

Original Research Article

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Evaluation of Native AMF Population for Drought Stress Ameliorating Potential in Finger Millet

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ABSTRACT

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Finger millet is a very important crop because of its resilience against adverse climatic conditions. It is well adapted to higher elevations, drought tolerant, disease resistant and able to grow on marginal lands with poor soil fertility, which reflects in having highest productivity among millets. Also, it is a good source of protein, fat, and minerals. All these factors make it suitable especially among the segment of low- income group populations. Arbuscular mycorrhizal fungi (AMF) are capable of reducing the adverse effects of drought on plants. Recently, there is a growing interest in the Finger millet-AMF symbiosis in relation to enhancing water uptake, growth, plant nutritional status and yield. However, to our knowledge, there is no study performed related to the application of native mycorrhizal population in improving drought tolerance of finger millet. Native mycorrhiza inoculation resulted enhanced biomass production compared to than non-mycorrhizal finger millet plants, irrespective of soil water status. In addition, AMF inoculation led to significantly higher nutrient and biochemical status of plants. Our results support the idea of exploiting native mycorrhizal population in improving the drought tolerance of finger millet plants.

Introduction

Among all the rhizospheric microbes, arbuscular mycorrhizal fungi (AMF) are the most interesting, incapable of living without their host plants, they form the most abundant of symbiotic association on earth. The AMF belong to the phylum Glomeromycota and are represented by 230 species (Oehl *et al.*, 2011). The mutualistic association of AMF with most of the vascular plant roots results in increased

uptake of water, inorganic nutrients, enhanced tolerance to many environmental stress and protection from pathogens for the host and in reward of this, fungus gets the carbon assimilates from host, essential for its survival (Smith and Read, 1997). It is evident by many studies that AMF are crucial for the functioning of terrestrial ecosystems. Not only their presence but also their genetic and functional diversities are of importance: AMF diversity can be decisive for both plant

community structure and ecosystem productivity (der Heijden and Scheublin, 2007).

Farmers can take better advantage of the AM symbiosis in two ways: through management of the community of AM fungi indigenous to their soils or through inoculation with effective isolates of AM fungi (Bagyaraj 1992). Also, AMF inoculation from somewhere else can suppress and can cause extinction of locally adapted species (Friberg, 2001). An alternative to purchasing inocula is for the farmer to produce it him/herself on-farm. These methods were first developed for the tropics (Gaur 1997; Gaur and Adholeya 2000; Maiti *et al.*, 2009).

Finger millet is an underutilized crop of tropics with high nutritional value. Recently, it has gained interest of researchers because of its ability to withstand water deficient condition. The current study was performed to evaluate the potential of native AMF population in ameliorating harmful effects of water stress condition in finger millet. It was considered if they are able to improve the growth and stress tolerance of a drought resilient crop, they will definitely be able to benefit susceptible crops. Monoculture of *R. intraradices* was selected as positive control because of its wide commercial application.

Materials and Methods

Inoculum preparation

The study was conducted using soil of six agricultural lands located at different altitudes of Kumaun Himalayas (Table 1). Four cores of 20 cm depth with 100 g of rhizosphere soil was taken from each sites, collected and pooled in polybags to form a composite sample. To maximize the inoculum potential and encourage sporulation by AMF species present in soil samples, we cultured AMF in

the greenhouse using a highly mycotrophic host plants, Maize, Sorghum and Cowpea. Since we had no information about the native AMF community we utilized the trap culture method using three plants instead of one crop to provide viable AMF propagules their best host for symbiosis to happen efficiently. Potting mixture included 200gm soil sample with autoclaved mixture of vermiculite and gravel (2:1). After 60 days, the plants were left for drying to enhance sporulation. Potting substrate after 8 such cycles was used as the inoculum for the study.

Pot experiment to evaluate the effect of indigenous mycorrhiza

Finger millet (var. PRM1) collected from Hill campus GBPUA&T, Ranichauri was selected for the study. The soil and sand (1:5) were sterilized (autoclaved for 2 h at 120 °C for three consecutive days) and used as potting mixture. The trap soil of each location after eight cycles was used as inoculum representing the native AMF diversity (Inoculum equivalent to 100 spores per seed was given).

Monoculture *Rhizophagus intraradices* was taken as positive control. Moisture level of potting substrate was maintained at 90 % for 55 days by weighing the pots and adding water according to the weight loss. Two sets of pots were set up for each inoculum one for irrigated and another for drought condition. Fertilization with Hoagland solution (Phosphorus strength- $\frac{1}{4}$) was done after one month of sowing seeds. After 55 days, one set was subjected to drought by withholding water till the water holding capacity (WHC) was at 50 % and harvesting was done. For biochemical parameters tissue was sampled on ice and stored at - 80 °C. Parameters measured were plant biomass, length, shoot nutrient uptake (N, P, K, Ca, Na), proline content, superoxide dismutase (SOD) activity,

Malonaldehyde (MDA) content and hydrogen peroxide (H₂O₂) level.

Plant sample collection and analysis

Plants were harvested after 60 days of sowing and washed thoroughly with tap water. After initial air drying, root/shoot length and root/shoot fresh weight were recorded. Plant samples (root and shoot) were kept in paper bags, dried at 65 °C in an electric oven until sample weight remained constant. The dry weight of shoots and roots was then assessed.

% Mycorrhiza frequency

Roots were first stained by trypan blue method. Root samples were cleared in KOH solution (2.5%) and stained using the Trypan Blue (0.05%) and stained as described by (Philips and Hayman 1970). Mycorrhizal colonization was determined by examining 1 cm root segments (n = 50 per each treatment) under the microscope according to Trouvelot *et al.*, (1986). Results are expressed as percentage frequency.

Mineral nutrition

Dried plant tissues were used for nutrient estimation. The nitrogen content of the leaves was estimated according to Kjeldahl method using the KJELPLUS System (Pelican, India). Diacid digestion of the sample was carried out using 5:1 mixture of HNO₃: HClO₄. Phosphorus content was measured by the vando-molybdate phosphate method. Sodium, potassium, and calcium were measured using flame photometer (Systronics Flame Photometer 128).

Determination of water holding capacity (WHC) of soil

Whatman no. 1 filter paper was placed in the bottom of a porous cup. It was weighed and

weight was recorded. Oven dried soil was filled in the cup gently and weight was recorded. Cup was placed into a shallow pan of water for 24 h allowing only the bottom of the cup to become wet. Soil was allowed to become saturated from the bottom of the cup to the surface. Cup was removed from the pan of water and placed in a humid enclosure for 24 h until drainage was completed and weight was recorded.

WHC of soil (%)

$$= \frac{\left(\text{Weight of the water contained in the saturated soil} \right) \times 100}{\text{Weight of the saturated soil}}$$

Where,

Weight of the water contained in the saturated soil = Weight of saturated soil - Weight of dry soil

Weight of the saturated soil = Weight of cup, filter paper and saturated soil - weight of cup and filter paper

Biochemical stress markers

Superoxide dismutase (SOD) activity

0.5 g leaf samples (fresh weight) was homogenized with a pestle in an ice-cold mortar in 5 ml cold buffer containing: 50 mM potassium phosphate buffer (pH 7.0), 1 mM ethylene diamine tetra acetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone (PVP). Whole extraction procedure was carried out at 4°C. The homogenate was centrifuged at 10,000 x g for 30 min at 4°C and the supernatant collected was used to assay enzyme activity. Protein concentration in the enzyme extract was determined by the method of Bradford (1976) using BSA as a standard.

