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Growth and Carbon metabolism assessment in finger millet (*Eleusine coracana* L. Gaertn) seedlings undergone PGPR treatment subjected to NaCl stress.

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Abstract
Salinity stress adversely effects the plant growth and productivity. To study the effect of salinity and to investigate the influence of PGPR (Plant Growth Promoting Rhizobacteria) *Acinetobacter calcoaceticus* on finger millet cultivars, we have grown two finger millet cultivars, VR-988 and VR-1076 in three sets of pots. First set of pots are controls (0 NaCl and no PGPR treatment), second set contained NaCl stressed seedlings and the third set consisted of PGPR treated seedlings subjected to NaCl stress. All the three sets of pots included cultivar VR-988 and VR-1076 maintained separately. After 48 hours seed germination was recorded. Root, shoot growth and dry weights were observed after 5th day. Chlorophyll-a, Chlorophyll-b, total Chlorophyll, total carbohydrate, starch content, reducing and non-reducing sugars, total soluble sugars (TSS) and α-amylase activity were studied on 4th, 6th and 8th day for all treatments in VR-988 and VR-1076.

Keywords: α-amylase, Chlorophyll, Plant Growth Promoting Rhizobacteria, Salinity stress.

Introduction
Plants are exposed to various environmental conditions. Plants which develop mechanisms to tolerate the adverse climatic conditions will succeed in the nature. Most of the crop plants are sensitive to the fluctuations in the environment. Majority of the crops show reduced growth and yields under abiotic stresses (Mahajan and Tuteja, 2005). Global population is raising and the food requirements of the growing population are not satisfied because of the limited availability of fertile land for cultivation due to pollution, urbanization and industrialization. This is the challenge facing the globe. One such crop that ensures food security is finger millet (*Eleusine coracana* L. Gaertn), a millet crop widely grown in arid and semi-arid areas of Asia and Africa. Nutrient deficiencies, drought and salinity are the major abiotic stresses which are severely effecting the crop growth and production (Maharajan *et al*., 2018). To attain better yields crop must first withstand and tolerate the adverse conditions like
salinity. Microorganisms growing in saline environments might serve as source to make the plants halotolerant. Scientists are focusing on this area as it is an eco-friendly approach. There were several works in various plants using PGPR, they provided evidence that these halotolerant PGPR promoted growth under saline conditions (Ramadoss *et al*., 2013; Kang *et al*., 2014). Early stages of the life cycle such as seed germination and seedling growth are very much sensitive to stresses like salinity. Hence the seedling stages were selected for most of the research works. If these stages acquire tolerance it might be advantageous for the plant to tolerate in the later developmental stages. The main objective of this work is to study the seedling growth and carbon metabolism of finger millet salt sensitive (VR-988) and salt tolerant (VR-1076) seedlings under controlled, salt stressed and bacterial (PGPR) treated under salt stress condition.

Materials and Methods
We have obtained the seed material from Agriculture Research Station, Vizianagaram, Andhra Pradesh, India. The experiment was conducted in the Physiology laboratory of

Botany department, Andhra University, Visakhapatnam. Salt tolerance assay was conducted for twelve finger millet cultivars. From the 12 cultivars two cultivars VR-988 and VR-1076 were identified as salt sensitive and salt tolerant cultivars respectively based on their performance (seed germination, seedling root length, seedling shoot length and dry weight) under NaCl stress.

Total six bacterial isolates were obtained from the soil samples. Among six isolates MGST-02 showed growth up to 8% NaCl concentration. This isolate was further identified as *Acinetobacter calcoaceticus* based on 16S rRNA sequencing and phylogenetic analysis.

Plant growth promoting assay of selected finger millet cultivars using the halotolerant PGPR *Acinetobacter calcoaceticus*

Healthy, uniform sized seeds of finger millet cultivars VR-988 and VR-1076 (salt sensitive and salt tolerant respectively) were selected then sterilized with hypo chlorite solution and washed with distilled water thrice. For evaluation of germination the seeds were grown in Petri dish containing Whatman No.1 filter paper. To study the growth parameters the seeds were grown in pots of diameter 10 cm. 20 seeds were taken in each pot.

