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## Effect of various bio-inoculants on leaf area of heat stressed maize (*Zea mays* L.)

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### Abstract

The present investigation was carried out during *Rabi* season of 2017-18 on a clay loam soil at the Agricultural College Farm, Bapatla to study the effect of various bio-inoculants on leaf area of heat stressed maize (*Zea mays* L.). The experiment was laid out in split-plot design with four different days of sowing of maize (M<sub>1</sub>: December 20<sup>th</sup>, M<sub>2</sub>: January 10<sup>th</sup>, M<sub>3</sub>: January 30<sup>th</sup> and M<sub>4</sub>: February 20<sup>th</sup>) as main plot treatments and five bio-inoculants (Control (S<sub>1</sub>), AMF (S<sub>2</sub>), PSB (S<sub>3</sub>), KRB (S<sub>4</sub>) and AMF+PSB+KRB (S<sub>5</sub>)) as sub plot treatments. Mean values for maize heat stress management via., bio-inoculants revealed that leaf area of maize increased with the increase in age of the crop. Mean values for days of sowing revealed that highest leaf area values of maize were recorded with December 20<sup>th</sup> (M<sub>1</sub>). With the increase in temperatures during delayed sowings decline in leaf area was seen. The maximum leaf area recorded with application of AMF+PSB+KRB (S<sub>5</sub>) in combination in all the sowings (normal and delayed), but however, it was comparable with AMF (S<sub>2</sub>). Thus, Jan 10<sup>th</sup> (M<sub>2</sub>), Jan 30<sup>th</sup> (M<sub>3</sub>) and Feb 20<sup>th</sup> (M<sub>4</sub>) with AMF+PSB+KRB (S<sub>5</sub>) is a sustainable approach to enhance the leaf area of maize during heat stress if sowings are delayed.

**Keywords:** Maize, bio-inoculants, leaf area and heat stress

### Introduction

Maize, one of the world's most widely cultivated crop, is sensitive to heat stress. Unfortunately, extreme heat waves are projected to increase in coming years and rise in temperatures due to global warming. Heat stress will become major factor along with drought that threatens the stability of maize management and production (Song *et al.*, 2018 and Zhang and Lin, 2016) [8, 14]. In India, maize production is 30 Million metric tons from the area of 9.70 Million ha with the productivity of 3.09 Metric t ha<sup>-1</sup>, respectively (USDA, 2021) [9]. Maize hybrids extract more nutrients, particularly N, P and K, than do rice or wheat (Yadvinder-singh *et al.*, 2005) [14]. Maize crop experiences heat stress particularly during *rabi* as the harvests of previous *kharif* crop gets delayed due to late on set of monsoon.

Plant leaves are the critical sites for photosynthesis and transpiration, which (Knight and Ackerly, 2003) [4] play an essential role in material and energy exchange within the soil-plant-atmosphere continuum (SPAC). Morphological and physiological adaptations of leaves are critical for plants to acquire and use limited available resources (e.g. nutrients and soil moisture) when suffering from abiotic stress particularly heat stress. In fact, plants could regulate their carbon dioxide assimilation and nutrient absorption capacity *via.*, the plasticity of leaf area and related traits (Marron *et al.*, 2003) [5] to improve their adaption and competitiveness to climate change (Yin *et al.*, 2018) [12].

Bio-inoculants (mycorrhizal fungi and rhizobacteria) actively colonize plant roots and increase plant growth and yield (Wu *et al.*, 2005) [10]. The mechanism by which rhizobacteria promote plant growth is by solubilisation of mineral phosphates, asymbiotic N<sub>2</sub> fixation (Salantur *et al.*, 2006) [7]. Inoculation of plants with arbuscular mycorrhizal fungi (AMF) and rhizobacteria (PSB - Phosphorus solubilizing bacteria and KRB - Potassium releasing bacteria) is a major asset for biological agriculture and is also need of the time. Environmental biotechnology is also receiving attention as a way to reduce chemical fertilizer doses without affecting crop yield and improving nutrient availability. It can then be evaluated as a component of integrated management strategies in agriculture (Adesemoye *et al.*, 2009, Zemrany *et al.*, 2006; Fuetes-Ramirez and Caballero-Mellado, 2006) [2, 13, 3].