SOD (EC 1.15.1.1) activity was assayed spectrophotometrically as the inhibition of

photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971). The reaction mixture (3 ml) consisted of 50 mM Na-phosphate buffer (pH 7.8), 13 mM L-methionine, 75 μ M NBT, 10 μ M EDTA, 2.0 μ M riboflavin and 0.1 ml enzyme extract. Test tubes containing the reaction mixture were incubated for 10 min in 4,000 lux at 35°C. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT reduction measured at 560 nm.

Total proline content

The total proline content was estimated using the method of Bates *et al.*, (1973). Leaf sample (0.2 g) was homogenized in 2.0 ml of 3% sulphosalicylic acid (w/v) and the residue was removed by centrifugation. Then, 2.0 ml of extract was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin (prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) for 1 h at 100°C. The reaction was terminated by immersion in an ice-bath before the reaction mixture was extracted with 4 ml of toluene. The chromophore containing toluene was warmed to room temperature and its absorbance was measured at 520 nm and the amount of proline was determined from a standard curve. Results were expressed in μ g of free proline g^{-1} fresh weight.

MDA content

Leaf material (0.3 g) was homogenized in 4 ml of 0.1 % trichloroacetic acid (TCA). Homogenate was centrifuged at 10,000 x g for 10 min at 4°C. Procedure of Heath and Packer (1968) was followed to measure the MDA content. Crude extract (0.3 ml) was mixed with 1.2 ml of 0.5% (w/v) 2-thiobarbituric acid (TBA) prepared in 20% (w/v) trichloroacetic acid (TCA). The mixture was incubated at 95°C for 30 min. Reaction was

terminated by placing the tubes in ice bath immediately and then centrifuged at 10000 x g for 10 min. Absorbance of the supernatant was measured at 532 nm and 600 nm. Absorbance at 600 nm is subtracted from the absorbance at 532 nm for non-specific absorbance. The concentration of MDA was calculated by using an extinction coefficient of 155 $mM^{-1} cm^{-1}$.

H₂O₂ content

Hydrogen peroxide was measured spectrophotometrically after reaction with potassium iodide (KI) according to Alexieva *et al.*, (2001). Reaction mixture was consisted of 0.5 ml of supernatant, 0.5 ml of 0.1 M potassium phosphate buffer and 2 ml of 1 M KI reagent. The blank had 0.1% TCA in place of leaf extract. The reaction was carried out for 1 h in darkness and absorbance was measured at 390 nm. The amount of H₂O₂ was calculated using standard curve prepared with different dilutions of a working standard of 100 μ M of H₂O₂.

Results and Discussion

% mycorrhizal frequency

Drought stress did not seem to reduce the % frequency of mycorrhiza occurrence in finger millet roots. All the inoculum resulted in colonization with above 80% mycorrhizal frequency at the time of harvesting in both well-watered and water stressed conditions (Fig. 1).

Agronomical parameters

All the inoculum significantly enhanced the shoot length with equal extent (Fig. 2) highest recorded in Nachini with 19 % increase over control plants in drought condition and 15 % increase over control plants in irrigated condition. Inoculation of

finger millet plants with AMF (both native population and *R. intraradices*) significantly improved shoot fresh weight under both drought (8 % - 34 %) and irrigated conditions (10 % - 29.7 %). Nachini, Dwarson and *R. intraradices* were the most effective inoculum (Fig. 3). In terms of shoot dry weight also, Nachini (40 % increase in drought condition and 31 % in irrigated conditions) and *R. intraradices* (24 % increase in drought condition and 37 % in irrigated conditions) were the most effective inoculum (Fig. 4). AMF inoculation resulted in enhanced root parameters irrespective of water conditions over non-mycorrhized control plants. Dharanaula (12.3 %) and *R. intraradices* (14.1 %) being the most effective inoculum in improving root length (Fig. 5). On the other hand, root fresh weight (Fig. 6) was recorded highest in Dwarson (28.7 % increase) in drought condition followed by Nachini (22.7 %) and under irrigated conditions, *R. intraradices* treated plants recorded 36 % increase over control followed by Nachini (31.6 %). Root dry weight (Fig. 7) of plants inoculated with Nachini inoculum showed highest increase over control plants under drought conditions, however, under irrigated conditions, *R. intraradices* was most effective followed by Chandak and Dwarson.

Mineral nutrition

Nutrient analysis of irrespective of inoculum, shoot tissue showed that the water stress resulted in significant reduction of N (18.1%), P (20%), K⁺ (9.6%), Ca⁺⁺ (13.8%) and Na⁺ (27.2%) levels compared to irrigated plants. However, inoculation with both native and exotic AMF significantly enhanced N (27.4-36.3%), P (11-37.1%), K⁺ (24.3-34.9%), Ca⁺⁺ (2.3-45.5%) contents of finger millet under drought stress. Similarly, in non-stressed conditions as well the

inoculation with AMF resulted in increased foliar nutrient content, N (19.8-23.3%), P (4.0-44.5%), K⁺ (6.7-29.8%), Ca⁺⁺ (12.0-36.7%) and Na⁺ of finger millet as compared to non-mycorrhized control plants (Fig. 8-12).

Biochemical stress markers

Superoxide dismutase (SOD)

Under irrigated conditions not much variation was observed in the activity of SOD between inoculated and uninoculated treatments. An increase in the activity of SOD (1.06-1.17-fold), was recorded in AMF treated plants under water stress compared to uninoculated control plants (Fig. 13).

Malondialdehyde (MDA) content

The plants inoculated with native mycorrhiza and monoculture *R. intraradices* on exposure with water stressed conditions, showed reduced MDA levels by 51.3%-18% as compared to water-stressed control plants, indicating that these strains imparted considerable protection against oxidative damage of lipids created by drought.

Highest response was observed in Dwarson inoculum followed by Nachini and *R. intraradices*. In the absence of water stress, MDA content following by AMF inoculation did not show any significant difference over control plants (Fig. 14).

Total proline (Pro) content

Proline plays crucial role in the process of osmotic adjustment under drought and salinity stress, helps in maintaining favourable osmotic potential for water uptake, thus, alleviating the stress injury. Therefore, proline accumulation is prominent indicator used by researchers for evaluation of stress faced by

the plants (Wu *et al.*, 2017). Inoculation with AMF, both native and exotic significantly improved proline concentration (up to 1.7-fold) in water-stressed plant indicating that AMF improved drought stress tolerance by modulating the proline production. In irrigated conditions as well, there was higher proline content in inoculated plants than the control plants (Fig. 15). Similar findings were reported by Tyagi *et al.*, (2018) in finger millet on inoculation with *R. intraradices*.

