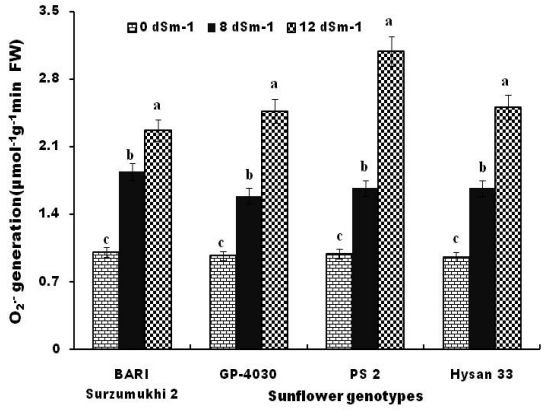


Fig. 7c: Effect of salinity on $O_2^{\bullet-}$ generation in sunflower leaves

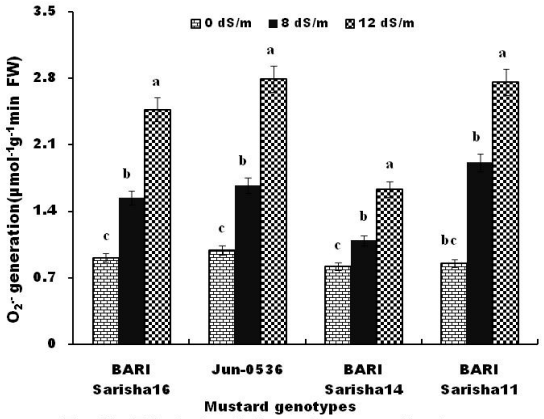


Fig. 8b: Effect of salinity on $O_2^{\bullet-}$ generation in mustard leaves

Fig 7 c, d: Superoxide generation ($O_2^{\bullet-}$) in sunflower and mustard genotypes under different levels of salinity

Activities of anti-oxidant enzymes:

Super oxide dismutase (SOD):

Activity of SOD varied significantly with genotypes under salt stress as well as control condition (Fig. 8a, b). In comparison to respective control of tolerant genotypes, the activity was significantly increased in Hysan-33, BARI Sarisha-11 and selected genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16. (Fig. 8a, b). On the other hand, in sensitive genotype PS-2 and BARI Sarisha-14 the activity increased only at 12 dSm^{-1} salt level. It was important that salt sensitive genotype had comparatively lower SOD activity under salinity as compared to other genotypes (Fig. 8a, b). Here also increased activity was observed in Hysan-33, GP-4030, BARI Surjamukhi-2, BARI Sarisha-11, Jun-0536 and BARI Sarisha-16 and slightly increased in sensitive genotype PS-2 and BARI Sarisha-14 at 12 dS m^{-1} salt stress.

Scavenging of ROS is essential for survival of plant under salinity (Shao et al. 2007). Among the ROS metabolizing enzymatic antioxidants, SOD converts $O_2^{\bullet-}$ to H_2O_2 and excessive accumulation of H_2O_2 is one of the indicator of oxidative stress (Apel and Hirt 2004). It gets dismutate to water and oxygen with the help of antioxidant enzymes like CAT, POD, APX and GPX (Gill and Tuteja 2010). Higher SOD activity in GP-4030, BARI Surjamukhi-2, Hysan-33, Jun-0536 and BARI Sarisha-16 as compare to sensitive PS-2 and BARI Sarisha-14 genotypes under salt stress are capable to provide better protection for $O_2^{\bullet-}$ mediated oxidative damage. Comparatively higher induction in SOD activity by salinity has been reported in other plant species (Hadiye et al., 2007; Fedina et al., 2009; Zagorchev et al. 2014; Rohman et al. 2016a).

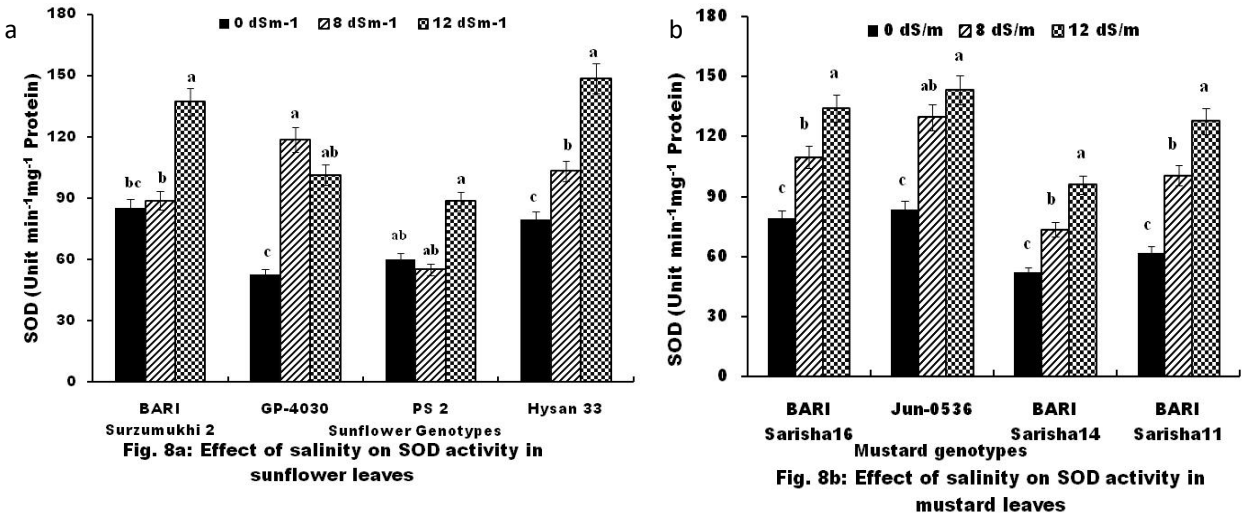


Fig. 8a: Effect of salinity on SOD activity in sunflower leaves

Fig. 8b: Effect of salinity on SOD activity in mustard leaves

Fig. 8a, b: Superoxide dismutase activity of sunflower and mustard genotypes under different levels of salinity

Catalase (CAT):

Differential CAT activities in response to salt stress were found among the genotypes (Fig. 9a, b). CAT which is through to be the most powerful enzyme to decomposed H_2O_2 induced in different concentration in tolerant genotypes. The susceptible genotype also induced CAT activity but it was comparatively lower than other genotypes. Here also higher increased activity was observed in tolerant than salt sensitive genotype which supported the specific activities in Fig. 9a, b.

