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Full Length Article

Can Catalase and Exopolysaccharides Producing Rhizobia Ameliorate Drought Stress in Wheat?

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Abstract

Catalase and exopolysaccharides producing ability of rhizobia increases its survival in arid and semiarid climates. Rhizobial exopolysaccharides activity increases root colonization and nutrient or water holding capacity of the rhizosphere. For the reason, a series of experiments were conducted to sort out efficient rhizobial isolates (belonging to *Lens culinaris* L., *Vigna radiata* L. and *Cicer arietinum* L. cultivated in Chakwal (arid) and Faisalabad (semiarid) areas which can ameliorate the impact of water deficit stress on wheat seedlings. Among 35 isolates, *Rhizobium leguminosarum* (LR-30), *Mesorhizobium ciceri* (CR-30 and CR-39), and *Rhizobium phaseoli* (MR-2) improved the growth, biomass and drought tolerance index of the wheat seedlings under PEG-6000 simulated drought. Selected isolates were producers of indole acetic acid, which enhanced the root length of the seedlings to dilute the drought impact. However, isolates LR-26, MR-17 and CR-34 remained deleterious for seedling growth whether drought was applied or not. In conclusion, rhizobial capability to survive under drought and improving seedling growth under drought is a good criterion to select beneficial bacteria for rescuing wheat seedlings growth under water deficit conditions. © 2014 Friends Science Publishers

Keywords: Inoculation; Water deficit; Stress tolerance; Exopolysaccharides; Catalase

Introduction

Generally drought stress occurs when the available water in soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Jaleel *et al*., 2009). Up to 45% of the world's agricultural lands are subjected to continuous or frequent drought wherein 38% of the world human population resides (Bot *et al*., 2000). The agricultural regions that are affected by drought can experience yield loss up to 50% or more (Jenks *et al*., 2005). As far as Pakistan's situation is concerned, we are facing water shortage problem since decades due to global warming, low rain fall, high temperature and reduced reservoir capacity (Anonymous, 2012). Pakistan is an agriculture based economy and agriculture is the prime sector to be affected by the increased shortage and scarcity of fresh water (FAO, 2007).

Drought tolerance is the ability of plant to grow, flower and display economic yield under suboptimal water supply (Farooq *et al*., 2009) and wheat is known as moderately drought tolerant crop. However, manipulation of microbial cohabitants can be used to improve adaptive mechanisms and productivity of crop plant (Tikhonovich and Provorvo, 2011), where bacterial inoculation biotechnology could help these plants to induce drought tolerance or sustain the growth and productivity under stress. Certain bacteria are known as beneficial to plant growth while living in association with the roots of the plants and these are termed as plant growth promoting rhizobacteria (PGPR) (Arshad and Frankenberger, 1998). They comprise of many genera including *Pseudomonads*, *Azospirillum*, *Serretia*, *Azotobacter* and *Rhizobium*. Rhizobia are nodule forming bacteria in legumes where they fix atmospheric nitrogen while living in symbiosis. However, they can survive in bulk soil and also in the rhizosphere of the plants. Therefore, they may also act as PGPR with non-nodulating plants. In such plants, although they cannot fix nitrogen but even then they can improve plant growth through certain other activities (Noel *et al*., 1996; Hussain *et al*., 2009) like phytohormones (Etesami *et al*., 2009), enzymes (Bajgiran *et al*., 2008; Zahir *et al*., 2008) and exopolysaccharides (Alami *et al*., 2000) production, nutrient solubilization (Zaidi *et al*., 2009), improvement in rhizosphere soil structure (Sandhya *et al*., 2009), and induced stress tolerance (Yang *et al*., 2009).

Rhizobia probably produce antioxidants (catalase), osmolytes, stress proteins, and exopolysaccharides to survive under harsh environments particularly drought (Goyal *et al*., 1986; Vanderlinde *et al*., 2010). However, rhizobia have been observed to survive under drought up to -3.5 MPa (Abolhasani *et al*., 2010). The ability of these rhizosphere bacteria to tolerate severe water deficit condition can be utilized to ameliorate drought impact on plants (Goyal *et al*., 1986). They can induce stress tolerance

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in plants by producing physical and chemical changes in plants (Yang *et al*., 2009). Different stress ameliorating and plant growth improving mechanisms of rhizobia have been observed including production of chaperons and sugars (Berjak, 2006), synthesis of stress enzyme 1-aminocyclopropane 1-carboxylic acid (Zahir *et al*., 2009), exopolysaccharides production (Alami *et al.,* 2000), production of low molecular weight organic compound like trehalose (Zahran, 1999), phosphate solubilization (Zaidi *et al*., 2009), improved nutrient availability (Hussain *et al*., 2009), production of siderophores (Arora *et al*., 2001), phytohormones production (Khalid *et al*., 2006) and enhanced root respiration (Volpin and Phillips, 1998) by influencing plant physiology.

Up till now, rhizobial inoculation has been tested for improving growth and productivity of cereals (rice, maize and wheat) in irrigated conditions (Mehboob *et al*., 2008; 2011; Hussain *et al*., 2009). But rhizobial inoculation to non-legumes under stress particularly drought has rarely be studied. However, some sporadic studies on rhizobial capability for improving plant growth of non-legumes are found in the literature but comprehensive information is scanty for their potential to ameliorate the drought impact on non-legumes. Therefore we hypothesized that rhizobia having capability to produce catalase and exopolysaccharides would be less susceptible to drought stress and helpful in ameliorating the impact of drought on wheat seedlings growth by developing micro-aggregates around the plant roots, improving nutrient or water uptake and improving drought tolerance index of the seedlings under drought. To confirm the hypothesis a series of studies was conducted and efficient plant growth promoting rhizobia (exopolysaccharides and catalase producing) were selected on the basis of their drought tolerance and ability for improving the growth of wheat seedlings under drought.

Materials and Methods

Rhizobial Isolation from Nodules

Leguminous plants lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.) and mung bean (*Vigna radiata* L.) were uprooted at flowering stage without damaging their nodules, from arid (Chakwal) and semiarid (Faisalabad) regions of the Punjab, Pakistan. These plants were transferred to the Soil Microbiology and Biochemistry Laboratory, University of Agriculture, Faisalabad in polythene bags. The plant along with roots and rhizosphere soil was left in flooded condition in a plastic bucket for 2-3 h. After the removal of soil, the roots were washed with tap water to remove the soil adhering to the roots. Nodules were cut from the roots with a sterilized razor and collected in Petri plate. These nodules were surface-disinfected by dipping in 95% ethanol for 5 seconds followed by 10 min dipping in 5% sodium hypochlorite and washing with sterilized distilled water for 4 times. Disinfected nodules were crushed in sterilized test tube with a sterilized glass rod. The suspension attained was used to inoculate Petri plates containing sterilized yeast extract mannitol agar (YMA) media with a sterilized inoculating needle. Inoculated plates were incubated at 28 ± 1ºC. Further, 3 to 4 times streaking was done to get the pure culture of the isolates. A total of 35 isolates capable to produce catalase (bubbling in response to 35% H_2O_2 was observed in the culture) and exopolysaccharides (mucoid growth of bacterial culture on the RCV-glucose medium) were identified qualitatively following standard protocols given by MacFaddin (1980) and Ashraf *et al.* (2004), respectively, selected, coded (Table 1) and preserved in 50% glycerol at -20 ± 1ºC. There were 10 isolates from lentil, 12 from chickpea and 13 from mung bean nodules.