Hydrogen peroxide (H₂O₂)

Drought causes severe oxidative damage and causes accumulation of cellular H₂O₂ content, it is another representative of level of stress faced by plants. Inoculation with AMF resulted in reduction of H₂O₂ content ranging from a 1.12-1.51-fold under water stressed conditions in comparison to control, however, in irrigated conditions, there was no marked difference between control and AMF inoculation. The accumulation of H₂O₂ content was lowest in the Dwarson followed by Nachini (Fig. 16).

There are very limited studies conducted on the interaction of finger millet and AMF. The few that have been performed are based on monoculture AMF. Tewari *et al.*, (1993) observed growth promotion of five finger millet varieties on inoculation with six AMF species, the result showed varied response among the varieties and the effectiveness of different fungi, *Glomus caledonium* was found to be the most promising potential.

Since, even with single AMF species variable results are observed, it is understandable that inoculum with mixed AMF population will have differential response. Our results are complying of results reported by Tyagi *et al.*, (2018) showing drought alleviation of finger millet seedlings after AMF inoculation due to

improved enzymatic and non-enzymatic activities of mycorrhized plants over non-mycorrhized control. There are no reports of using native AMF for finger millet growth promotion, however as discussed before, studies on several other crops have reported native AMF are better equipped in combating the stressed conditions over exotic species (Querejeta *et al.*, 2006; Petipas *et al.*, 2017; Harris-Valle *et al.*, 2018).

Our results showed enhanced proline content in mycorrhized plants, in addition to acting as an osmoprotectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, as a solute that protects macromolecules against denaturation, and as a means of reducing acidity in the cell (Kishor *et al.*, 2005). Plants have evolved several antioxidants mechanisms to avoid oxidative damage linked to stressful conditions. One such enzyme is SOD it rapidly convert superoxide to H₂O₂. Enhanced SOD activity under duress helps in mitigating the damage caused by reactive oxygen species, our findings further establishes this in finger millet plants as SOD activity was superior in mycorrhized finger millet plants as compared to non-mycorrhized plants. In addition to increased level of proline and SOD activity, mycorrhized finger millet plants also showed reduction in cellular MDA content, an indicator of reduced lipid peroxidation. The results are in concordance with Zhang *et al.*, 2010, where AMF inoculation in *Casuarina equisetifolia* decreased the MDA content under drought stress but didn't altered its status under well-watered condition. Essahibi *et al.*, (2018) found drought-stressed AM plants showed decreased hydrogen peroxide and malondialdehyde contents associated with increased activities of superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase compared to their relative non mycorrhized carob plants.

Fig.1 AMF % mycorrhizal frequency in roots of finger millet plants after the experiment

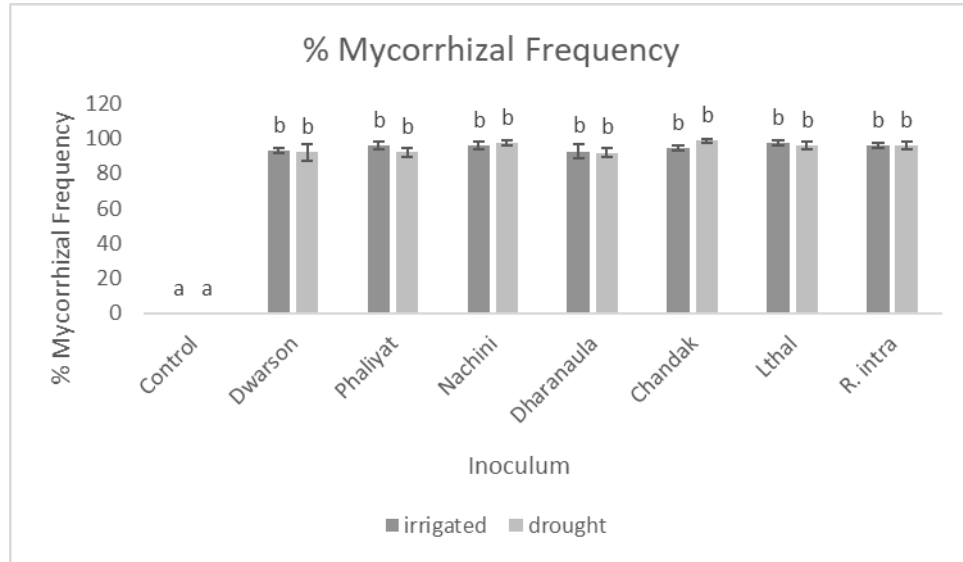


Fig.2 Effect of inoculum developed by trap culturing of native AMF population on the shoot length of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

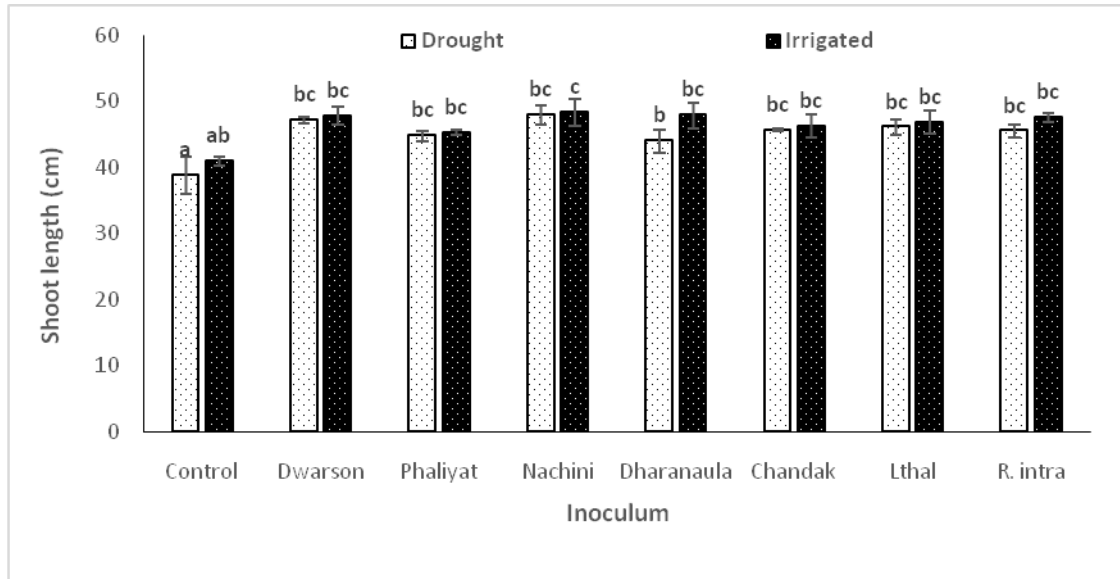


Fig.3 Effect of inoculum developed by trap culturing of native AMF population on the shoot fresh weight of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