We hypothesize that by combining AMF with PSB and KRB (N<sub>2</sub> fixation, P solubilization, K release), we expect additive or synergistic effects resulting from their combination and hence better improved of the crop growth particularly leaf area.

In this context, we assessed the effects of three bio-inoculants singly and in combination (AMF, PSB, KRB and AMF+PSB+KRB) on maize leaf area under heat stress in field conditions.

### Materials and Methods

An experiment was conducted with four dates of sowing M<sub>1</sub>: December - 20<sup>th</sup> (Normal sowing), M<sub>2</sub>: January - 10<sup>th</sup> (Delayed sowing), M<sub>3</sub>: January - 30<sup>th</sup> (Moderately delayed sowing) and M<sub>4</sub>: February - 20<sup>th</sup> (Extremely delayed sowing) as main plot treatments and five bio-inoculants treatments (S<sub>1</sub> - Control, S<sub>2</sub> - AMF (Arbuscular mycorrhizal fungi), S<sub>3</sub> - PSB (Phosphorus solubilizing bacteria), S<sub>4</sub> - KRB (Potassium releasing bacteria) and S<sub>5</sub> - AMF+PSB+KRB) as sub plot treatments which was replicated thrice. It was carried out on clay loam soils of Agricultural College Farm, Bapatla during *rabi*, 2017-18. The soil was slightly alkaline in nature, low in organic carbon and low in available nitrogen, medium in available phosphorus and normal in available potassium. During the crop growth period, the weekly mean maximum temperature ranged from 29.9 to 35.6 °C and minimum temperature ranged from 16.5 to 27.5 °C. A total rainfall of 54.0 mm in 3 days was received by M<sub>1</sub> and M<sub>2</sub> and 71.2 mm in 5 rainy days by M<sub>3</sub> and M<sub>4</sub> sowings during the crop growth period. The test hybrid used for sowing was P3396 and crop was sown at 60 cm and 20 cm inter and intra row distance, respectively and adopted all the standard package of practices. Application of nutrients was done as per the standard recommended dose in the form of urea, single super phosphate and murate of potash respectively. Maize crop was sown at 20 days interval as per treatment in the main plots so that the delayed and extremely delayed sown maize experiences heat stress. Bio-inoculants was applied to the soil of particular treatment plots. The data on Leaf area was recorded as per standard procedures at 20 days interval using Systronics leaf area meter 211. Statistical analysis of all the collected data are subjected to an analysis of variance (ANOVA) least significance difference (LSD) test at probability level 0.05 for split plot design as outlined by Panse and Sukhatame, 1985.

### Results and Discussion

Production and maintenance of leaf area is important for dry matter production and yield. The leaf area was greatly influenced by delayed sowings along with bio-inoculants application. Leaf area per plant increases exponentially up to 60 DAS and thereafter a gradual decline was observed at 80 DAS.

The calculated mean data related to leaf area at 20, 40, 60, 80 DAS and as affected by heat stress and bio-inoculants application have been summarized and presented in Table 1 and detailed interaction of bio-inoculants with different dates of sowing for heat stress was presented in Table 2. The interaction at 20, 40, 60 DAS was found to be non-significant. Significant results in interaction was observed in 80 DAS.

Highest leaf area was found in M<sub>1</sub> sowing at 20, 40, 60 and 80 DAS (619, 1493, 3169 and 2666 cm<sup>2</sup>, respectively) whereas the lowest was observed in M<sub>4</sub> sowing at 20, 40, 60 and 80 DAS (491, 1223, 1550 and 817 cm<sup>2</sup>, respectively). With the delay in sowing of maize leaf area gets reduced. At 20 DAS the decline in the leaf area from 619 to 491 cm<sup>2</sup> was observed. At 40 DAS the leaf area reduced from 1493 to 1238 cm<sup>2</sup>. At 60 DAS leaf area gets reduced from 3169 to 1550 cm<sup>2</sup>. At 80 DAS the decline in leaf area of all the sowings was observed and it ranged from 2666 to 817 cm<sup>2</sup>.