Drought Tolerance Assay

All the isolates were tested for drought tolerance capability on the basis of their population growth at different drought levels simulated by polyethylene glycol (PEG) 6000 in yeast extract mannitol broth (YMB) media (Busse and Bottomley, 1989). Fresh inoculum of each isolate was prepared in 100 mL conical flask containing 50 mL sterilized YMB and incubated for 3 days in orbital shaking incubator at 28 ± 1ºC and 100 rpm. Culture cells were harvested by centrifugation at 4000*g* and 4ºC for 15 min. Inoculum of uniform cell density 0.5 (10^{6-8} cells mL^{-1}) was prepared in sterilized YMB using optical density meter. Growth media were developed for different drought levels by adding 0, 150, 250 and 350 g PEG L^{-1} of YMB. Osmotic potential of these media were -0.04, -0.70, -1.67 and -2.18 MPa, respectively, measured by Cryoscopic Osmometer (OSMOMAT-030-D, Gonotec, Germany). Osmotic potential of the media containing PEG was measured before and after autoclaving to check the change in developed potential. Freshly prepared inocula (0.5 OD) of each isolate was inoculated (0.5 mL) in triplicated set of sterilized test tubes containing seven mL of YMB with different PEG concentrations and incubated in orbital shaking incubator at 28 ± 1ºC and 100 rpm. Un-inoculated control set of test tubes at each PEG concentration was also maintained with three repeats. After 4 days of incubation, OD was measured by spectrophotometer at 600 nm (0.5 OD = 10^{6-8} cells mL^{-1}). Isolates showing more absorbance (OD) were supposed drought tolerant.

Plant Growth Promoting Activity of Rhizobial Isolates under Simulated Drought

Rhizobial isolates were further tested for their ability to promote seedling growth of wheat (*Triticum aestivum* L.) cultivar Sahar-2006 under PEG-6000 simulated drought. The experiment was performed by dividing isolates into three groups with respect to the host legume crop from which they were isolated. Three separate trials were conducted with rhizobial isolates of lentil, chickpea and

mung bean. Fresh inoculum was prepared for each experiment as described in drought tolerance assay. After maintaining the uniform cell density, two sterilized filter paper sheets were soaked with the respective inoculum. Three surface sterilized pre-germinated seeds were dipped in the inoculum for 10 min and placed in between the sheets soaked in respective inoculum. The filter paper sheets were rolled and put in sterilized glass jars (Asghar *et al*., 2002). Sterilized broth was used as control treatment. Each treatment was replicated thrice. Different drought levels (No PEG, 15 and 25% PEG having potentials -0.02, -0.62 and -1.23 MPa, respectively) were maintained in sterilized Hoagland solution (1/2 strength) by using PEG-6000. Light and dark periods were adjusted at 10 and 14 h, respectively, and suitable temperature (20-25^{o}C) was maintained. After five days of germination, drought was imposed by applying PEG solutions. Plants were harvested after 15 days of drought treatment and data regarding root or shoot length, and fresh or dry biomass were recorded.

Drought Tolerance Index

Drought or stress tolerance index of the wheat seedlings was derived by dividing the shoot length of inoculated and stressed (SLIN and S) seedling with the shoot length of un-inoculated and unstressed (SLUN and US) seedling (Ahmad, 2011).

$$\mathrm{DTI} = \frac{(\mathrm{SLIN}\ \&\ S)}{(\mathrm{SLUN}\ \&\ US)}$$

Where, SLIN = shoot length of inoculated, SLUN = shoot length of un-inoculated, S = stressed. US = unstressed.

Rhizobial isolates showing better performance for drought tolerance, plant growth promoting activity under drought and improving DTI were pronounced as prominent isolates.

Plant Growth Promoting Mechanisms and Identification of Selected Rhizobia

Selected rhizobial isolates were identified using BIOLOG® identification system (Microlog System release 4.2, Biolog Inc., USA) and confirmed by inoculation to their respective legumes (*Lens culinaris* L., *Vigna radiata* L. and *Cicer arietinum* L.) in axenic conditions where they produced nodules. Plant growth promoting mechanisms which in one or the other way supported plant growth and survival under stress were measured following their standard protocols. Bacterial aggregation ability was determined by measuring the turbidity of cell suspension (OD1) after 20 min settling of cell aggregates in test tube and second turbidity (OD2) after 1 min vortex. Using the formula:

$$\%\text{aggregation} = [(\mathrm{OD2} - \mathrm{OD1})/\mathrm{OD2}] \times 100$$

The aggregation ability was calculated (Madi and Henis, 1989; Burdman *et al*., 1998). Organic acid producing ability was identified with change in the colour of bromothymol blue dyed agar media from blue to yellow/orange (Vincent, 1970). Indole acetic acid production with or without L-tryptophan using salkowski reagent was measured at 535 nm on spectrophotometer and IAA concentration was calculated using standard curve (Sarwar *et al*., 1992). Siderophores activity was recorded by observing the change in the colour of chrom azurol S (CAS) agar medium due to the inoculation of bacteria (Schwyn and Neilands, 1987).

Statistical Analysis

Data collected was analyzed statistically following completely randomized design in two factor factorial settings by two way analysis of variance (ANOVA) (Steel *et al*., 1996) and treatment means were compared using Tukey's test. The statistical software "Statistix" version 8.1 was used for analysis (Copy right 2005, Analytical Software, USA).

Results

Drought Tolerance of Rhizobia from Different Legumes

Rhizobia showed significant capability to withstand the simulated drought conditions (Table 1). Among the isolates from lentil, LR-29, LR-32 and LR-30 were prominent at 15, 25 and 35% PEG-6000 containing YMB, respectively. Whereas isolate LR-19 was following LR-29 at 15% PEG stress and LR-29 was following LR-32 and LR-30 at 25 and 35% PEG induced drought, respectively. Isolate MR-18 showed highest optical density among isolates followed by MR-2 at 15% PEG (Table 1). At 25% PEG, highest OD was recorded by MR-13 followed by MR-6. Whereas, in the most desiccated condition (35% PEG), MR-2 remained prominent among the isolates, whereas MR-13 was at second place. When chickpea rhizobial isolates were compared for drought tolerance, then CR-29 was fair enough to stand at 35% PEG induced drought followed by CR-26, CR-38 and CR-39 as compared to other isolates (Table 1). At 25% PEG, the isolate CR-25 showed highest OD followed by CR-29. On the other hand, isolate CR-25 gave highest OD among the isolates followed by CR-26 at 15% PEG concentration.

Overall, rhizobial isolates from chickpea were more tolerant, keeping lentil nodular isolates at second place but the isolates from the nodules of mung bean were least tolerant to desiccation compared to isolates from chickpea and lentil.