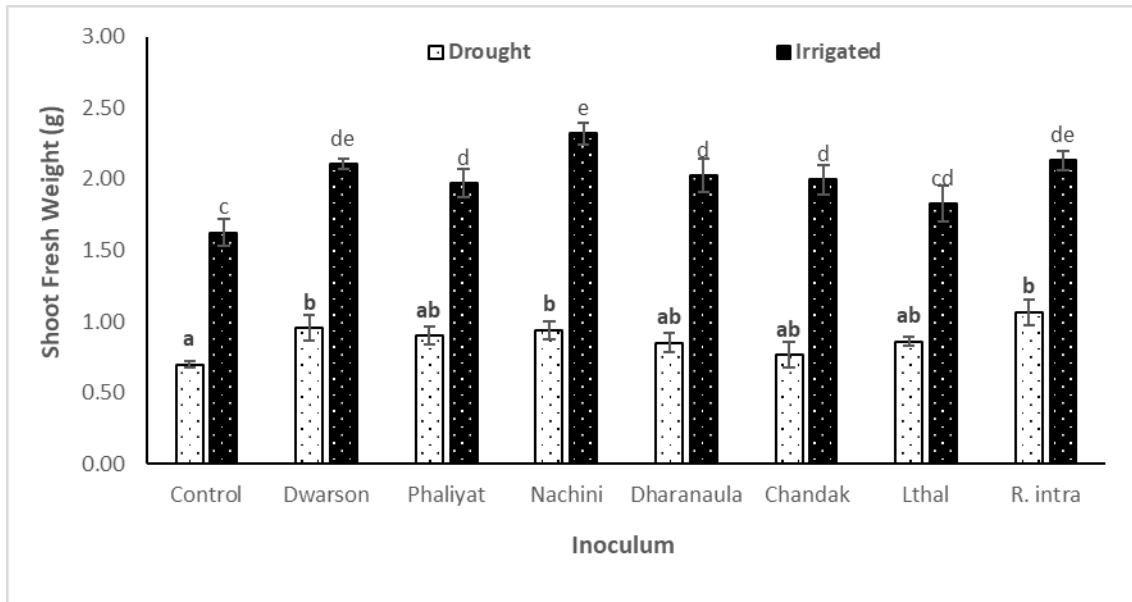


Fig.4 Effect of inoculum developed by trap culturing of native AMF population on shoot dry weight of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

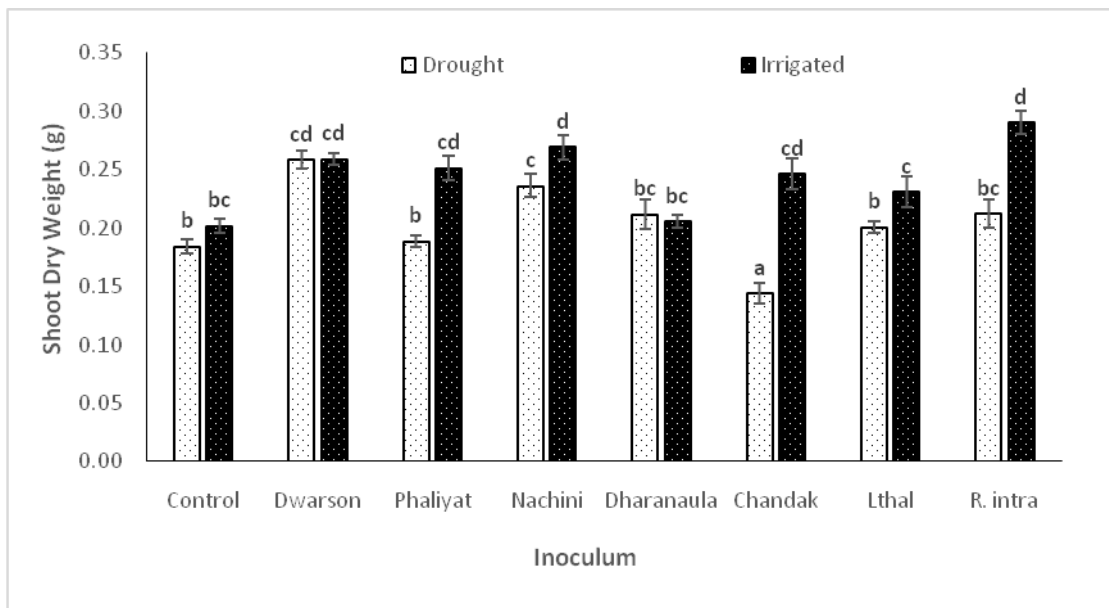


Fig.5 Effect of inoculum developed by trap culturing of native AMF population on the (I) root length of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

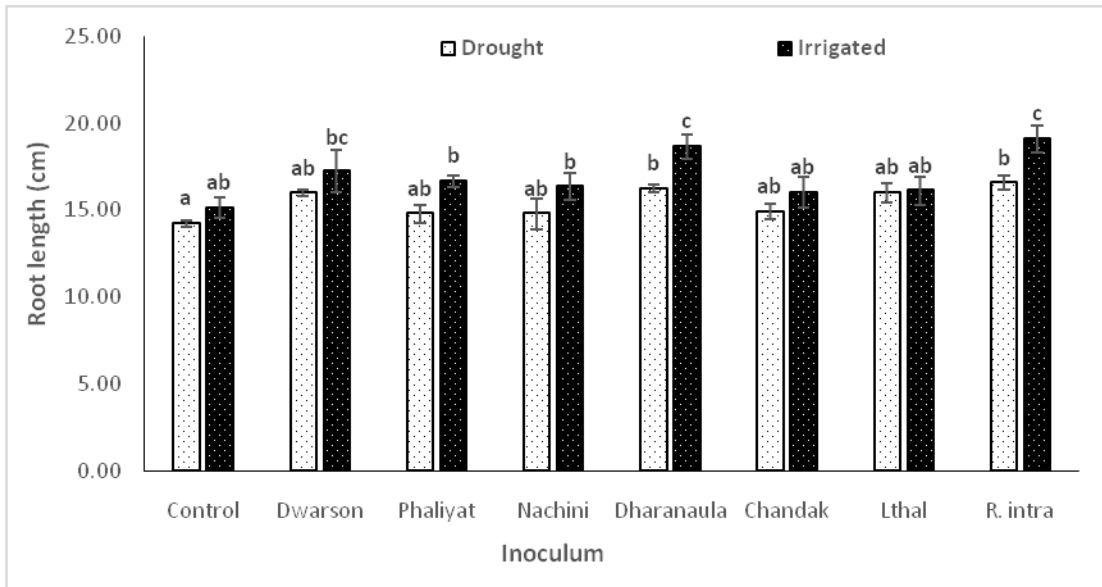


Fig.6 Effect of inoculum developed by trap culturing of native AMF population on the root fresh weight of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

