Supplementary information for:

Enhancing maize tolerance to combined drought and heat stress through catalase 2 and dehydrin gene modulation: Single vs. combined rhizobacterial inoculum

Iviwe Notununu[1,2,3], Lucy Moleleki[1], Ashira Roopnarain[2,4] And Rasheed Adeleke[5*]

[1]*Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Lynnwood Rd, Hatfield, Pretoria, 0002, South Africa.*

[2]*Microbiology and Environmental Biotechnology Research Group, Agricultural Research Council−Soil, Climate and Water, Pretoria, 0083, South Africa*

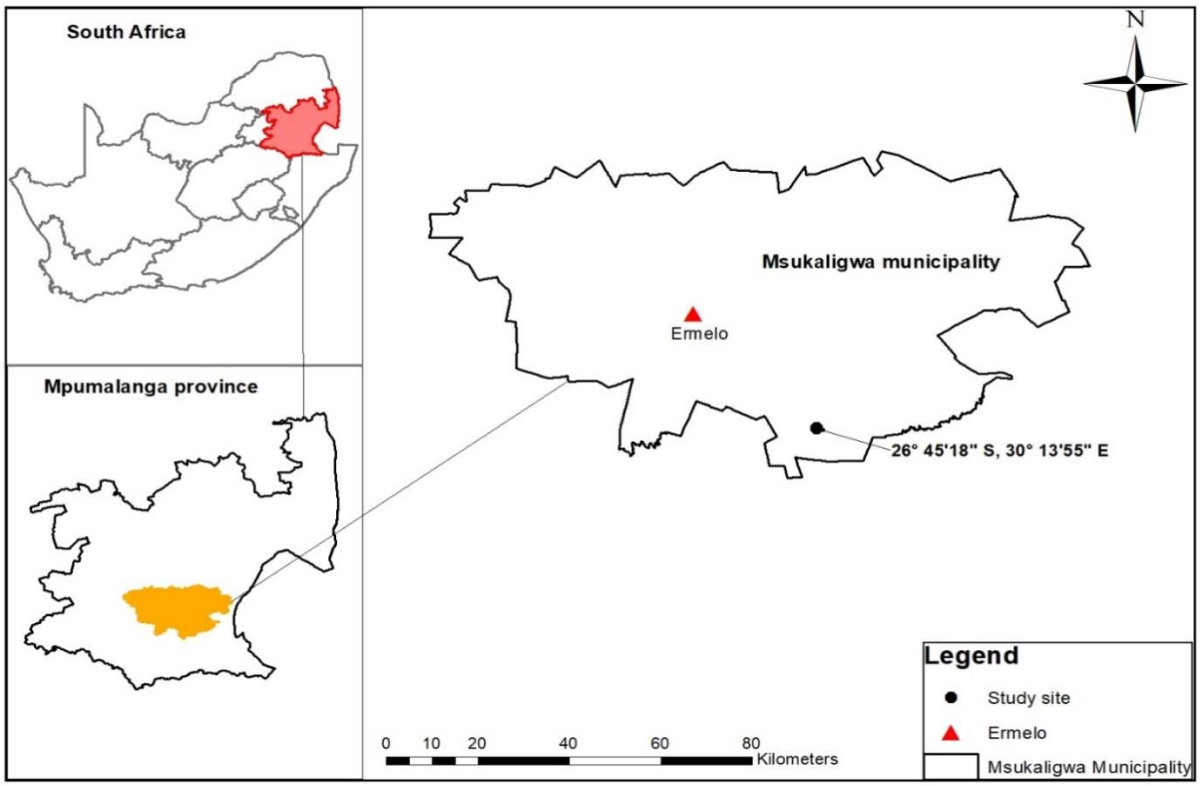
[3]*Department of life and consumer sciences, College of Agriculture and Environmental Sciences, University of South Africa, Florida Park, Roodepoort, 1709.*

[4]*Department of Environmental Sciences, College of Agriculture and Environmental Sciences,*

University of South Africa, Florida Park, Roodepoort, 1709.