Screening of Rhizobia having Potential to Improve Plant Growth under Drought

***Rhizobium leguminosarum*:** Rhizobial isolates from lentil nodules showed a significant capability to reduce/ameliorate the impact of drought on wheat seedlings and improved their growth (root or shoot length and fresh or dry weight)

Table 1: Optical density (OD_{600}) of rhizobial isolates from lentil, mung bean and chickpea nodules after 96 hours incubation in YEM without and with 15, 25 and 35% PEG (osmotic potential, -0.04, -0.70,-1.67 and -2.18 MPa, respectively)

Isolates	Lentil rhizobia (n = 3) (optical density (OD) at 600 nm)			
	-0.04 MPa	-0.70 MPa	-1.67 MPa	-2.18 MPa
LR-15	0.50 ± 0.00	0.31 ± 0.01	0.17 ± 0.01	0.07 ± 0.00
LR-18	0.79 ± 0.01	0.26 ± 0.00	0.16 ± 0.00	0.09 ± 0.00
LR-19	1.11 ± 0.01	0.47 ± 0.00	0.22 ± 0.00	0.09 ± 0.01
LR-21	0.53 ± 0.01	0.25 ± 0.00	0.14 ± 0.00	0.09 ± 0.00
LR-23	0.50 ± 0.01	0.28 ± 0.01	0.13 ± 0.00	0.07 ± 0.00
LR-26	0.53 ± 0.00	0.22 ± 0.00	0.14 ± 0.00	0.10 ± 0.00
LR-29	1.02 ± 0.01	0.70 ± 0.00	0.23 ± 0.00	0.14 ± 0.00
LR-30	0.41 ± 0.01	0.31 ± 0.00	0.22 ± 0.00	0.16 ± 0.01
LR-32	0.79 ± 0.01	0.45 ± 0.00	0.26 ± 0.01	0.12 ± 0.01
LR-33	0.68 ± 0.01	0.24 ± 0.00	0.20 ± 0.00	0.08 ± 0.01
	Mung bean rhizobia (n = 3) (optical density (OD) at 600 nm)			
MR-2	0.94 ± 0.00	0.37 ± 0.01	0.21 ± 0.01	0.17 ± 0.01
MR-3	0.49 ± 0.00	0.29 ± 0.01	0.22 ± 0.02	0.06 ± 0.01
MR-6	0.44 ± 0.00	0.36 ± 0.03	0.23 ± 0.03	0.05 ± 0.00
MR-8	0.44 ± 0.01	0.12 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
MR-11	0.60 ± 0.00	0.20 ± 0.01	0.11 ± 0.00	0.11 ± 0.01
MR-12	0.36 ± 0.01	0.16 ± 0.00	0.15 ± 0.00	0.10 ± 0.00
MR-13	0.73 ± 0.01	0.20 ± 0.01	0.26 ± 0.00	0.12 ± 0.00
MR-16	0.60 ± 0.00	0.21 ± 0.01	0.08 ± 0.00	0.08 ± 0.01
MR-17	0.47 ± 0.00	0.13 ± 0.00	0.09 ± 0.01	0.08 ± 0.01
MR-18	0.61 ± 0.01	0.39 ± 0.00	0.12 ± 0.01	0.08 ± 0.00
MR-22	0.42 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
MR-42	0.50 ± 0.00	0.24 ± 0.00	0.19 ± 0.01	0.11 ± 0.00
MR-43	0.72 ± 0.01	0.18 ± 0.00	0.15 ± 0.00	0.11 ± 0.00
	Chickpea rhizobia (n = 3) (optical density (OD) at 600 nm)			
CR-21	0.37 ± 0.00	0.25 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
CR-24	0.51 ± 0.00	0.22 ± 0.01	0.17 ± 0.00	0.14 ± 0.00
CR-25	0.52 ± 0.01	0.33 ± 0.01	0.24 ± 0.01	0.14 ± 0.00
CR-26	0.87 ± 0.01	0.31 ± 0.00	0.17 ± 0.00	0.15 ± 0.01
CR-28	0.64 ± 0.00	0.25 ± 0.00	0.15 ± 0.00	0.07 ± 0.00
CR-29	0.31 ± 0.01	0.23 ± 0.01	0.20 ± 0.00	0.19 ± 0.01
CR-30	0.72 ± 0.00	0.25 ± 0.00	0.12 ± 0.00	0.12 ± 0.01
CR-31	0.54 ± 0.01	0.22 ± 0.00	0.14 ± 0.00	0.12 ± 0.00
CR-34	0.43 ± 0.00	0.23 ± 0.01	0.17 ± 0.00	0.11 ± 0.00
CR-35	0.86 ± 0.00	0.22 ± 0.01	0.13 ± 0.00	0.12 ± 0.01
CR-38	0.29 ± 0.01	0.19 ± 0.00	0.15 ± 0.00	0.15 ± 0.00
CR-39	0.50 ± 0.00	0.22 ± 0.00	0.18 ± 0.00	0.15 ± 0.00

Standard error of each mean has been mentioned as (mean ± SE) after calculation on Microsoft Excel sheet

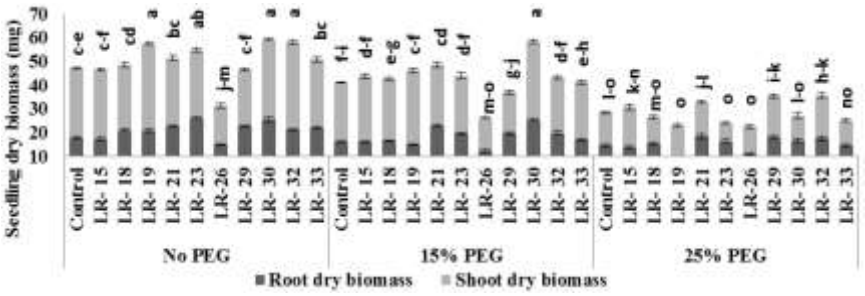

Fig. 1: Dry biomass of wheat seedlings influenced by the inoculation of rhizobia isolated from lentil nodules under normal condition and drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment and certain bars are invisible due to very small value. Different letters indicate significantly different seedling dry biomass at $p \leq 0.05$

in contrast to un-inoculated control (Table 2; Fig. 1). However, few isolates caused reduction in seedling growth including LR-26, LR-33 and LR-15. Root length of the wheat seedlings was significantly improved with the inoculation of LR-19, LR-23 and LR-32 as compared to un-inoculated control under un-stressed condition. When inoculated plants were irrigated with half strength Hoagland solution containing 15% PEG, LR-30 inoculated seedlings showed significant improvement (20%) in root length over respective control. However, isolate LR-15

Table 2: Plant growth promotion of wheat seedlings under drought (No PEG, 15 and 25% PEG) when inoculated with *Rhizobium leguminosarum* isolated from lentil nodules in gnotobiotic conditions

