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Enhancing Drought Tolerance in Wheat Using Encapsulated *Lelliottia amnigena* MSR-M49

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drought is considered one of the most important stresses that raise concern and is responsible for changes in the soil that have an impact on living organisms and plants. In a three –factor field experiment, encapsulated PGPR *Lelliottia amnigena* MSR-M4 was used to study the effect of their bio-inoculation on two wheat resistance Shandawil 1 and sensitive Gemmeiza 12 varieties under 80% and 60% from crop evapotranspiration (ETC). The results showed that drought conditions led to a decrease in the total chlorophyll and carotenoids but the values of pigment contents increased with encapsulated PGPR inoculants. Inoculation with PGPR *Lelliottia amnigena* MSR-M4 enhanced the IAA and ABA content in shoots of two genotypes wheat plants especially in genotype

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Gemmeiza 12. Application of encapsulated *Lelliottia amnigena* MSR-M4 achieved the lowest ethylene content compared to un-inoculated wheat; there is also higher reduction of proline accumulation in two verities of wheat plants and achieved higher relative water content under 80% and 60% from ETC.As well as there is an improvement in yield-related traits and wheat productivity due to the improvement in dehydrogenase soil enzyme. Accumulative of ant oxidative enzymes in wheat can be indication of their relative tolerance to drought stress. Encapsulated MSR-M4 approves greater enhancement in yield-related quality of wheat productivity, as well as (N, P, and K %) in grains of two genotype wheat plants.

Keywords: Drought stress; Lelliottia amnigena; MSR-M4; wheat-yield.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the world's important food crops for more than 3rd of the world's population. It added a huge amount of energy of protein over other grain crops in the global diet **(**Ahmad et al., 2020). Recently, there has been a decrease in the soil nutrients, but there is a decrease in fertility worldwide as to extensive natural resource usage also even dangers to the ecosystem like biotic and abiotic pressure (Waraich et al., 2020; Ahmad et al., 2018). Drought is one of the most important environmental stresses that affect in plant growth and productivity. Tolerance to water pressure is a complex parameter in which crops 'creation can be affected by several characteristics (Arash et al., 2013). Drought is expected that by the year 2025, about 1.8 billion people will face absolute water shortage and 65% of the world's population can live under water-stressed environments. Plant tolerance to drought is divided into two parts: the plant avoids drought and dehydration tolerance (Kramer and Boyer 1995). Drought avoidance contains root deepness, use the plant for available water and life style to plant for using rainfall. Dehydration tolerance consists of the ability of the plant to grow under partially dry conditions when rain falls continues (Salekdeh et al., 2002). However, drought could stop protein production and under water-narrow conditions few mRNAs kinds are made by plants. Proline production is too sometimes well-known under water limited stress (Seyahjani et al., 2020). Water deficiency can similarly retard the increase of different proteins in the meantime boosts protein creation (Mohtashami et al., 2020). Inoculation with useful microorganisms could increase drought toleranceof plants growing in arid or semi-arid regions (Marulanda et al., 2007). The use of plant growth-promoting microorganisms (PGPM: bacteria, fungi and algae) could increase the uptake of nutrients and water by plants (Stamenkovi´c et al., 2018). Increased crop production is correlated to the capability of microorganisms to fix nitrogen, solubilize minerals, or products phytohormones (auxins and gibberellins). These mechanisms adjust nutrient cycling then increase soil quality (Bakhshandeh et al., 2020). Bacteria improve plant growth under abiotic stress conditions for example drought (Olenska´ et al., 2020). So, it is essential to able to the existence of the soil make known to microorganisms and increases their accessibility to the cultivated crops. For this study, numerous formulations or carriers are applied to confirm their long-term existence, where strains of microorganisms are encapsulated to formula a microbial inoculant. Final formula of inoculants could be liquid or solid (powder or granules). Granular inoculants are applied in the soil, liquid and powder forms are most frequently usage for seed coating before sowing **(**Nwachukwu et al., 2021 ;de Moraes et al., 2021). Carrier could be cheap, available, ecologically friendly, and able to delivering microorganisms nearly the root method. Generally, it could emphasize bacterial survival and protection through long-term storage (further than one year). Polymers used to encapsulate PGPR must also stay biodegradable and afford organized bacteria release in the soil (Bashan et al., 2013).The objective of current study was to evaluate the affectivity of encapsulated *Lelliottia amnigena* MSR-M49 inoculation of two resistance and sensitive wheat cultivars by evaluating growth, physiological and biochemical status of plants under water stress field conditions.

2. MATERIALS AND METHODS

2.1 Laboratory Part

2.1.1 Bacterial strain and characterization

The strain used in this study namely: *Lelliottia amnigena* MSR-M49 with accession no. MN494098, it was isolated from El-Arish city, North Sinai Gvernorate, it was grown in LB broth

medium for 48h at 30°C on a shaker incubator at 120 rpm.

2.1.2 Encapsulation processing

Strain *Lelliottia amnigena* MSR-M49 inoculated onto LB broth for 48 hours at 28°C with constant shaking at 150 rpm to reach the extreme growth (10^8cfu/ mL) and cell pellets were collected by centrifuging at 6000 rpm for 10 minutes after that the cells were washed in sterile distilled water and re-suspended in 400 ml phosphate buffer pH 7. Capsules were prepared by processing similar to that described by (Bashan et al., 2002). Survival of MSR-M49 was tested according to methods described by Abo-Koura and Maie (2016). Scanning electron microscopy was used to investigate the morphology of *Lelliottia amnigena* MSR-M49 in capsules using the (SEM, Quanta FEG 250, FEI Company, Eindhoven, and the Netherlands) in accordance with the manufacturer's instructions.

2.1.3 Preparation of bacterial inoculum

Lelliottia amnigena MSR-M49 was grown in LB broth medium for 48 hours at 28°C to exponential phase (10^8cfu mL^{-1}). Two forms of inoculum were prepared, the capsules beads as described done above was mixed with the seeds of each variety of wheat separately,and the other form was liquid, its carried with sterilized peat and sterilized sugar solution (10%) according to the protocol followed by Sajid et al.,(2016).

2.2 Experimental Design

The experiments were conducted in field at agricultural Research Center (ARC) Giza Govern., Egypt, during season (2022 -2023). The site is located at (30°01'13.6"N 31°12'30.4"E: 30°01'11.3"N 31°12'20.5"E. The seeds of two local winter wheat genotypes (resistant cultivar) Shandawil 1 (*Triticum aestivum* L.) and (sensitive cultivar) Gemmeiza 12 (*Triticum aestivum* L*)* were obtained from field Crops Research Inst., Agricultural Research Center, Giza, Egypt, and were used in the experiments for study the effect of inoculation with either capsules or liquid *Lelliottia amnigena* MSR-M49 under two levels from ET crop. Bulk density, physical and chemical properties of the soil at the experimental site were determined according to Klute (1986) and Page et al., (1982), whereas particle size was according to Piper, (1950) and chemical properties according to Ryan et al.,(1996). In order to estimate the effects of drought stress on physiological and morphological characteristics of two cultivars of wheat plants a factorial experiment based on split split plot design was conducted with four replications, the plot area was 6 × 7m each. The treatments were; main plots (variety of wheat) while the sup plots was (irrigation regimes based on crop evapotranspiration):100%, 80% and 60% ET crop. Sub sub plots were control (without inoculation) by capsules and liquid of bacteria. We take six plants to determine the growth criteria, plants were fertilized with mineral fertilization as Nitrogen (N), phosphors (P), potassium (K) was applied as recommended dose for Egyptian Ministry of Agriculture.

2.2.1 Total chlorophyll and carotenoids

Total chlorophyll and carotenoids content was assessed as described by Hiscox and Israelstam (1979). 100mg of leaf sample was taken randomized from the top of three plants and cut into pieces, were placed in tubes and homogenized in 5 mL of acetone (80% V/V), and stored in a refrigerator at 4^0C for 4 days, to extract the pigments. The total chlorophyll and carotenoids were calculated as modified by Arnon equations (Wellburn 1994) and expressed in mg/ g. fresh weight.