Catalase is one of the vital enzymes in scavenging H_2O_2 in plant cells exposed to various abiotic stresses due its higher turnover rate of reaction (Garg and Manchanda 2009). The role of CAT in scavenging H_2O_2 was observed in several studies (Hasanuzzaman et al. 2011a; Hasanuzzaman and Fujita 2013). In this study, CAT activity was significantly decreased upon exposure to salt stress in sensitive genotype PS-2 and BARI Sarisha-14 and this decrease in CAT activity in PS-2 and BARI Sarisha-14 under salt stress might be due to its inactivation by the accumulated H_2O_2 induced by water shortage or ineffective enzyme synthesis or change in assembly of enzyme sub-units (Gupta et al. 2009). On the other hand, CAT activity was slightly increased with the increase NaCl stress in selected sunflower and mustard genotypes GP-4030, BARI Surjamukhi-2, Jun-0536 and BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 (Fig. 9a, b). This trend was supported by earlier reports (Azooz et al. 2009; Hasanuzzaman et al. 2014).

a b

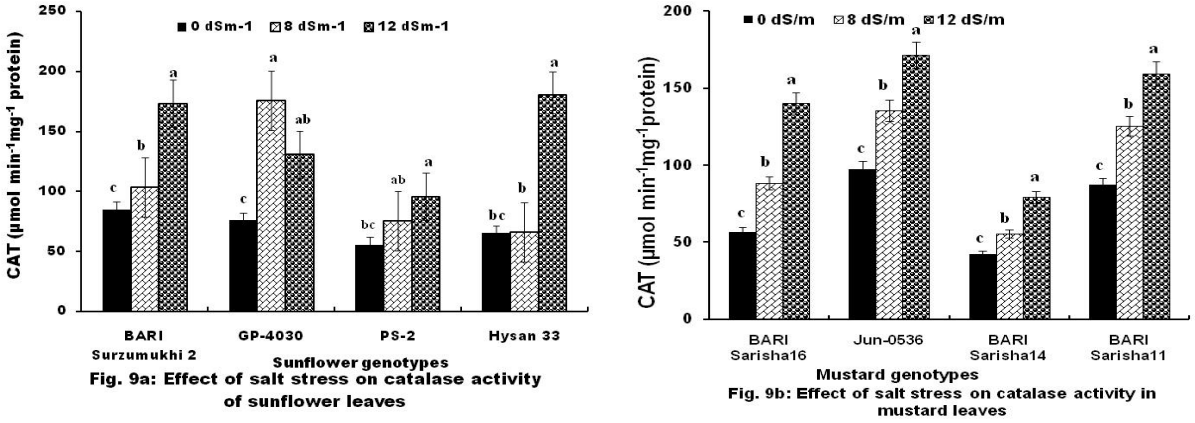


Fig. 9a, b: Catalase activity of sunflower and mustard genotypes under different levels of salinity

Peroxidase (POD)

The POD activity varied sunflower and mustard genotypes under both control and salinity (Fig. 10a, b). However, there was no harmony in the induction of the activity except Hysan-33. In comparison to control of selected tolerant genotypes remained almost similar under salinity and the activity increased in genotypes BARI Surjamukhi-2 at 8 dSm^{-1} but no increased in genotype GP-4030 compared to the control under salt stress (Fig. 10a, b). On the other hand, the activity slightly increased in salt sensitive genotype at 8 dSm^{-1} under salt stress.

Increasing POD activity by salinity stress has been reported in various plants (Meloni et al. 2003; Li et al., 2014; Rohman et al., 2015). In higher plants, H_2O_2 is scavenged by the non-specific PODs (Miller et al. 2010). POD along with CAT and APX are reported to scavenge H_2O_2 to water in plant species (Gill and Tujeta 2010; Miller et al. 2010). The increased activities of POD under salt stress played important role in H_2O_2 scavenging (Rohman et al. 2016a). In this study, selected genotypes, GP 4030, BARI Surjamukhi-2, Jun-0536 and BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 had higher specific POD activity (Fig. 10a, b), suggesting its role in H_2O_2 metabolism in this genotypes.

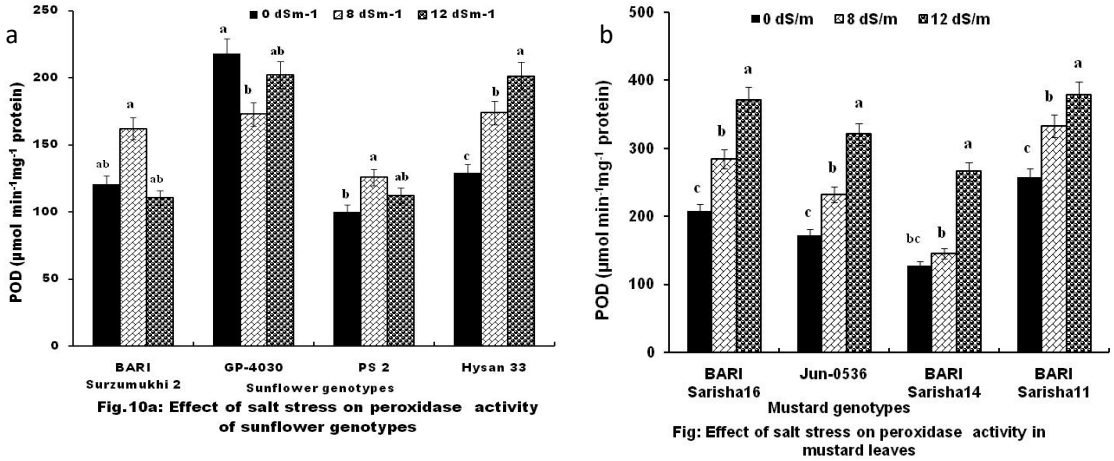


Fig 10a, b: POD activity of sunflower and mustard genotypes under different levels of salinity

