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Genotype-Specific Biochemical and Root Architectural Responses of Wheat to Actinobacteria Under Moisture Stress

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Abstract

Drought stress severely restricts wheat productivity by impairing physiological and metabolic processes, necessitating the exploration of microbe-assisted strategies for enhancing tolerance. This study investigated the genotype-specific responses of twenty bread and durum wheat genotypes to Actinobacteria under controlled moisture-stress conditions. Seeds were treated with two Actinobacteria isolates (AUDT 545 and AUDT 862), a microbial consortium of both isolates and a microbe-untreated control. After 25 days of regular irrigation, drought stress was imposed by withholding water, reducing soil moisture to 9%. Biochemical traits (proline, total soluble sugars, peroxidase and superoxide dismutase activity) and root architectural parameters (root length, volume, surface area and diameter) were quantified to assess stress adaptation. The microbial consortium elicited the most pronounced improvements in osmotic adjustment, antioxidant activity and root system development, with distinct variation among genotypes. The findings highlight the potential of Actinobacteria-based inoculants particularly consortium formulations to enhance drought resilience in wheat through coordinated biochemical and root architectural modifications.

***Key words*:** Wheat, Actinobacteria, Drought tolerance, Biochemical traits, Root architectural traits, Microbial inoculation

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated staple food crops globally and plays a crucial role in the food and nutritional security of millions, especially in South Asia. In India, wheat occupies a significant share of the total cropped area and is a key component of the rabi cropping system [1]. However, wheat productivity is increasingly threatened by abiotic stresses, among which moisture stress has emerged as a major constraint in rainfed and limited-irrigation areas. Water deficit limits plant growth by disturbing water balance, triggering oxidative stress through accumulation of reactive oxygen species (ROS), reducing photosynthetic efficiency and impairing nutrient uptake, ultimately lowering grain yield and quality [2-3]. To cope with such stress, wheat plants deploy a combination of strategies, including the accumulation of osmolytes, activation of antioxidant defense systems and modification of root architecture, all of which help maintain cellular stability, water uptake and metabolic functions under adverse conditions [4-5].

In recent years, plant growth promoting microbes, particularly Actinobacteria, have emerged as effective tools to enhance crop resilience to drought in a sustainable and environmentally friendly manner [6-7]. Actinobacteria are capable of improving stress tolerance by promoting the accumulation of osmolytes like proline and soluble sugars, stimulating antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) and reducing oxidative damage in plants exposed to water deficit [8-9]. These microbes also influence plant growth by producing phytohormones, enhancing nutrient availability and forming biofilms or exopolysaccharides that improve soil water retention and root–

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soil contact, thereby contributing to better stress adaptation [10-11].

Root system traits are critical for drought tolerance, as roots enable plants to access deeper soil moisture. Enhanced root surface area, length, volume and diameter improve water and nutrient acquisition and support sustained plant growth during moisture stress [12-13]. Inoculation with drought-tolerant Actinobacteria has been shown to positively influence root morphology, biomass and root/shoot ratio, which are vital for maintaining plant performance under water-limited conditions [10], [14]. Despite the documented benefits of microbial inoculation, genotype-specific responses remain poorly characterized. Wheat genotypes vary in their capacity for osmolyte accumulation, antioxidative activity and root plasticity, influencing how effectively they respond to microbial treatment under drought [15]. Understanding these genotype-dependent interactions is essential for identifying superior lines that can maximize the benefits of microbial-assisted stress mitigation [16].

This study aims to evaluate the effects of Actinobacteria inoculation under moisture stress on twenty wheat genotypes with contrasting genetic backgrounds (G1-G20). We integrated biochemical assessments (proline, total soluble sugars, antioxidant enzyme activities) with detailed analyses of root architecture (surface area, length, volume, diameter) to identify genotypes that exhibit strong stress resilience when assisted by beneficial microbes. The findings are expected to inform breeding and microbial-assisted strategies for developing drought-resilient wheat, contributing to sustainable crop production under increasing water scarcity.

