Microbes Mediated Mitigation of Abiotic Stresses in Agriculture

S.S. Bobade, S.P. Dhekane, P.A. Salunke, S.G. Mane, S.S. Dhawan, R.J. Marathe, R.B. Deshmukh1, Y.B. Phatake

ABSTRACT

Background: Crop yields are limited by major biotic and abiotic stresses. Various studies had been suggested that abiotic stresses like drought, flood and salinity play a major role in limiting crop yield. Heavy metal contamination is also a major problem in the agriculture sector.

Methods: A pot experiment was conducted to elucidate the effect of inoculating bacterial strains on the wheat plant under various stress conditions. The bacteria were isolated and screened from drought, flood and heavy metal stressed soil samples. The selected strains were identified by morphological, biochemical and molecular methods. The ability of Acinetobacter junni S1, Acinetobacter junni S2, Leclercia adecarboxylata and Klebsiella variicola to stimulate the growth of plants were determined by pot experiment using a completely randomized design. The positive effect of isolates on seed germination percent, shoot and root length of the treated wheat plant were recorded. Analysis of soil samples from pots was carried out for evaluation of the presence of macro and micronutrients.

Result: The pots inoculated with selected isolates showed a significant increase in pH 7.77, EC 2.11, carbon 0.78, nitrogen 30.83 kg/ha, phosphorus 2.95 kg/ha, potassium 535.32 kg/ha, zinc 0.15 ppm, manganese 0.376 ppm, iron 0.53 ppm and copper 0.15 ppm as compared to control. The chlorophyll content estimation was carried out by using Arnon’s method. The chlorophyll a, b and total chlorophyll was found to be 14.39, 39.74 and 38.75 respectively.

Key words: A. junni S1, A. junni S2, K. variicola, L. adecarboxylata, Pot assay, Wheat.

INTRODUCTION

A twenty-first-century challenge is the production of sufficient food to meet population demands despite reductions in the quantity and quality of arable land and water and increasingly variable weather patterns that are associated with climate change. Crop losses due to extreme environmental conditions have risen steadily over the past several decades (Bailey-Serres et al., 2012) and climate models predict an increased incidence of floods (Hirabayashi et al., 2013), droughts (Pryor et al., 2009) and extreme temperatures (Bita and Gerats, 2013, Gourdji et al., 2013). Integrated climate change and crop production models project decline in the yields of major crops such as corn, wheat and rice with serious ramifications on global food production this century (Rosenzweig et al., 2014). Abiotic stresses are one of the major areas of concern to fulfill the required food demand. (Wani et al., 2013). The major abiotic stresses worldwide causing risks to food security are high salinity, drought and cold (Sanghera et al., 2011). Climatic change with elevated temperature is major agricultural problem in many areas in the world (Lobell and Gourdji, 2012). An unfavorable environment comprising extreme high or low temp, salinity and drought poses a complex set of stress conditions. Plants can sense and react to stresses in many ways that favor their sustenance (Jiang et al., 2016).

It is generally believed that abiotic stresses are considered to be the main source of yield reduction (Reynolds and Tuberosa, 2008). The estimated potential yield losses are 17% due to drought, 20% due to salinity, 40% due to high temp.,15 % due to low temp. and 8% by other factors (Ashraf et al., 2008). Multiple stress factors produce complex defense signals in plants and therefore the result of plant-microbe interaction can be decided by prioritization of physiological pathways in plants (Schenk et al., 2012).

Soil constituents a complex matrix that include pathogenic and beneficial microorganisms (Raaijmakers et al., 2009). PGPR having ACC-deaminase enzyme that could improve the plant growth under stress conditions (Nadeem et al., 2006). PGPR can improve plant growth through several mechanisms, such as increased nutrient uptake, suppression of pathogen by producing antibiotic or siderophore or bacterial or fungal antagonistic substances, phytohormone production and nitrogen fixation (Almaghrabi...
et al., 2013). Inoculation of soil with a beneficial microorganism that is adopted to adverse conditions promotes plant growth and protects the plant against the deleterious effects of some environmental stresses (Marulanda et al. 2008). Most commonly bacteria such as Azospirillum, Herbaspirillum, Acetobacter, Azotobacter and Azorarcus are used as biofertilizers, mainly because of their ability to fix atmospheric nitrogen. Rhizobacteria enhancing the tolerance of plants to high concentration of a pollutant and/or promoting plant growth could provide a useful tool for making the process of phytoremediation more efficient (Dimkpa et al., 2009). P. fluorescens, P. putida, Bacillus spp. and Arthrobacter spp. have been shown to enhance resistance against various soil-borne pathogens and also mitigate salt, as well as drought stress in different plants (Barriuso et al., 2008). The present study aimed to isolate and study potent bacteria from stressed soil samples and evaluation of their effects by mitigation of abiotic stresses in agriculture.