Ascorbate per oxidase (APX)

Salinity increased APX activity in all sunflower and mustard genotypes (Fig. 11a, b). Importantly, all the tolerant genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536 and BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 maintained higher APX activity than salt sensitive genotypes PS-2 and BARI Sarisha-14 (Fig. 11a, b).

The enzymes APX of the ascorbate-glutathione (AsA-GSH) cycle work with AsA, GSH and NADPH and together detoxify H_2O_2 in a series of cyclic reactions and again regenerate AsA and GSH (Apel and Hirt 2004; Noctor et al. 2012). Ascorbate peroxidase catalyzes the reduction of H_2O_2 to H_2O by using AsA (Xu et al. 2008). Thus higher inductions in APX activity in tolerant genotypes (Fig. 11a, b) are capable to decompose H_2O_2 to H_2O by using AsA. However, the increased activities of APX might have important role in AsA recycling in the tolerant genotypes thus they helped in APX mediated H_2O_2 metabolism to confer tolerance in this genotypes. Moreover, H_2O_2 is reduced to water by APX and plays a role in cell defense mechanism (Kangasjarvi et al., 2008; Ashraf, 2009). Though several studies reported to increase APX activity in plants under salinity stress (Nounjan et al., 2012), while in some other its activity decreased (Azooz et al., 2009; Sharma and Dubey, 2005) and another study no change in APX activity was observed (Bartoli et al., 1999).

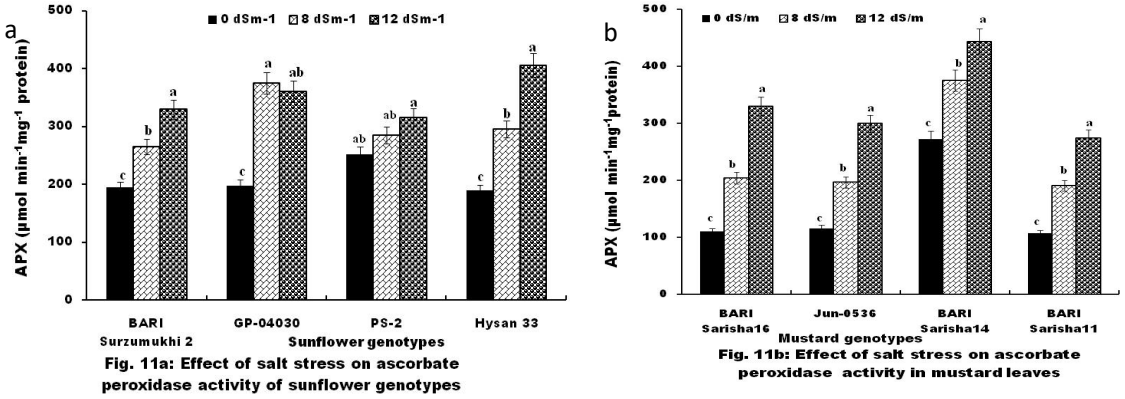


Fig. 11a: Effect of salt stress on ascorbate peroxidase activity of sunflower genotypes

Fig. 11b: Effect of salt stress on ascorbate peroxidase activity in mustard leaves

Fig 11 a, b: APX activity of sunflower and mustard genotypes under different levels of salinity

Glutathione S-Transferase (GST)

The GST which shows both GPX and GST activity increased significantly with salt level in all the genotypes (Fig. 12a, b). However, the activity was comparatively lower in salt sensitive genotype PS-2 and BARI Sarisha-14. In comparison to salt sensitive genotype, the activity of GST was significantly increased in tolerant genotypes Hysan-33 and BARI Sarisha-11 as well as selected genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536 and BARI Sarisha-16 at 12 dSm^{-1} salinity stress (Fig. 12a, b).

Plant GSTs, multifunctional family enzyme, catalyze conjugation of glutathione with xenobiotic compounds for detoxification and stress tolerance is often correlated with enhanced activity of GST (Nocter et al. 2012). Detoxify hydro-peroxide directly have important role in growth and development (Gong et al. 2005). The GST activity might have also role in leaf senescence in

sunflower and mustard genotypes under salt stress. In our experiment, GST activity significantly increased under salt stress where comparatively higher activity was observed in salt tolerant Hysan-33, and BARI Sarisha-11 and selected genotypes GP-4030, BARI Surjamukhi-2, Jun-0536 and BARI Sarisha-16 than in salt sensitive sunflower PS-2 and BARI Sarisha-14 (Fig. 15). Our results are partially supported by Hasanuzzaman et al. (2014). However, the higher GST activity in tolerant under higher salt concentration might also be involved in growth of sunflower (Gong et al. 2005).