2.2.2 IAA and ABA

10 gm. of plant tissue sample was taken from each treatment, ground with70% (v/v) methanol, and then stored overnight at 4°C to estimate the hormone in the plant. Samples were analyses on HPLC after filtering using Millipore filter as described by Durley et al., (1982) and Wurst et al., 1984).

2.2.3 Ethylene Content

3 leaves / plant after 45 days sowing were putted in 10-mL jars then added 1 mL of the arranger solution (50 mM Na2HPO4/NaH2PO4, pH 6.8), we closed the jars with a rubber septum then incubated for 24 h at 25^0C in the dark. The production of ethylene content was determine using gas chromatography (Ribaudov et al., 2006).

2.2.4 Relative water content in leaves (RWC %) and Proline content

We take three leaves randomly from the wheat plants from each plots then weighed it directly to obtain (fresh mass FM) after that we determine the turgid mass (TM) by floating the leaves inside the distilled water. After the imbibition period, distributing water was removed gently on the

surface of the leaf using tissue paper then the leaves were weighted. After the process of impregnating the leaves, it was placed in a hot oven at 70°C for 48 h in order to obtain the dry mass (DM). Water content in leaves was assessed by measuring relative water content (RWC) according to Kaya and Higgs (2003) as follows:

RWC % = (FM-DM / TM-DM) X 100

While the proline content in fresh leaves of wheat plants was determined according to Bates et al., (1973).

2.2.5 Antioxidant Enzymes and Dehydrogenase activity (DHA)

Catalase (CAT) enzyme activity and Acerbate Peroxidase (APX) were determined after 45 days from sowing as described by Aebi (1974) and (Jebara et al., 2005) respectively, while dehydrogenase activity (DHA) (µg TPF/g dry soil /day) in rhizosphere soil for each treatment was determined according to (Somasegaran and Hoben 1994) after 45 days from sowing.

2.2.6 Yield parameters

In the end of experiments, we collected the samples of plants using 1 m2 wooden frame in order to determine wheat yield and its components. We take 10 plants randomly from two internal rows to measure plant height (cm) ,spike length (cm),1000 grain wt. (g) ,straw and grain yield (Ton/ fed).Samples of grains was oven dried at 70°C up to a constant dry weight, grounded then prepared for digestion method as described by Jackson (1973). Digests samples were prepared for measurement the NPK. Nitrogen content was determined by Kjeldahel method while potassium content was determined by Flame photometer as pronounced by Jackson (1973) on the other hand the phosphorous was determined to the method outlined by (Allen, et al., 1974).Biological yields (Ton/ he) were determined from the whole area of experimental unit. Harvest index was calculated are described by the following Equation as described by Kozak and Mdry (2006) as follow: HI= grain yield / Biological yield x 100.

2.2.7 Water relations

CROPWAT model was used to calculate reference evapotranspiration with Penman - Monteith.

2.2.7.1 Crop evapotranspiration (ETc):- (Allen 1998)

ETC. = ETo × Kc

Where:-
ETC = Crop evapotranspiration.
ETo = Reference evapotranspiration.
Kc = Crop coefficient (from FAO 56)

2.2.7.2 Applied irrigation water (AIW)

A furrow surface irrigation method was used to conduct this treatment. Applied irrigation water was measured by a flow meter installed in the main pumping unit of irrigation water. The depth of applied irrigation water (AIW) to the experimental plots was calculated according to the following equation:

$$AIW=\frac{ETc}{Ea}$$

Where:
ETc. = water consumptive use (CU, mm/d), or actual evapotranspiration (ETc.).
Ea. = application efficiency (fraction) = 0.5 for surface system at the site. A submerged flow orifice with fixed dimensions was used to measure the amount of water to be applied to the experimental plots. The discharge of the orifice is calculated according to the following equation (Michael, 1978).

$$Q = CA\sqrt{2gh}$$

Where:
Q = discharge through orifice, (cm^3/sec)
C = coefficient of discharge (0.6 up to 0.8).
A = cross-sectional area of the orifice (cm2)
g = acceleration of gravity (981 cm/sec).
h = head of water causing discharge through the orifice (cm).

2.2.7.3 Water consumptive use (CU)

On determining water consumptive use, soil samples were collected using a regular auger just before and 48 hours after each irrigation and at harvest time in 15cm increment from soil surface down to 60 cm of soil profile. Water consumptive use was calculated according to Israelsen and Hansen (1962) as follows:

$$\mathrm{CU\ (m)} = \frac{\theta 2 - \theta 1}{100} \mathrm{x\ BdxERZ}$$

Where:
CU = water consumptive use (m).

Θ2 =Soil moisture percentage by weight, determined 48 hours after irrigation.
Θ1 = Soil moisture percentage by weight, determined before the following irrigation.
Bd = Bulk density (kg m-3)
ERZ= Effective root zone (0.6 m).
Water consumptive use as (m3fed-1) was obtained by multiplying the value of CU (m)

2.2.7.4 Water Use Efficiency (WUE)

Water use efficiency was calculated according to Jensen (1983) as follows:

$$\mathrm{WUE} = \frac{\mathrm{Y}}{\mathrm{CU}}$$

Where: WUE = kg seeds m-3 water consumed. Y = Seed yield (kg fed-1).
CU = Seasonal water consumptive use (m^3 fed^{-1}).

2.2.7.5 Water Productivity (WP)

Water productivity is an efficiency term calculated as a ratio of product output over water input. The output could be biological goods such as crop grain, fodder….etc. So, water productivity, in the present study, is expressed as kilogram of wheat seed obtained per the unit of applied irrigation water. The water productivity values (kilograms of wheat grains m-3 of applied water) were calculated as follows:

$\mathrm{WP}\ (\mathrm{kg}^{-3})\ \frac{\text{Seed yield (Kg ha}-1)}{\text{Applied water (M3 ha}-1)}$= FAO (2003).

2.3 Statistical Analyses

The experiment was designed with system split split plots. It was used Least significant difference test for comparison means using the statistical analysis software; CoStat (CoHort Software, U.S.A) version 6.4. The values of probability $p<0.05$ were considered statistically significant. Based on the least significant difference test.

3. RESULTS AND DISCUSSION

3.1 Agro-meteorological data

Monthly the average of agro-meteorological data at the experimental site and class A pan (E pan) values for the seasons are presented in (Table 1).

3.2 Physical and Chemical Properties of Experimental Soil

The characterized of the experimental site were determined to: clay loam soil 40% clay, 35.4% silt and 23.8% sand respectively. The total organic matter with approximately 1.84%, salinity of soil was 0.99 dSm^{-1} with neutral/ slit alkane pH 7.75, which is average of this region (Table 2).

Table 1. Monthly average meteorological data of Giza research weather station

Months	Temperature (°C)		Relative humidity (%)	Wind speed (m/sec)	Sun shine (h)	ETo (mm/day)
	Max.	Min.				
November	20.0	14.5	54	2	10.4	2.05
December	20.4	8.9	66	3	11.0	1.49
January	17.6	6.6	89	3	10.3	1.44
February	20.1	7.6	66	2	11	2.03
March	24.2	9.6	60	3	11.8	3.04
April	26.9	11.9	56	3	12.8	4.02

Table 2. Physical and chemical properties of soil at the experimental site

Season	Particle size distribution*			Textural class	Chemical properties**					
	Clay %	Silt	Sand		O.M. (%)	EC dSm^{-1} (1:5)	Available (ppm) N	P	K	pH (1:2.5)
2022-2023	40.8	35.4	23.8	Clay loam	1.84	0.99	45.00	12.5	191.90	7.75

Table 3. Soil water constants and bulk density at the experimental site

Depths (cm)	Field capacity (%)	Wilting point (%)	Available moisture (%)	Bulk density (g cm $^{-3}$)	Available moisture (mm/layer)	
0-15	35.8	18.8	17.0	1.21	31.0	59.1
15-30	33.4	17.3	16.1	1.18	28.1	
30-45	31.9	15.1	16.8	1.25	31.5	65.5
45-60	31.7	16.8	14.9	1.52	34.0	