[5]*Unit for Environment Science and Management, North-West University (Potchefstroom Campus), Potchefstroom, 2520, South Africa.*

Corresponding Author Email Address: Rasheed.adeleke@nwu.ac.za



Supplementary Fig. S1: The study site in Mpumalanga Province of South Africa

Supplementary Table S1: Candidate stress response genes and the reference genes, their primer sequences and product sizes.

Gene Name	Primer Name	Primer Sequence	Product size	Reference
Dehydrin 2	dhn2-624F	5'-ACGAAGACTCAGACCCCACCA-3'	104	(Capelle *et al*., 2010)
	dhn2-727R	5'-GCGTCTTCCGGCTTCTTGT-3'		
Heat Shock Protein 70	Hps70F	5'-AGCTAAGACTGGGTGGCTGA- 3'		Designed
	Hsp70R	5'-GTCGTCTTCTCCCTGTGCTC-3'		
Catalase 2	Cat2_F	5'-TCTCTGTCTGCTTTCGCTCA-3'	151	Designed
	Cat2_R	5'-GGACACAGCCAGCCATTATT-3'		
Tubulin beta (β-Tub)	β-TUB_F	5'- CTACCTCACGGCATCTGCTATGT-3'	139	Lin *et al*., 2014
	β-TUB_R	5'- GTCACACACACTCGACTTCACG-3'		
Elongation factor 1 alpha (EF1a)	EF1a_F	5'- TGGGCCTACTGGTCTTACTACTGA-3'	135	Lin *et al*., 2014
	EF1a_R	5'- ACATACCCACGCTTCAGATCCT-3'		

Supplementary Table S2: Screened bacterial isolates for drought (40 % PEG 6000) and heat stress (42 ℃) cultured on TSB broth. Bacterial isolates that exhibited an OD above 0.40 for both drought and heat stress were considered drought and heat tolerant. Bacterial isolates coloured green exhibited tolerance to both drought and heat stress

Sample No:	SAMPLE ID	Heat stress		Drought stress	
		Average Optical density (OD)	Standard Deviation	Average Optical density (OD)	Standard Deviation
1	21MN1A	1.09	0.04	0.08	0.18

2	32MN1B	1.10	0.01	0.50	0.09
3	15MN6B	0.71	0.04	0.16	0.05
4	15MN5	1.34	0.10	0.10	0.13
5	32MN2B	1.23	0.10	0.03	0.04
6	14MN3B	1.05	0.17	1.15	0.90
7	22MN2A	0.87	0.11	0.02	0.05
8	14MN3A	1.00	0.10	0.38	0.22
9	14MN5A	1.12	0.00	0.49	0.10
10	14MN5B	0.92	0.08	0.01	0.03
11	22MN2B	1.02	0.03	0.45	0.04
12	11MN3	0.93	0.01	0.42	0.06
13	21MN1B	0.94	0.14	0.45	0.14
14	14MN5A	0.27	__	0.45	0.10
15	11MN1	1.07	0.04	0.53	0.06
16	15MN6B	0.70	0.04	0.15	0.05
17	32MN1A	1.07	0.04	0.37	0.10
18	21MN3	1.00	0.01	0.35	0.10
19	11MN2	1.14	0.04	0.50	0.08
20	32MN3	0.95	0.02	0.37	0.07
21	32MN4	1.10	0.04	0.10	0.06
22	14MN1	1.09	0.01	0.28	0.17