Isolates	Root length (cm) (n = 3)			Shoot length (cm) (n = 3)		
	-0.02 MPa	-0.62 MPa	-1.23 MPa	-0.02 MPa	-0.62 MPa	-1.23 MPa
Control	16.1±0.28d-i	14.8±0.15f-k	10.3±0.37no	19.2±0.19e-g	16.0±0.29kl	11.0±0.26no
LR-15	14.8±0.42f-j	14.0±0.29h-m	11.3±0.25m-o	17.7±0.44h-j	17.2±0.24i-k	11.8±0.27m-o
LR-18	16.5±0.55d-h	16.2±0.42d-h	13.3±0.73i-m	20.8±0.60b-d	18.2±0.44g-i	12.2±0.60mn
LR-19	20.0±0.29ab	16.8±0.44c-g	10.0±0.58o	21.7±0.44b-d	20.3±0.73b-f	11.5±0.87m-o
LR-21	17.3±0.44b-f	16.3±0.44d-h	12.8±0.44j-n	19.8±0.44d-f	17.2±0.60i-k	12.5±0.29m
LR-23	19.3±0.44a-c	15.2±0.44e-j	12.0±0.58k-o	21.5±0.29b-d	17.7±0.60h-j	7.5±0.29p
LR-26	15.6±0.34d-j	14.4±0.80g-l	10.3±0.67no	20.0±0.76c-f	15.8±0.38kl	11.2±0.47m-o
LR-29	17.8±0.44a-e	15.3±0.60d-j	11.3±0.60m-o	21.3±0.60bc	16.3±0.33j-l	15.0±0.58l
LR-30	18.0±0.29a-d	17.7±0.44b-e	11.7±0.67l-o	23.5±0.76a	20.7±0.33b-e	10.5±0.76o
LR-32	20.5±0.29a	14.7±0.88f-k	10.5±0.29no	24.7±0.44a	19.0±0.58f-h	11.8±0.43m-o
LR-33	15.5±0.29d-j	14.8±0.43f-k	11.5±0.00m-o	18.0±0.58g-i	16.0±0.58kl	11.5±0.29m-o
HSD	0.48			1.44		
	Root fresh weight (mg/plant) (n = 3)			Shoot fresh weight (mg/plant) (n = 3)		
Control	177±8.8cd	127±8.8e-h	63±3.3j-l	190±2.9ef	153±2.0hi	47±1.8n
LR-15	147±3.3c-f	120±5.8f-i	35±2.9kl	163±2.0gh	114±3.5l	37±2.0n-p
LR-18	233±3.3ab	173±6.7c-e	43±12.0j-l	217±2.9d	170±2.3g	30±2.9op
LR-19	150±5.8c-f	137±8.8d-g	30±5.8l	217±2.9d	213±3.7d	27±1.2p
LR-21	153±14.5c-f	120±5.8f-i	80±5.8h-k	167±3.3gh	143±1.8ij	47±2.0n
LR-23	237±14.5ab	140±5.8d-f	37±12.0kl	244±2.1c	213±3.3d	47±0.9no
LR-26	120±5.8f-i	90±5.8g-j	40±5.8kl	177±3.3fg	123±3.8kl	37±1.5n-p
LR-29	180±5.8cd	140±10.0d-f	73±8.8i-l	170±3.5g	83±3.3m	37±2.4n-p
LR-30	257±8.8a	190±5.8bc	40±5.8kl	267±2.4b	187±2.0f	40±1.7n-p
LR-32	253±14.5a	137±8.8d-g	45±8.7j-l	303±3.3a	203±2.4de	30±0.6op
LR-33	135±8.7d-g	140±11.6d-f	45±8.7j-l	130±1.2jk	120±2.3kl	30±1.2op
HSD	8.61			14.17		

Means sharing similar letters are statistically similar to each other at $p \leq 0.05$. The table represents the interaction effect of drought and rhizobial inoculation on different growth parameters of wheat seedlings under axenic conditions. HSD represents honestly significant difference among treatment means and standard error of each mean has been mentioned as (mean ± SE) after calculation on Microsoft Excel sheet

reduced root growth up to 5% of the un-inoculated control at 15% PEG solution treatment but remained statistically similar to control. Application of 25% PEG solution affected root growth severely but root length of the most of the inoculated seedlings was at par with un-inoculated control except LR-18, which improved root length up to 30%. As far as, shoot length is concerned, LR-18, LR-19, LR-23, LR-29, LR-30 and LR-32 inoculation significantly improved shoot length over control in normal conditions. But LR-15 caused non-significant decline in shoot length as compared to control in un-stressd condition (Table 2). At 15% PEG, LR-18, LR-19, LR-23, LR-29, LR-30 and LR-32 inoculation showed significantly high shoot length over respective control. In the meantime, isolates LR-15, LR-21, LR-26, LR-29 and LR-33 were statistically similar with the respective un-inoculated control at 15% PEG. Similar trend was observed at 25% PEG simulated drought where inoculation remained at par with respective un-treated control but LR-29 and LR-21 produced significant increase of 36 and 13% in shoot length, respectively, compared to respective un-inoculated control. Rhizobial isolates LR-18, LR-23, LR-30 and LR-32 significantly improved root fresh weight of the wheat seedlings from 32-45% over control at normal condition and remained at par with each other (Table 2). Isolate LR-26 influenced root fresh weight negatively but non-significantly by reducing it up to 32% compared to control. Isolate LR-30 inoculation gave significant improvement in the root fresh weight of the wheat seedlings as compared to un-inoculated control at 15% PEG simulated drought. The seedlings inoculated with LR-21 showed highest root fresh weight among the treatments followed by LR-29 at 25% PEG induced drought however, the differences were non-significant. At 25% PEG, inoculation of isolates from lentil nodules could not develop significant differences from the control for shoot fresh biomass (Table 2). Inoculation of rhizobial isolates LR-18, LR-19, LR-23, LR-30 and LR-32 gave significant improvement in the shoot fresh weight of the wheat seedlings under normal as well as drought induced by 15% PEG over respective un-inoculated controls. At 25% PEG induced drought, LR-21 and LR-32 increased root and shoot dry biomass up to 27 and 30%, respectively, in contrast to the respective controls followed by LR-29 (about 25%) for root and shoot dry biomass, respectively (Fig. 1). Under 15% PEG induced drought, LR-30 showed highest root dry biomass followed by LR-21 and LR-30 again showed maximum shoot dry mass followed by LR-19. However, seedling dry biomass was maximum by LR-30 and LR-32 inoculation at 15 and 25% PEG induced drought, respectively, followed by LR-21 and LR-29. Isolate LR-26 remained non beneficial and reduced seedling dry biomass up to 37 and 20% at 15 and 25% PEG induced drought, respectively, compared to the respective controls.

***Rhizobium phaseoli*:** Rhizobial isolates from mung bean nodules performed significantly for ameliorating the impact of drought on seedling growth of wheat (Table 3).