C: control - 5 day old seedlings maintained at room temperature 28 ± 2^0 C and 60-65% relative humidity throughout the experiment were treated as control seedlings.

S: salt stressed -5 day old seedlings grown in pots supplemented with 1500 ppm NaCl solution were considered as salt stressed seedlings.

S+B: bacterial treated under salt stress- 5 day old seedlings treated with *Acinetobacter calcoaceticus* bacterium grown in pots supplemented with 1500 ppm NaCl solution.

Chlorophyll estimation

Total chlorophyll contents (Chlorophyll-a, Chlorophyll-b and total Chlorophyll) were estimated using the method of Arnon (1949). The amount of Chlorophyll-a, Chlorophyll-b and total Chlorophyll were measured as milligrams of Chlorophyll content per gram of plant tissue.

Total Carbohydrate estimation

100 mg of the sample was weighed and taken into a boiling tube. Hydrolysed by keeping it in a boiling water bath for three hours with 5 mL of 2.5 N HCl and cooled to room temperature. It was neutralized with sodium carbonate made up to 100 mL volume and centrifuged. The supernatant was collected and 0.5 and 1 mL aliquots were taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. '0' served as blank. The volume was made upto 1 mL in all the tubes including the sample tubes by adding distilled water. Then 4 mL of anthrone reagent was added. It was heated for eight minutes in a boiling water bath. Rapidly cooled and read the green to dark green colour at 630 nm.

Starch and Total Soluble Sugars (TSS) estimation

The starch and total soluble sugars were estimated according to the method of Mc Cready *et al*., (1950) as modified by Clegg (1956). Soluble sugars were separated by alcohol extraction and the residue containing starch was brought into solution with perchloric acid.

Estimation of Reducing Sugars

Total reducing sugars were estimated according to the phenol-sulfuric acid method of Dubois *et al*., (1956) as followed by Smyth and Dugger (1980).

Estimation of Non-Reducing Sugars

The reducing sugar content subtracted from the total soluble sugar content was considered as non- reducing sugars.

Estimation of α -amylase activity

Amylase activity was estimated by the method of Filner and Varner (1967) as followed by Kapoor and Sachar (1979).

The readings were taken on 4^{th}, 6^{th} and 8^{th} day.

Statistical analysis

The results obtained in our studies were subjected to statistical analysis on SPSS statistical tool, Standard Errors were calculated in order to signify the results statistically.

Results

Table- 1: showing seed germination and seedling growth of selected finger millet cultivars under various conditions.

Cultivar	Treatment	Percent germination	Root length (cm)	Shoot length (cm)	Dry weight (mg)
VR-988	C	98.5 ± 0.05[a]	3.6 ± 0.33[b]	4.8 ± 0.45 [a]	22.5 ± 0.17 [a]
VR-988	S	56.1 ± 0.28[b]	3.1 ± 0.35[c]	2.4 ± 0.03[b]	13.1 ± 0.09[b]
VR-988	B+S	89.0 ± 0.09 [a]	4.3 ± 0.29 [a]	4.3 ± 0.03 [a]	21.4 ± 0.05 [a]
VR-1076	C	100 ± 0.00[a]	3.9 ± 0.35 [ab]	4.5 ± 0.07 [a]	23.9 ± 0.99[b]
VR-1076	S	65.2 ± 0.43[b]	3.4 ± 0.42[b]	2.9 ± 0.13[b]	16.4 ± 0.05[c]
VR-1076	B+S	93.6 ± 0.84[a]	4.8 ± 0.09 [a]	4.1 ± 0.19 [a]	25.2 ± 0.34 [a]

Note: Different letter indicates the significant difference between different treatments ($p \leq 0.01$). All the results were the average of three replicates. Standard Errors were calculated for the data for significance.