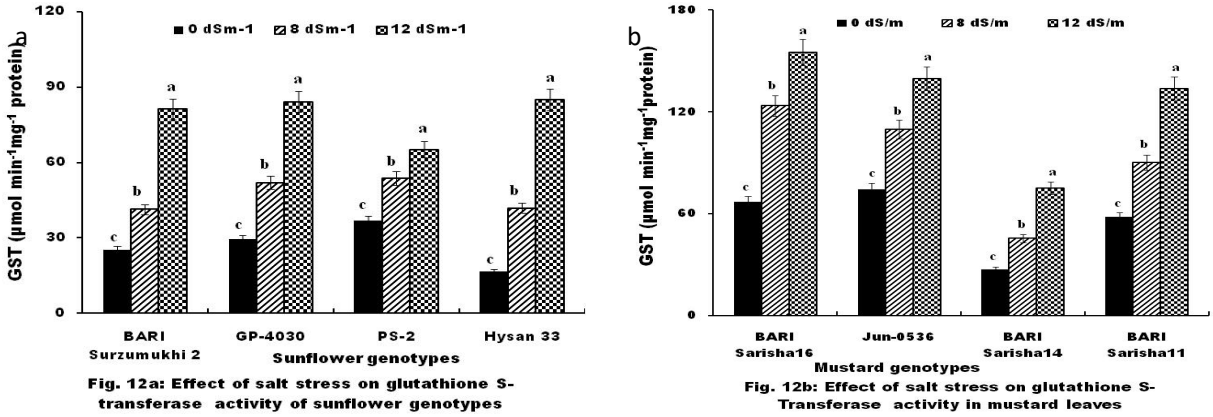


Fig. 12a: Effect of salt stress on glutathione S-transferase activity of sunflower genotypes

Fig. 12b: Effect of salt stress on glutathione S-Transferase activity in mustard leaves

Fig. 12a, b: GST activity of sunflower and mustard genotypes under different levels of salinity

Glutathione reductase (GR)

Glutathione reductase activity increased in all sunflower and mustard genotypes under salt stress compared to control (Fig. 13a, b). However, the increment was comparatively higher in tolerant genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 than the sensitive genotypes, PS-2 and BARI Sarisha-14 (13 a, b).

Glutathione reductase is another important enzyme of glutathione-ascorbate (GSH-AsA) cycle which is important for maintaining GSH pool in plant cells. On the other hand, GSH is used by GPX, GST and other metabolic system. Therefore, GR is necessary for accelerating the H_2O_2 scavenging (Pang and Wang 2010) as well as to enhance plant tolerance against oxidative stress (Khanna Chopra and Selote 2007). In our experiment, data showed that tolerant genotypes had higher increment in GPX activity than the salt sensitive genotype, suggesting their better maintain of GSH under salt stress. Higher activity of GR in stress tolerant plants was observed in several studies (Aghaei et al. 2009; Rohman et al. 2016a).

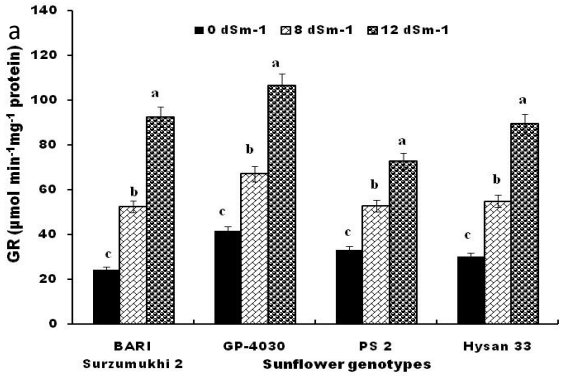


Fig. 13a: Effect of salt stress on glutathione reductase activity of sunflower genotypes

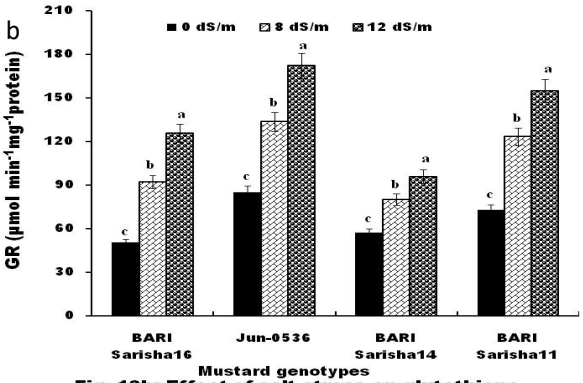


Fig. 13b: Effect of salt stress on glutathione reductase activity in mustard leaves

Fig. 13a, b: GR activity of sunflower and mustard genotypes under different levels of salinity

Guaiacol peroxidase (GPX)

Glutathione peroxidase activity varied in both control as well as stress condition in all genotypes (Fig. 14a, b). The increment was comparatively higher in sensitive genotypes, PS-2 and BARI Sarisha-14 than the tolerant genotypes, GP-4030, BARI Surjamukhi-2, Jun-0536, BARI Sarisha-16 as well as Hysan-33 and BARI Sarisha-11 (14 a, b).

The GPX is another vital enzyme of antioxidant defense system and due to substrate specifications and stronger affinity for H_2O_2 it can efficiently scavenge, especially, H_2O_2 and thus provide protection against salt stress (Noctor et al. 2012). In this experiment, salt tolerant Hysan-33 and BARI Sarisha-11 as well as sensitive PS-2 and BARI Sarisha-14 showed higher activities of GPX. Salinity can inhibit the enzymes low GSH availability. However, higher GPX activity in

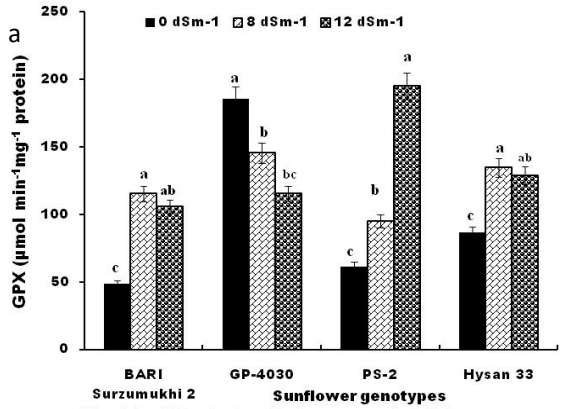


Fig. 14a: Effect of salt stress on glutathione peroxidase activity of sunflower genotypes