**Materials and Methods**

**Collection of different soil samples**

In the present study to isolate abiotic stress tolerating microorganisms, different soil samples were collected from MIDD, Baramati (Pune, lies at N 18° 11’ 0.44”, E 74° 36’ 9.57”, located at altitude 550m above MSL) and Phaltan (Satara, lies at N 17° 59’ 26.3475”, E 74° 25’ 47.4608”, located at altitude 563m above MSL). These soil samples were naturally affected by different abiotic stresses such as drought, flood and heavy metal (Mayak et al., 2004; Siddaramappa et al., 1973; Siddikee et al., 2010). The collected samples were brought to the laboratory and the research work was carried out in the department of Microbiology (Shardabai Pawar Mahila Arts, Commerce and Science College, Sharda Nagar, Baramati-413 115 during the year 2019-2020).

**Enrichment, isolation and screening**

For the enrichment, one gram of each soil sample was inoculated in nutrient broth and incubated at room temp. for 72 hrs on a rotary shaker incubator. After enrichment serial dilution was performed and 10^-1, 10^-4, 10^-5 and 10^-6 dilutions were selected for inoculation on a sterile nutrient agar plate. Inoculated Petri dishes were incubated at 37°C for 24 hrs. After incubation well-isolated colonies were selected and screened on the basis of their ability to grow in presence of heavy metals like Cr (Chromium) and Zn (Zinc) at 20mg and 35mg conc. respectively (Kasana et al., 2008; Marakana et al., 2018; Sandhya et al., 2009).

**Characterization of isolates**

All the four screened isolates were successfully characterized by using the morphological character. Colony characters of the isolates were recorded such as size, shape, color, margin, elevation, opacity and consistency, etc. The cell character of the isolates was also determined by using the standard gram staining procedure. The motility of the isolates was determined by using the Hanging drop method (Benedetto et al., 2019). The ability of isolates to produce capsule and spore were also checked successfully (Oktari et al., 2017; Jasmin, 1945).

All the four selected isolates were further characterized by using biochemical tests viz., catalase test, oxidase test, sugar fermentation test, IMViC and nitrate reduction tests, based on Bergey’s Manual for determinative bacteriology upto the genus level (Apun et al., 2000). Finally, the results were confirmed and bacteria were identified up to species level by MALDI-TOF technique.

For preparation the sample for MALDI-TOF, microbial growth from a pure colony was mixed with the matrix solution, followed by preparation of smear on target plate, air drying and loading of the dried plate inside instrument. The sample was then exposed to source of ionization. After ionization, ionized peptides and proteins travel towards detector in a vacuum tube and they get separated based on their mass to charge ratio (m/z). A mass spectrum of the strains under study were then generated, which were compared with that of the other strains present in the reference database. The database includes biomarkers detected in MALDI spectra of intracellular proteins primarily in the range of 2 to 20 kDa (Rahi et al., 2016).

**Evaluation of plant growth promoting abilities by pot assay**

**Microorganism used**

In the present study, four selected and identified strains of isolates Acinetobacter junni S1, Acinetobacter junni S2, Leclercia adecarboxylata and Klebsiella varicola were used (Chun et al., 2014; Sang et al., 2019).

**Plant used**

Pot assay was performed by using Triticum aestivum, L., Cv. Lokwan belonging to family Poaceae/ Graminae. This cultivated variety is locally known as “Lokwan Gehu”, was studied for the effect of isolated strains on plant growth, development and changes in soil properties under stress conditions (Gang et al., 2013; Khan et al., 2013).

**Preparation of potting mixture**

The soil sample was collected from village Vadgaon-Nimbalkar, Pune, Maharashtra (18° 7’ 47N, 74° 21’ 43E) and air-dried under sunlight. Visible roots and debris were removed from the soil and discarded. The soil aggregations were broken down gently by crushing them using a wooden hammer. The grounded soil sample was then passed through a 2 mm stainless steel sieve and sterilized at 15 psi for 15 min. The sieved sample was mixed thoroughly for making the composite sample and preserved in plastic bags (Marathe et al., 2017).