C- Control
S-Salt stressed
B+S- Bacterial treated under salt stress

Table -2: showing the effect of NaCl and the influence of *Acinetobacter calcoaceticus* on chlorophyll content and carbohydrates in finger millet cultivars VR-988 and VR-1076.

Cultivar	Treatment	Day	Chl-a mg/L	Chl-b mg/L	Total Chl mg/L	Total Carbohydrate
VR-988	C	4^{th}	36.12 ±0.40 [a]	45.18±0.11 [a]	81.30±0.31 [a]	4.88±0.05[b]
		6^{th}	36.58±0.18 [a]	48.52±0.31 [a]	85.10±0.11 [a]	5.16±0.01[b]
		8^{th}	37.00±0.21 [a]	50.11±0.33 [a]	87.12±0.26 [a]	5.28±0.03[b]

VR-988	S	4th	22.30±0.33c	21.13±0.19 a	43.44±0.29c	6.87±0.02 a
		6th	23.37±0.29c	23.3 ± 0.17c	46.74±0.83c	6.99±0.05 a
		8th	23.80±0.27c	23.87±0.19c	47.67±0.39c	7.07±0.05 a
VR-988	B+S	4th	33.51±0.46b	38.47±0.05b	71.98±0.28b	4.91±0.01b
		6th	34.12±0.26b	41.54±0.28b	75.66±0.28b	5.39±0.03b
		8th	35.73±0.33b	42.25±0.54b	77.99±0.93b	5.52±0.08b
VR-1076	C	4th	35.58±0.13b	46.6±0.29b	82.28±0.54b	4.70±0.03c
		6th	36.30±0.40b	51.90±0.09b	88.21±0.98b	4.84±0.03c
		8th	37.13±0.11b	53.13±0.28b	90.27±0.84b	4.95±0.02c
VR-1076	S	4th	32.10±0.35c	40.09±0.33c	72.19±0.33c	5.95±0.08 a
		6th	33.38±0.15c	42.69±0.33c	76.07±0.35c	6.03±0.02 a
		8th	34.37±0.26c	45.61±0.85c	79.98±0.34c	6.14±0.01 a
VR-1076	B+S	4th	36.89±0.11 a	51.03±0.08 a	87.93±0.35 a	5.06±0.11b
		6th	38.71±0.19 a	52.11±0.13 a	76.07±0.35c	5.11±0.03b
		8th	38.37±0.19 a	57.06±0.27 a	95.43±0.54 a	5.51±0.03b

Note: Different letter indicates the significant difference between different treatments ($p \leq 0.01$). All the results were the average of three replicates. Standard Errors were calculated for the data for significance.

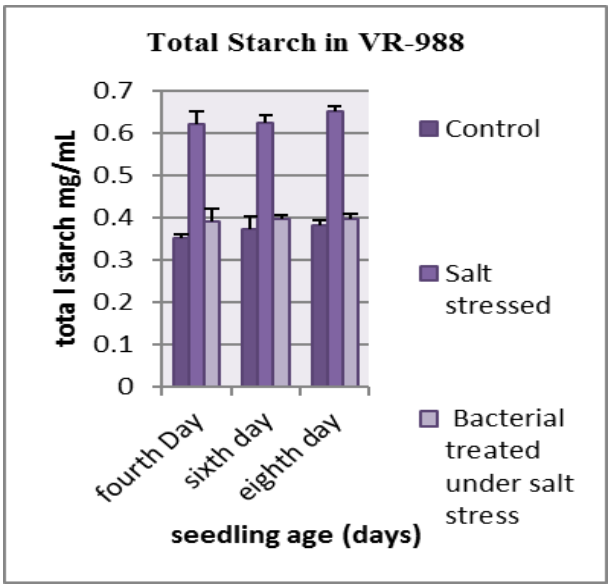

Fig. 1: Total starch content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

