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Pseudomonas-aided zinc application improves the productivity and biofortification of bread wheat

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Table S1. Analysis of variance for effect of different Zn application methods with or without *Pseudomonas* sp. MN12 addition on photosynthetic traits of wheat cultivars

SOV	DF	Photosynthesis (*A*)		Transpiration (*E*)		Intercellular CO_2 (*Ci*)		Stomatal conductance (*Gs*)	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
Cultivar (C)	1	33.2**	3.16*	0.935**	0.144ns	705.2*	1870.2**	0.01405**	0.01209*
Microbe (M)	1	12.6**	56.3**	0.957**	3.200**	20981.6**	44434.2**	0.02244**	0.03283**
Zinc (Zn)	4	22.4**	15.4**	0.718**	0.739**	14503.8**	13467.3**	0.01215**	0.01469**
C× M	1	3.19*	0.23ns	0.088ns	0.002ns	2049.3**	1175**	0.00002ns	0.00016ns
C× Zn	4	1.35ns	0.31ns	0.054**	0.015*	324.8*	231.8*	0.00057**	0.00009ns
M× Zn	4	0.70ns	1.10ns	0.031*	0.063**	847.8**	399.5**	0.00241**	0.00157**
C× M × Zn	4	0.23ns	0.90ns	0.016ns	0.017*	34.9ns	213.2**	0.00033*	0.00027**
Error	60	0.80	0.46	0.010	0.005	111.4	70.1	0.00011	0.00006
Total	79								

SOV= Sources of variation; DF= Degree of freedom; ns= non-significant; *$p \leq 0.05$; **$p \leq 0.01$.

Table S2. Effect of *Pseudomonas* sp. MN12 addition on photosynthetic rate and intercellular CO_2 of two wheat cultivars

Treatments	Lasani-2008	Faisalabad-2008	Mean (M)	Lasani-2008	Faisalabad-2008	Mean (M)
	Photosynthesis (µmol CO_2 m^{-2} s^{-1}) (2013-14)			**Photosynthesis (µmol CO_2 m^{-2} s^{-1}) (2014-15)**		
No Microbes	12.94c	14.62ab	13.78B	14.3	14.6	14.4B
Microbes	14.13b	15.02a	14.57A	15.9	16.4	16.1A
Mean (C)	13.53B	14.82A		15.1 B	15.5 A	
HSD ($p \leq 0.05$)	C= 0.40; M= 0.40; C × M= 0.74			C=0.30; M=0.30		
	Intercellular CO_2 (µmol mol^{-1}) (2013-14)			**Intercellular CO_2 (µmol mol^{-1}) (2014-15)**		
No Microbes	262a	266a	264A	281a	279a	280A
Microbes	240b	224c	232B	241b	224c	233B
Mean (C)	251A	245B		261A	251B	
HSD ($p \leq 0.05$)	C= 4.71; M= 4.71; C × M= 8.82			C=3.74; M=3.74; C × M=6.99		

Means sharing same case letter for main effects and interaction for a parameter do not differ significantly at HSD ($p \leq 0.05$); M= microbe application; C= wheat cultivar.

Table S3. Effect of different Zn application methods on intercellular CO_2 and transpiration rate of two wheat cultivars

Treatments	Lasani-2008	Faisalabad-2008	Mean (Zn)	Lasani-2008	Faisalabad-2008	Mean (Zn)
	Intercellular CO_2 (µmol mol^{-1}) (2013-14)			**Intercellular CO_2 (µmol mol^{-1}) (2014-15)**		
Hydropriming	299a	288a	293A	313a	296b	305A
Seed priming	232de	229def	230C	250cd	233ef	241C
Seed coating	269b	253bc	261B	262c	260cd	261B
Soil application	212f	219ef	216D	234ef	232f	233D
Foliar application	244cd	238cd	241C	247de	235ef	241CD
Mean (C)	251A	245B		261A	251B	
HSD ($p \leq 0.05$)	V= 4.71; Zn= 10.49; V × Zn= 17.33			V=3.74; Zn=8.32; V × Zn=13.75		
	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$) (2013-14)					
Hydropriming	1.58e	1.67e	1.63D			
Seed priming	1.90bc	2.28a	2.09A			
Seed coating	1.73de	1.88cd	1.81C			
Soil application	2.00bc	2.29a	2.15A			
Foliar application	1.88cd	2.05b	1.97B			
Mean (C)	1.82B	2.04A				
HSD ($p \leq 0.05$)	V= 0.04; Zn= 0.09; V × Zn= 0.16					

Means sharing same case letter for main effects and interaction for a parameter do not differ significantly at HSD ($p \leq 0.05$); C= wheat cultivars; Zn= zinc application methods