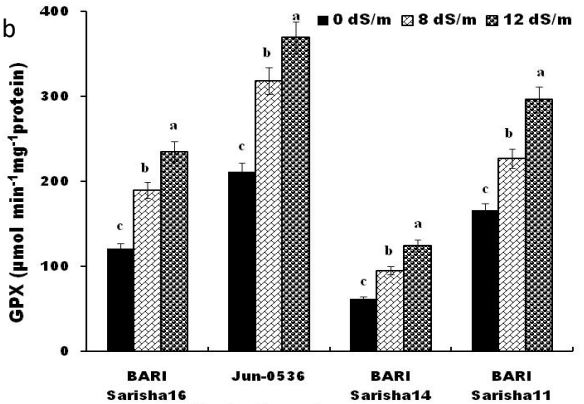


Fig. 14b: Effect of salt stress on glutathione peroxidase activity in mustard leaves

Fig 14a, b: GPX activity of sunflower and mustard genotypes under different levels of salinity

sensitive PS-2 and BARI Sarisha-14 are important of survival mechanism for these genotypes. Increased GPX activity has been reported by Zhang *et al*. (1995) and Rohman *et al*., (2015) in maize under salt stress. Differential response of GPX activity in salt sensitive and tolerant varieties was also reported in many plant studies (Hasanuzzaman et al. 2011a, b; Sai Kachout et al. 2013).

At all, the differential growth of selected sunflower and mustard cultivars under saline conditions was found to be attributable to differential physiological and biochemical parameters, hence these could be used as potential selection criteria for screening sunflower and mustard germplasm for salt tolerance. Such information would be useful in sunflower and mustard breeding programs for improving salinity to tolerance.

Oil quality relation under salt stress of selected sunflower and mustard genotypes

Oil content

Oil contents of different sunflower (40-48%) and mustard (35-44%) genotypes ranged between 32 to 50%. The results of oil extraction from sunflower and mustard under this study are presented in the Table 1. Abdullahi *et al.,* (1991) reported that sunflower and mustard seeds are good source of oil. The oil content of the seeds varied from 35 to 48%. The results of present study are in genotypes with the conclusion of Mohammed and Hamza, (2008). Nzikou et al. (2009) reported that sunflower and mustard seed contain 49% and 44%, respectively which indicated that sunflower and mustard seed as an excellent source of oil.

Fatty acid composition

The fatty-acid compositions of sunflower and mustard samples for each variety are presented in Table 2. There is no significant difference between control and 12 dS/m saline stress in sunflower and mustard genotypes (Table 2) in fatty acid composition.

The fatty acid peaks of sunflower and mustard oil that contained only 10-16% total saturated fatty acid (SFA) and 44-60% mono unsaturated fatty acid (MUFA) and 25-35% for mustard and 55-65% polyunsaturated fatty acid (PUFA). The main saturated fatty acids in sunflower and mustard seed oil were palmitic (8.67%), stearic (2-6%) acids and 5-7% to 0.5-1.0% arachidic acid as illustrated by Nzikou *et al.* (2009). The oil samples of sunflower and mustard contained saturated and unsaturated acids (14.90% and 85.10%), respectively. Sunflower and mustard oil are predominantly made up of oleic and linoleic acids, respectively. The results are in agreement with reports by Nzikou *et al.* (2009). Palmitic acid is the predominant saturated fatty acid of sunflower and mustard. The oleic and linoleic acid balance in the genotype increases the oil stability (Arslan et al. 2007). The level of MUFA depends on the level of oleic acid. The European Commission reports that erucic acid 22:1 in vegetable oils must be present at the maximum value of 5.0% for human health as studied Citil et al. (2011). In this study, erucic acid was found to be between 35-48%. The long-chain *ω-3* and *ω-6* fatty acids are commonly called PUFAs. Long-chain *ω-3* PUFAs cannot be readily synthesized by the human body and are mostly obtained through the diet and *ω3/ω6* ratios are considered to be important as reported Alasalvar et al. 2002; Pigott and Tucker, (1987). Citil et al. (2011) noted that an increase in the *ω3/ ω6* PUFA ratio increased the availability of *ω3* PUFAs, which are beneficial for human health. However, fatty acid composition of edible oil, SFA, MUFA and PUFA (1:1-3:1), and linoleic/ linolenic (*ω3/ ω6)* (5-10:1) ratio are good quality oil for human health.

Table:2 Oil content (%) and fatty acid composition of selected sunflower and mustard genotypes under salt stress