**Inoculation of seeds**

For inoculation, collected seeds of T. aestivum were surface-sterilized with 0.5 % sodium chloride (NaCl) solution for 1-2
min, rinsed in sterilized distilled water and dried under a sterile air stream (Deshwal and Kumar, 2013). A loopfull suspension of all four isolates were inoculated and cultured under continuous shaking (100 rpm) conditions in nutrient broth (500ml) and incubated at 37°C for 24 hrs. After incubation 50 ml fresh culture was inoculated in each 250gm soil sample which was used for pot assay (Marathe et al., 2017).

**Pot preparation**

Sterile and air-dried soil samples were added into each pot amounting 250g/pot. For assessment, different abiotic stresses viz. flood, drought and heavy metal were selected to conduct the experiment four different abiotic stress conditions were maintained each having seven treatments, which includes positive control, negative control, isolated I, II, III, IV and consortia of all the organisms. (Islam et al., 2014; Marathe et al., 2017; Rangeshwaran et al., 2013; Sarma and Saikia, 2014). The artificially contaminated soil was prepared by mixing control soil with heavy metals. To study the effect of heavy metals, pots were supplemented with 2mg and 0.35mg of Cr (Chromium) and Zn (Zinc) respectively (Barbosa et al., 2015; Khan et al., 2013). In every pot previously sterilized seeds were inoculated in equal distance and depth. All pots were arranged in a completely randomized design on a terrace to get equal exposure to sunlight and proper aeration (Khan et al., 2013). The pots were manually irrigated with distilled water regularly. To study the effect of drought and flood stress on the selected plants, pots were watered with 20 ml and 5 ml water respectively. The plants were harvested manually by uprooting them carefully from the pots for analysis of root and shoot length. The effect of isolates on percent germination of seeds was also recorded (Gang et al., 2013). The experiment was performed in triplicates. The mean values were used for calculating standard errors.

**Assessment of physicochemical properties of soil**

At the end of pot assay (after 60 days), soil samples from selected pots were used for assessment of physicochemical properties. For determination of pH and EC of soil, 10 gm of soil was taken in 50 ml of beaker then 25 ml distilled water was added and stirred well for 10 min. The pH was recorded and allowed it to settle down for 1hr after settling down all soil particles, the supernatant liquid was taken and the EC value was recorded (Sillanp, 1982; Gogoi et al., 2016).

Organic carbon was determined by a modification of Alten’s method (Tares and Sippola, 1978). An amount of 30-100 mg soil, 25 ml 0.25 M potassium dichromate and 40 ml H$_2$SO$_4$ were added to a 400 ml flask. The mixture was kept for 1.5 hrs on a hot water bath, allowed to cool for 30 min and 175 ml water was added. After standing overnight, the solution was measured colorimetrically (630nm) and readings were compared with standard (Sillanp, 1982; Singh et al., 2017).

For the determination of available nitrogen from the soil, the alkaline permanganate method was used. 20gm of soil sample was taken in the Kjeldahl flask and 20ml water was added. 1 ml of liquid paraffin and few glass beads were added to prevent frothing and bumping during distillation. In the Kjeldahl flask 100 ml 0.32% KMnO$_4$ and 2.5 % NaOH were added and connected to the ammonia distillation apparatus. The content was distilled in Kjeldahl assembly at a steady rate and the liberated ammonia was collected in a flask containing boric acid solution with indicator. With the absorption of ammonia, the pink color of the boric acid solution turns to green. The content was titrated with 0.02 N H$_2$SO$_4$ to get the original shade pink.

For the determination of phosphorus, 2.5gm of soil sample was mixed with one teaspoon of activated carbon (Darco-G.60) in a 250 ml conical flask. 50 ml of 0.5 M NaHCO$_3$ solution (extracting solution) was then added to it. After addition, the flask was shaken for half an hour on a shaker and suspension was filtered immediately after shaking. 5 ml filtrate was transferred into a 25 ml volumetric flask in which drop by drop 5 ml ammonium molybdate was added with constant shaking. It was followed by the addition of 1ml dilute solution (working solution) of SnCl$_2$. Finally, the color intensity was measured on a photoelectric colorimeter. Available P in Kg/ha was then calculated by using the following formula (Marathe et al., 2017).