Table 1 List of twenty durum and bread wheat genotypes used for study

S. No.	Name	Species	Pedigree
1.	UAS 446	*Triticum durum*	DWR 185/ DWR2006/UAS419
2.	Amruth	*Triticum durum*	ANLC/GAZA
3.	DWR-2006	*Triticum durum*	SULA/CREX/AAZ
4.	AKDW-2997-16	*Triticum durum*	CPAN6140/RAJ1555
5.	Bijaga yellow	*Triticum durum*	MYSORE LOCAL/GAZA
6.	UAS 428	*Triticum durum*	GREEN-14/YAV-10/AUK/UAS402
7.	UAS 347	*Triticum aestivum*	(TOB/ERA/TOB/CNO67/#/PLO/S/VE E#5/5/KAUZ/6/FRET2YDWR162
8.	UAS 375	*Triticum aestivum*	*UAS 320/GW 322//LOK*
9.	HD-2888	*Triticum aestivum*	C306/*T.spaerococcum*//HW 2004
10.	UAS 304	*Triticum aestivum*	SERI/CEP80120//KAUZ / PBW 343
11.	GDP 40	*Triticum durum*	CPAN6140/RAJ1555
		List of advanced breeding lines used in the field experiment	
12.	UAS 3020	*Triticum aestivum*	UAS 320 X (QUAIU#3//MILAN/AMSEL)
13.	UASBW 11421	*Triticum aestivum*	ZAKA/3/AJAIA_12/F3LOCAL (SEL.ETHIO.135.85) //PLATA_13/4/SOOTY_9/RASCON_37//WODUCK/CHAM_3/5/SOOTY_9/RASCON_37//GUAYACAN INIA/3/BSET/PNIO_3/4/PLATA_7/ILBOR_1//SOMAT_3/3/OUET_1/8/BZEL/CALAMON_1//TEJE_1/7/DON JESUS/6/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNF
14.	UASBW 12982	*Triticum aestivum*	TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/ZAMP_2/4/CANELO_8//SORA/2*PLATA_12/5/CBC 501 CHILE/GUANAY//GAUK_5/7/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RASCON_37//WODUCK/CHAM_3/8/GUAYACAN INIA/2*SNITAN/3/SOMAT_3
15.	UASBW 13039	*Triticum aestivum*	LOK-62 X (BOW/VEE /5/ND/VG9144 //KAL/BB/3 /YACO/4/CHIL/6/CASKOR/3/CROC_1 /AE. SQUARROSA (224) //OPATA/7/PASTOR // MILAN/KAUZ/3/BAV92)
16.	UASBW 12380	*Triticum aestivum*	MUNAL #1*2//SOKOLL/WBLL1
17.	UASDW 30805	*Triticum durum*	SOOTY_9/RASCON_37//GUAYACAN INIA/4/BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL (SEL.ETHIO.135.85) //PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3
18.	UASDW 31156	*Triticum durum*	BYBLOS/6/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOMBRA_20/4/SILVER_14/MOEWE/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL (SEL.ETHIO.135.85) //PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3
19.	UASDW 30820	*Triticum durum*	BD00088.504/4/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN/5/D04340B/CIRNO C 2008
20.	UASDW 31138	*Triticum durum*	INIA/3/DUKEM_12/2*RASCON_21/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL (SEL.ETHIO.135.85) //PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3

MATERIALS AND METHODS

Twenty wheat genotypes, including bread and durum wheat types, were used as experimental material (Table 1). Actinobacteria isolates (AUDT 545 and AUDT 862) (Fig 1-2) were obtained from the Department of Microbiology, UAS, Dharwad. Seeds were surface-sterilized with 0.1% sodium hypochlorite for 10 minutes and rinsed 2–3 times with sterile water. The microbial consortium made by mixing equal quantity of isolate and then treated to seeds. The genotypes were sown in pots under two treatments: microbe-treated seeds inoculated with Actinobacteria and microbe-untreated seeds maintained as controls. Plants were irrigated regularly for 25 days, after which drought stress was imposed by withholding irrigation. Soil moisture content was monitored to quantify drought intensity and moisture levels declined to 9% [17].