Isolate MR-2 gave significantly high improvement in the root length of the seedlings up to 37% compared to control under normal conditions but remained at par with other isolates. Similar trend was followed at 15 and 25% PEG solution treatment where rhizobial inoculation produced 22-42% and 29-65% increase in root length of seedlings, respectively, compared to respective controls. Maximum shoot length was recorded by MR-2 inoculation among the isolates followed by MR-13 and MR-6 compared to un-inoculated control under normal conditions (Table 3). However, MR-22 gave 49 and 78% more shoot length with respect to control at 15 and 25% PEG, respectively. The isolates MR-12 and MR-2 were following MR-22 for shoot length at 25 and 15% PEG induced water stress, respectively. Root fresh weight was significantly affected due to the inoculation of rhizobia but the results of many isolates remained indifferent from each other (Table 3). Under highest drought level (25% PEG), isolates MR-2, MR-6, MR-12, MR-13 and MR-16 were prominent for improving root fresh weight as compared to un-inoculated control. At 15% PEG induced drought, significantly high improvement (up to 69%) in root fresh weight was observed by MR-2 inoculation over respective control followed by MR-6, MR-13 and MR-43. A marked decrease in root biomass was observed with MR-8 (26%) as compared to control under normal conditions whereas, its inoculation could not make significant differences at 15 and 25% PEG induced drought as compared to respective controls. Isolate MR-2, MR-6, MR-13 and MR-22 inoculation significantly improved the shoot fresh biomass over respective control under normal conditions. At 15% PEG induced drought, isolate MR-13 inoculation gave significant improvement in shoot fresh weight (up to 23%) followed by MR-2 and MR-6. Inoculation of MR-2 gave highest significant increase in the shoot fresh weight up to 22 and 124% as compared to corresponding control at normal and 25% PEG stressed conditions, respectively (Table 3). Isolates MR-11 and MR-18 caused highest decrease in shoot fresh biomass compared to control under normal conditions followed by MR-16 and MR-17 at 15% PEG induced stress in contrast to respective control. However, MR-16 caused maximum reduction in shoot fresh weight compared to respective control when irrigated with 25% PEG solution. Seedling dry biomass was improved due to rhizobial inoculation under PEG induced drought conditions where maximum biomass was recorded i.e. 31.0 and 27.5 mg due to isolate MR-2 inoculation at 15 and 25% PEG induced water deficit, respectively followed by isolates MR-3 (30.9 mg) and MR-

Table 3: Plant growth promotion of wheat seedlings under drought (No PEG, 15 and 25% PEG) when inoculated with *Rhizobium phaseoli* isolated from mung bean nodules in gnotobiotic conditions

Isolates	Root length (cm) (n = 3)			Shoot length (cm) (n = 3)		
	-0.02 MPa	-0.62 MPa	-1.23 MPa	-0.02 MPa	-0.62 MPa	-1.23 MPa
Control	11.1±0.35i-k	9.9±0.36k	7.7±0.24l	16.2±0.38p-t	14.1±0.32u-w	10.6±0.23x
MR-2	15.2±0.22a	14.1±0.38a-d	12.6±0.32d-i	24.9±0.31a	21.0±0.36d-i	15.2±0.25q-v
MR-3	13.5±0.29a-g	12.80.32c-i	9.9±0.35k	22.5±0.29b-d	20.2±0.35f-k	12.3±0.35wx
MR-6	14.7±0.22ab	13.7±0.29a-g	11.5±0.24h-k	23.3±0.33a-c	20.4±0.31e-j	15.4±0.30q-v
MR-8	13.6±0.37a-g	12.0±0.39g-j	11.1±0.22i-k	21.7±0.31c-g	18.4±0.32k-o	14.4±0.19t-v
MR-11	14.5±0.29a-c	12.9±0.15c-h	11.2±0.38h-k	19.6±0.22i-m	17.8±0.36m-p	15.0±0.29r-v
MR-12	14.2±0.27a-d	13.5±0.38a-g	11.3±0.32h-k	22.0±0.23c-f	20.3±0.33e-j	16.3±0.33p-s
MR-13	14.8±0.39ab	13.9±0.09a-f	12.2±0.33e-j	24.0±0.40ab	19.6±0.36i-m	15.2±0.37q-v
MR-16	13.9±0.24a-e	12.8±0.30c-i	11.1±0.39i-k	18.9±0.31j-m	16.6±0.38o-r	14.7±0.39r-v
MR-17	14.5±0.03a-c	12.2±0.32e-j	10.6±0.38jk	19.8±0.33h-l	18.2±0.38l-o	14.4±0.30t-v
MR-18	14.0±0.09a-d	12.6±0.35d-i	11.3±0.15h-k	20.8±0.27d-i	17.0±0.33n-q	14.5±0.26s-v
MR-22	14.2±0.27a-d	13.3±0.36b-g	11.1±0.34i-k	22.2±0.33b-e	21.1±0.38d-i	18.8±0.22j-n
MR-42	13.9±0.38a-f	12.2±0.17f-j	11.2±0.29h-k	21.5±0.26c-h	20.0±0.32g-l	15.8±0.29q-u
MR-43	13.9±0.32a-f	12.2±0.36e-j	11.5±0.29h-k	21.6±0.32c-h	21.0±0.27d-i	13.8±0.38vw
HSD	1.74			1.84		
	Root fresh weight (mg/plant) (n = 3)			Shoot fresh weight (mg/plant) (n = 3)		
Control	86±2.1cd	45±2.6g-k	30±1.7k	175±3.7c-e	135±3.7hi	45±3.3l-o
MR-2	120±2.9a	77±2.0de	50±1.7f-i	213±2.9a	165±2.3d-g	100±2.3j
MR-3	80±3.5d	57±2.4fg	30±1.7k	190±1.7bc	140±1.7hi	27±3.3p
MR-6	107±0.9ab	63±3.3ef	50±3.5f-i	197±3.3b	160±3.5e-g	60±1.7kl
MR-8	63±3.3ef	47±3.8g-j	33±3.3jk	170±2.1d-f	127±3.3i	33±0.9op
MR-11	97±3.3bc	60±5.8fg	40±0.6h-k	130±1.2i	127±2.4i	53±3.3k-m
MR-12	100±1.7bc	57±1.8fg	47±3.3g-j	157±3.3fg	130±1.7i	50±0.6k-n
MR-13	120±1.7a	63±3.8ef	50±2.9f-i	200±2.3ab	167±3.3d-f	63±0.9k
MR-16	87±3.8cd	47±2.4g-j	47±3.3g-j	163±3.3e-g	103±3.3j	30±1.7op
MR-17	103±3.8b	50±0.6f-i	33±1.8jk	173±2.4de	103±2.9j	40±2.3m-p
MR-18	80±2.3d	53±3.3f-h	33±3.5jk	130±3.5i	130±1.2i	57±3.8kl
MR-22	103±2.4b	50±0.2f-i	37±2.0i-k	197±2.7b	150±0.6gh	50±2.9k-n
MR-42	87±3.3cd	53±2.3f-h	37±1.8i-k	167±3.8d-f	133±2.0i	37±3.3n-p
MR-43	107±2.9ab	63±3.3ef	37±3.3i-k	180±3.5cd	137±3.3hi	53±0.9k-m
HSD	16.20			15.55		

Means sharing similar letters are statistically similar to each other at $p \leq 0.05$. The table represents the interaction effect of drought and rhizobial inoculation on different growth parameters of wheat seedlings under axenic conditions. HSD represents honestly significant difference among treatment means and standard error of each mean has been mentioned as (mean ± SE) after calculation on Microsoft Excel sheet

Table 4: Plant growth promotion of wheat seedlings under drought (No PEG, 15 and 25% PEG) when inoculated with *Mesorhizobium ciceri* isolated from chickpea nodules in gnotobiotic conditions