\[
\text{Available Phosphorus (P) of soil (Kg/ha)} = \frac{AX \text{volume of (extractant/volume of aliquot)} X (1/wt. of soil)}{X 2.24 X 10^6}
\]

For determination of potassium, sodium, iron, zinc and copper, 5gm of soil was taken in a 100 ml conical flask and 25 ml ammonium acetate was then added. The contents of the flask was then shaken for 5 min and immediately filtered through Whatman no.1 filter paper. The first few ml of the filtrate was rejected. Finally, by using known standard solutions of potassium, sodium, iron, zinc and copper present in the extract were determined by flame photometer using K, Na, Fe, Zn and Cu filtrate respectively (George et al., 2013; Marathe et al., 2017).

For determination of available Calcium, 5gm of soil was taken in 250 ml conical flask and 50 ml of 1 N HCl was added to it. It was kept on a shaker for 30 min and was filtered through filter paper. 5 ml of filtrate was taken in a flask and 4 drops of phenolphthalein indicator was dded and titrated against 0.5 N NaOH solution. End point was the appearance of pink colour. Blank was also run without taking the soil sample (Sillanp, 1982). For determination of available manganese; 10gm of soil was mixed with 25 ml of diethylene triamine pentaacetic acid solution. After shaking filtered it through the Whatman filter paper and readings were recorded on the atomic absorption spectrophotometer at 279 nm (Sillanp, 1982; Doddabasawa et al., 2020).

**Estimation of Chlorophyll**

The leaf samples collected from different treatment were analyzed for chlorophyll content by using Arnon’s method (Arnon, 1949). 0.1 gm of fresh plant material (leaf sample) was collected in a clean beaker. The sample was ground to produce fine pulp with an addition of 10 ml of 80% acetone.
The extract was centrifuged at 5000 rpm for 5 min. The supernatant was transferred to a test tube which was covered in black marble paper to avoid photo-oxidation of pigment. Finally, the absorbance of the solution was recorded at 663 nm (chlorophyll a) and 645 nm (chlorophyll b) against the solvent (80% acetone) as blank (Rajalakshmi and Banu, 2015). Chlorophyll in mg per gm of tissue was then calculated by using the following formula (Kumar et al., 2014; Marathe et al., 2017; Robert, 2002).

1. Chlorophyll a (mg/gm tissue) = 12.7(A663) – 2.69(A645) x (V/1000 x W)
2. Chlorophyll b (mg/gm tissue) = 22.9(A663) - 4.68 (A645) x (V/ 1000 x W)
3. Total chlorophyll (mg/gm tissue) = 20.2(A663) + 8.02(A645) x (V/ 1000x W)

Where,
A = absorbance at specific wavelength.
V = final volume of chlorophyll extract in 80% acetone.
W = fresh weight of tissue extract.

RESULTS AND DISCUSSION
Collection of different soil samples
Heavy metal, flood and drought stressed soil samples were successfully collected from different regions of Baramati and Phaltan.

The soil samples collected from Baramati MIDC (Pune) and Phaltan (Satara) regions showed presence of a notable amount of bacterial diversity. Siddaramappa et al., also used flood soil samples for isolation of microorganisms (Siddaramappa et al., 1973). They found Bacillus spp. and Pseudomonas spp. from a collected soil sample. Mayak et al., used soil sample collected from drought region for isolation, they found the presence of ACC deaminase producing PGPR Achromobacter piechaudii ARV8. Several other authors preferred stress soil samples like drought, salinity, flood, heavy metal contaminated soil samples for screening and isolation of potent plant growth promoting microorganisms (Muhammad et al., 2004; Sandhya et al., 2009; Siddikee et al., 2010; Yandigeri et al., 2012).

Enrichment, isolation and screening
After enrichment, bacterial growth was observed in nutrient broth. A loopfull suspension from serially diluted tubes was streaked on sterile nutrient agar plates. Well isolated, white colored colonies were selected and screened (Fig 1) based on their ability to grow in presence of heavy metals like Cr and Zn, total four isolates showing significant growth in presence of the selected heavy metals were screened for further studies (Siddikee et al., 2010).

After enrichment and isolation, the characterization of isolates was done by using the morphological, biochemical and molecular method (MALDI TOF MS). Marakana et al. and Rahman et al., used a similar approach for the identification of microorganisms from saline stressed soil (Marakana et al., 2018 and Rahman et al., 2017). They identified the organisms successfully by using Bergey’s Manual of determinative bacteriology up to the genus level.