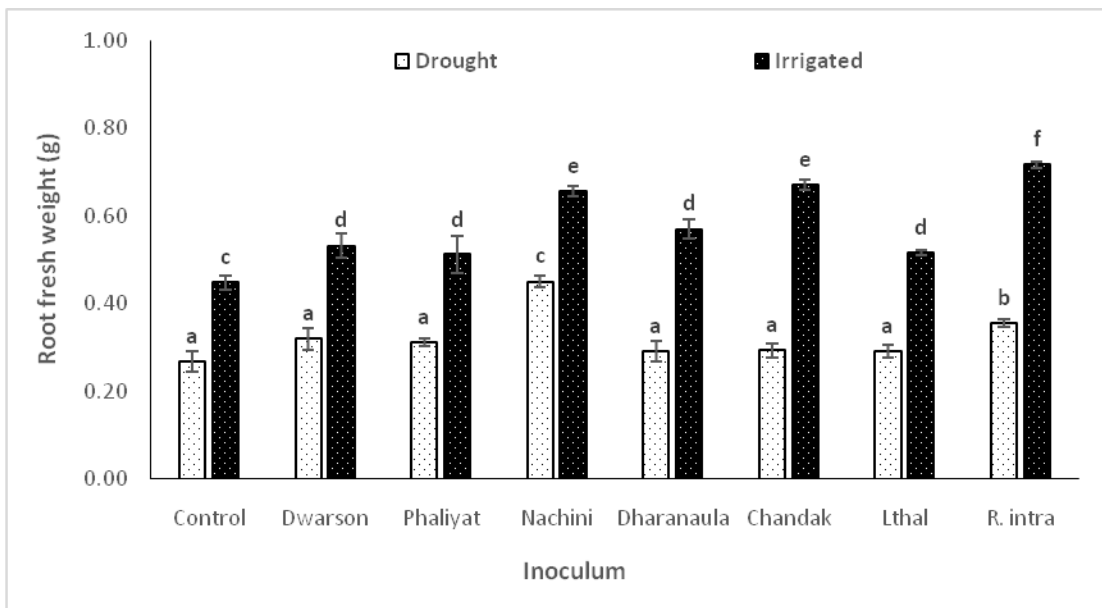


Fig.7 Effect of inoculum developed by trap culturing of native AMF population on the root dry weight of finger millet plants under irrigated and water stressed conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

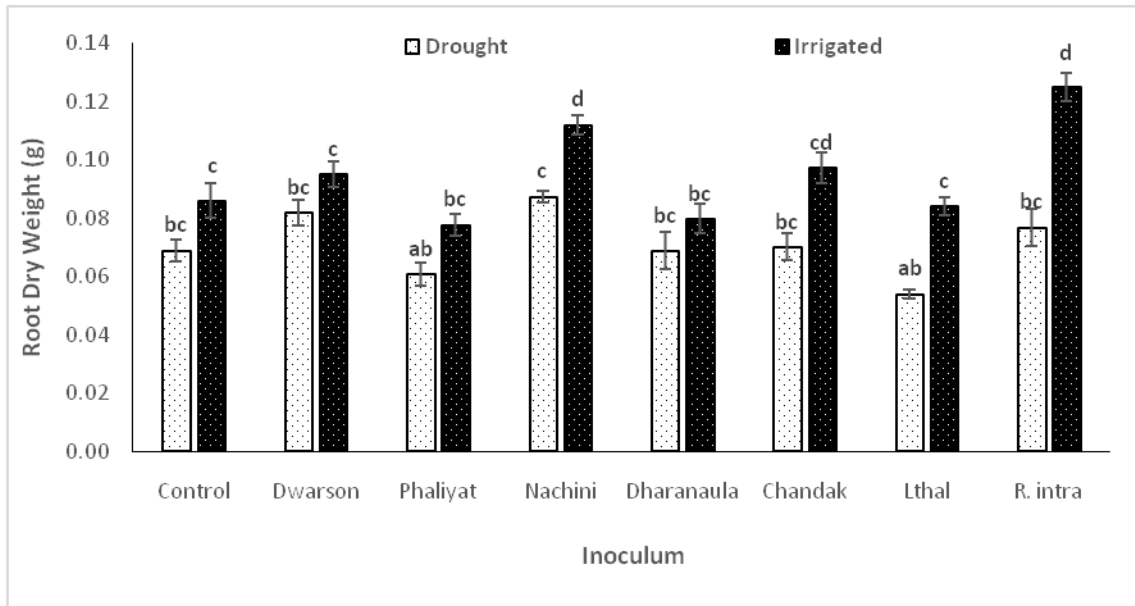


Fig.8 Effect of inoculum developed by trap culturing of native AMF population on the Nitrogen (%) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

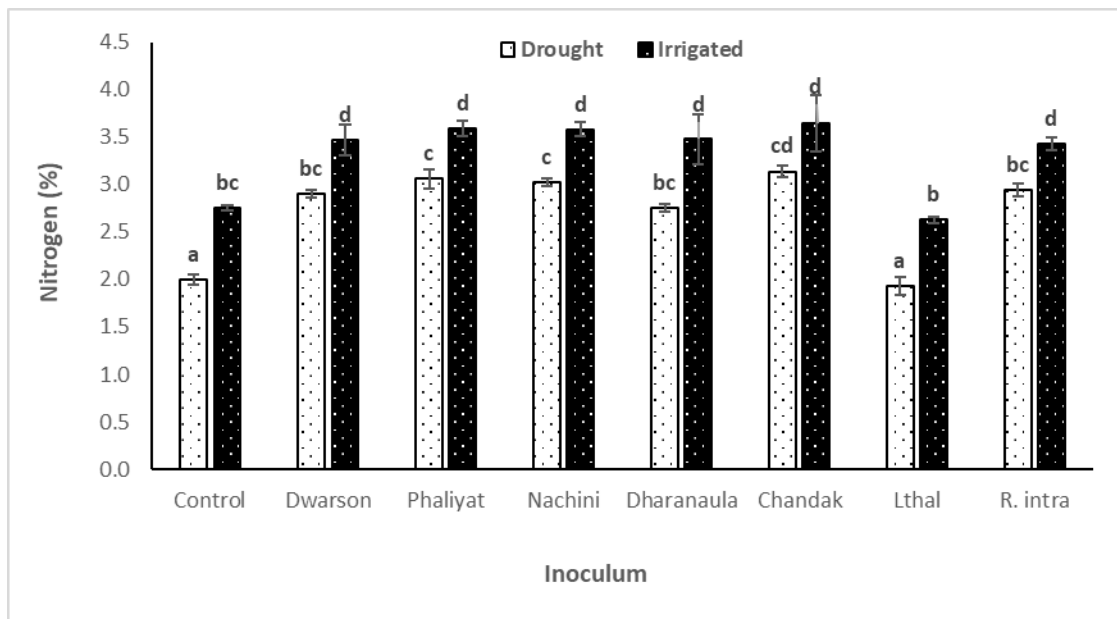


Fig.9 Effect of inoculum developed by trap culturing of native AMF population on the Phosphorus (%) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