Isolates	Root length (cm) (n = 3)			Shoot length (cm) (n = 3)		
	-0.02 MPa	-0.62 MPa	-1.23 MPa	-0.02 MPa	-0.62 MPa	-1.23 MPa
Control	10.7±0.35r	8.6±0.39s	6.9±0.38t	18.0±0.29j	15.7±0.18k	7.6±0.09r
CR-21	16.3±0.29a-d	14.8±0.29d-j	12.5±0.25m-q	23.8±0.32a	19.0±0.32g-j	12.0±0.29op
CR-24	16.8±0.12ab	14.5±0.25e-k	12.6±0.23m-p	20.7±0.27d-f	18.5±0.20ij	12.2±0.29n-p
CR-25	14.8±0.36d-j	13.4±0.23j-o	11.8±0.29o-r	20.3±0.35e-h	17.6±0.33j	9.7±0.15q
CR-26	15.1±0.30c-j	14.3±0.29f-l	13.6±0.15j-n	21.9±0.35b-d	18.4±0.29ij	11.5±0.18op
CR-28	15.9±0.23a-e	13.9±0.35h-n	11.8±0.25p-r	19.7±0.27f-i	17.7±0.38j	9.7±0.35q
CR-29	16.5±0.29a-c	14.8±0.39d-j	12.7±0.15l-p	21.3±0.31c-f	18.9±0.35g-j	14.4±0.38k-m
CR-30	16.2±0.30a-d	15.4±0.18b-h	11.8±0.27o-r	23.0±0.32ab	22.5±0.29a-c	13.7±0.33l-n
CR-31	14.0±0.29g-m	13.7±0.27i-n	12.4±0.19n-q	21.8±0.27b-e	14.5±0.29k-m	11.3±0.33pq
CR-34	15.2±0.31c-i	14.5±0.32e-k	10.9±0.30qr	21.0±0.29c-f	13.0±0.29m-o	11.3±0.35pq
CR-35	15.8±0.24a-f	13.0±0.26k-p	10.3±0.33r	20.3±0.33d-g	18.6±0.23h-j	12.3±0.27n-p
CR-38	17.2±0.32a	14.6±0.15e-j	12.4±0.23n-q	20.6±0.30d-f	18.2±0.17ij	11.7±0.37op
CR-39	16.3±0.18a-d	15.6±0.22b-g	12.9±0.21l-p	22.5±0.06a-c	18.1±0.29ij	15.2±0.22kl
HSD	1.57			1.65		
	Root fresh weight (mg/plant) (n = 3)			Shoot fresh weight (mg/plant) (n = 3)		
Control	61±3.3e-g	51±3.3f-i	21±2.4l	161±2.0d-f	126±3.7h	25±2.0o
CR-21	90±2.3a-c	53±2.9f-i	27±2.0j-l	193±0.9b	110±1.2i	20± 1.7o
CR-24	87±3.5a-c	63±3.3e-g	37±3.3i-l	163±3.3c-f	137±3.8gh	67± 1.5lm
CR-25	83±3.3b-d	53±3.3f-i	50±0.6f-i	173±3.8cd	133±3.3h	40±2.3n
CR-26	73±3.3c-e	50±1.7f-i	40±2.3i-k	150±3.5fg	100±3.5ij	40±3.5n
CR-28	83±3.3b-d	63±3.3e-g	27±3.7j-l	153±2.4ef	127±2.0h	23±2.4o
CR-29	83±3.8b-d	67±3.4d-f	50±2.5f-i	157±2.0ef	137±1.8gh	87±1.8jk
CR-30	90±3.5a-c	83±2.9b-d	50±1.2f-i	197±1.9ab	150±3.1fg	70±3.5lm
CR-31	90±2.3a-c	50±3.5f-i	40±1.2i-k	210±1.2a	57±2.4m	27±2.0no
CR-34	97±3.4ab	37±2.4i-l	23±3.5kl	153±3.3ef	70±0.6lm	33±0.9no
CR-35	87±3.3a-c	67±2.0d-f	47±3.4g-i	167±3.8c-e	130±1.2h	57±3.7m
CR-38	90±3.8a-c	47±2.2g-i	43±2.0h-j	163±3.5c-f	100±0.6ij	33±2.9no
CR-39	103±3.3a	77±3.3c-e	60±1.7e-h	197±1.8ab	177±2.4c	77±2.9kl
HSD	16.70			14.78		

Means sharing similar letters are statistically similar to each other at $p \leq 0.05$. The table represents the interaction effect of drought and rhizobial inoculation on different growth parameters of wheat seedlings under axenic conditions. HSD represents honestly significant difference among treatment means and standard error of each mean has been mentioned as (mean ± SE) after calculation on Microsoft Excel sheet

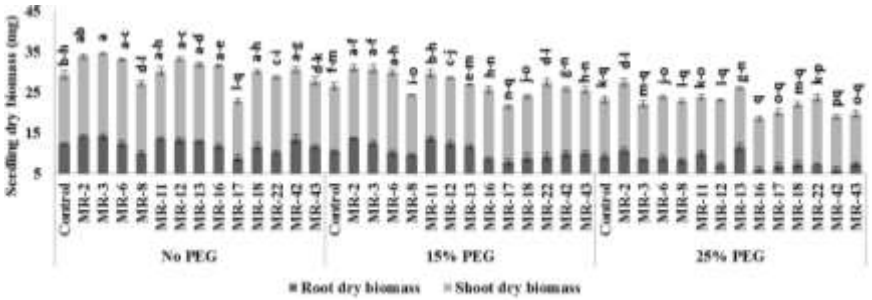

Fig. 2: Dry biomass of wheat seedlings influenced by the inoculation of rhizobia isolated from mung bean nodules under normal condition and drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment and certain bars are invisible due to very small value. Different letters indicate significantly different seedling dry biomass at $p \leq 0.05$

13 (26.0 mg) at respective 15 and 25% PEG induced droughts (Fig. 2). However, shoot dry biomass was maximally produced by isolate MR-6 (19.7 mg) followed by MR-3 (18.4 mg) at 15% PEG induced drought. But root dry matter at 15% PEG induced water limited condition was higher with MR-2 (13.8 mg) inoculation followed by MR-11 (13.6 mg). At 25% PEG induced drought MR-13 (11.5 mg) and MR-2 (16.7 mg) caused maximum root and shoot dry biomass production, respectively, followed by MR-2 (10.8 mg) and MR-22 (16.5 mg), respectively. Maximum decrease in seedling dry biomass was observed by MR-16 and MR-17 up to 20 and 19% at 25 and 15% PEG induced drought, respectively, in contrast to respective controls.

