

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.811.045>

Screening of AM Fungi based on Peroxidase Activity under Irrigation Regime of -500kPa in Soybean (*Glycine max* L. Merrill)

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ABSTRACT

Keywords

Peroxidase,
Soybean, Hydro-
meteorological,
Crops

Article Info

Accepted:
04 October 2019
Available Online:
10 November 2019

Using pressure plate and membrane apparatus, irrigation regime of -500kPa was calculated. Forty AM fungal isolates were tested based on their peroxidase activity in soybean plant. Among the AMF, the soybean plants which received UASDAMFS1 (3.86 U mg⁻¹ protein) exhibited less peroxidase activity compared to non mycorrhized soybean plants which recorded the highest peroxidase activity (5.06 U mg⁻¹ protein) at irrigation regime of -500 kPa. Total dry matter accumulation in plants was also recorded.

Introduction

Moisture stress is a serious problem in India. The severity and the extent of moisture stress not only depend on low rainfall but also on the other hydro-meteorological factors like infiltration and moisture retention capacity of the soil. Aridity of an area depends on rainfall in relation to potential or actual evapotranspiration and the moisture holding capacity of the soil. Potential evaporation is a measure of the maximum possible evaporation from the soil and the transpiration from vegetation, if the soil is fully saturated. It is a

measure of consumptive use of water by crops.

To ameliorate the adverse affects of moisture stress microorganisms could play an important role in adaptation strategies and increase of tolerance to abiotic stresses in agricultural plants, such as plant growth promoting microorganisms which colonizes the portion of soil attached to the root surface and influenced by root exudates termed as rhizosphere soil (Bent, 2006; Lugtenberg and Kamilova, 2009).

Some of the beneficial microorganisms can also enter into the root system of their hosts and enhance their beneficial effects with an endophytic lifestyle such as arbuscular mycorrhizal fungi (AMF) as well as plant growth promoting rhizobacteria (Das and Varma, 2009). Measuring of the Peroxidase activity is one of the important parameter to be studied during stress conditions.

Materials and Methods

Experimental design and statistical analysis

The data were subjected to analysis following Completely Randomised Design (CRD) as defined by Gomez and Gomez (1984). -500kPa was measured by using Pressure plate and membrane apparatus. Moisture in the plastic cups was maintained by measuring daily evapotranspiration rate. This experiment was conducted at department of agricultural microbiology, University of Agricultural Sciences, Dharwad.

Soil and biological material

The soil which used for the experiment was Red soil and it was collected from University of Agricultural Sciences, Dharwad farm. The AM fungal species used were isolated from the Dharwad and Belgaum districts.

Parameters measured

The plastic cups were filled with sterilized soil and sand in the ratio of 1:1 and inoculated with forty native AM fungi isolates @ 20 g per cup. Soybean seeds were sown. The treatment details were encompassing the native AMF isolates and a control without AMF.

These treatments were laid out in a CRD design, replicated four times. The plants were tested for peroxidase activity and dry biomass

accumulation. The soybean plants were raised under irrigation regime of -500 kPa. Water was added to the plants on daily evapotranspiration rate base.

Red soil

Irrigation regime -500kPa - 24.76 % (-500 kPa)

This can be calculated by “Pressure plate and Membrane apparatus.”

Volume of the soil = 350 cc

Water to be present to maintain irrigation regime at -500kPa

$$= \frac{350 \times 24.76}{100} = 86.66 \text{ c.c}$$

1 cubic centimetre = 1 ml

86.66 cubic centimetre = **86.66** ml.

Surface area of the pot which is exposed to sunlight = πr^2
= $3.14 \times 4.25 \times 4.25$

$$= 56.71 \text{ cm}^2$$

Evapotranspiration rate = 4.07 mm/unit area

$$\therefore 1 \text{ mm} = 0.1 \text{ cm}$$

Total surface evapotranspiration rate from the pot which is exposed to sunlight

$$= 0.407 \times 56.71$$

$$= 23.08 \text{ cm}^3 (23.08 \text{ ml}) \therefore 1 \text{ cm}^3 = 1 \text{ ml}$$

Amount of water remaining in the water = 86.66 - 23.08
= 63.58 ml

Amount of water to be added to maintain irrigation regime of -500kPa = 23.08 ml.

Parameters measured

Peroxidase activity

Peroxidase activity was done by the following the procedure given by Maehly and Chance (1954).