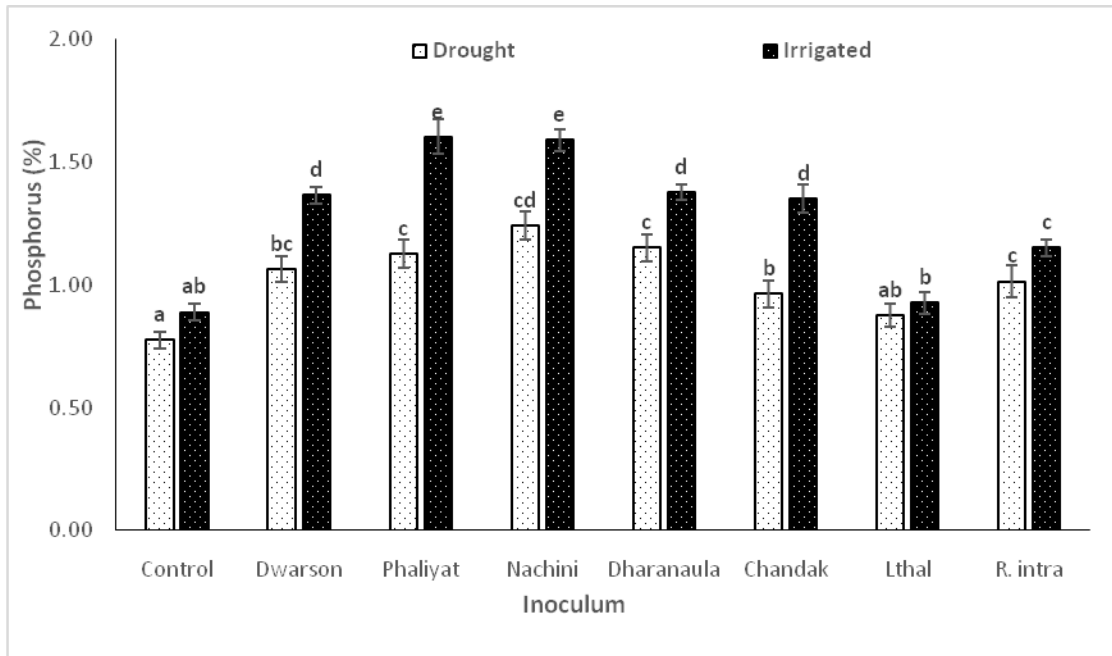


Fig.10 Effect of inoculum developed by trap culturing of native AMF population on the Potassium (%) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

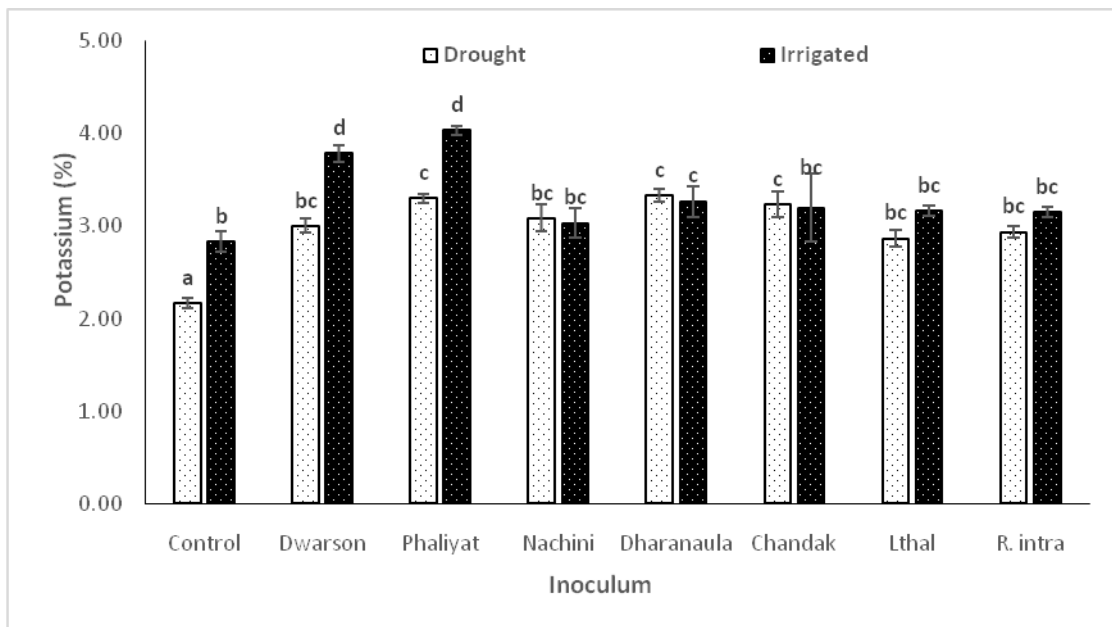


Fig.11 Effect of inoculum developed by trap culturing of native AMF population on the Calcium (%) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

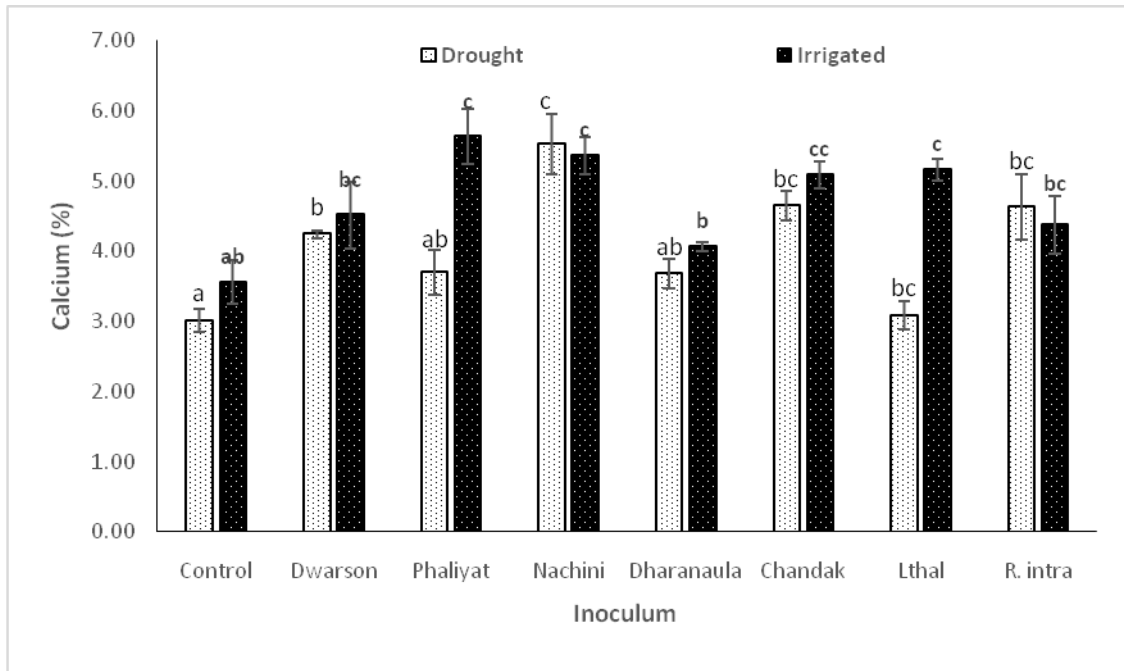


Fig.12 Effect of inoculum developed by trap culturing of native AMF population on the Sodium (%) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