23	22MN2	0.90	0.04	0.33	0.08
24	31MN1B	0.66	0.04	0.50	0.19
25	21MN3S	1.16	0.06	0.34	0.07
26	35MN3	0.89	0.03	0.37	0.14
27	31MN1S	1.30	0.02	0.12	0.02
28	23MN5	0.91	0.03	0.37	0.08
29	14MN2	1.12	0.03	0.24	0.15
30	35MN1	0.85	0.04	0.10	0.09
31	26MP3	1.47	0.08	0.03	0.09
32	20MP2S	1.24	0.03	0.00	__
33	27MP1	0.11	0.03	0.03	0.08
34	33MP1	0.87	0.04	0.54	0.21
35	15MP4	1.00	0.06	-0.06	0.03
36	14MP3	1.06	0.02	0.29	0.06
37	26MP3	1.07	0.01	0.08	0.06
38	30MP4	1.15	0.09	0.14	0.10
39	16MP1	0.87	0.01	0.04	0.16
40	15MP2	0.18	0.04	0.01	0.04
41	36MP8	0.64	0.01	0.96	0.30
42	14MP4	1.22	0.15	0.07	0.04
43	20MP2B	1.14	0.19	0.21	0.14
44	18MP2	1.20	0.05	0.10	0.09
45	12MP2	1.04	0.05	-0.02	0.06
46	23MP1	1.13	0.05	0.16	0.08
47	36MP1	0.74	0.05	0.40	0.20
48	26MP4Y	0.87	0.08	0.22	0.11

49	28MP1W	0.37	0.04	0.14	0.03
50	30MP5	1.00	0.03	0.37	0.05
51	31MP1	0.59	0.04	0.06	0.10
52	30MP3Y	0.93	0.03	0.18	0.03
53	23MP3	0.29	0.07	0.03	0.03
54	34MP2	0.86	0.05	0.48	0.11
55	36MP4	0.89	0.03	0.33	0.12
56	26MP2	0.91	0.03	0.14	0.09
57	21MP2Y	0.01	__	0.14	0.05
58	19MP4Y	0.77	0.41	-0.02	__
59	21MP1	1.08	0.01	0.21	0.04
60	21MP2	-0.08	__	-0.03	0.01
61	19MP4W	0.27	0.02	-0.02	0.00

Supplementary Table S3*:* 16S rDNA gene sequence similarity (%) of test isolates to known isolates

Isolate ID	Sequence analysis results	% Similarity
36MP8	***Leclercia_sp._strain_T3196-2***	**100**
34MP2	***Leclercia_sp._strain_T3196-2***	**99**
33MP1	***Lelliottia_amnigena_strain_NCTC12124***	**99**
32MN1B	***Bacillus_cereus _ strain 24195***	**100**
31MN1	***Bacillus_ cereus _ strain 24195***	**100**
21MN2	***Bacillus_ cereus _ strain 24195***	**100**
21MN1B	***Bacillus_pseudomycoides_strain_MF-68***	**100**

14MN5A	***Bacillus_ cereus _ strain 24195***	**100**
14MN3B	***Acinetobacter_sp._*DSM30007**	**99**
11MN3	***Bacillus_ cereus _ strain 24195***	**100**
11MN2	***Bacillus_ cereus _ strain 24195***	**100**
11MN1	***Bacillus_ cereus _ strain 24195***	**100**

Sequence data for species identification

>32MN1B

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GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGC

GCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGA

AACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCG

TAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAG

GCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT

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>11MN1

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CGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATT

GGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAAT

GCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACT

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>11MN2

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AGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGA

GTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGA

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>11MN3

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>21MN2B

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>14MN5A

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AAC

>14MN3B

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CT

>33MP1

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>34MP2

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ACCGCCTGGGGAGTACGGCCGC

>36MP8

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CAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC

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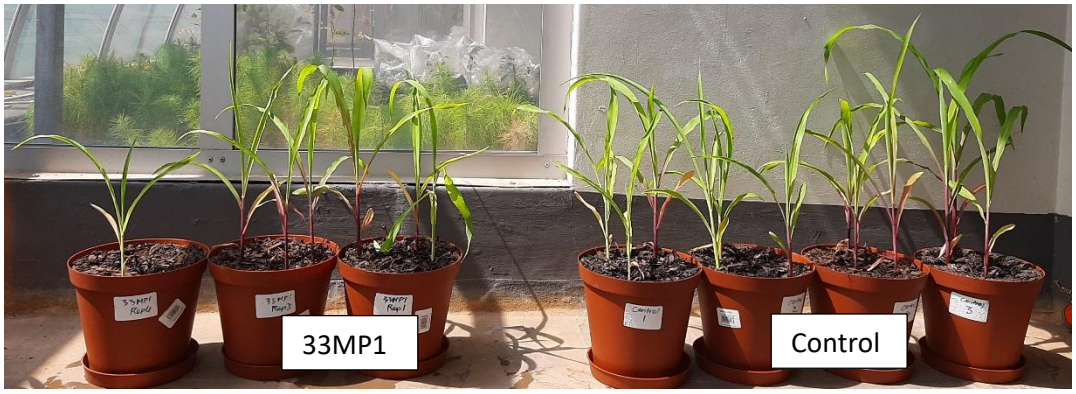
11MN1
Control


21MN1B
Control


33MP1
Control

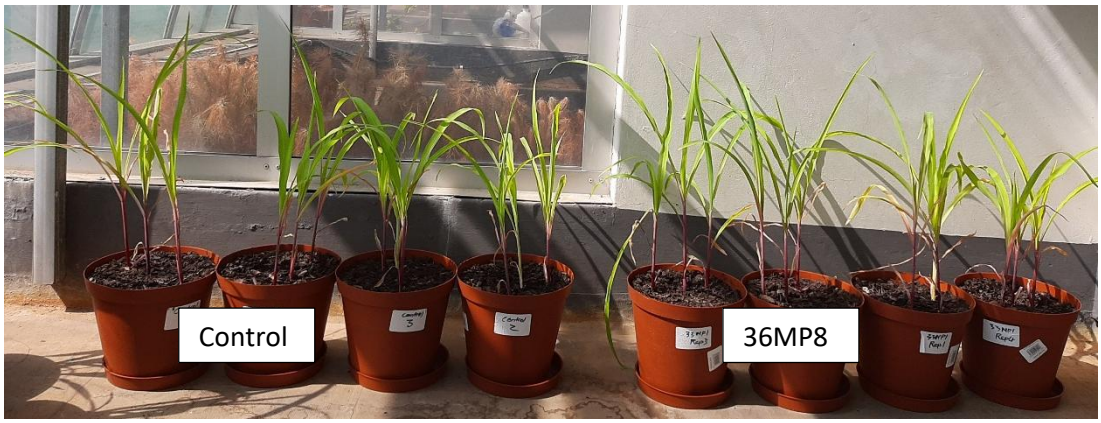

36MP8
Control

Supplementary Fig. S2: Visual depiction of bacterized maize seeds grown under ambient conditions with soil moisture maintained at 80 % WHC and the temperature at 25/23 °C after 32 days.