Characterization of isolates
Morphological characteristics
The selected isolates were further identified by using the morphological method. The following Table 1 shows the result obtained.

Biochemical test
All four isolates were further identified upto the genus level by using biochemical tests selected by Bergey’s Manual of determinative bacteriology (Table 2).

Based on the result obtained from biochemical test it was concluded that four isolates show similarities with Acinetobacter spp., Leclercia spp. and Klebsiella spp.

| Table 1: Morphological characterization of the selected isolates. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Organism       | Size   | Shape   | Color     | Margin | Elevation | Opacity | Consistency | Gram character | Motility       |
| Isolate I      | 3mm    | Circular | White    | Entire | Elevated  | Opaque  | Smooth      | Gram negative rod | Non motile     |
| Isolate II     | 1mm    | Circular | White    | Entire | Elevated  | Opaque  | Smooth      | Gram negative rod | Non motile     |
| Isolate III    | 2mm    | Irregular | White   | Entire | Elevated  | Opaque  | Rough       | Gram negative rod | Non motile     |
| Isolate IV     | 3mm    | Circular | White    | Entire | Elevated  | Opaque  | Smooth      | Gram negative rod | Non motile     |

Enrichment Serial dilution Screening
Fig 1: Enrichment and screening of the potent microorganisms from selected soil samples.
Table 2: Biochemical characterization of the selected isolates.

| Test                              | Isolate I | Isolate II | Isolate III | Isolate IV |
|----------------------------------|-----------|------------|-------------|------------|
| Indole test                       | -         | -          | +           | -          |
| Methyl red                        | +         | +          |             | -          |
| Voges-Prousker                    | -         | -          |             | -          |
| Catalase                          | +         | +          | +           | +          |
| Xylose and Rhamnose               | -         | -          | (A+G)       | (A+G)      |
| Sucrose                           | -         | -          |             | -          |
| Lactose and Glucose               | -         | -          | (A+G)       | (A+G)      |
| Maltose and Mannose               | -         | -          | (A+G)       | (A+G)      |
| Galactose and Fructose            | -         | -          | (A+G)       | (A+G)      |
| Sorbitol and Mannitol             | -         | -          | (A+G)       | (A+G)      |
| Cellobiose, Salicin and Trehalose | -         | -          | (A+G)       | (A+G)      |
| Oxidase and citrate utilization   | -         | -          |             | -          |
| Starch hydrolysis                 | -         | -          |             | -          |
| Nitrate reduction test            | -         | -          |             | -          |

(A+G) = Acid and gas production, (-) Negative, (+) Positive.

Fig 2: MALDI-TOF MS spectra of *Acinetobacter junni* S1.

Fig 3: MALDI-TOF MS spectra of *Acinetobacter junni* S2.

Fig 4: MALDI-TOF MS spectra of *Leclercia adecarboxylata*.

Fig 5: MALDI-TOF MS spectra of *Klebsiella variicola*. 
Molecular analysis (MALDI-TOF MS)

Finally, the four isolates were identified up to species level by using MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectroscopy). This technique is very effective and reproducible for characterization of the microorganism.

In the MALDI-TOF MS, organisms were identified based on score value. For first isolate match score was 2.402 and 2.32 for *Acinetobacter junni* (S1) DSM 1532 DSM (Fig 2), for isolate second match score was 2.534 and 2.448 for *Acinetobacter junni* (S2) DSM 1532 DSM (Fig 3), for isolate third match score was 2.447 and 2.237 for *Leclercia adecarboxylata* CCM 4443 CCM (Fig 4) and Fig 5 shows the match score (2.279 and 2.172) for isolate four (*Klebsiella variicola* 37924).

Urquiza et al. in 2017 successfully used the MALDI-TOF MS technique for the identification of microorganisms up to species level from the soil. They isolated *Bacillus muralis* CA9, *Bacillus simplex* CA15, *Bacillus simplex* CA16a, *Bacillus simplex* CA22, *Bacillus muralis* CA16b etc. from stressed soil. According to Yonetani et al. (2016), MALDI-TOF MS is a very cheap and time saving technique for identification of clinical samples, they isolate Aerococcus spp., Enterococcus spp., Streptococcus spp., Micrococcus spp., Achromobacter spp. from clinical samples. According to Vargha et al., MALDI-TOF MS technique is a very sensitive method of characterization of Arthrobacter spp. from the soil (Vargha et al., 2006). According to Shazia et al., MALDI-TOF MS technique can be successfully used for subtyping the strains based on their protein profile and for identification of ureolytic bacteria from different soils (Shazia et al., 2018).