Biochemical observations were recorded after stress imposition. Proline content was estimated following Bates *et al*. [18]. Fresh leaf tissue (0.5 g) was homogenized in 3% sulpho salicylic acid and reacted with acid ninhydrin. Absorbance was read at 520 nm and proline concentration was expressed as µmoles g^{-1} fresh weight:

µ moles of proline / g fresh leaf tissue = (Proline mL × mL of toluene × 5) / (115.2 × Weight of sample)

Superoxide dismutase (SOD) activity was assayed according to Beyer and Fridovich [19]. One unit of SOD = Volume of enzyme extract required to cause 50% inhibition of NBT reduction. Total soluble sugars (TSS) were quantified using the phenol sulphuric acid method [20].

TSS (mg/g fresh weight) = (OD × Standard factor × Dilution) / Weight of sample.

Peroxidase (POD) activity was measured using the 4-methyl catechol method described by Gorin and Heidema [21]:
POD Activity (Units/min/mg protein) = (Change in absorbance at 420 nm per minute) / 0.01

For root growth analysis, the plants were gently removed from the pots per treatment per genotype. Then roots were washed and scanned in root scanner Winrhizo and data on root traits such as total root length, surface area and diameter were measured [22]. The observation on following parameters was taken root surface area, root length, root diameter and root volume.

Fig 1 Colony growth of AUDT 545

Fig 2 Colony growth of AUDT 862

RESULTS AND DISCUSSION

Biochemical responses to moisture stress and actinobacteria treatment

Proline accumulation

Proline content (µg g^{-1} FW) showed a consistent increase across all genotypes following Actinobacteria inoculation under moisture stress, indicating enhanced osmotic adjustment (Table 2). On average, proline concentration increased from 102.48 µg g^{-1} FW in untreated plants to 111.65 µg g^{-1} FW under treated conditions. The maximum proline accumulation was recorded in UASBW 12982 (G14), which increased from 148.00 to 157.20 µg g^{-1} FW, while genotypes such as UASBW 13039 (G15) and UAS-334 (G11) also maintained high proline levels following treatment. In contrast, comparatively lower proline accumulation was observed in AKDW-2997-16 (G4) and UASDW 30805 (G17), despite moderate improvement after inoculation. The observed enhancement of proline content under Actinobacteria treatment supports its role in stabilizing cellular membranes, maintaining enzyme activity, and improving osmotic buffering under dehydration stress, as reported earlier in wheat and other cereals [23-24].

Total soluble sugars (TSS)

Total soluble sugars (TSS; mg g^{-1} FW) also increased substantially in response to Actinobacteria inoculation, reflecting improved osmotic balance and carbon reserve formation under moisture stress (Table 2). The mean TSS content increased from 41.11 mg g^{-1} FW in untreated plants to 44.27 mg g^{-1} FW under treated conditions. Higher TSS accumulation was recorded in genotypes such as UASBW 12982 (G14) and UAS-334 (G11), with values exceeding 49 mg g^{-1} FW, whereas untreated Amruth (G2) and UAS-3020 (G12) exhibited relatively lower sugar levels (≈31–32 mg g^{-1} FW), though both showed clear improvement following treatment. Enhanced soluble sugar accumulation under microbial inoculation has been associated with improved photosynthate stabilization, maintenance of cellular turgor, and protection of metabolic processes during moisture stress [25].

Table 2 Mean performance of genotypes for proline (µg g^{-1} FW) and TSS (mg g^{-1} FW) under microbe treated and microbe untreated condition