Table S4. Analysis of variance for the effect of different Zn application methods with or without *Pseudomonas* sp. MN12 addition on the yield components of wheat cultivars

		Grains per spike	100-grain weight	Grain yield		Harvest index	
SOV	**DF**	**Pooled data**	**Pooled data**	**2013-14**	**2014-15**	**2013-14**	**2014-15**
Cultivar (C)	1	37.84**	0.0177ns	0.9095**	0.3781**	1107.2**	9.18ns
Microbe (M)	1	31.40*	0.0383*	0.9483**	0.4961**	696.9**	0.99ns
Zinc (Zn)	4	144.42**	0.0640**	1.6809**	0.7767**	595.8**	124.80**
C× M	1	0.87ns	0.0005ns	0.6178**	0.0551**	56.4ns	55.95ns
C× Zn	4	7.98ns	0.0023ns	0.0354ns	0.0259**	20.4ns	100.81**
M× Zn	4	5.36ns	0.0018ns	0.0358ns	0.0283**	32.5ns	83.02*
C× M × Zn	4	2.03ns	0.0038ns	0.0782*	0.0073ns	159.5*	92.07*
Error	60	3.71	0.0086ns	0.0266	0.0056	48.9	27.03
Total	79						

SOV= Sources of variation; DF= Degree of freedom; ns= non-significant; *$p \leq 0.05$; ** $p \leq 0.01$.

Table S5. Effect of *Pseudomonas* sp. MN12 addition on grains per spike and grain yield of two wheat cultivars

Treatments	Lasani-2008	Faisalabad-2008	Mean (M)	Lasani-2008	Faisalabad-2008	Mean (M)
	Grains per spike (pooled data)			Grain yield (g plant^{-1})(2014-15)		
No Microbes	37.3	38.9	38.1B	2.33c	2.41b	2.37B
Microbes	38.7	39.9	39.3A	2.44b	2.62a	2.53A
Mean (C)	38.0B	39.4A		2.38B	2.52A	
HSD ($p \leq 0.05$)	C= 0.86; M= 0.86; V × M=1.60			C=0.33; M=0.33; C × M=0.062		

Means sharing same case letter for main effects and interaction for a parameter do not differ significantly at HSD ($p \leq 0.05$); M= microbe application; C= wheat cultivar.

Table S6. Effect of different Zn application methods on grain yield of two wheat cultivars

Treatments	Lasani-2008	Faisalabad-2008	Means (Zn)
	Grain yield (g plant^{-1}) (2014-15)		
Hydropriming	2.11e	2.32cd	2.21D
Seed priming	2.62ab	2.70a	2.66A
Seed coating	2.29cd	2.48b	2.39B
Soil application	2.68a	2.74a	2.71A
Foliar application	2.21d	2.34c	2.27C
Mean (C)	2.38B	2.52A	
HSD ($p \leq 0.05$)	M= 0.25; Zn= 0.55; M × Zn= 0.92		

Means sharing same case letter for main effects and interaction for a parameter do not differ significantly at HSD ($p \leq 0.05$); C= wheat cultivars; Zn= zinc application methods.

Table S7. Analysis of variance for the effect of different Zn application methods with or without *Pseudomonas* sp. MN12 addition on grain minerals composition of wheat cultivars

		Protein		Phytate		Bioavailable Zn		Phytate/Zn ratio	
SOV	**DF**	**2013-14**	**2014-15**	**2013-14**	**2014-15**	**2013-14**	**2014-15**	**2013-14**	**2014-15**
Cultivar (C)	1	0.8508ns	0.0405ns	0.0003ns	0.435**	0.016ns	0.0001ns	14.50ns	7.38ns
Microbe (M)	1	0.0567ns	0.0245ns	0.7801**	2.211**	0.128**	0.9461**	61.95**	255.26**
Zinc (Zn)	4	0.9659*	3.6982**	5.1039**	8.602**	2.750**	4.2992**	1283.63**	1242.03**
C× M	1	0.0690ns	0.0005ns	0.0673ns	0.210**	0.002ns	0.4651**	2.66ns	64.62**
C× Zn	4	0.3008ns	0.1705ns	0.0038ns	0.040**	0.007ns	0.0614**	9.18ns	24.37**
M× Zn	4	0.1436ns	0.0808ns	0.0307ns	0.121**	0.004ns	0.0530**	5.01ns	6.11ns
C× M × Zn	4	0.1670ns	0.2443ns	0.0188ns	0.117**	0.041**	0.1751**	18.22*	23.36**
Error	60	0.3148	0.1429	0.0205	0.009	0.009ns	0.0079	4.30	2.68
Total	79								

SOV= Sources of variation; DF= Degree of freedom; ns= non-significant; *$p \leq 0.05$; **$p \leq 0.01$.