***Mesorhizobium ciceri*:** Isolates from the nodules of chickpea performed good for improving the seedling growth of wheat under PEG induced water deficit conditions (Table 4). Root length of the seedlings was affected seriously due to drought but the inoculation of rhizobia prominently increased the root length at 15 and 25% PEG induced drought as well as under normal conditions. Under normal conditions and at 25% PEG induced drought, rhizobial inoculation significantly promoted the shoot length

over control (Table 4). At 15% PEG induced drought, except isolates CR-31 and CR-34, all the inoculated rhizobia gave significant improvement in the shoot length of the wheat seedlings as compared to un-inoculated control. At 25% PEG induced stress, isolate CR-39 showed maximum significant improvement in root fresh weight followed by CR-25, CR-26, CR-29, CR-30, CR-31, CR-35 and CR-38. At 15% PEG induced drought, CR-30 inoculation gave significantly high increase (63%) in the root fresh weight over control followed by CR-39 (up to 51% increase). Shoot fresh weight improvement was also significant due to CR-39 inoculation at 15% PEG induced drought with 41% increase over respective control followed by CR-30 with 19% increase. In the same lines, CR-29 showed maximum significant shoot fresh weight at 25% PEG stress followed by CR-39, CR-30, CR-24, CR-35, CR-25 and CR-26. Inoculated isolate CR-29 showed maximum seedling dry biomass followed by CR-30 at 25% PEG induced drought compared to control (Fig. 3). At 15% PEG induced drought, isolate CR-30 remained most prominent with 33.3 mg seedling dry biomass followed by CR-29 (31.5 mg) with respect to control (26.8 mg). When only root dry matter was compared with respect to inoculated treatments then CR-29 and CR-30 inoculation produced maximum biomass (14.2 and 11.2 mg) at 15 and 25% PEG induced drought followed by CR-39 with 14 and 10.8 mg root dry biomass, respectively, as compared to the respective controls. As well as, shoot dry matter was maximally improved by CR-30 (20.1 mg) and CR-29 (16.5 mg) at 15 and 25% PEG induced drought, respectively, followed by CR-29 (17.3 mg) and CR-30 (15.2 mg) at respective drought treatments with respect to respective controls.

Drought Tolerance Index

Rhizobial inoculation helped wheat plants to sustain their growth under water deficit stress. Therefore, change in drought tolerance index (DTI) of the plants was derived to test the potential of inoculant bacteria for improving drought abiding ability in plants. Isolates of rhizobia belonging to lentil nodules, significantly improved DTI under 15 and 25% PEG induced drought in contrast to un-inoculated control (Fig. 4). Isolates LR-19 and LR-30 remained prominent for improving DTI of wheat seedling at 15% PEG induced drought (up to 27-29%) in contrast to the respective control, whereas LR-29 was showing maximum DTI under 25% PEG induced drought with 37% increase over respective control. A negative interaction of LR-23 was observed with 32% reduction in DTI of wheat seedlings as compared to respective un-inoculated control at 25% PEG induced drought. Mung bean nodular isolates have also performed positively for enhancing DTI of wheat seedlings at all drought levels but most of the isolates were statistically at par with each other or un-inoculated control (Fig. 5). Even then MR-2, MR-22 and MR-43 improved tolerance index up to 16% followed by MR-3, MR-6 and MR-12 with 12% increase at 15% PEG induced drought over respective control. However, 6-8% decrease in DTI of seedlings was recorded due to MR-18 and MR-16 over control at -0.62 MPa (25% PEG) water potential. At -1.23 MPa, isolate MR-22 remained prominent for enhancing DTI about 31% over control, while MR-3 caused 15% decrease in tolerance index in contrast to control. Rhizobial isolate CR-30 from chickpea enhanced drought tolerance index of wheat seedlings up to 43% at 15% PEG induced drought compared to the respective control

Table 5: Identification and plant growth promoting characterization of selected rhizobial isolates

Characteristics		*R. phaseoli*	*R. leguminosarum*	*Mesorhizobium ciceri*	
		MR-2	LR-30	CR-30	CR-39
Aggregation (%)		4.61	5.63	2.68	6.93
Catalase		+	++	++	++
Exopolysaccharides		++	++	+++	++
Siderophores		-	+	-	-
Organic acid		++	-	++	++
IAA production (mg L^{-1})	Without L-TRP	1.25	2.61	1.70	3.77
	With L-TRP	19.63	27.39	16.57	34.26

Average of three repeats, (+) for possession and (-) for absence of the character

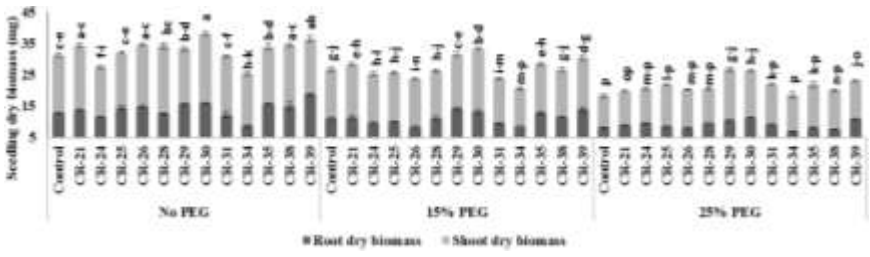

Fig. 3: Dry biomass of wheat seedlings influenced by the inoculation of rhizobia isolated from chickpea nodules under normal condition and drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment and certain bars are invisible due to very small value. Different letters indicate significantly different seedling dry biomass at $p \leq 0.05$

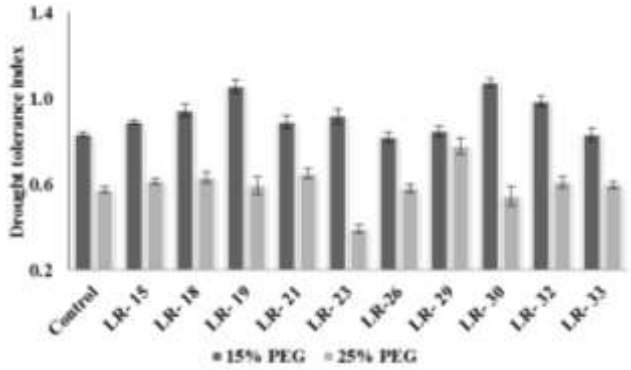

Fig. 4: Drought tolerance index of wheat seedlings on the basis of shoot length influenced by the inoculation of rhizobia isolated from lentil nodules under drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment

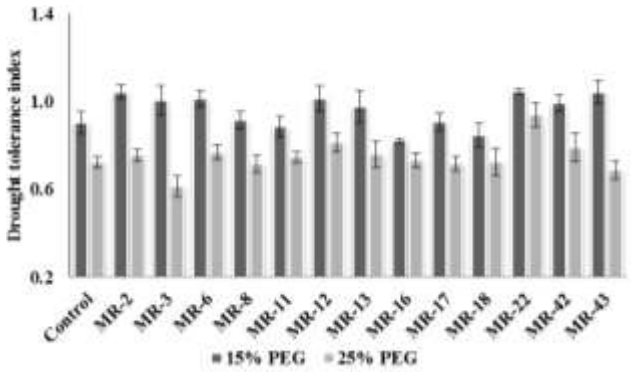

Fig. 5: Drought tolerance index of wheat seedlings on the basis of shoot length influenced by the inoculation of rhizobia isolated from mung bean nodules under drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment

followed by CR-21 with 20% increase in DTI (Fig. 6). Isolates CR-34 and CR-31 caused reduction in DTI up to 18 and 9%, respectively at 15% PEG solution treatment compared to respective control. At 25% PEG induced stress CR-39 was prominent followed by CR-29 with an increment up to 100 and 90%, respectively, over un-inoculated control.