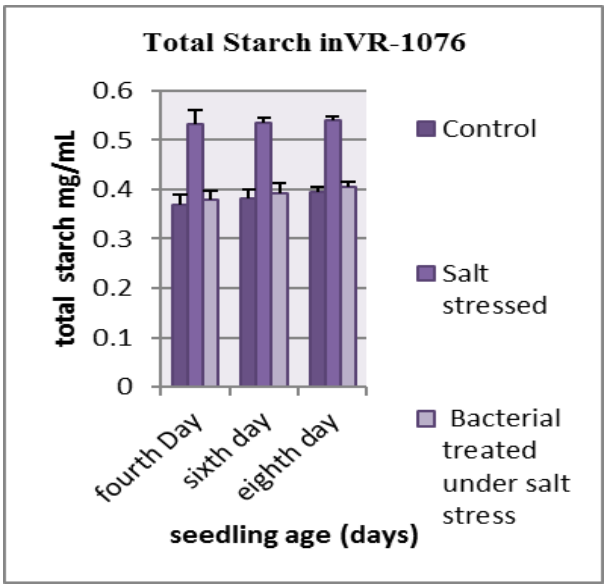

Fig. 2: Total starch content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

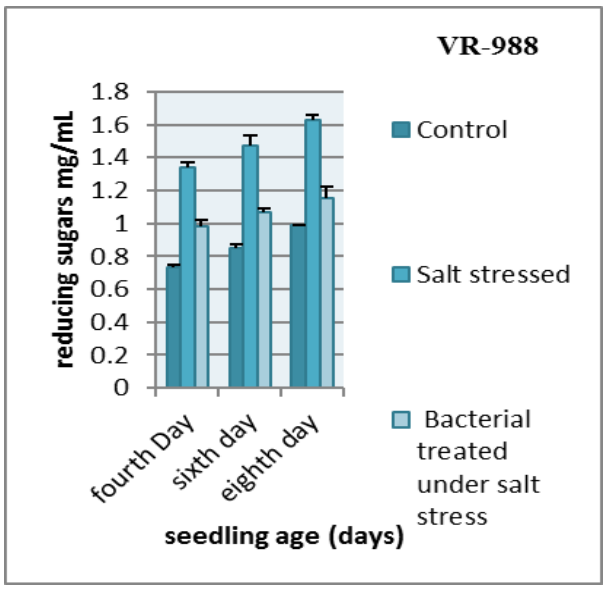

Fig. 3: Reducing sugars content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

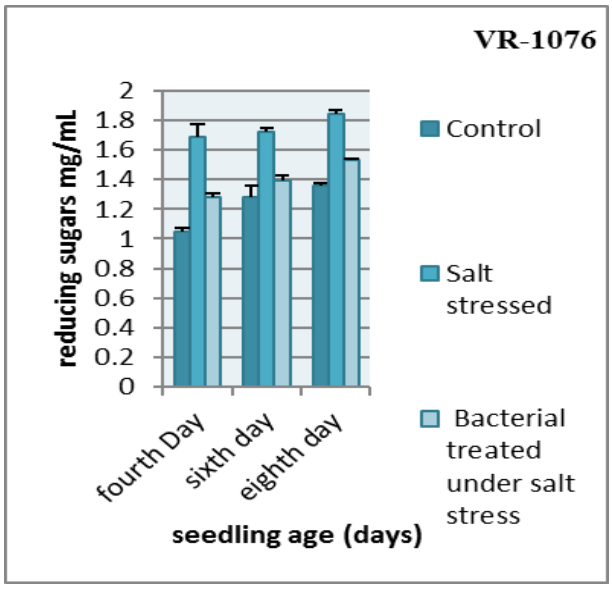

Fig. 4: Reducing sugar content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