Sample name	Oil/Fat (%)	Saturated fatty acid (%)							Unsaturated fatty acid (%)						
									Mono				Poly		
		Myristic ($C_{16:0}$)	Palmitic ($C_{16:0}$)	Stearic ($C_{18:0}$)	Arachidic ($C_{20:0}$)	Behenic ($C_{22:0}$)	Lignoceric ($C_{24:0}$)	TSFA	Palmitolic ($C_{16:1}$)	Oleic/ *ω-9* ($C_{18:1}$)	Erucic ($C_{22:1}$)	TMUFA	Linoleic/ *ω-6* ($C_{18:2}$)	Linolenic/ *ω-3* ($C_{18:2}$)	TPUFA
Jun-0536 (Control)	**35**	0.11	4.48	1.41	6.12	1.13	1.55	**14.8**	0.49	12.61	35.68	**48.78**	25.71	7.76	**33.47**
Jun-0536 (12 dS/m)	**32**	0.48	5.18	1.25	5.48	1.15	2.52	**16.06**	0.52	10.74	35.05	**46.31**	26.56	7.99	**34.55**
BARI Sarisha 14 (Control)	**39**	0.11	1.69	0.86	5.72	1.79	0.19	**10.36**	0.61	21.79	44.42	**66.82**	10.92	7.76	**18.68**
BARI Sarisha 14 (12 dS/m)	**38**	0.36	3.89	2.65	7.63	0.63	0.34	**15.5**	0.41	18.79	37.94	**57.14**	19.27	4.98	**24.25**
BARI Sarisha 16 (Control)	**34**	0.12	4,31	0.84	4.88	1.06	2.11	**9.01**	0.41	10.39	39.97	**50.77**	24.65	8.32	**32.97**
BARI Sarisha 16 (12 dS/m)	**33**	0.12	4.72	1.70	5.64	2.78	1.48	**16.44**	1.44	13.16	37.71	**52.31**	23.16	6.58	**29.74**
BARI Sarisha 11 (Control)	**39**	0.11	3.48	1.37	7.13	0.77	1.16	**14.02**	0.41	13.63	45.89	**59.93**	17.68	6.95	**24.63**
BARI Sarisha 11(12 dS/m)	**40**	0.23	4.33	1.63	7.59	1.11	1.32	**16.21**	0.43	15.35	41.64	**57.42**	18.65	6.55	**25.2**
GP-4030 (Control)	**41**	0.06	6.69	4.42	0.26	0.61	0.14	**12.18**	0.07	23.87	23.94	**47.88**	63.88	0.22	**64.00**
GP-4030 (12 dS/m)	**43**	0.09	7.22	4.69	0.54	0.98	0.21	**13.73**	0.11	25.21	25.32	**50.64**	65.33	0.19	**65.52**
BARI Sun 2 (Control)	**44**	0.21	12.23	3.78	0.22	0.51	0.17	**17.12**	0.33	30.01	30.34	**60.68**	51.76	0.26	**52.02**
BARI Sun 2 (12dS/m)	**42**	0.11	6.63	5.42	0.29	0.55	0.13	**13.13**	0.06	22.33	22.39	**44.78**	64.52	0.08	**64.60**
PS 2 (Control)	**50**	0.07	6.86	5.61	0.31	0.71	0.15	**13.71**	0.09	32.51	32.60	**65.2**	53.63	0.12	**53.75**
PS 2 (12 dS/m)	**49**	0.09	7.22	6.11	0.29	0.91	0.21	**14.83**	0.22	34.33	34.55	**69.1**	54.55	0.19	**54.74**
Hysan 33(Control)	**48**	0.05	6.61	6.34	0.43	0.95	0.11	**14.49**	0.08	23.84	23.92	**47.84**	61.49	0.11	**61.60**
Hysan 33(12dS/m)	**49**	0.09	7.01	7.11	0.53	0.85	0.19	**15.78**	0.16	28.55	28.71	**57.42**	65.64	0.21	**65.85**

[TSFA= Total saturated fatty acid; TMUFA= Total mono unsaturated fatty acid; TPUFA= Total poly unsaturated fatty acid]