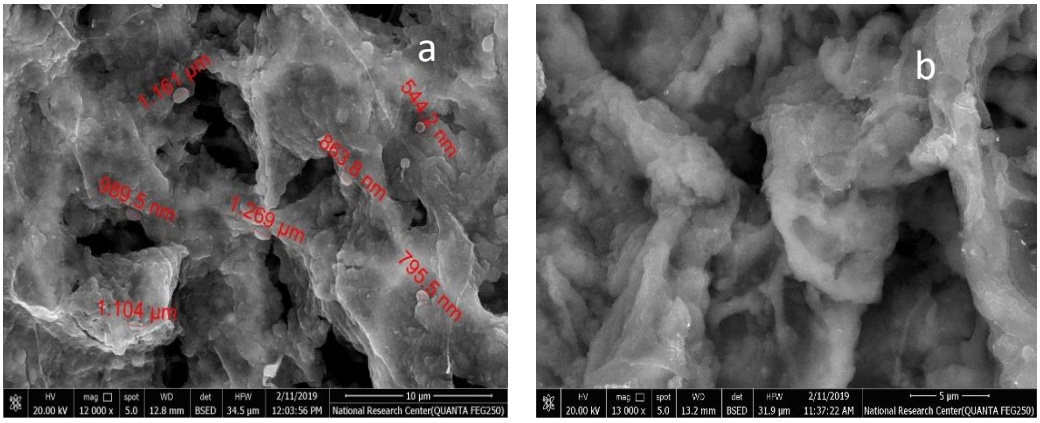


Fig. 1. SEM image of the surface of microcapsules *Lelliottia amnigena* MSR-M49: a (encapsulated beads of MSR-M49), b (encapsulated media with sodium alginate).

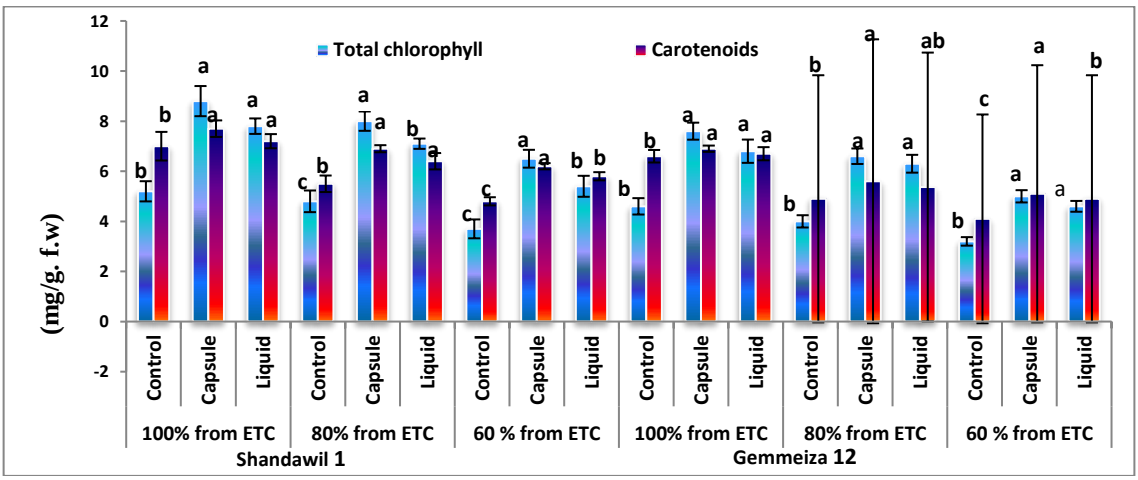


Fig. 2. Total Chlorophyll and carotenoids of two genotype wheat plants Shindawil 1and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates

3.3 Water Capacity of the Exponential Filed

Field capacity, wilting point, bulk density and available moisture were determined in different soil depth as inducted in the (Table 3). The wilting point was 18.8 % in 0-15 depths where it is decreased in depth 45-60 recorded 16.8% while the bulk density increased with increasing the depth.

3.4 Survival of *Lelliottia amnigena MSR-M49* in the capsules by Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) using for investigated the shape and purity the bacteria inside the capsules (Figs. 1 a, b). SEM showed the presence of MSR-M49 inside of capsules without any contamination and the shape of bacterial cells are clear, and the bacteria

continued to survive inside the alginate. SEM of microcapsules indicated a less consolidated surface structure by improved surface hardness and porosity, characteristic that are known to reduce mass transfer resistance (Lu et al ,2007). Therefore, porosity improved and extra canals were shaped in the beads containing alginate, resulting in better bacterial release.

3.5 Total Chlorophyll and Carotenoids

In general, the TChl and carotenoids pigments (Fig. 2) are deficient when wheat plants are exposed to drought at irrigation levels 80 and 60 % from ETC than control 100% ETC. In the 60% ETC conditions, wheat inoculated with either capsules or liquid MSR-M49 had increased significantly TChl and carotenoids than control plants ($p \leq 0.05$). While the, Gemmeiza 12 was more sensitive than the Shindawil 1 variety when exposed to drought stress. MSR-M49 inoculations improved the TChl and carotenoids pigments in wheat plants compared to untreated plants (control).The genotype Shindawil 1 was verified maximum TChl and carotenoids (mg/g. F.W) relative to Gemmeiza 12 wheat genotype. The reduction in the concentration of TChl and carotenoids under drought stress at 60 % from ETC were 6.5 and 6.2 mg/g. F.W, respectively in wheat inoculated with capsules compared to control in genotype Shindawil 1 while in Gemmeiza 12 recorded 6.6 and 5.6 mg/g. F.W, respectively in TChl and carotenoids. A reduction in chlorophyll content and carotenoids are a common condition detected under drought stress (Zhuang et al., 2020). Wheat inoculated with the capsules and liquid MSR-M49 exposed to drought stress conditions exhibited greater in total chlorophyll content and carotenoids than untreated wheat under the similar conditions (Zhang et al 2019). Samaneh (2020) quantified that extended-time drought stress decreased total chlorophyll content, which was greater in sensitive wheat cultivars than drought resistant cultivars. The results showed that the components of photosynthetic are significantly damaged in drought sensitive plant genotypes compared to drought resistant genotypes because the chlorophyll content F,, F,/F, and F,/F, in drought resistant varieties are much higher than in sensitive varieties under drought stress (Khayyat et al., 2014). Notably, the drought-induced reduction in carotenoids content was much more in the sensitive variety than tolerant variety. Carotenoids act as protecting pigment and scavengers of ROS (Singh et al., 2017). Carotenoids as well play a significant role in the mechanisms defending the photosynthetic device against several dangerous environmental factors (Strzałka et al., 2003).

3.6 IAA and ABA Content

Drought stress condition reduced the IAA and ABA concentration in shoot tolerant and sensitive varieties of wheat (Fig. 3) where the reduction is was more significant in the sensitive Gemmeiza 12 variety than the tolerant Shandawil 1 variety. Inoculation of with capsules and liquid bacteria increased the IAA and ABA in all treatment under 80 and 60 % from ETC. Inoculation with capsules of MSR-M49 recorded higher value of IAA compared with liquid MSR-M49 in two genotype varieties of wheat, it was196.7µg/g F.W in genotype Shandawil 1 wheat while it was 79.1µg/g F.W in genotype Gemmeiza 12 wheat under 80 % form ETC, the ABA was higher in plants inoculated either capsules or liquid bacteria in both varieties wheat, recorded 145.4 and 150.7 µg/g F.W in tolerant Shandawil 1, where in Gemmeiza 12 were 362.5 and 378.9 µg/g F.W under 80 % from ETC. Plant hormones play an important role in the multiplication process, and these hormones are present in the mother plant and in the cuttings, the rooting procedure will be affected either positively or deleteriously (Kelen and Demirtas 2001). IAA was lower in the sensitive genotypes under drought stress conditions as compared to un-inoculated (control) plants. The tolerant variety resisted the adversative influence of drought to a superior extent. All the treatments inoculated with bacteria either in capsules or liquid culture has ameliorative effects, PGPR-stimulated increase in IAA was greater in the sensitive genotypes variety. In the rhizosphere soil, there are a residual effects to capsules treated plants affected in drought stressed plants, these results are in harmony with Panichikkal et al., (2021) who found that encapsulated *Pseudomonas* sp. rhizobacteria professionally manufacture phytohormones (IAA) and increase soil enrichment then tolerate deleterious environmental effects (Stella et al., 2019). In this experimentation, we found that, highest concentrations of ABA in un-inoculated plants in the drought condition. These indications that drought stress conditions producing a signalling pathway that led to ABA biosynthesis. This is normal result, conferring to Salinas and González (2003), who exposed that a diminution in the hydric prospective of corn leaves increased the ABA production. Under drought stress conditions, this method avoids the plant