S No.	Genotypes	Proline (µg g^{-1} FW)		TSS TSS (mg g^{-1} FW)	
		Untreated	Treated	Untreated	Treated
1.	UAS 446 (c)	88.09	92.06	35.67	37.17
2.	Amruth (c)	81.50	94.98	31.50	36.14
3.	DWR-2006 (c)	94.22	111.80	38.04	45.13
4.	AKDW-2997-16 (c)	70.20	96.50	32.50	49.14
5.	Bijaga yellow (c)	90.20	96.08	36.41	38.80
6.	GDP 40	114.77	120.90	46.34	48.82
7.	UAS-428 (sc)	115.06	116.50	46.28	42.67
8.	UAS-347 (c)	91.94	113.03	37.12	45.63
9.	UAS-375 (c)	97.69	101.55	39.25	41.00
10.	HD-2888 (c)	121.32	122.50	48.98	45.13
11.	UAS-334 (sc)	122.74	122.10	49.56	49.30
12.	UAS-3020	79.22	104.20	31.98	42.07
13.	UASBW 11421	90.34	105.22	36.48	42.48
14.	UASBW 12982	148.00	157.20	52.07	55.22
15.	UASBW 13039	111.97	117.78	45.15	47.55
16.	UASBW 12380	111.28	119.10	44.93	48.09
17.	UASDW 30805	86.40	89.90	34.89	36.29
18.	UASDW 31156	109.11	109.60	44.00	44.25
19.	UASDW 30820	121.23	122.50	48.94	42.22
20.	UASDW 31138	104.40	119.58	42.15	48.28
	Mean	102.48	111.65	41.11	44.27
	Minimum	70.20	89.90	31.50	36.14
	Maximum	148.00	157.20	52.07	55.22

c- Check, sc- Susceptible check

Table 3 Mean performance of genotypes for peroxidase (unit/g/min FW) and SOD (unit/g FW) under microbe treated and microbe untreated condition

S No.	Genotypes	Peroxidase (unit/g/min FW)		SOD (unit/g FW)	
		Untreated	Treated	Untreated	Treated
1.	UAS 446 (c)	564.65	590.05	492.85	522.75
2.	Amruth (c)	571.35	618.50	503.00	553.60
3.	DWR-2006 (c)	668.00	698.35	565.40	593.00
4.	AKDW-2997-16 (c)	562.65	605.15	477.70	507.85
5.	Bijaga yellow (c)	587.23	627.40	490.15	508.35
6.	GDP 40	724.85	763.35	579.50	596.50
7.	UAS-428 (sc)	632.85	636.05	532.30	543.35
8.	UAS-347 (c)	677.23	715.70	553.00	573.20
9.	UAS-375 (c)	607.65	632.95	520.50	533.05
10.	HD-2888 (c)	667.35	671.18	558.70	576.70
11.	UAS-334 (sc)	730.00	764.25	594.55	601.55
12.	UAS-3020	632.05	672.50	475.30	504.75
13.	UASBW 11421	631.50	666.75	529.00	555.60
14.	UASBW 12982	780.15	823.85	631.00	674.00
15.	UASBW 13039	704.85	725.60	568.50	592.50
16.	UASBW 12380	713.30	747.10	574.65	600.15
17.	UASDW 30805	550.15	575.00	466.25	479.25
18.	UASDW 31156	652.60	663.25	541.10	553.60
19.	UASDW 30820	623.75	628.65	525.40	534.40
20.	UASDW 31138	716.00	745.65	580.00	614.00
	Mean	649.91	678.55	537.94	560.90
	Minimum	550.15	575.00	466.25	479.25
	Maximum	780.15	823.85	631.00	674.00

c- Check, sc- Susceptible check

Peroxidase activity (POD)

Peroxidase activity (unit g^{-1} min^{-1} FW) increased consistently across genotypes following Actinobacteria inoculation under moisture stress, indicating an enhanced antioxidative defense mechanism (Table 3). The mean peroxidase activity increased from 649.91 under untreated conditions to 678.55-unit g^{-1} min^{-1} FW after treatment. The highest enzyme activity was recorded in UASBW 12982 (G14), which increased markedly from 780.15 to 823.85 unit g^{-1} min^{-1} FW, followed by UAS-334 (G11) (730.00 to 764.25 unit g^{-1} min^{-1} FW) and GDP 40 (G6) (724.85 to 763.35 unit g^{-1} min^{-1} FW). In contrast, lower peroxidase activity was observed in UASDW 30805 (G17), although a moderate increase was evident following treatment (550.15-to-575.00-unit g^{-1} min^{-1}

FW). The enhanced peroxidase activity under Actinobacteria inoculation suggests improved scavenging of hydrogen peroxide, thereby protecting cellular components from oxidative damage during moisture stress.