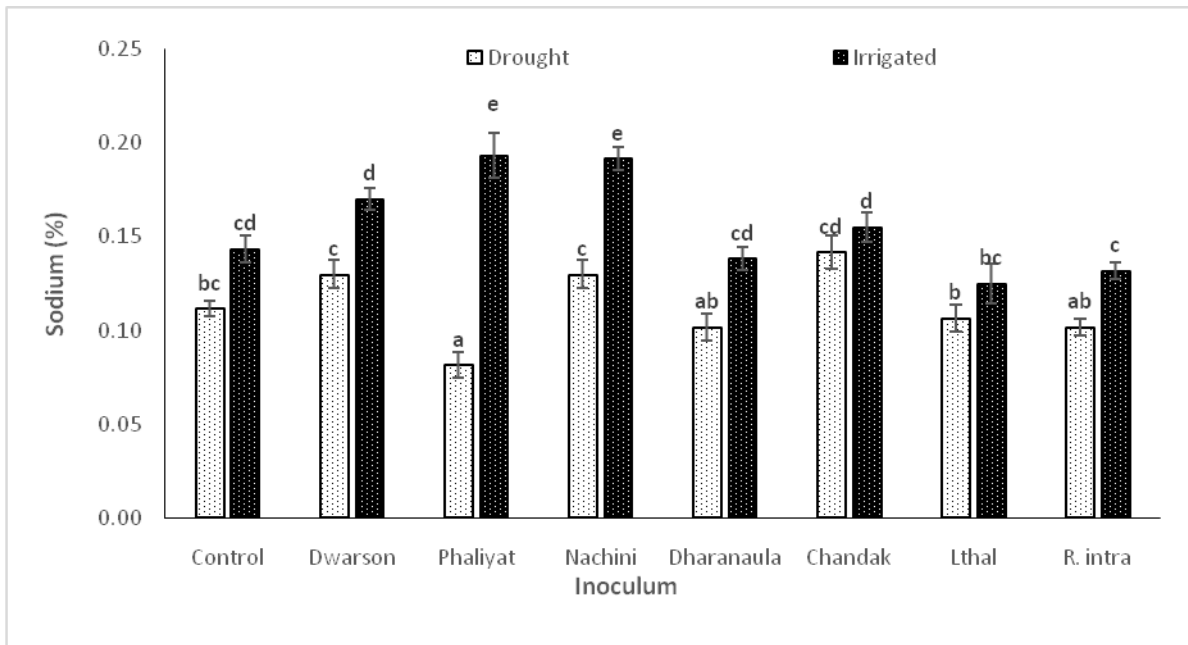


Fig.13 Effect of inoculum developed by trap culturing of native AMF population on the SOD (Unit/mg FW) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

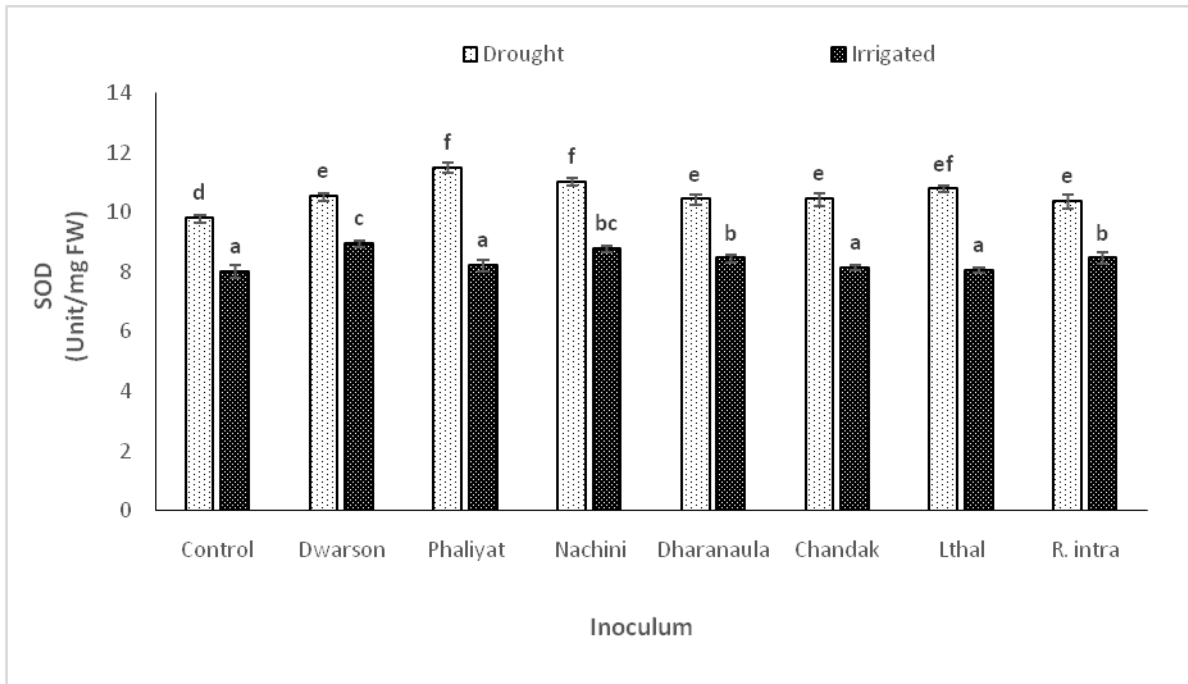


Fig.14 Effect of inoculum developed by trap culturing of native AMF population on the MDA ($\mu\text{g/g}$ FW) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

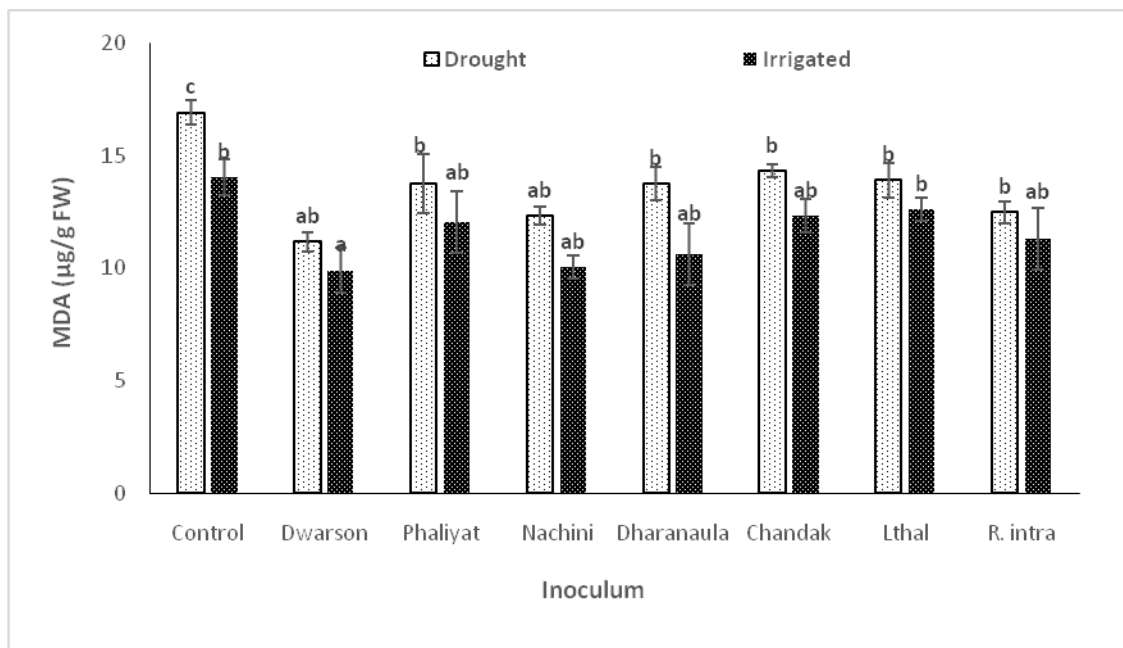


Fig.15 Effect of inoculum developed by trap culturing of native AMF population on the Proline content ($\mu\text{g/g}$ FW) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$