from losing water through transpiration .This lead to a deficiency in the photosynthesis rate that leads to loss the colour (chlorophyll) besides slow growth of the plant. In the drought condition, non-inoculated plants exhibited an increased in ABA concentration, while inoculated plants either in capsules or liquid MSR-M49 did not appearance a similar reaction. The results indicated that inoculation with MSR-M49 had a decreased effect on ABA pathway in plants. It recorded a lower level in ABA concentration. Gagné-Bourque et al., (2015) reported that *Bacillus subtilis* strain B26, advises resistance in contrast to drought stress in Brachypodium besides this is related to the organize of expression of numerous drought-reacting genes besides the modification of the DNA methylation method.

3.7 Ethylene Content

Fig. 4 shows the ethylene content in two genotype shoots wheat plants under drought stress. Control plants recorded higher ethylene compared to all treatments, and there is no significant difference between treatments in two genotype wheat. Application of capsules achieved the lowest among the other treatments followed by application of liquid MSR-M49 in two genotype wheat, whereas the value of ethylene content was lower in genotype sensitive Gemmeiza 12. Ethylene is one of the modest organic molecules through organic activity, and can play important role as plant growth organizer at very little concentrations. The ethylene content recorded lower concentration in inoculated plants, due to the effects of the ACC deaminase formed by the bacteria which might alleviate the harmful effect of ethylene, thus dropping the pressure level in the plant and stimulating plant growth (Glick 2005; Tim and Nevo 2011).

3.8 Relative Water Content (R.W.C %) and Proline Content

R.W.C was diminished with increasing drought stress in shoots of two genotype wheat plants (Fig. 5); plants treated with capsules MSR-M49 achieved higher RWC under two levels from ETC as compared to respective un-inoculated plants. Inoculations with capsules MSR-M49 stimulated 35% increasing in RWC while the increasing was 10% in inoculation with liquid MSR-M49 under 80% from ETC. Under 60% the stimulated in RWC was low compared to 80%. Overall plants of two genotype wheat executed significantly better with inoculations either with capsules or liquid MSR-M49 at both 80 and 60% from ETC. There was a significant increase in proline content in shoots of both wheat verities (Fig. 6), proline content recorded higher increased in Gemmeiza-12 than Shindawil 1 under drought stress. The increases in proline content was 21% under 80% ETC and 50% under 60% ETC respectively, in Shindawil 1, whereas it was 26 % under 80% ETC and 45% under 60% ETC respectively, in Gemmeiza-12. Inoculation with MSR-M49 reduced the proline content in shoots of wheat. Capsules of MSR-M49 recorded higher reduction of proline content in two verities, recorded (4mg/g d.w) followed by (6.0 mg/g d.w) in liquid MSR-M49 in 80% ETC as compared to control. Conferring to the results, drought stress significantly declined the RWC in the two genotypes wheat (Fig. 5). RWC is normally measured as a biological indicator to evaluate the water stability of plants (Lata et al., 2011). The decrease in RWC might be connected to low water accessibility under drought conditions. Reduction in RWC under water stress must too been detected in numerous crops (Hodaei et al., 2018; Saad et al.,—2020). Inoculation with capsules of *Lelliottia amnigena* MSR-M49 helps in better preservation of plant water stability in two genotypes wheat under drought stress. It has been stated that capsules PGPR inoculation makes well-advanced root system, which therefore leads to improvement in water uptake ability (Saad et al., 2019; Saad et al.,–2020). Arzanesh et al., (2011) stated that PGPR inoculation could raise leaf relative water content by decreasing leaf water potential. It seemed that non-inoculated plants might not usage this physiological device to preserve the RWC for the reason that the control plants were not inflated and, in several cases, had razed. These results are in arrangement with those obtained by (Paleg et al., 1983). Proline is an important amino acid in all plants, until when not stressed. In this study, there was a significant increase in proline content in shoots of both wheat verities (Fig. 6). Ashraf and Foolad (2005), who indicated that proline creation, could be told as indicator of stress in plants, taking into account their way of stimulation. It has too been stated that plants that were inoculated with Pseudomonas sp. bacteria showed a reduction in proline production when they were under water stress (Tiwari et al., 2016). This can be in harmony with our results, the plants inoculated with *A. brasilense* and *H. seropedicae* might have a minimize scale of stress in the drought stress than un-inoculated plants. Wheat treated with capsules A. *chroococcum* exhibited decrease in proline

accumulations in shoots (Saad et al.,–2020). Although there are several reports that PGPR prevent proline accumulation (Tiwari et al., 2016), other scholars have stated that PGPR have encouraging influence on over creation of proline (Sandhya et al., 2010). These differences might as to mechanism of bacterial statement, differences in the bacterial species with plant, contact among bacteria and the concentration of drought stress.

3.9 Antioxidant Enzymes Activities

The drought stress significantly increased antioxidant enzymes activities in the shoots of two verities wheat plants (Fig. 7 a,b) , the amounts of the enzymes was higher in Gemmeiza 12 than genotype Shindawil 1 under 80% and 60% ETC. With an increase in exposure to drought stress 80%, there is an increase in these enzymes in the shoots of the wheat plant,but in the case of inoculation with MSR-M49 in two forms,there is a noticeable decrease in the level of peroxidase (APX) and catalase (CAT) under drought stress conditions (Fig. 7 a,b). In APX enzyme inoculation with capsules MSR-M49 reduced the amount of enzyme recorded 2.7 µmol H_2O_2 mg^{-1}protein min^{-1}at 80% ETC, while was 5.4 µmol H_2O_2 mg^{-1}protein min^{-1} in Shandawil 1at 60% ETC. Inoculation with capsules MSR-M49 recorded 6.7 µmol $H_2O_2mg^{-1}$protein min^{-1} in CAT while inoculation with liquid MSR-M49 recorded 11.2 µmol H_2O_2 mg^{-1}protein min^{-1} under 80 and 60 % ETC respectively, in Shandawil 1, while in Gemmeiza 12 was 11.4 and 13.0 µmol H_2O_2 mg^{-1}protein min^{-1} respectively, in wheat inoculated with capsules under 80 and 60 % from ETC. The antioxidant capability of inoculated wheat under drought stress was assessed by studying CAT and APX activities in leaves. Presence of CAT and APX enzymes eliminates dangerous H_2O_2 by changing to oxygen and water then avoids the plants from cellular destruction and prevents the harmless effects of hydrogen peroxide. This information is verified with report of (Malleswari and Bagyanarayana 2013). Hassanpour et al., (2014) found that, there are increased in the activity of ascorbate peroxidase and peroxidase under drought stress conditions. In our study, *Lelliottia amnigena* MSR-M49 participate plant growth promotion and biochemical in wheat crop under drought condition (Fig. 7 a and b) therefore, we find that there is a decrease in the activity of theses enzymes in two genotypes verities. These characteristics important for plant against stress produced damages by improved water relations, decreased antioxidant enzymatic activity (Kalam et al., 2020). Also, the amounts of antioxidant enzymes produced in wheat Gemmeiza 12 were higher than the Shindawil 1 cultivar. Hojati et al., (2011) informed that the high levels of enzymes in plants indicate improved tolerance to drought stress. In this trial, the activity of CAT in drought-sensitive cultivar Gemmeiza 12 began to reduction before the arrival to irrigation under extended-term drought at −80 from ETC. Xinpeng et al., (2019) indicated that the activity of peroxidase enzymes in drought resistant cultivars was higher than susceptible cultivars. Saad et al.,–(2020) found that capsules *A. chroococcum* is informed to progress catalase activity under drought stress condition in wheat plants (*Triticum aestivum* L.). Similarly, capsules containing *Paenibacillus polymyxa* MSRH5, *Bacillus nakamurai* MSRH1 and *Bacillus pacificus* MSR H3 consortium induce high activity of peroxidase and ascorbate peroxidase in wheat plants (*Triticum aestivum* L.) (Saad et al.,-2019), as well consortium of PGPR containing *P. jessenii* R62, *P. synxantha* R81 and *A. nitroguajacolicus* strainYB3 and YB5 enhanced growth of plant along with inducing catalase (CAT), peroxidase (PX), ascorbate peroxidase (APX) in Sahbhagi (drought tolerance) and IR-64 (drought sensitive) rice crop (Gusain et al., 2015).