Superoxide dismutase (SOD)

Superoxide dismutase (SOD; unit g^{-1} FW) activity also showed a significant enhancement in response to Actinobacteria treatment, reflecting strengthened detoxification of superoxide radicals under stress conditions (Table 3). On average, SOD activity increased from 537.94 unit g^{-1} FW in untreated plants to 560.90 unit g^{-1} FW following inoculation. The highest SOD activity was observed in UASBW 12982 (G14), increasing from 631.00 to 674.00 unit g^{-1} FW, while genotypes such as UAS-334 (G11) (594.55 to 601.55 unit g^{-1} FW) and UASDW 31138 (G20) (580.00 to 614.00 unit g^{-1} FW) also exhibited pronounced enhancement. Conversely, relatively lower SOD activity was recorded in UASDW 30805 (G17) (466.25 to 479.25 unit g^{-1} FW), indicating limited antioxidative capacity. The coordinated increase in SOD and peroxidase activities under microbial inoculation highlights an efficient reactive oxygen species detoxification system, contributing to improved stress tolerance in wheat under moisture deficit conditions.

Across all biochemical traits, Actinobacteria-treated plants consistently outperformed untreated ones. The combined increase in proline, sugars, POD and SOD indicates that Actinobacteria improve both osmotic adjustment and ROS detoxification. Genotypes UASBW 12982, UASBW 13039, UAS-334 and UASDW 31138 emerged as the most biochemically resilient under drought. These results align with reports emphasizing that Actinobacteria enhance stress signaling pathways and oxidative buffering [6], [9].

Root architectural responses under moisture stress and actinobacteria treatment

Root surface area

Root surface area (cm^2) increased consistently in most genotypes following Actinobacteria inoculation under moisture stress, indicating improved soil exploration and water uptake potential (Table 4). The mean root surface area increased from 81.69 cm^2 under untreated conditions to 89.48 cm^2 after treatment. A pronounced enhancement was observed in UASBW 12380 (G16), which recorded the highest values, increasing from 100.98 to 115.25 cm^2, followed by UASBW 13039 (G15) (85.44 to 98.67 cm^2) and UAS-375 (G9) (91.75 to 99.55 cm^2). In contrast, UASDW 30820 (G19) exhibited the lowest root surface area under both conditions, though a modest increase was evident following inoculation (57.49 to 61.90 cm^2). The overall increase in root surface area under microbial treatment suggests enhanced root proliferation and adaptive plasticity, which are critical for improved moisture acquisition under drought stress.

Table 4 Mean performance of genotypes for root surface area and root average diameter under microbe treated and microbe untreated condition

S No.	Genotypes	Root surface area (cm^2)		Root diameter (mm)	
		Untreated	Treated	Untreated	Treated
1.	UAS 446 (c)	89.25	95.40	0.40	0.44
2.	Amruth (c)	85.71	90.50	0.35	0.41
3.	DWR-2006 (c)	83.63	93.40	0.47	0.54
4.	AKDW-2997-16 (c)	73.63	81.78	0.41	0.44
5.	Bijaga yellow (c)	94.09	97.00	0.34	0.50
6.	GDP 40	71.15	78.10	0.48	0.54
7.	UAS-428 (sc)	88.95	93.90	0.34	0.39
8.	UAS-347 (c)	72.00	85.78	0.36	0.41
9.	UAS-375 (c)	91.75	99.55	0.33	0.39
10.	HD-2888 (c)	81.95	89.63	0.34	0.38
11.	UAS-334 (sc)	87.60	92.62	0.32	0.41
12.	UAS-3020	77.62	85.41	0.41	0.50
13.	UASBW 11421	72.37	81.46	0.33	0.37
14.	UASBW 12982	66.62	82.00	0.30	0.38
15.	UASBW 13039	85.44	98.67	0.34	0.37
16.	UASBW 12380	100.98	115.25	0.40	0.44
17.	UASDW 30805	77.12	83.25	0.46	0.53
18.	UASDW 31156	84.60	90.64	0.40	0.51
19.	UASDW 30820	57.49	61.90	0.57	0.64
20.	UASDW 31138	91.80	93.35	0.32	0.40
	Mean	81.69	89.48	0.38	0.45
	Minimum	57.49	61.90	0.30	0.37
	Maximum	100.98	115.25	0.57	0.64