Among the bio-inoculants the highest leaf area was recorded with S<sub>5</sub> (AMF+PSB+KRB) followed by S<sub>2</sub> (AMF) > S<sub>3</sub> (PSB) > S<sub>4</sub> (KRB) at 20, 40, 60 and 80 DAS in all the sowings and lowest leaf area was observed in S<sub>1</sub> untreated control or with no application of bio-inoculant.

Among the interactions, significant differences were observed at 80 DAS. At 80 DAS, the highest leaf area was recorded with the normal sown crop applied with AMF+PSB+KRB (M<sub>1</sub>S<sub>5</sub> - 2967 cm<sup>2</sup>) followed by (M<sub>1</sub>S<sub>2</sub> - 2723 cm<sup>2</sup>) and the lowest leaf area was obtained with the late sown crop with no application of bio-fertilizer (M<sub>4</sub>S<sub>1</sub> - 765 cm<sup>2</sup>). AMF+PSB+KRB application to the extremely late sown maize crop recorded leaf area of (M<sub>4</sub>S<sub>5</sub> - 882 cm<sup>2</sup>). The decline in 21.5% leaf area of extremely delayed sown (M<sub>4</sub>S<sub>1</sub>) over the normal sown crop without bio-inoculant application (M<sub>1</sub>S<sub>1</sub> - 2416 cm<sup>2</sup>) and it was statistically at par with the normal sown crop without bio-inoculant application. Extremely late sown maize crop applied with AMF+PSB+KRB (M<sub>4</sub>S<sub>5</sub> - 882 cm<sup>2</sup>) increased the leaf area by 23.6% over the late sown crop without bio-inoculant application (M<sub>4</sub>S<sub>1</sub>) in the present study.

The rapid decline in functional leaf area in the delayed sowing crops might be due to the rapid senescence induced by increased temperatures whereas it was observed with the AMF+PSB+KRB and AMF application, that more leaf area over control was retained in the treated plants even under delayed sowing.

It is revealed that leaf area in normal sown maize is higher in the study. Higher leaf area contributes towards higher photosynthesis and biomass. Increase in the temperature during the crop growth in delayed sowings reduced the leaf area due to leaf firing and leaf senescence which indicated that delayed sowings in maize is sensitive towards higher temperature exposure. This increase in leaf area may be due to better production and metabolism of auxin, hormones responsible for the cellular elongation or cytokinin, hormones that stimulate the cellular division (Anzala *et al.*, 2006) triggered by bio-inoculant application.

Delayed sowing resulted in plants with fewer leaves and slower rates of leaf appearance in sweet corn (Williams, 2008). Asif *et al.* (2007) improved mean leaf area in maize at higher K level could be attributed to the activation of several enzymes, increase in protein synthesis, N uptake and utilization.

**Table 1:** Leaf area (cm<sup>2</sup>) means at different growth stages of maize as influenced by heat stress and bio-inoculants during *rabi*, 2017-18