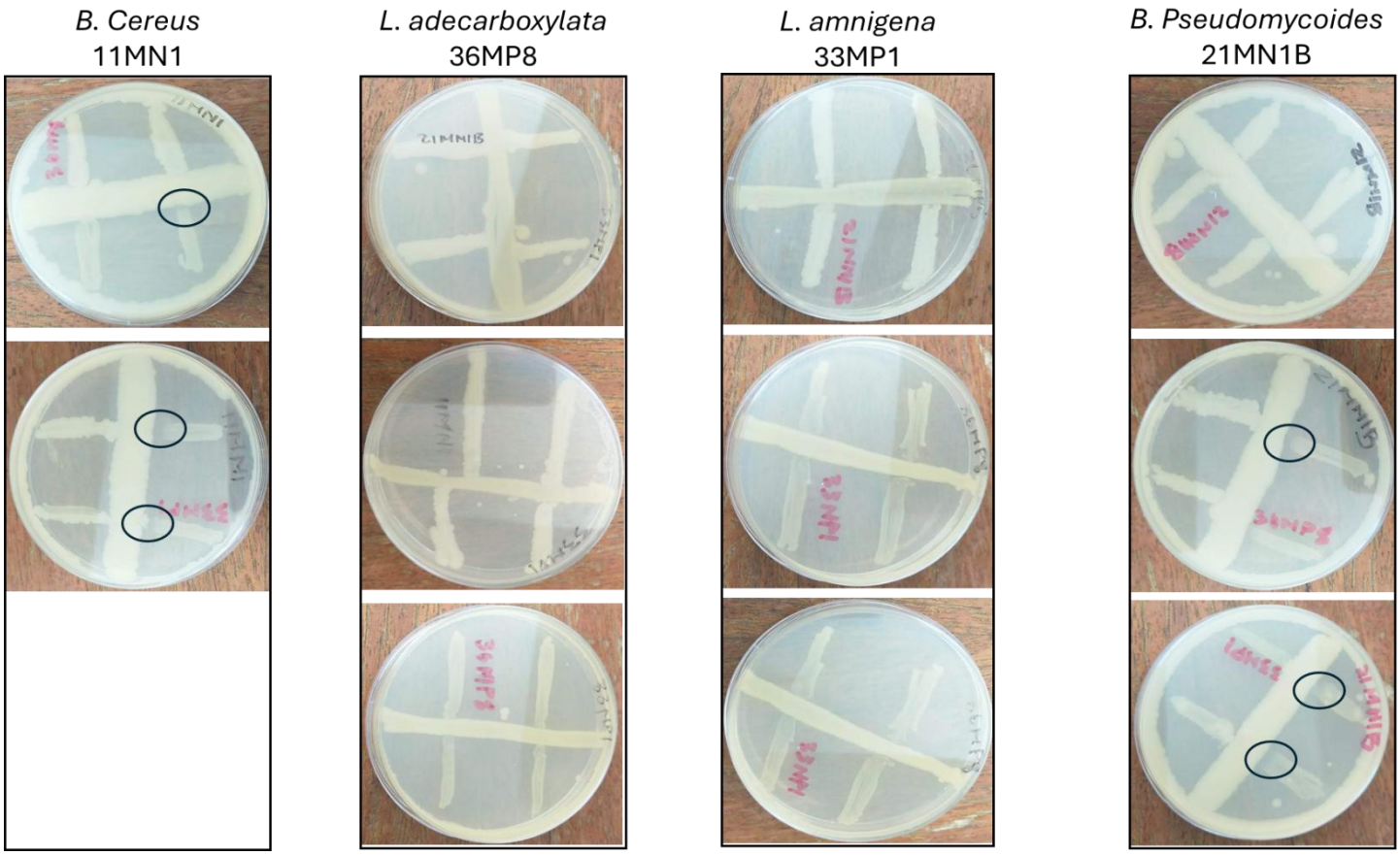

Control
11MN1


Control
21MN1


Control
33MP1


Control
36MP8

Supplementary Fig. S3: Visual depiction of bacterized maize seeds grown under the concurrent stress of drought (40 % WHC) and heat (32/28 °C) stress after 32 days.



Supplementary Fig 4: The compatibility of the bacterial isolates tested on TSA plates was determined using the cross-streak dual culturing method. The primary isolate was streaked first and incubated at 32 °C for 24 h, then the second microbial isolate was streaked perpendicularly, growing outward from the emerged colonies of the initial streak and incubated for another 24 h 32 °C. *B. cereus* and *B. pseudomycoides* showed antagonism against *L adecarboxylata* and *L. amnigena*.

Sinawo, Tsipinana, corrected final, Disser

Supplementary Fig. 5: Dissertation “Tsipinana, S., 2019. The impact of fertilizer application, tillage systems and crop rotation on soil health and rhizosphere microbial community structure under maize and soybean plantation. University of South Africa, Pretoria”.