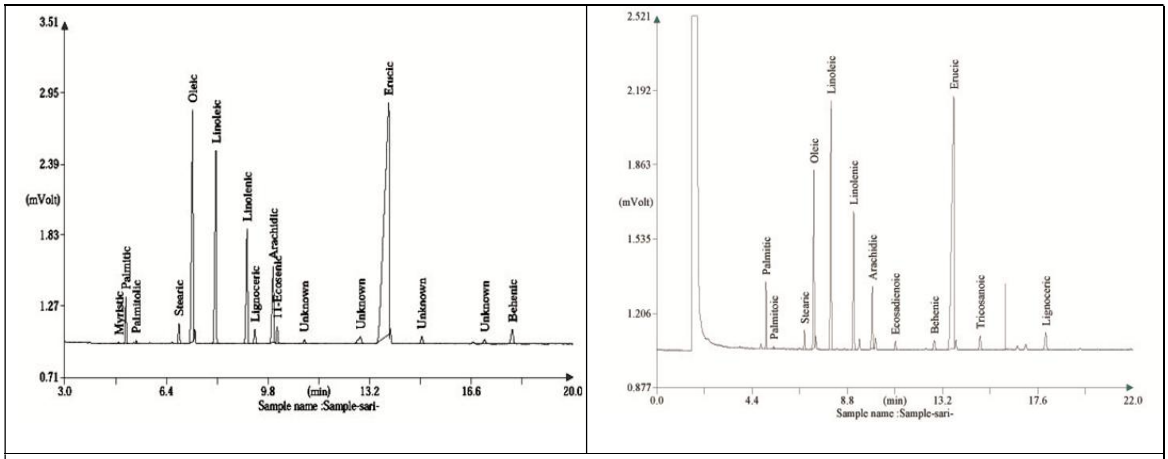


Fig. 15a, b Fatty acid composition of mustard seed by Gas Chromatography

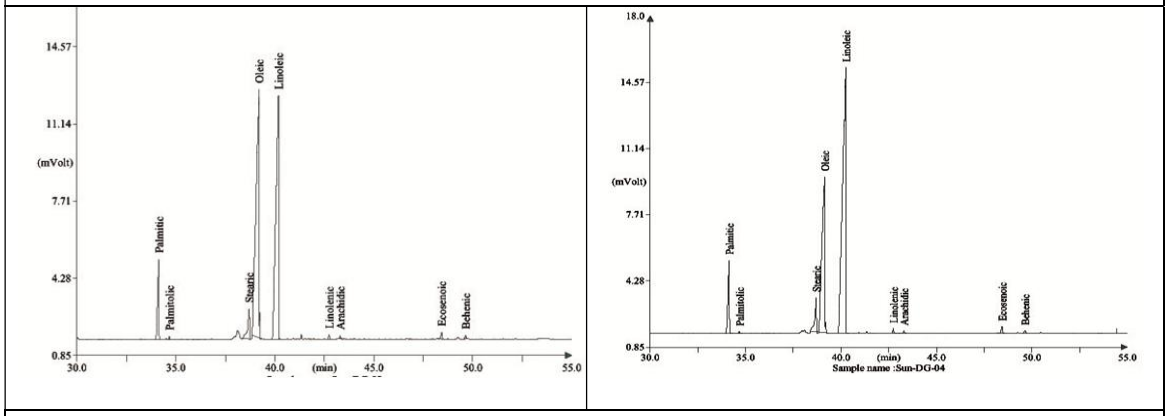


Fig. 16a, b: Fatty acid composition of sunflower seed by Gas Chromatography

12. Research highlight/findings:

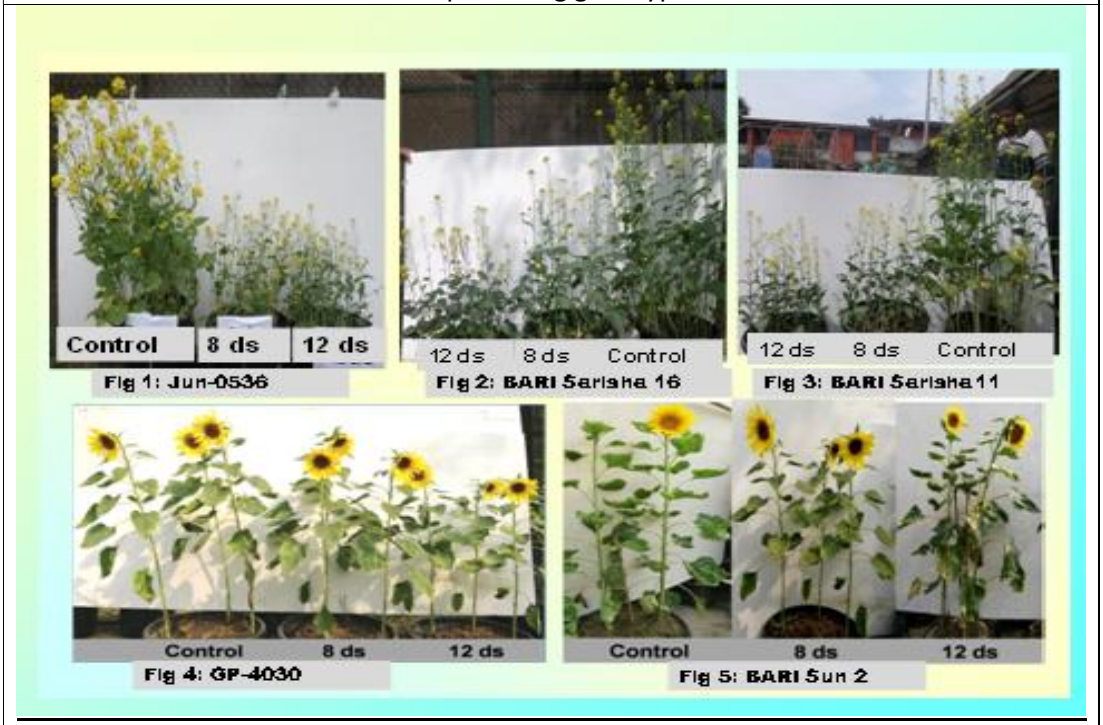
a) Two sunflower (GP-4030 and BARI Surjamukhi-2) promising genotypes have been selected against salinity after screening of forty sunflower genotypes.

b) Three mustard (Jun-0536, BARI Sarisha-16 and BARI Sarisha-11) genotypes have been selected against salinity after screening of sixty five mustard genotypes.

c) Anaerobic enzyme measurement protocols are found highly efficient and existing ones. The salt tolerant genotypes may have differential growth, better osmotic adjustment, and protection from free radicals by increased accumulation of proline and MDA content, H_2O_2, higher K^+/Na^+ and antioxidant activities, hence these could be used as potential selection criteria for screening of sunflower and mustard germplasm for salt tolerance.

d) Oil content and fatty acid profile/composition protocol have been developed for assessing oil content and quality and awaring farmers and customers in recognizing pure oils.

Major Findings based on objectives
Three mustard and two sunflower promising genotypes have been selected.



B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	One Computer and Accessories	59,500	One Computer and Accessories	59,500 (100%)	Achieved or completed
(b) Lab and field equipment	Soil EC meter = 1 Na^+ ion meter = 1 K^+ ion meter = 1	2,37,700	Soil EC meter = 1 Na^+ ion meter = 1 K^+ ion meter = 1	2,37,700 (100%)	Achieved or completed
(c) Other capital items	N/A	N/A	N/A	N/A	N/A

2. Establishment/renovation facilities: (Not Applicable)

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
N/A	N/A	N/A	N/A	N/A	N/A

3. Training/study tour/ seminar/workshop/conference organized: (Not Applicable)

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	N/A	N/A	N/A	N/A	N/A
(b) Workshop	N/A	N/A	N/A	N/A	N/A