**Evaluation of plant growth promoting ability by pot assay**

The completely randomized design for pot assay was selected. The pots were prepared using standard procedure. Fig 6, 7, 8 and 9 shows the sets of the pot assay for drought, flood, heavy metals (Zn and Cr) stress respectively.

![Fig 6: Pot assay for drought stress.](image)

![Fig 7: Pot assay for flood stress.](image)

![Fig 8: Pots assay for heavy metal stress (Zn).](image)

![Fig 9: Pot assay for heavy metal stress (Cr).](image)

**Table 3: Effect of organism on seed germination time and %.

| Pot name          | Germination time (days) | Germination (%) | Pot name          | Germination time (days) | Germination (%) |
|-------------------|-------------------------|-----------------|-------------------|-------------------------|-----------------|
| DC (Positive control) | 10                      | 50              | HMC₁ (positive control) | 8                      | 100             |
| DS I (isolate I)    | 10                      | 50              | HMS₁ (isolate I)   | 8                      | 50              |
| DS II (isolate II)   | 10                      | 75              | HMS₂ (isolate II)  | 8                      | 75              |
| DS III (isolate III)| 10                     | 100             | HMS₃ (isolate III) | 8                      | 50              |
| DS IV (isolate IV)   | 10                     | 75              | HMS₄ (isolate IV)  | 8                      | 50              |
| DS (consortium)      | 10                     | 100             | HMS₅ (Consortium)  | 8                      | 75              |
| DC (negative control)| 10                     | 50              | HMC₁ (negative control) | 8                      | 50              |
| FC (positive control)| 6                      | 100             | HMC₂ (positive control) | 6                      | 50              |
| FS I (isolate I)     | 6                       | 100             | HMS₁ (isolate I)   | 6                      | 100             |
| FS II (isolate II)    | 6                       | 100             | HMS₂ (isolate II)  | 6                      | 50              |
| FS III (isolate III)| 6                      | 50              | HMS₃ (isolate III) | 6                      | 50              |
| FS IV (isolate IV)    | 6                       | 75              | HMS₄ (isolate IV)  | 6                      | 75              |
| FS (Consortium)       | 6                       | 100             | HMS₅ (Consortium)  | 6                      | 100             |
| FC (negative control)| 6                      | 75              | HMC₂ (negative control) | 6                      | 50              |
Pot assay was performed to study the effect of four isolates namely A. junni (S1), A. junni (S2), L. adecarboxylata and K. variicola on the growth and development of wheat under different stress conditions. Huddedar et al., also used Acinetobacter strain for pot assay by using a wheat plant. According to them, it helps to induce the production of IAA to promote plant growth and development (Huddedar et al., 2002). Khan et al., also used PGPR for pot assay by using a wheat plant. They report that PGPR having ACC-deaminase enzyme could improve the plant growth under heavy metal stress (Khan et al., 2013).

**Effect of organisms on root length and shoot length**

It was found that selected strains have a notable positive effect on seed germination, root length and shoot length as compared with negative control under different stress conditions. In drought stress condition L. adecarboxylata strongly influences root and shoot length. In flood condition A. junni S2 and in heavy metal stress (Chromium and Zinc), A. junni S2 and S1 significantly influence seed germination, root length and shoot length. The effect of these organisms on seed germination, root and shoot length is shown in the following Fig 10,11,12 and 13.

Egamberdiyeva and Hoflich also used root colonizing salt tolerating bacteria for pot assay by using the wheat plant. They found that salt tolerating bacteria induce the production of IAA and stimulate the growth of the plant (Egamberdiyeva and Hoflich, 2003). Marathe et al., also set pot assay experiment to check the effect of Pseudomonas aeruginosa on the growth and development of soyabean plant (Glycine max). They found that organism shows a positive effect on germination time, root and shoot length and chlorophyll content of plant when compared with control (Marathe et al., 2017). Khan et al., also designed pot assay to study microbial inoculants effect on wheat growth in Cr contaminated soil. They found that microbial inoculants significantly increase root and shoot length in chromium contaminated soil (Khan et al., 2013).