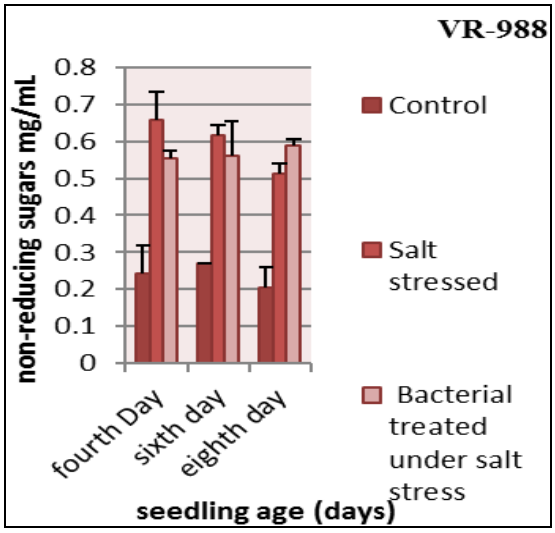

Fig. 5: Non-reducing sugars content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

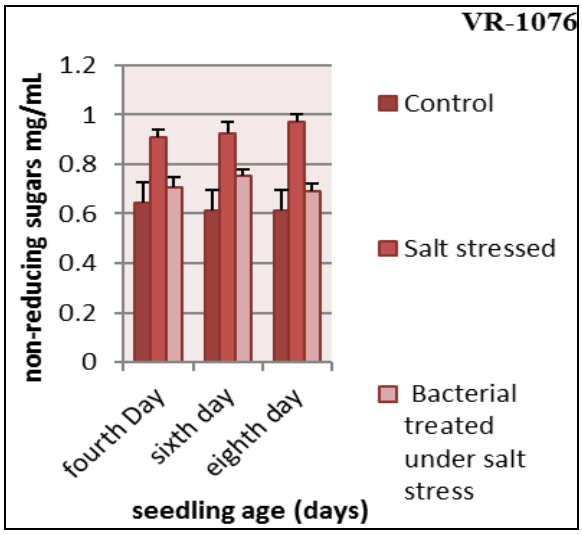

Fig. 6: Non-reducing sugar content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

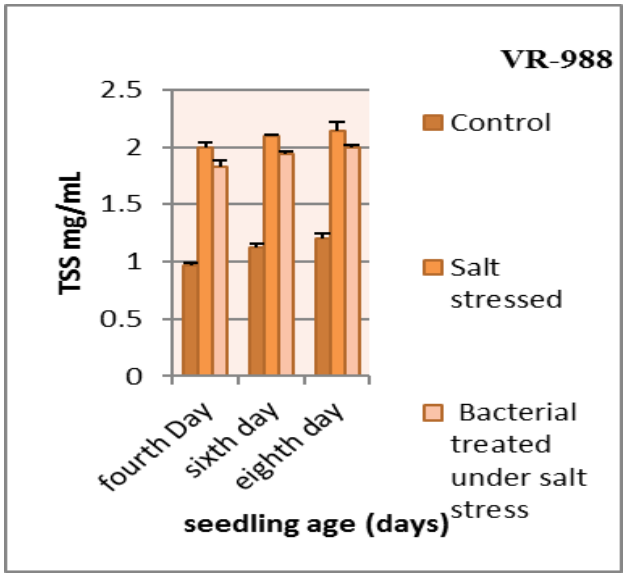

Fig. 7: Total soluble sugars in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

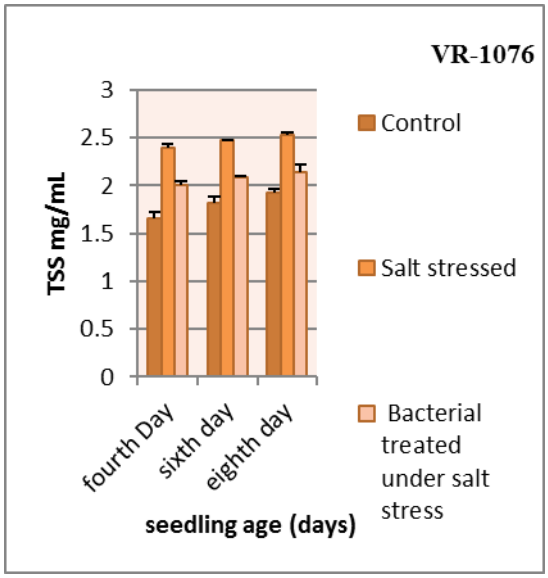

Fig. 8: Total soluble sugars in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