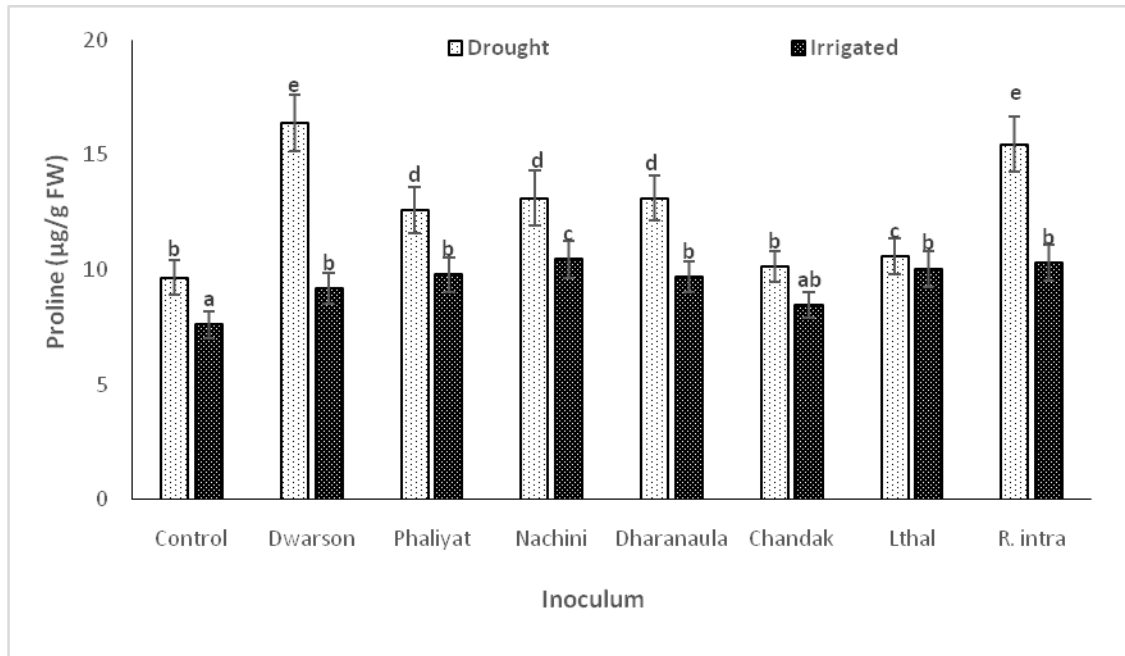
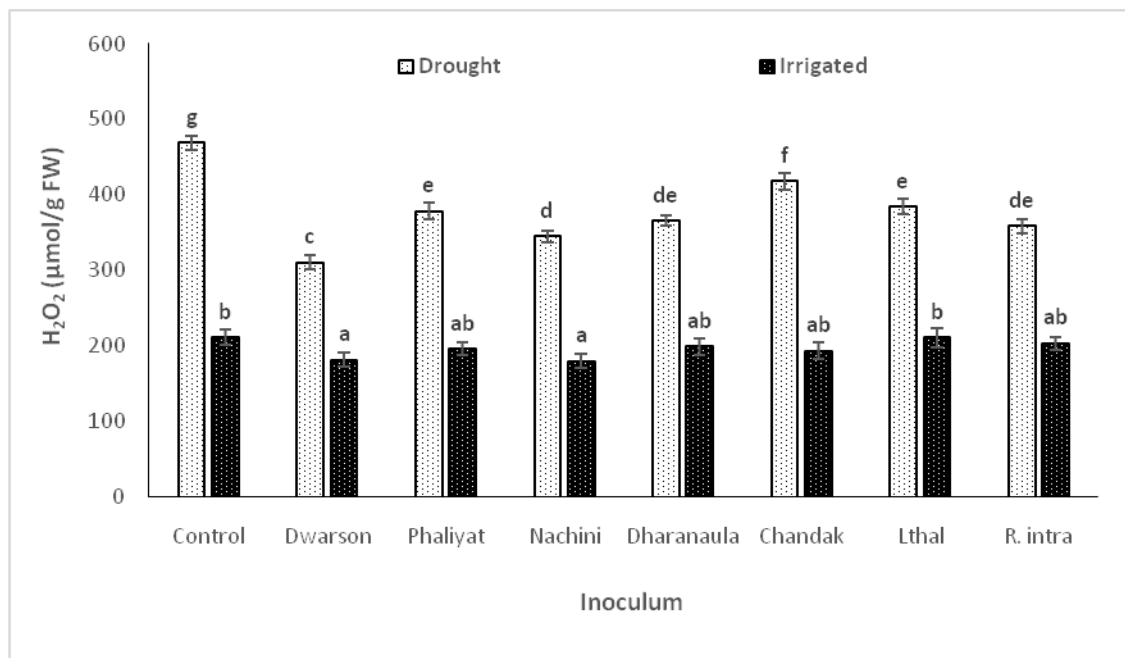


Fig.16 Effect of inoculum developed by trap culturing of native AMF population on the H_2O_2 level ($\mu\text{mol/g}$ FW) of finger millet plants under drought and irrigated conditions. Values are the means of 3 replicates \pm S.E. Values with different alphabets are significantly different at $p < 0.05$



Thus, AMF strengthened the mechanisms involved in drought tolerance of carob by improving water relations, increasing cell wall rigidity, and enhancing oxidative stress alleviation.

The results showed the extent of drought implications on growth of finger millet. Water stress resulted in reduced biomass, nutritional and biochemical status of finger millet plants. On the other hand, the developed native mycorrhizal inoculums elevated the morphological, nutritional and the biochemical status of finger millet plants under both well-watered and water stressed conditions. We found enhanced phosphorous in mycorrhized plants under both well-watered and severe stress conditions, and similar finding was previously observed in many other plant species (Burghelea *et al.*, 2015). The results also indicate if not better, native AMF are capable of growth promotion of same extent as commercially exploited *R. intraradices*. The response varied among the inoculums, however, Dwatson and Nachini remained the most effective inoculum as evident by the enhancement of most of the parameters studied by their inoculation.

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