3.10 Dehydrogenase Activity (DHA)

We note from the results that water stress at 80% and 60% ETC had a harmful effect on the dehydrogenase enzyme in the soil and led to a decrease in the enzyme activity recorded a reduction 12 % and 42 % respectively, at 80 % and 60 % from ETC in Shandawil 1 (Fig. 8), while the reduction was higher in Gemmeiza 12, recorded 25 % and 44 % respectively, at 80 % and 60 % from ETC. The dehydrogenase activity in the wheat inoculated with MSR-M49 increased under 80 % from ETC, recorded 92.1(µg TPF g dry $soil^{-1}$ day^{-1}) in capsules while in liquid was 78.9 (µg TPF g dry $soil^{-1}$ day^{-1}) in Shandawil 1 compared with un-inoculated plants. In Gemmeiza 12 was 88.6 (µg TPF g dry $soil^{-1}$ day^{-1}) in capsules and was 83.6 (µg TPF g dry $soil^{-1}$ day^{-1}) in Liquid MSR-M49 at 80 % from ETC. The control treatment exhibited lower dehydrogenase activity than the un- inoculated plants. One of the characteristics of water-deficient soil is that it has a weak structure, poor aeration, has surface peeling, has a high pH, and a low rate of filtration. These conditions affect the roots of plants and growth. It also increases osmotic

pressure and leads to a decrease in the activity of soil enzymes (Piromyou et al., 2011). DHA activity was increased in the soils inoculated with PGPR (Fig. 8). In this experiment, we find that there is activity of the DHA, and this activity increases due to the dry condition of the soil, especially the soil inoculated with capsules MSR-M49, and one of these explanations for this is the adaptation of the native soil community, physiologically and genetically, to environments with limited water. Soil microorganisms have several tools to survive dryness in soil. For example, *Lelliottia amnigena* MSR-M49 have been informed to synthesize exopolysaccharides (Ibrahim et al., 2020) to increase their survival through periods of little external water potential. Polysaccharides are characterized by hydroscopic and so could keep higher water content in the colony microenvironment than in the bulk soil for example water potential deny (Roberson and Firestone, 1992). Our results are harmony with (Alharbi et al., 2022) who found that inoculation of PGPRs lead to in higher levels of dehydrogenase than un-inoculated plants.

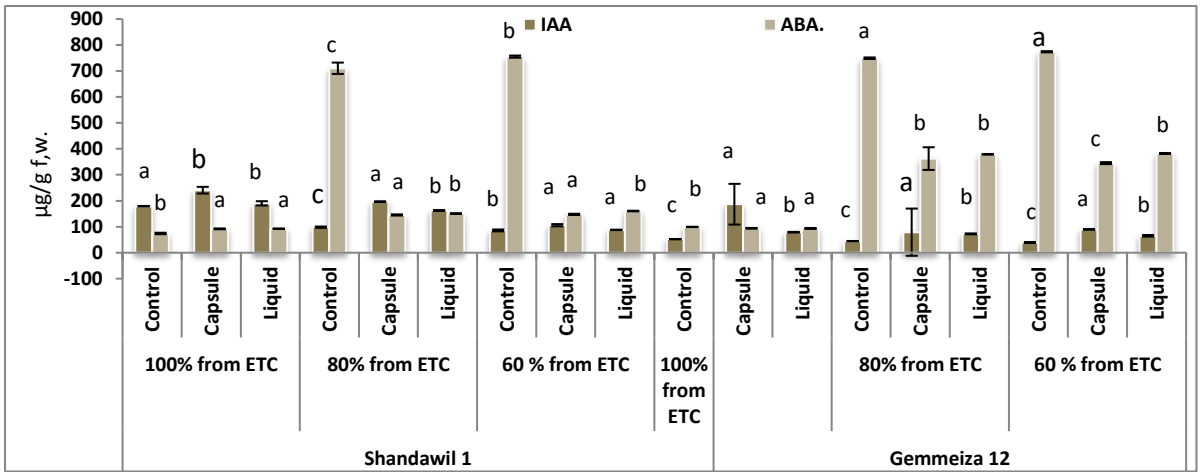


Fig. 3. IAA and ABA content of two genotype wheat plants Shindawil 1 and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates.

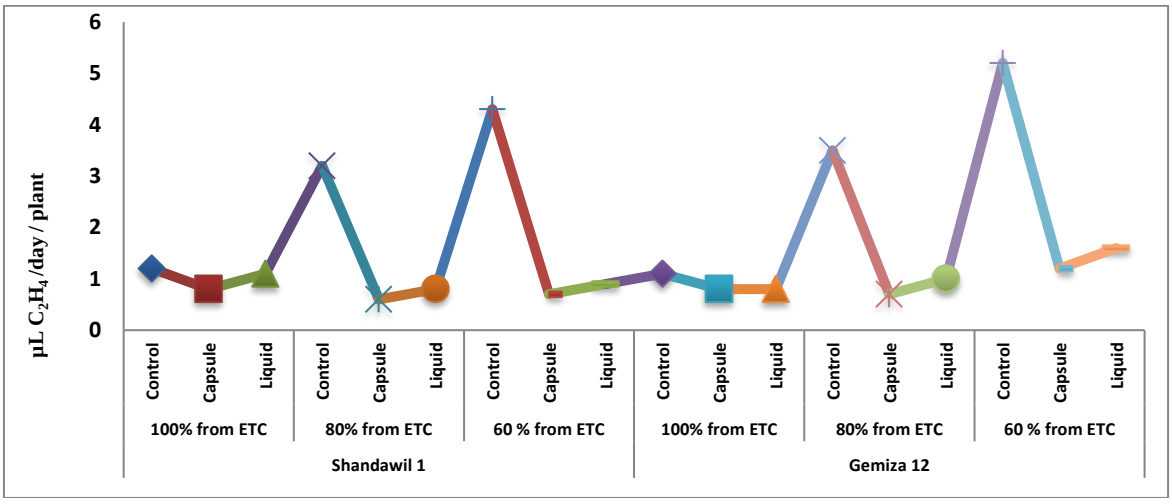


Fig. 4. Ethylene content of two genotype wheat plants Shindawil 1and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates

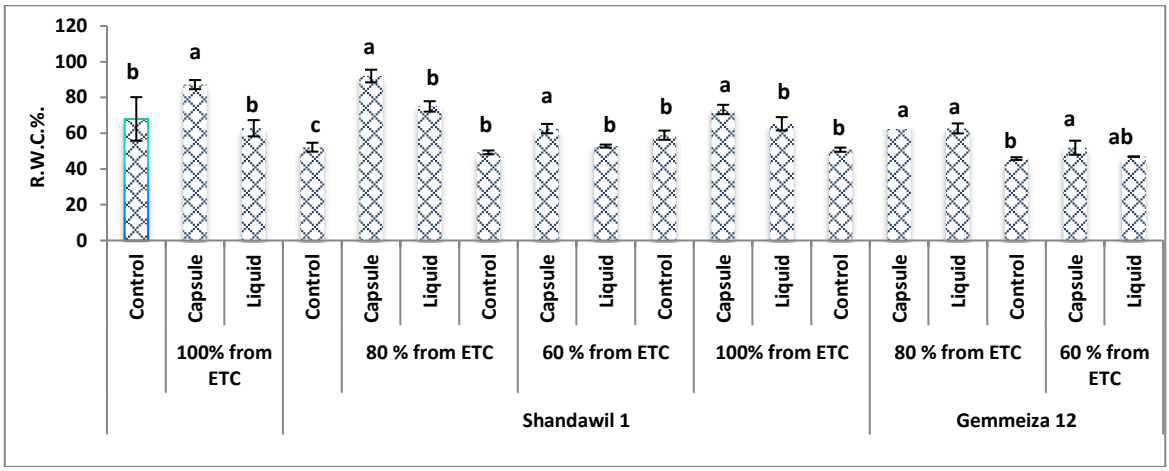


Fig. 5. Relative water content (R.W.C %) of two genotype wheat plants Shindawil1and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates

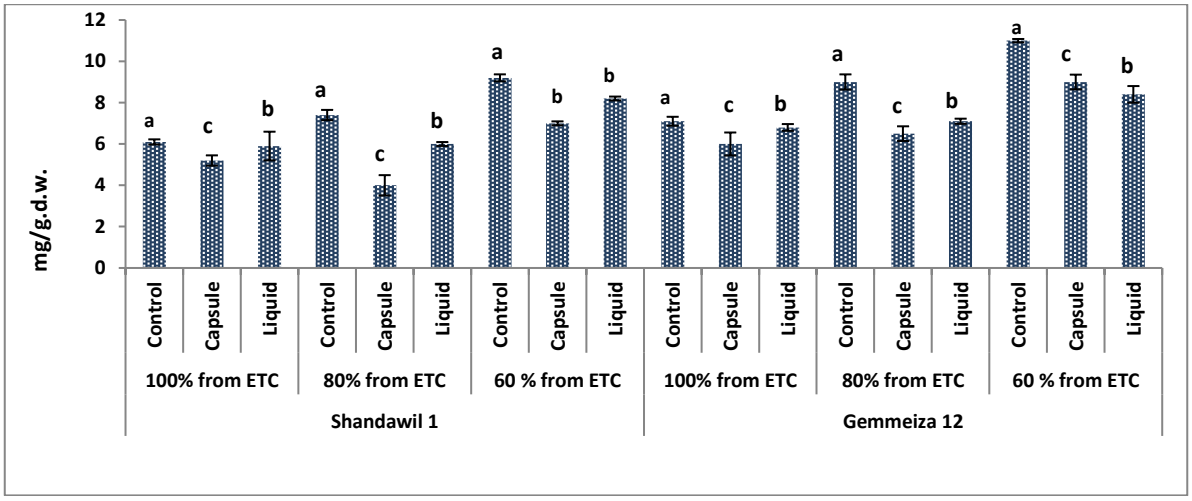
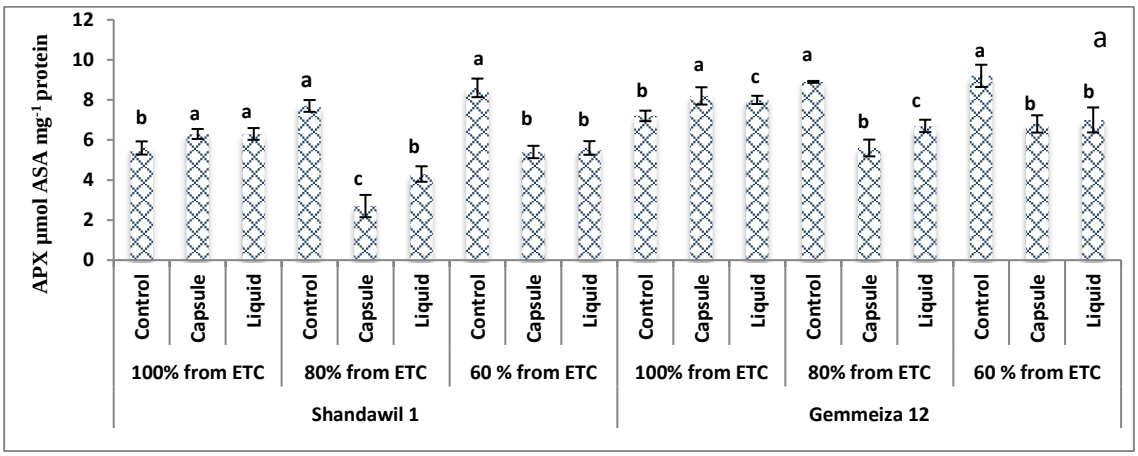


Fig. 6. Proline content of two genotype wheat plants Shindawil1 and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates



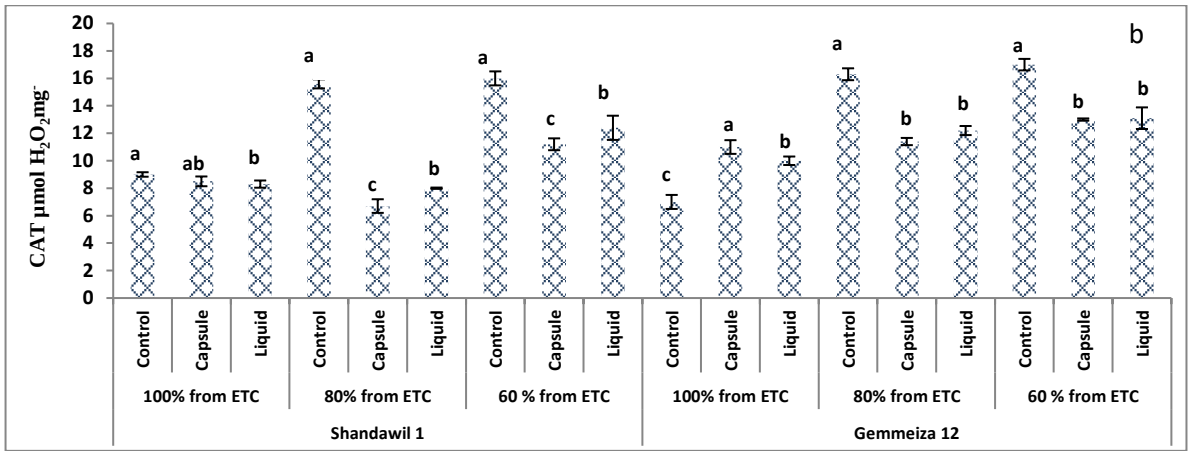


Fig. 7. Antioxidant enzyme (a and b) in shoots of two genotype wheat plants Shindawil 1 and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates.

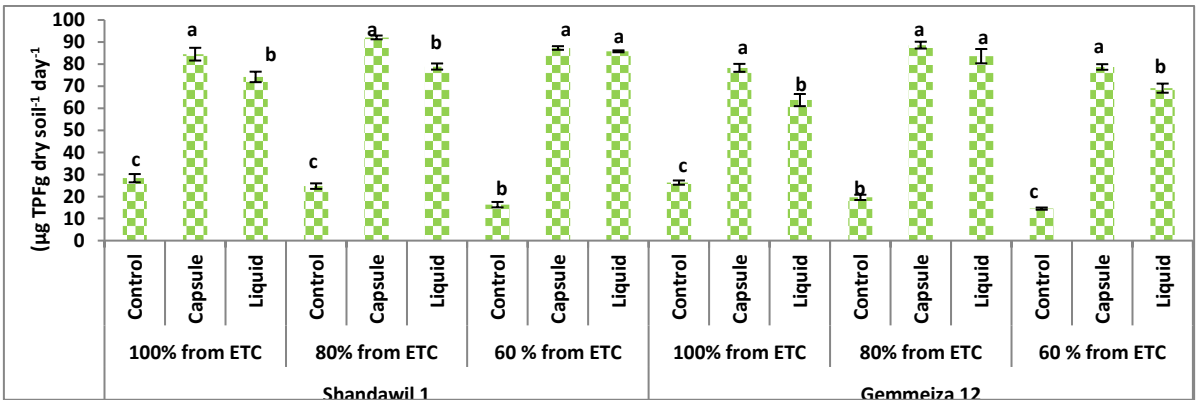


Fig. 8. Dehydrogenase activity (DHA) in rhizosphere of soil two genotype wheat plants Shindawil1and Gemmeiza12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49. Error bars represent the standard deviation between 3 replicates