Identification of Factors Promoting Wheat Growth by Rhizobial Inoculation

Rhizobial isolates (MR-2, LR-30, CR-30 and CR-39) selected from the series of experiments were further tested for their possible mechanisms of action which could be responsible for the improved growth of seedlings under drought (Table 5). All the four isolates were capable to produce exopolysaccharides and catalase. Isolates MR-2, CR-30 and CR-39 had the ability to produce organic acids but unable to produce siderophores. On the contrary, LR-30 can produce siderophores but had no capacity to produce organic acids. When these isolates were quantified to make aggregates, CR-39 had highest aggregation capacity

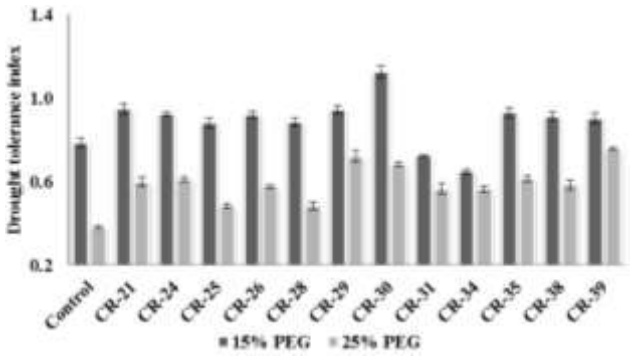

Fig. 6: Drought tolerance index of wheat seedlings on the basis of shoot length influenced by the inoculation of rhizobia isolated from chickpea nodules under drought induced by 15 and 25% PEG solutions. Vertical bars represent the standard error of each treatment

followed by LR-30, MR-2 and CR-30, respectively. Quantification of indole-3-acetic acid (IAA) production in the presence and absence of precursor L-tryptophan (T-TRP) showed enormous ability of CR-39 up to produce IAA. Other isolates including MR-2, LR-30 and CR-30 were following CR-39 for producing IAA in the absence and presence of L-TRP, respectively. Isolates CR-30 and CR-39 were identified as *Mesorhizobium ciceri*, MR-2 as *Rhizobium phaseoli* and LR-30 as *Rhizobium leguminosarum* by BIOLOG® identification system. Further, upon inoculation they caused nodulation to respective crops i.e. mung bean (MR-2), chickpea (CR-30, CR-39) and lentil (LR-30).

Discussion

Rhizobial capability to survive water deficit conditions has been proved by the findings of drought tolerance assay (at -0.70, -1.67 and -2.18 MPa osmotic potential) of the isolates from the nodules of three legume species (*Lens culinaris* L., *Vigna radiata* L. and *Cicer arietinum* L.). Their ability to produce catalases and possibly oxidases are helpful in saving cellular membranes and nucleic acid from destruction and sustaining metabolism under stress (Goyal *et al*., 1986; Boumahdi *et al*., 1999). Rhizobia are producers of exopolysaccharides which helps in the development of biofilm, where they get protection from extremities and have the availability of water and nutrients (Vanderlinde *et al*., 2010). Accumulation of osmolytes and change in cellular morphology has previously been identified as probable mechanisms to avoid damage from drought stress by rhizobia (Busse and Bottomley, 1989; Smith and Smith, 1989). One more surprising characteristics of bacteria is the siderophores production which has been reported to enhance its survivability under drought (Arzanesh *et al*., 2011). Amrani *et al*. (2010) recorded similar results, while growing rhizobial species up to 25% PEG in YMB media. Similarly, 60% of the 45 *Rhizobium*

sullae stains were reported to tolerate drought up to -0.9 MPa by Fitouri *et al.* (2012). These findings lead them to suggest that rhizobia can be utilized as inoculant to crops in marginal lands.

Inoculation of symbiotic bacteria to non-nodulating crop (wheat) seeds resulted into beneficial, neutral and harmful associations. Isolates producing beneficial association with wheat seedlings have improved the root or shoot lengths and fresh or dry biomass of the seedlings under water deficit conditions. These improvements could be due to their ability to colonize roots hugely (bacterial aggregation ability), production of phytohormones (IAA), exopolysaccharides or catalase production and increased mobilization of nutrients through lowering rhizosphere pH (organic acid) or binding the nutrients (siderophores production) (Table 5). Rhizobial inoculation has rescued plant growth through improving drought tolerance index of the seedlings, which could be due to the production of osmolytes and antioxidants in the rhizosphere. Findings of these experiments have been supported by the results of certain researchers where they used to inoculate non-legumes with rhizobium under water limited conditions. Moderately drought tolerant *Rhizobium sullae* from the semi-arid region of Tunisia significantly improved the shoot dry biomass of inoculated plants under drought (Fitouri *et al*., 2012). Similarly, exopolysaccharides producing rhizobial strain YAS34 was inoculated to sunflower under reduced water system and improvement in dry biomass, nitrogen nutrition and water uptake of plants was observed (Alami *et al*., 2000). Rhizobia (KYGT207) isolated from drying soils improved the soil aggregation in the rhizosphere of wheat seedlings due to exopolysaccharides production and thus rescued plant growth under stress (Kaci *et al*., 2005). Whereas, the auxin and cytokinin producing capability of *Rhizobium* and *Bradyrhizobium* is an important mechanism for improving dry biomass of inoculated sorghum under water deficit situation (Rashad *et al*., 2002).

Isolates LR-26, MR-17 and CR-34 showed negative association with the inoculated plants and resulted in the reduction of root or shoot lengths and fresh or dry biomasses either in normal or water stressed conditions. This harmful correlation could be due to the excessive production and accumulation of toxins like cyanides, growth regulators (auxins) and nitric oxides in the rhizosphere (Perrine *et al*., 2005; Francine *et al*., 2007).

On the basis of drought tolerance assay and plant growth promotion trials, isolates MR-2, LR-30, CR-30 and CR-39 were selected as proficient rhizobia for supporting wheat seedlings growth under drought stress. Overall, the rhizobial isolates from chickpea nodules performed better than the rhizobial isolates from mung bean and lentil nodules; therefore, two isolates were selected from the group of chickpea rhizobia. Selected isolates were identified as *Rhizobium phaseoli* (MR-2), *Rhizobium leguminosarum* (LR-30), and *Mesorhizobium ciceri* (CR-30 and CR-39) using BIOLOG® identification system (Table 5). Inoculation of the respective legumes with selected rhizobia caused nodulation to confirm their identity.

Catalase producing ability (Table 5) of the rhizobia makes them capable to stand in water deficit conditions and beneficial to host plants under stress. However, exopolysaccharides, siderophores and organic acid (Table 5) producing ability could be responsible to improve water and nutrient holding capacity and nutrient bioavailability of the rhizosphere. Auxin production (Table 5) by the selected rhizobia might have improved the root length, which enhanced the nutrient and water capturing capacity of the seedlings under stress. Microbial aggregation ability (Table 5) of the selected isolates highlights their capability to colonize the plant roots and they have good ability to live on the rhizoplane of the plants.

Inoculation of catalase and exopolysaccharides producing *Rhizobium* and *Mesorhizobium* species has proved beneficial interaction to wheat (non-legume) under water stress conditions. Further, it can be concluded that rhizobial capability to produce catalase and exopolysaccharides not only a key to their drought tolerance property but also a reliable attribute to select efficient rhizobial isolates as PGPR for non-legumes. These experiments have opened up new avenues to explore the mechanisms of rhizobia to associate and support non-legumes growth under stress environments. Anyhow, *Rhizobium phaseoli* (MR-2), *Rhizobium leguminosarum* (LR-30), and *Mesorhizobium ciceri* (CR-30 and CR-39) are potential rhizobia to rescue wheat growth under drought but evaluation trials are suggested to confirm their efficiency.

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