**Assessment of physicochemical properties of soil**

**Drought stressed soil**

Selected pots which showed the highest growth of plant were tested for all physicochemical properties of soil including micronutrient and macronutrient. Under stress condition pH 7.8, EC 1.80 and OC 1.03 were slightly increased as compared with negative control 7.7, 1.6, 0.48 and positive control 7.74,0.5 and 1.11, respectively. Table 4 show the results of soil analysis for physicochemical properties under drought stress.

**Flood stressed soil**

Under flood stress condition, pH 8.11, EC 3.07 and OC 1.28 were slightly increased as compared with negative control 7.38, 0.60, 0.82 and positive control 7.83, 0.48, 2.06 respectively. The results of physicochemical properties of soil under flood stress are shown in Table 5.
Heavy metal stress condition (Chromium)

The pot which showed the highest growth of wheat was tested for all physicochemical properties of soil including micronutrient and macronutrient. Table 6 shows the result obtained under heavy metal stress condition. Under stress condition pH 7.73, EC 2.61 and OC 1.20 were slightly increased as compared with negative control 7.2, 1.5, 0.47 and positive control 7.64, 1.07, 0.43 respectively.

Heavy metal stress condition (Zinc)

Table 7 shows result obtained from soil under heavy metal (Zinc) stress conditions. Under Zn stress condition pH 7.77, EC 2.61 and OC 1.20 were slightly increased as compared with negative control 7.2, 1.5, 0.47 and positive control 7.64, 1.07, 0.43 respectively.

Based on soil analysis studies, pots inoculated with isolates exhibit slightly alkaline pH when compared with negative control. The soil from the pot containing DSIII, FSII, HMSII, HMSI, showed a significant increase in the level of carbon, nitrogen, phosphorous and potassium when compared with the soil of negative control, while all seven pots from each set did not show a significant difference in zinc, copper, manganese, iron and calcium content. The treatment of isolates stimulates the activities and physicochemical properties of soil.

In the present study, isolates showed a positive effect on carbon, nitrogen, phosphorous and potassium content while no significant effect on sodium, zinc and copper content. Our results were similar to Mengual et al. (2014). Maximum value of chlorophyll is the indication of optimum growth of the plant, so in the present study we used this parameter to

Estimation of chlorophyll

The yield of Chlorophyll a, Chlorophyll b and total chlorophyll were calculated from different test plants (Fig 14). Maximum value of chlorophyll is the indication of optimum growth of the plant, so in the present study we used this parameter to
check the positive impact of the isolates on plant growth and development. The pots inoculated with different isolates such as DSIII, FSII, HMSII, HMSI showed the most prominent effect on all three types of pigments when compared with a negative control.

We determined the chlorophyll a, chlorophyll b and total chlorophyll content in different stress conditions and compared with control. Tiwari et al. determined chlorophyll content of wheat. They found total chlorophyll content of stressed plant leaves was maximum when treated with Halomonas spp. (Tiwari et al., 2011). Marathe et al., found that plant treated with Pseudomonas aeruginosa shows highest chlorophyll b than chlorophyll a and total chlorophyll compared with control (Marathe et al., 2017). In the present study out of four isolates, isolate III (L. adecarboxylata) showed a positive impact on total chlorophyll in drought conditions. Minimum effect on chlorophyll a, b and total chlorophyll content was observed in the pot which was inoculated with isolate II (A. junni S2) under heavy metal chromium stress.

CONCLUSION

Biotic and abiotic stresses significantly affect final crop yield. The study found that abiotic stresses like drought, flood and heavy metals significantly decrease overall plant growth. In the present study pot experiment was used to assess the effect of bacterial inoculum on the growth and development of wheat plants under various stress conditions. The study concluded that drought, flood and heavy metal stressed soil samples were the rich source of the diverse microbial communities including bacteria. The isolates can be readily identified up to species level by morphological, biochemical and molecular methods as Acinetobacter junni S1, Acinetobacter junni S2, Leclercia adecarboxylata and Klebsiella variicola. The isolates showed a positive effect on seed germination percent, shoot, root length, chlorophyll content, macro and micronutrient content of the treated wheat plant when compared with control.

So this study also concludes that different isolates from the stresses region can have great potential which can be explored for mitigation of various abiotic stresses and improving crop yield significantly.

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