3.11 Yield Attributes

Data in (Table 4) shows a remarkable decrease in yield component in two genotypes varieties wheat plants under drought stress conditions, whereas the inoculation with bacteria in two forms led to improve the growth and productivity under drought in comparison to the non-inoculated control plants. The recorded increase in plant height were 28 %, 34 % in spike length and 43 % in 1000 grain; respectively, in case of inoculation with capsules of MSR-M49 in Shandawil 1 at 80% ETC. Also, straw and grain yield in two genotype wheat plants were significantly affected with bacteria compared to non-inoculated plants. As well as wheat inoculated with capsules showed maximum biological yield followed by inoculated with liquid bacteria in two different genotype wheat plants. Furthermore results had been recorded low impact on components of yield in genotype Gemmeiza-12. Morphological modifications in plant under drought stress include reduced size, the plant height, spike length, grain 1000 grain, as well as grain and straw yield due to stimulus of the ABA precursor called ACC that inhibits the growth of root, through early maturity (Lamaoui et al., 2018).The results of this study exposed an increase in the plant height, yield component of wheat treated with either capsules or liquid bacteria in drought conditions compared to non-treated. Encapsulated bacteria increase soil productivity and endure harmful environmental effects (drought conditions) (Stella et al., 2019). Alginate beads containing bacteria significantly improved beneficial plant growth parameters (sorghum) (Trejo et al., 2012). Established on the direct influence on plant growth a positive influence of coated bacteria could be showed that produced minor differences in plant height

has been renowned: of 25% (Meftah et al., 2020), 19% (Joe et al., 2012), 13% (Hern´and ez-Montiel et al., 2017), comparing to the control. Harvest index (HI) indicates the stability among the productive measures of the plant and have reservations. It shows the incidence of good dividing of biological yield. The harvest index of the two varieties was significantly increased by the inoculation of capsules bacteria inoculants over control. Application of capsules MSR-M49 improved the yield of wheat plants in comparison with stressed untreated plants. These conclusions exhibited that, encapsulated bacteria have the ability to increase plant growth, and subsequently capitalize on yield and productivity (Hafez et al., 2019). MSR-M49 produce different plant hormones in the rhizosphere of wheat as secondary metabolites that encourage plant development directly, the mechanisms by which MSR-M49 could stay alive besides adapt to extreme drought surroundings are related with excretion of EPS (Vurukonda et al., 2016) and other studies have detected the same thing, this results are agreement with (Daniel et al., 2022)

3.12 Macronutrients of Wheat Grains

Application of bacterial either capsules or in liquid culture improvement the macronutrients (N, P and K %) in grains of two genotype wheat plants under drought stress conditions (Table 5). MSR-M49 in all two forms alleviated the dangerous effects made by drought stress compared to un treated plants (control), maximum N, P and K % in grains were obtained in wheat treated with capsules MSR-M49, was 2.1, 1.04 and 0.58 % respectively, under 80% from ETC in Shandawil 1. Whereas in Gemmeiza12, recorded 1.5, 0.91 and 0.47% respectively, under 80% from ETC. Minimum N, P, and K% were noticed in treatments in drought stress in two genotype wheat under 60 % from ETC. Inoculation of either capsules or liquid MSR-M49 inoculants significantly enhanced grain N, P and K of the two wheat varieties over control (Table 5). Mantelin and Touraine (2004) informed that plants inoculated with PGPR significantly improved the nutrient elements like Ca, K, Fe, Cu and Zn among proton pump ATPase. Furthermore, Karlidag et al., (2007) stated that inoculation of Bacillus inoculants enhanced the mineral elements by apple plants. Besides, Kumar et al., (2017) stated that co-inoculation of Enterobacter with *S. marcescens* and *M. arborescens* enhanced grain N and P uptake of wheat variety in the field experiment. In general, this study established that the capsules MSR-M49 use was able of improving the growth, yield related parameters, and grain nutrient of the two varieties. Though, the encapsulated bacterial exhibited a marked difference in their influence on numerous structures of growth and productivity of Dukem (Dz-01-974) teff variety. Saad et al., (2020) found that inoculation wheat with capsules *A. chroococcum* significantly enhanced N, P, and K contents in grains under water defiant 80% from actual evapotranspiration.

3.13 Crop Water Relations

The amounts of irrigation water applied (m3 ha^{-1}) were determined for all treatments. The total amount of water applied throughout the growing season for both wheat varieties under different treatments is presented in (Table 6) also the applied irrigation water (AIW), water consumptive use (Cu) water use efficiency (WUE) and water productivity (WP) are presented in (Table 6). Results indicate that the amount of water consumptive use increased in case of 100% treatments of both wheat varieties. Water-use efficiency (WUE) was significantly influenced by the irrigation treatments and their inoculants. The lowest WUE was found in the 100% treatments of both wheat varieties, while the highest WUE was observed in the 60% and 80% irrigation treatments with capsule inoculants for both wheat varieties. The Shandawil 1 variety, in particular, exhibited the highest WUE under these conditions, primarily due to its higher productivity compared to the Gemmeiza 12 variety. The results indicated that the amounts of water applied were 5601, 4350, and 3200 m^3/ha respectively, for the treatments under study, corresponding to 100%, 80%, and 60% of the ETC. The higher grain yield were found at 100% of ETC treatment (5601 m^3/ha) for both wheat varieties with capsule inoculants compared with the other irrigation treatments. The increase in water consumptive use depends on the growth yield or availability of soil moisture in maize root zone. These results are in agreement with those obtained by of Hafiz and Ewis (2015) and Farré and Faci, (2006). According to Morsy and Abdel Latif (2012), increased water usage in onion production resulted in reduced water productivity across all varieties. Data indicate that bacterial inoculation with capsule at varying irrigation levels (100%, 80%, and 60% ETC) enhanced water productivity relative to both the control and full irrigation (100% ETC. Specifically, the findings reveal that the 60% and 80% irrigation treatments yielded the highest water productivity

Table 4. Yield component in two genotype wheat plants Shindawil 1and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49.

Wheat cultivar (A)	ETC % (B)	Inoculants (C)	Plant height (cm)	Spike length (cm)	1000 grain wt. (g)	Straw yield (Ton/ hac)	Grain yield (Ton/ hac)	Biological yield (Ton/ hac)	Harvest index (HI %)
Shandawil 1	100% from ETC	Control	76.6	10.8	47.2	7.8	7.7	15.5	49
		Capsule	102	13.3	66.5	12.7	10.6	23.3	45
		Liquid	94.6	11.1	64.2	12	8.9	20.9	42
	80% from ETC	Control	71.6	9.0	43.5	17.3	6.9	24.2	33
		Capsule	92	12.1	62.5	12.3	9.8	22.1	44
		Liquid	85.3	12.1	57.8	12.3	7.9	20.2	39
	60 % from ETC	Control	70.3	8.1	41.6	6.9	6	12.9	46
		Capsule	81.6	11.2	50.8	9	7.9	16.9	46
		Liquid	79.0	10.5	46.5	8.4	6.2	14.6	42
Gemmeiza 12	100% from ETc	Control	72.6	8.9	42.9	6.7	6.9	13.6	50
		Capsule	90.3	10.0	61.1	12.4	9.4	16.3	57
		Liquid	89.3	9.53	55.1	10.3	8.4	18.7	44
	80% from ETC	Control	70.3	8.5	40.5	6	6.7	12.7	52
		Capsule	86.0	10.4	54.4	10.8	7.9	18.7	42
		Liquid	82.3	9.7	44.4	10.6	7.7	18.3	42
	60 % from ETC	Control	68.6	7.9	39.8	5.8	6.2	12.0	51
		Capsule	80.3	9.7	52.4	9.4	7.7	17.1	45
		Liquid	77.0	8.9	49.6	10.3	7.2	17.5	41
L.S.D at 0.05									
A			*	*	*	*	*	*	*
B			1.107	0.3160	0.3160	0.1263	0.05954	--------	---------
C			1.149	0.1476	0.6237	0.1476	0.1109	----------	---------
AXBXC			2.813	0.2556	0.3614	0.3614	0.1921	----------	---------