A 0.5 g of fresh leaf sample was weighed and ground well in a mortar with little quantity of chilled phosphate buffer at pH 6.6 and filtered through a double layered muslin cloth to remove the pulp, made up to 25 ml and centrifuged for 30 minutes at 2,000 rpm at 4 °C. The clear extract was used as enzyme source. The same enzyme was used for the estimation of soluble protein. 3 ml of 0.05 M guaiacol solution was pipetted out into a test tube to which 0.1 ml of enzyme extract was added. Then 0.5 ml of 1 per cent hydrogen peroxidase was added, mixed the contents rapidly and the absorbance was measured in calorimeter at 470 nm at an interval of 20 seconds.

Enzyme activity was calculated by taking the average difference of O. D. (optical density) between two consecutive time intervals and enzyme activity was expressed in terms of $\Delta OD \text{ sec}^{-1} \text{ mg}^{-1} \text{ protein}$ (*i.e.* specific activity).

Dry matter accumulation

Five randomly selected plants were uprooted at the time of harvest and dried in a hot air oven at 70 °C for 48 h to achieve constant weight. The shoot dry weight and root dry weight were recorded separately and total dry matter accumulation in plant was recorded in terms of gram per plant (g plant^{-1}).

Results and Discussion

The rhizosphere soil samples of soybean were collected and used for the isolation of AMF. Among the AMF, the soybean plants which

received UASDAMFS1 exhibited less peroxidase activity followed by UASDAMFS5, UASDAMFS7, UASDAMF10, UASDAMFS15, UASDAMFS19, UASDAMFS25, UASDAMFS27, UASDAMFS33 and UASDAMFS36 (3.86, 4.12, 4.17, 4.01, 3.55, 3.92, 3.12, 4.03, 3.88 and 3.27 $\text{U mg}^{-1} \text{ protein}$ respectively) compared to non mycorrhized soybean plants which recorded the highest peroxidase activity ($5.06 \text{ U mg}^{-1} \text{ protein}$) at irrigation regime of -500 kPa. Highest total dry biomass was recorded in the soybean plants received UASDAMFS1 followed by UASDAMFS5, UASDAMFS7, UASDAMF10, UASDAMFS15, UASDAMFS19, UASDAMFS25, UASDAMFS27, UASDAMFS33 and UASDAMFS36 respectively when compared to the uninoculated control (Table 1).

Uninoculated plants showed more peroxidase activity. This is in agreement with the findings of Saeedesh and Alireza (2017) who also reported that the lower oxidative damage in the AM plants seems to be a consistent effect of AM symbiosis, regardless of the fungal species involved in the association (Ruiz-Lozano *et al.*, 2001b and Porcel *et al.*, 2003).

AMF contribution to plant drought tolerance might also have occurred through drought avoidance mechanisms such as hyphal water uptake (Marulanda *et al.*, 2003) or increased water uptake related to mycorrhizal changes in root morphology (Kothari *et al.*, 1990) or soil structure (Auge *et al.*, 2001a). Such mycorrhizal effects could allow plants to remain more hydrated than non-AM plants as soil dries (Auge *et al.*, 2001b). AM formation contributes to the production of scavenging peroxy radicals, buffering cellular free-radicals and producing a powerful ROS-scavenging system (Ashraf and Foolad, 2007) (Table 2).

Table.1 Potential evapotranspiration rate data of October and November months (P. Et in mm)

| Sl. No | Date | P. Et in mm | Water to be added (ml) |
|---------------|-------------|--------------------|-------------------------------|
| 1 | 15/10/2017 | 4.07 | 23.08 |
| 2 | 16/10/2017 | 3.80 | 21.54 |
| 3 | 17/10/2017 | 4.29 | 24.32 |
| 4 | 18/10/2017 | 4.03 | 22.85 |
| 5 | 19/10/2017 | 4.55 | 25.80 |
| 6 | 20/10/2017 | 4.48 | 25.40 |
| 7 | 21/10/2017 | 4.01 | 22.74 |
| 8 | 22/10/2017 | 3.89 | 22.06 |
| 9 | 23/10/2017 | 3.73 | 21.15 |
| 10 | 24/10/2017 | 3.31 | 18.71 |
| 11 | 25/10/2017 | 4.15 | 23.53 |
| 12 | 26/10/2017 | 4.49 | 25.46 |
| 13 | 27/10/2017 | 4.69 | 26.59 |
| 14 | 28/10/2017 | 4.54 | 25.74 |
| 15 | 29/10/2017 | 4.69 | 26.59 |
| 16 | 30/10/2017 | 4.21 | 23.87 |
| 17 | 31/10/2017 | 4.75 | 26.93 |
| 18 | 1/11/2017 | 4.01 | 22.74 |
| 19 | 2/11/2017 | 4.93 | 27.95 |
| 20 | 3/11/2017 | 4.95 | 28.07 |
| 21 | 4/11/2017 | 5.26 | 29.82 |
| 22 | 5/11/2017 | 4.25 | 24.10 |
| 23 | 6/11/2017 | 4.82 | 27.33 |
| 24 | 7/11/2017 | 5.48 | 31.07 |
| 25 | 8/11/2017 | 5.35 | 30.33 |
| 26 | 9/11/2017 | 5.49 | 31.13 |
| 27 | 10/11/2017 | 4.97 | 28.18 |
| 28 | 11/11/2017 | 4.35 | 24.66 |
| 29 | 12/11/2017 | 4.09 | 23.19 |
| 30 | 13/11/2017 | 4.76 | 26.65 |