c- Check, sc- Susceptible check

Root diameter

Root diameter (mm) also showed a noticeable increase in response to Actinobacteria treatment, reflecting improved structural robustness and conductive capacity of roots under moisture deficit (Table 4). The mean root diameter increased from 0.38 mm in untreated plants to 0.45 mm following treatment. The maximum root diameter was recorded in UASDW 30820 (G19), increasing from 0.57 to 0.64 mm, followed by DWR-2006 (G3) and GDP 40 (G6), both reaching 0.54 mm under treated conditions. Conversely, genotypes such as UASBW 12982 (G14) and UAS-334 (G11) maintained relatively thinner roots under untreated conditions (0.30 and 0.32 mm, respectively), though both exhibited clear thickening after inoculation. The coordinated enhancement of root surface area and diameter under Actinobacteria treatment highlights microbial-mediated modulation of root architecture, facilitating improved water absorption, mechanical stability, and drought resilience in wheat.

Table 5 Mean performance of genotypes for Root length and Root volume under microbe treated and microbe untreated condition

S No.	Genotypes	Root volume (cm^3)		Root length (cm)	
		Untreated	Treated	Untreated	Treated
1.	UAS 446 (c)	0.72	0.90	699.60	955.68
2.	Amruth (c)	0.76	0.91	775.67	899.95
3.	DWR-2006 (c)	0.82	0.96	529.44	725.86
4.	AKDW-2997-16 (c)	0.90	1.02	600.56	654.26
5.	Bijaga yellow (c)	0.89	1.00	728.57	892.28
6.	GDP 40	0.83	0.94	445.37	519.93
7.	UAS-428 (sc)	0.90	1.01	650.87	735.00
8.	UAS-347 (c)	0.72	0.83	557.77	640.82
9.	UAS-375 (c)	0.78	0.9	700.84	745.74
10.	HD-2888 (c)	0.80	0.93	692.67	838.57
11.	UAS-334 (sc)	0.75	0.87	897.99	970.05
12.	UAS-3020	0.88	0.99	642.05	778.22
13.	UASBW 11421	0.78	0.92	600.87	828.26
14.	UASBW 12982	0.76	0.86	842.44	979.64
15.	UASBW 13039	0.75	0.87	814.38	1014.92
16.	UASBW 12380	0.76	0.94	661.53	861.47
17.	UASDW 30805	0.83	0.93	631.29	722.70
18.	UASDW 31156	0.80	0.92	508.94	688.56
19.	UASDW 30820	0.81	0.97	608.85	773.88
20.	UASDW 31138	0.73	0.91	839.81	916.92
	Mean	0.80	0.93	671.47	807.13
	Minimum	0.72	0.83	445.37	519.93
	Maximum	0.90	1.02	897.99	1014.92