Table 5. Macronutrients of grains in two genotype wheat plants Shindawil 1 and Gemmeiza 12 with irrigation intervals 80 and 60 % from ETC treated with either capsules or liquid MSR-M49

Wheat cultivar (A)	ETC % (B)	Inoculants (C)	Grains		
			N%	P%	K%
Shandawil 1	100% from ETC	Control	1.8	0.75	0.43
		Capsule	2.1	0.92	0.57
		Liquid	2.0	0.9	0.56
	80% from ETC	Control	1.2	0.64	0.39
		Capsule	2.1	1.04	0.58
		Liquid	1.7	0.98	0.54
	60 % from ETC	Control	0.8	0.60	0.35
		Capsule	1.7	0.98	0.48
		Liquid	1.1	0.85	0.52
Gemmeiza 12	100% from ETC	Control	1.5	0.73	0.37
		Capsule	1.7	0.83	0.42
		Liquid	1.7	0.81	0.43
	80% from ETC	Control	1.2	0.67	0.32
		Capsule	1.5	0.91	0.47
		Liquid	1.5	0.85	0.42
	60 % from ETC	Control	0.73	0.63	0.29
		Capsule	0.9	0.88	0.43
		Liquid	0.7	0.81	0.39
L.S.D at 0.05					
A		*		*	*
B		0.1498		0.00076	0.0010
C		0.07844		0.021	0.00068
AXBXC		0.1921		0.053	0.0016

Table 6. Amounts of irrigation water applied, Water consumptive use, water use efficiency and water productivity under the adopted treatments in 2022/2023 season

			Shandawil 1			
ETC %	**Inoculants**	**Grain yield (Kg/ ha)**	**AIW m^3/ha.**	**WCU m^3/ha**	**WUE (kg m^{-3})**	**WP (Kg/m^3)**
100% ETC	Control	7700	5601	2902	2.65	2.65
	Capsule	10600		3000	3.53	3.53
	Liquid	8900		2950	3.02	3.02
80% ETC	Control	6900	4350	2322	2.97	2.97
	Capsule	9800		2465	3.98	3.98
	Liquid	7900		2355	3.35	3.35
60 % ETC	Control	6000	3200	1741	3.45	3.45
	Capsule	7900		1800	4.39	4.39
	Liquid	6200		1778	3.49	3.49
			Gemmeiza 12			
100% ETC	Control	6900	5601	2862	2.41	1.23
	Capsule	9400		2950	3.19	1.68
	Liquid	8400		2890	2.91	1.50
80% ETC	Control	6700	4350	2297	2.92	1.64
	Capsule	7900		2350	3.36	1.94
	Liquid	7700		2310	3.33	1.89
60 % ETC	Control	6200	3200	1706	3.63	2.03
	Capsule	7700		1750	4.40	2.52
	Liquid	7200		1735	4.15	2.36

Table 7. Correlation coefficients between Biological yield and some traits investigated.

Wheat cultivar	ETC %	Variables	RWC	T.Chl	Ethylene	DHA
Shandawil 1	100% ETC	Biological yield	0.6	-0.8	-0.9	1.0
		RWC		0.6	-0.9	0.5
		T.Chl	0.6		-0.9	1.0
		Ethylene	-0.9	-0.9		-0.8
	80% ETC	Biological yield	1.0	1.0	-1.0	1.0
		RWC		1.0	-0.9	1.0
		T.Chl	1.0		-1.0	1.0
		Ethylene	-0.9	-0.98		-0.9
	60% ETC	Biological yield	1.0	1.0	-0.8	0.8
		RWC		0.9	-0.7	0.7
		T.Chl	0.9		-0.9	0.9
		Ethylene	-0.7	-0.9		-1.0
Gemmeiza 12	100% ETC	Biological yield	0.5	0.7	-0.9	0.7
		RWC		0.9	-0.8	1.0
		T.Chl	0.9		-1.0	1.0
		Ethylene	-0.8	-1.0		-1.0
	80% ETC	Biological yield	1.0	1.0	-1.0	1.0
		RWC		1.0	-1.0	1.0
		T.Chl	1.0		-1.0	1.0
		Ethylene	-1.0	-1.0		-1.0
	60% ETC	Biological yield	0.6	1.0	-1.0	1.0
		RWC		0.8	-0.7	0.7
		T.Chl	0.8		-1.0	1.0
		Ethylene	-0.7	-1.0		-1.0

compared to both the control and 100% irrigation treatments for both wheat varieties. In particular, the Shindawell 1 cultivar exhibited the highest water productivity (WP) values when subjected to inoculation and 60% ETC irrigation, surpassing both the control and the 100% ETC treatment. These enhancements in WP are primarily attributed to elevated grain productivity, particularly notable in the Shandawil variety 1. Morsy and Abd El- Latif (2012) found that increasing water applied for onion yield gave the lower water productivity for all varieties. Thus, hormone and exopolysaccharides producing *Lelliottiaa mnigena* MSR-M49 can improve the drought stress in two verities wheat especially sensitive Gemmeiza 12 and stimulate their growth. Furrow irrigation, a system wherever water is moved from a head channel to crop channels through siphons, it is simplest and earliest forms of irrigation transfer (Souss 2010). The efficacy of furrow irrigation is affected by field slope and length, and with water infiltration amounts. The water usage effectiveness was enhanced for plants with a high percentage of bacterial colonization. Inoculation plants with PGPR required less water than non-inoculated plants (Eulenstein et al., 2017). Increasing the irrigation water applied under 100% ETC could be return to the increase in direct evaporation. So, the seasonal irrigation water applied is higher under 100% ETC followed by 80% and 60% ETC for wheat through the growth season. The present results are in agreement with those achieved by the results of (Morsy et al., 2018). IWUE values were usually higher under lower irrigation conditions (Bozkurt et al., 2009), also showed that IWUE values reduced by lower water applications (Bozkurt and Mansuroglu, 2011). Saad et al., (2020) suggested that inoculated with capsules of *Azotobacter chroococcum* could improve drought tolerance and water use efficiency of plants under water deficit conditions and average values of water utilization efficiency (WUtE) were affected by irrigation and fertilizer treatments.

3.14 Correlation analysis between Biological Yield and Different Wheat Parameters

In 100% irrigations, RWC was strong correlated with biological yield the values was 0.6, but there were negative correlated with T.chl, ethylene and biological yield , the value were -0.8 and -0.9 respectively while the correlated was positive strong between the DHA and biological yields (Table 7) in Shandawil, while in 80% irrigations, there was positive strong correlation between biological yield and RWC, T.Chl and DHA ,its recorded 1.0,1.0 and 0.1 respectively, but with ethylene, it was negative correlated, the value was -0.1. In genotype Gemmeiza 12, the positive correlation was observed between biological yields with RWC, T.Chl and DHA under 100, 80 and 60 % irrigations, but there was negative correlated between biological yield and ethylene in all three irrigations treatments.

4. CONCLUSION

This study demonstrates that inoculating wheat plants with encapsulated *Lelliottia amnigena* MSR-M49 strain enhances their tolerance to drought stress. The encapsulation technique ensures a controlled, gradual release of bacterial cells, improving their ability to adhere to and colonize the roots, which enhances their role as plant growth- promoting. This method could serve as an effective and sustainable bio-fertilizer alternative for wheat cultivation, supporting its growth under drought conditions. Notably, the sensitive wheat variety *Gemmeiza* 12 was able to grow successfully under 80% and 60% of the crop evapotranspiration (ETC, demonstrating the strain's ability to help wheat plants survive in water-scarce conditions. The inoculation with encapsulated MSR-M49 also resulted in the highest water use efficiency, a critical factor under drought stress. Overall, the *Lelliottia amnigena* MSR-M49 strain offers a promising, eco-friendly solution for improving drought resistance in plants, contributing to sustainable agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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