Table S8. Effect of different Zn application methods on protein contents of wheat cultivars

Treatments	Lasani-2008	Faisalabad-2008	Means (Zn)
	Protein contents (%) (2014-15)		
Hydropriming	12.1	12.5	12.3C
Seed priming	13.0	12.9	13.0B
Seed coating	12.9	12.8	12.8B
Soil application	13.6	13.5	13.5A
Foliar application	13.4	13.5	13.4A
Mean (C)	13.0	13.0	
HSD ($p \leq 0.05$)	Zn = 0.37		

Means sharing same case letter for main effects and intercation for a parameter do not differ significantly at HSD ($p \leq 0.05$); C= wheat cultivar; Zn= zinc application methods

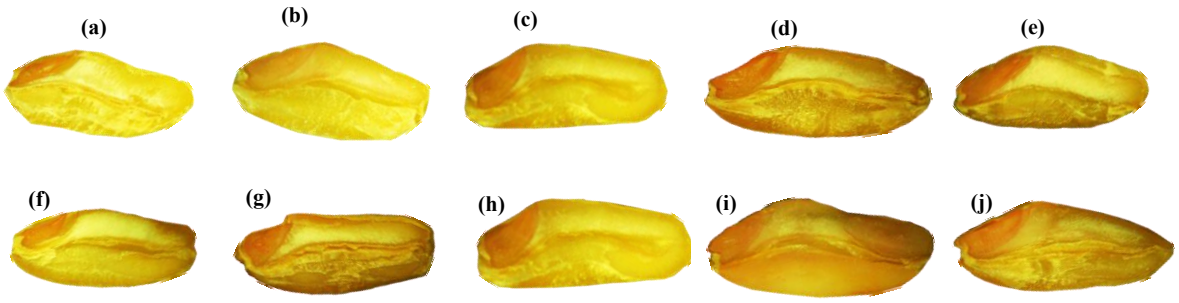

Figure 1S. Zinc localization in seed fractions of wheat using DTZ in wheat cultivar Lasani-2008 as influenced by Zn application methods and MN12 inoculation (a) Hydropriming (b) Seed priming with Zn (c) Seed coating with Zn (d) Soil application of Zn (e) Foliar application of Zn (f) Hydropriming + MN12 (g) Seed priming with Zn + MN12 (h) Seed coating with Zn + MN12 (i) Soil application of Zn + MN12 (j) Foliar application of Zn + MN12. Red colour is indicating the Zn deposition in different seed parts. Increase in intensity of red color shows the higher accumulation of Zn and vice versa. Most of the Zn is deposited in embryo > Aleurone >endosperm of seed.

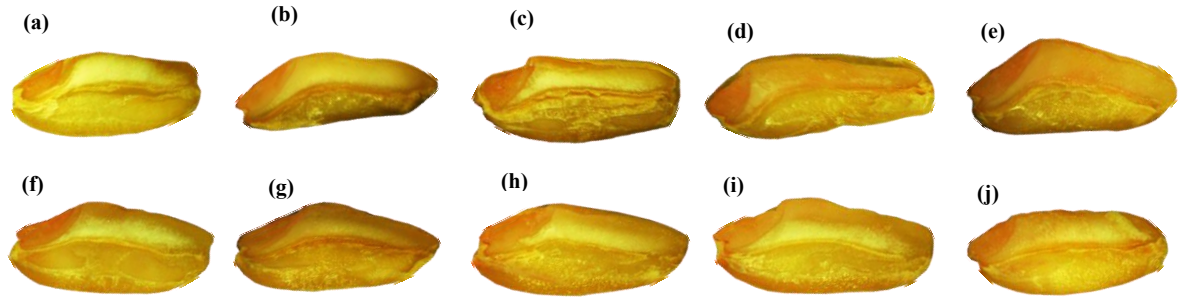

Figure 2S. Zinc localization in seed fractions of wheat using DTZ in wheat cultivar Faisalabad-2008 as influenced by Zn application methods and MN12 inoculation (a) Hydropriming (b) Seed priming with Zn (c) Seed coating with Zn (d) Soil application of Zn (e) Foliar application of Zn (f) Hydropriming + MN12 (g) Seed priming with Zn + MN12 (h) Seed coating with Zn + MN12 (i) Soil application of Zn + MN12 (j) Foliar application of Zn + MN12. Red colour is indicating the Zn deposition in different seed parts. Increase in intensity of red color shows the higher accumulation of Zn and vice versa. Most of the Zn is deposited in embryo > Aleurone > endosperm of seed.