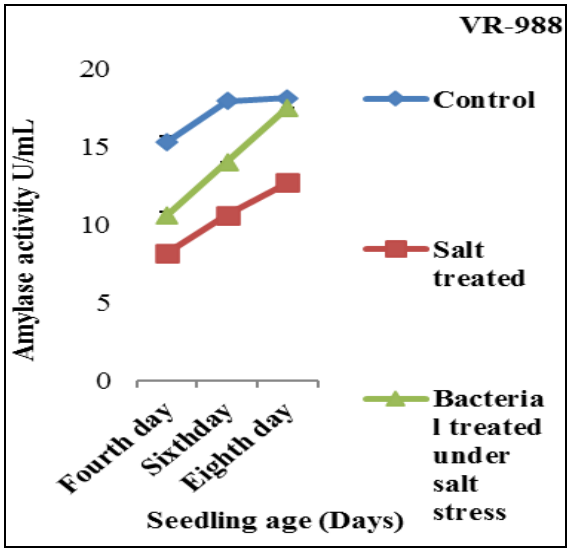

Fig. 9: Amylase activity in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

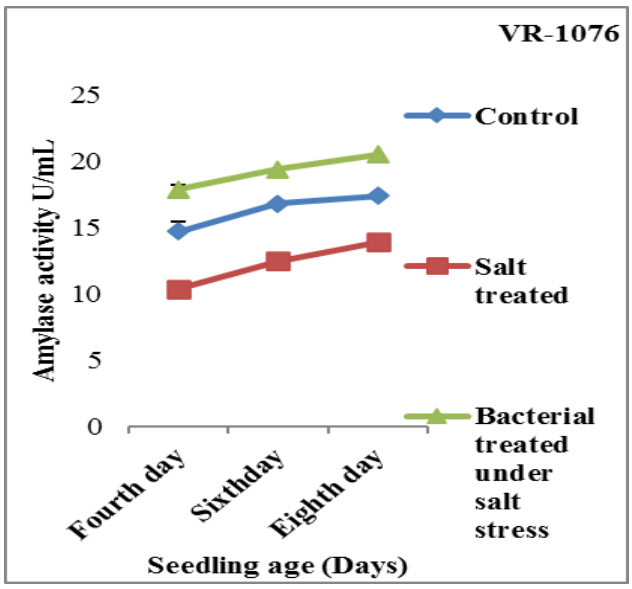

Fig. 10: Amylase activity in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

Discussion:

Seed germination and seedling growth:

Both the selected finger millet cultivars (VR-988 and VR-1076) showed reduced seed germination, root and shoot growth and dry weight under NaCl stress when compared to their respective control seeds. The seeds undergone bacterial treatment under NaCl stress improved their growth as compared to the seeds grown under NaCl stress. The effect of salt and the influence of the bacterium *Acinetobacter calcoaceticus* was more on salt sensitive finger millet cultivar, VR-988 than salt tolerant cultivar VR-1076.

Our results were in accordance with Kang *et al*., 2014, who worked in cucumber under saline soils using *Acinetobacter calcoaceticus.* Species belonging to the genus *Acinetobacter* were isolated from the rhizosphere of *Pennisetum glaucum* (pearl millet) were studied for their growth promoting activity and were observed that the application of *Acinetobacter* improved the shoot height, root length and dry weight (Rokhbakhsh-zamin *et al*., 2011). Bacteria isolated from natural saline habitats were reported to have the property of enhancing seed germination, seedling growth and dry weight in wheat was studied by Ramadoss *et al*., (2013).