Treatments	2017-18			
	20 DAS	40 DAS	60 DAS	80 DAS
<b>Dates of sowing</b>				
M <sub>1</sub> - Dec 20 <sup>th</sup>	619	1493	3169	2666
M <sub>2</sub> - Jan 10 <sup>th</sup>	596	1453	3091	2625
M <sub>3</sub> - Jan 30 <sup>th</sup>	524	1361	2989	2260
M <sub>4</sub> - Feb 20 <sup>th</sup>	491	1238	1550	964
S.Em	2.62	7.08	17.74	9.36
CD (p=0.05)	9.08	24.51	61.40	32.40
CV (%)	1.82	1.98	2.54	1.70
<b>Bio-inoculants</b>				
S <sub>1</sub> - Control	449.00	1207.60	2286.08	1823.45
S <sub>2</sub> - AMF	592.35	1462.55	2830.03	2125.48
S <sub>3</sub> - PSB	566.28	1399.73	2764.53	2039.55
S <sub>4</sub> - KRB	544.98	1354.10	2654.73	1939.85
S <sub>5</sub> - AMF+PSB+KRB	636.93	1509.65	2965.85	2324.80
S.Em	7.97	20.05	40.94	33.33
CD (p=0.05)	22.96	57.75	117.94	96.01
CV (%)	4.95	5.01	5.25	5.63
<b>Interaction</b>				
M X S	N.S	N.S	N.S	S
S X M	N.S	N.S	N.S	S

[M<sub>1</sub>-December 20<sup>th</sup> (Normal sowing), M<sub>2</sub>-January 10<sup>th</sup> (Moderately delayed sowing), M<sub>3</sub>-January 30<sup>th</sup> (Delayed sowing), and M<sub>4</sub>-February-20<sup>th</sup> (Extremely delayed sowing); S<sub>1</sub>-Control (No bio-inoculants applied), S<sub>2</sub>-AMF (Arbuscular mycorrhizae fungi), S<sub>3</sub>-PSB (Phosphorus solubilizing bacteria), S<sub>4</sub>-KRB (Potassium releasing bacteria) and S<sub>5</sub>-Mix (AMF+PSB+KRB)]

**Table 2:** Effect of high-temperature stress and bio-inoculants on leaf area (cm<sup>2</sup>) of maize at 80 DAS

Year	2017-18				
	(M <sub>1</sub> ) Dec-20 <sup>th</sup>	(M <sub>2</sub> ) Jan-10 <sup>th</sup>	(M <sub>3</sub> ) Jan-30 <sup>th</sup>	(M <sub>4</sub> ) Feb-20 <sup>th</sup>	Mean
(S <sub>1</sub> ) Control	2416	2312	1801	765	1823
(S <sub>2</sub> ) AMF	2723	2555	2401	824	2125
(S <sub>3</sub> ) PSB	2678	2489	2175	817	2040
(S <sub>4</sub> ) KRB	2548	2341	2069	801	1940
(S <sub>5</sub> ) AMF+PSB+KRB	2967	2816	2635	882	2325
Mean	2666	2502	2216	818	---

	2017-18		
	Main plots	Sub plots	Interaction
S.Em	16.08	33.33	66.66
CD @ 0.05	55.64	96.01	192.02
CV (%)	3.04	5.63	--

[M<sub>1</sub>-December 20<sup>th</sup> (Normal sowing), M<sub>2</sub>-January 10<sup>th</sup> (Moderately delayed sowing), M<sub>3</sub>-January 30<sup>th</sup> (Delayed sowing), and M<sub>4</sub>-February-20<sup>th</sup> (Extremely delayed sowing); S<sub>1</sub>-Control (No bio-inoculants applied), S<sub>2</sub>-AMF (Arbuscular mycorrhizae fungi), S<sub>3</sub>-PSB (Phosphorus solubilizing bacteria), S<sub>4</sub>-KRB (Potassium releasing bacteria) and S<sub>5</sub>-Mix (AMF+PSB+KRB)]

## Conclusion

Based on the above results it can be concluded that heat stress reduces the leaf area in maize. In normal sown conditions leaf area gradually increases from 20 DAS to 60 DAS, later decline in leaf area was observed as the assimilate produced were transferred to reproductive parts to attain the yield. Application of bio-inoculants in normal as well as in delayed sowing increases the leaf area of maize. Treatment of bio-inoculant in combination AMF+PSB+KRB (S<sub>5</sub>) increases the leaf area more pronouncedly as compared to individual application. AMF+PSB+KRB (S<sub>5</sub>) were found to be the most effective and sustainable approach to enhance the leaf area of the heat stressed maize.

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