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	2,06,173.38	2,06,173.38	2,06,173.38	0	100%	
B. Field research/lab expenses and supplies	10,68,315.00	10,68,315.00	10,68,315.00	0	100%	
C. Operating expenses	85,002.62	85,002.62	85,002.62	0	100%	
D. Vehicle hire and fuel, oil & maintenance	45,400	45,400	45,400	0	100%	
E. Training/workshop/ seminar etc.	-	-	-		-	
F. Publications and printing	63000	0	0	0	0%	**No fund release**
G. Miscellaneous	32200	29950	29950	0	100%	
H. Capital expenses	297200	297200	297200	0	100%	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
I) To select salt tolerant genotypes of sunflower and mustard for the coastal area of Bangladesh.	1) Collection and preparation of germplasm, fertilizer, layout and other inputs. 2) Sixty five mustard and forty sunflower genotypes have been screening against salinity 3) Screening and evaluation and data collection of sunflower and mustard genotypes against salinity.	Two sunflower (GP-4030, BARI surzumukhi 2) and three mustard (Jun-0536, BARI Sarisha 16, BARI Sarisha 11) promising genotypes have been selected against salinity.	Due to isolation of salt tolerant sunflower and mustard genotypes the area and production is expected to be raised in the coastal belt.
II) To determine the physiological mechanism of salt tolerant sunflower and mustard genotypes.	1) Selected seed preparation, design, layout, planting, fertilizing, intercultural operation and inputs ready. 2) To study the physiological parameters (shoot and root length, leaf area, fresh and dry weight, biomass, photosynthesis parameters, chlorophyll pigments,	1) The selected promising genotypes had good water status, osmotic adjustment and physiological parameters showed better tolerance to the salt stress. **2)** Physiological mechanism would be	The differential growth and physiological parameters of selected genotypes under salt stress could be used as potential selection criteria for salt tolerance improvement.

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
	proline, K^+/Na^+ ratio etc) of sunflower and mustard under salt stress.	developed.	
III) To study the biochemical characterization of sunflower and mustard genotypes and to develop oil quantity/content and quality protocol of sunflower and mustard for awaring farmers and customers.	To study the biochemical parameters (proline, MDA, H_2O_2, superoxide, antioxidant enzymes, CAT, SOD, POD, APX, GR, GPX, GST etc, oil content, fatty acid composition) of sunflower and mustard under salt stress.	1) Based on biochemical character selected genotypes showed better tolerance to the salt stress. 2) Oil quality (fatty acid profile***, ω-3, ω-6, ω-9*** fatty acids) and oil content determination protocol would be developed.	1) Biochemical parameters of selected genotypes could be used as potential selection criteria for salt tolerance improvement. 2) The finding can be useful in sunflower and mustard breeding programs for developing salt tolerant cultivars.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/**leaflet**/flyer etc.	Leaflet = 1		One leaflet would be publish after receiving fund
Journal publication	2		One journal paper has been submitted and another is under preparation.
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity and Non-commodity)

Non-commodity:
Newly selected salt tolerant mustard and sunflower genotypes/varieties would help to improve/increase area and productivity in the coastal area of Bangladesh. They would be eco-friendly and nutrition security in Bangladesh. Information about technologies is given in Sl. No. 12. of PCR.

ii. **Generation of new knowledge that help in developing more technology in future**

The knowledge of the present research provides a scope of improving cropping system or developing alternate cropping pattern rather than existing cropping system in coastal area, through development of salt tolerant and short duration oilseed varieties.

iii. Technology transferred that help increased agricultural productivity and farmers' income

In coastal and offshore areas of Bangladesh, the major parts of this land are affected by different level of salinity. Newly selected salt tolerant mustard and sunflower genotypes would show potential to increase area and production and consumption of edible oil in farmer's level awareness about nutritional aspect. Ultimately farmer's income would be increased.

iv. Policy Support

The findings of the project would help to take action plan or pilot production programme of salt tolerant sunflower and mustard variety in the coastal area of Bangladesh for increasing productivity to reduce the existing gap between production and consumption of edible oil. There is a possibility of growing salt tolerant mustard (short duration) and sunflower variety after aman rice harvest in the coastal areas for sustainable food and nutrition security. There is a scope of improving cropping system or developing alternate cropping pattern or sources rather than existing cropping system in coastal area. Government can take action plan regarding the relevant fact.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description and output of consultation meeting, monitoring Workshops/seminars etc.):

Report on sub-project (ID-459) activities is good on the basis Field monitoring, desk monitoring, monitoring workshop and evaluation workshop.

ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No)	Remarks
Technical Division/ Unit, BARC	14/03/2018	1	Satisfactory
PIU-BARC, NATP-2	14/03/2018	1	Satisfactory
Internal Monitoring (BARI)	6/02/2018 & 19/02/2018	2	Satisfactory

We joined one training program "Training on Public Procurement Management" organized by "Bangladesh Institute of Management (BIM), Dhaka" and funded by PIU, NATP-2, Dhaka

Field Monitoring, Team visit and Workshop on BARI funded by PIU, NATP-2, Dhaka


H. <u>Lesson Learned/Challenges (if any)</u>

i) Recession of stagnant water at late in coastal area makes delay of sowing the crops like mustard, sunflower, soybean, and maize etc. As a result crops cultivation is sometimes delayed as well as yield is reduced.

ii) Delaying of crop cultivation in coastal area is sometimes partially damaged by natural hazard like heavy rainfall, storm, hailing, flash flood etc.

iii) Delayed fund release hindered smooth running of the project activity.

iv) Unstable electricity supply hampered the lab work and quality of stored chemicals.

I. <u>Challenges (if any)</u>

i) Crop establishment is sometime delay due to late recession of water in the coastal area. Consequently, there is potential risk of being crop damaged partially or fully by natural hazard like heavy rainfall, storm, hailing, flash flood etc. Sometime excessive rainfall may cause early flood and can damage the crops.

ii) The supply of chemicals and backup supports for lab work are till time consuming.

Signature of the Principal Investigator
Date: 03.01.2019
Seal
Dr. Md. Shakawat Hossain
Principal Investigator (PI)
"Selection of Salt.....ID (CRG): 459
SSO, Central Lab, ORC, BARI, Gazipur

03.01.2019
Counter signature of the Head of the organization/authorized representative
Date:
Seal
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