Chlorophyll content:

Irrespective of treatment chlorophyll contents were increased from fourth day to eighth day in VR-988 and VR-1076. Salt stressed seedlings had lower chlorophyll contents than control and bacterial treated seedlings under salt stress. Control seedlings maintained relatively higher chlorophyll contents than salt stressed and seedlings undergone bacterial treatment under salt stress. Chlorophyll contents might be destroyed due to excessive NaCl. Han and Lee, (2005) reported that under NaCl stress lettuce plants inoculated with PGPR showed improved chlorophyll contents. Enhanced chlorophyll content under stress in presence of PGPR was also reported by Nadeem *et al*., (2006).

Carbohydrate, starch and TSS content:

Total carbohydrates, starch, reducing and non-reducing sugars and total soluble sugars (TSS) were greatly enhanced in salt stressed VR-988 and VR-1076. *A. calcoaceticus*

treatment in saline condition resulted in reduction of carbohydrates, starch and sugar contents in both the cultivars but the reduction was more in salt sensitive (VR-988) than salt tolerant (VR-1076) cultivar.

Studies conducted on rice (Amirjani, 2010) revealed that accumulation of carbohydrates was an effective mechanism to tolerate stress. Sugar accumulation under saline conditions provides defence against stress and it shows the extent of salt tolerance of plants (Bohnert and Jensen, 1996). Enhanced sugar accumulation was found during stress in groundnut (Shukla *et al*., 2012). Accumulation of starch after salt treatment was also observed in rice cultivars as reported by Pattanagul and Thitisaksakul (2008). One possible reason to accumulate starch is that the components required for synthesis of starch are simple sugars which are produced by the hydrolysis of sucrose due to the enzymatic activity of alkaline invertase. Under saline condition the enzymatic activity increased in turn leading to the accumulation of starch.

Basic levels of TSS were more in salt tolerant cultivar than salt sensitive cultivar under ideal (control) conditions. Salinity increased the reducing, non-reducing and total soluble sugars (TSS) in both selected cultivars. This demonstrates the role of sugars in providing protection against salinity. *A. calcoaceticus* treatment in saline condition resulted in lowering the sugar contents in VR-988 and VR-1076 than salt stressed seedlings but higher than in control seedlings of both cultivars. Based on this observation it was concluded that *A. calcoaceticus* applied seedlings did not register much stress and accordingly lower sugars were accumulated in bacterial treated seedlings under salt stress in VR-988 and VR-1076. Similar results were found in oat where plants produced high amounts of sugars under stress, salt stressed seedlings inoculated with *Klebsiella* produced fewer sugars than seedlings under stress but have higher levels than controls (Sapre *et al*., 2018).

α -amylase activity

As per the results α-amylase activity reduced in salt stressed seedlings of finger millet cultivars VR-988 and VR-1076 when compared to their control seedlings. Bacterial treated seedlings under salinity stress have showed improved activities of the enzyme.

The reduction in α-amylase activity may be due to NaCl toxicity or due to the less imbibition by the seeds because water as a solvent is necessary to stimulate the enzyme activity. The studies on various plants suggested that salinity has decreased the seed germination by reducing the α-amylase activity in the seeds during germination (Singh *et al*., 2001 in lentil). Amylase activity was observed to be more in salt tolerant cultivars under salt stress when compared to salt sensitive cultivars of rice (Govindaraju and Balakrishnan, 2002).

Conclusion

In our studies conducted in finger millet cultivars VR-988 and VR-1076 we observed that seed germination and seedling growth were reduced under NaCl stress where as the treatment with PGPR *Acinetobacter calcoaceticus* resulted in improved seed germination and seedling growth. Chlorophyll contents and enzymatic activity of α-amylase decreased when subjected to NaCl stress. PGPR treated seedlings under salt stress produced high quantities of chlorophyll when compared to seedlings subjected to NaCl stress without PGPR treatment and also showed improved α-amylase activity as compared to NaCl stressed seedlings. This result was similar to both VR-988 and VR-1076 but the improvement was more in VR-988 which was a salt sensitive cultivar.

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