Table.2 Screening of AM fungal isolates based on the peroxidase activity and total dry matter in soybean at -500 kPa

| SI. No | Isolate code | Peroxidase activity (U mg ⁻¹ protein) | Total dry matter (g plant ⁻¹) |
|--------|----------------------|---|--|
| 1 | UASDAMFS1 | 3.86 | 1.35 |
| 2 | UASDAMFS2 | 4.33 | 1.07 |
| 3 | UASDAMFS3 | 4.23 | 1.09 |
| 4 | UASDAMFS4 | 4.57 | 1.09 |
| 5 | UASDAMFS5 | 4.12 | 1.15 |
| 6 | UASDAMFS6 | 4.78 | 1.13 |
| 7 | UASDAMFS7 | 4.17 | 1.12 |
| 8 | UASDAMFS8 | 4.71 | 1.22 |
| 9 | UASDAMFS9 | 4.19 | 1.10 |
| 10 | UASDAMFS10 | 4.01 | 1.29 |
| 11 | UASDAMFS11 | 4.71 | 1.15 |
| 12 | UASDAMFS12 | 4.22 | 1.23 |
| 13 | UASDAMFS13 | 4.34 | 1.08 |
| 14 | UASDAMFS14 | 5.08 | 1.09 |
| 15 | UASDAMFS15 | 3.55 | 1.96 |
| 16 | UASDAMFS16 | 4.20 | 1.18 |
| 17 | UASDAMFS17 | 4.57 | 1.09 |
| 18 | UASDAMFS18 | 4.77 | 1.06 |
| 19 | UASDAMFS19 | 3.92 | 1.30 |
| 20 | UASDAMFS20 | 4.19 | 1.12 |
| 21 | UASDAMFS21 | 4.85 | 1.10 |
| 22 | UASDAMFS22 | 4.79 | 1.13 |
| 23 | UASDAMFS23 | 4.72 | 1.19 |
| 24 | UASDAMFS24 | 4.34 | 1.45 |
| 25 | UASDAMFS25 | 3.12 | 2.14 |
| 26 | UASDAMFS26 | 4.22 | 1.09 |
| 27 | UASDAMFS27 | 4.03 | 1.15 |
| 28 | UASDAMFS28 | 4.18 | 1.11 |
| 29 | UASDAMFS29 | 4.35 | 1.09 |
| 30 | UASDAMFS30 | 4.22 | 1.10 |
| 31 | UASDAMFS31 | 4.65 | 1.15 |
| 32 | UASDAMFS32 | 4.46 | 1.12 |
| 33 | UASDAMFS33 | 3.88 | 1.33 |
| 34 | UASDAMFS34 | 4.39 | 1.27 |
| 35 | UASDAMFS35 | 4.98 | 1.10 |
| 36 | UASDAMFS36 | 3.27 | 2.04 |
| 37 | UASDAMFS37 | 4.87 | 1.12 |
| 38 | UASDAMFS38 | 4.45 | 1.23 |
| 39 | UASDAMFS39 | 4.98 | 1.09 |
| 40 | UASDAMFS40 | 4.18 | 1.98 |
| 41 | UIC | 5.06 | 1.03 |
| | S.Em.± | 0.03 | 0.05 |
| | C.D. (p=0.01) | 0.10 | 0.13 |

UIC-Uninoculated control

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How to cite this article:

Suma R. Konnur, P. Jones Nirmalnath and Kuligod, V. 2019. Screening of AM Fungi based on Peroxidase Activity under Irrigation Regime of -500kPa in Soybean (*Glycine max* L. Merrill). *Int.J.Curr.Microbiol.App.Sci.* 8(11): 362-367. doi: <https://doi.org/10.20546/ijemas.2019.811.045>