c- Check, sc- Susceptible check

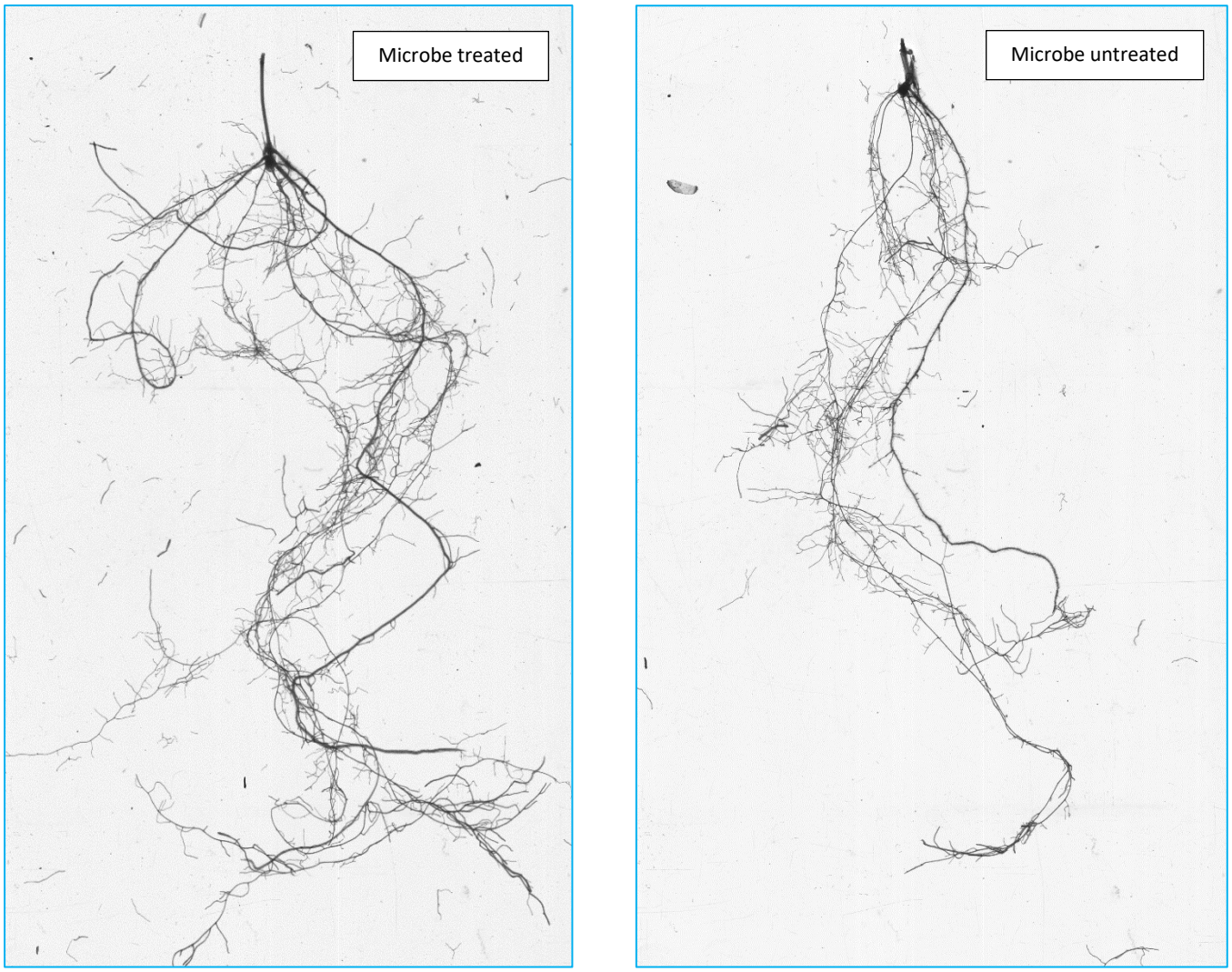

Fig 3 Root architecture of wheat under moisture stress with and without microbial treatment

Root volume

Root volume (cm^3) exhibited a consistent increase across genotypes following Actinobacteria inoculation under moisture stress, reflecting enhanced root biomass development and soil exploitation capacity (Table 5). The mean root volume increased from 0.80 cm^3 under untreated conditions to 0.93 cm^3 after treatment. The highest root volume under treated conditions was recorded in AKDW-2997-16 (G4) (1.02 cm^3), followed by UAS-428 (G7) (1.01 cm^3) and Bijaga yellow (G5) (1.00 cm^3). In contrast, relatively lower root volume was observed in UAS 446 (G1) and UAS-347 (G8) under untreated conditions (0.72 cm^3), though both genotypes showed clear enhancement following inoculation. The overall increase in root volume under microbial treatment suggests improved assimilate allocation belowground and greater capacity for water and nutrient uptake under moisture-limited conditions.

Root length

Root length (cm) showed a marked enhancement in response to Actinobacteria inoculation, indicating increased root elongation and exploratory ability under drought stress (Table 5). The mean root length increased substantially from 671.47 cm in untreated plants to 807.13 cm following treatment. Genotypes UASBW 13039 (G15) and UASBW 12982 (G14) recorded the longest root systems under treated conditions, reaching 1014.92 cm and 979.64 cm, respectively, followed closely by UAS-334 (G11) (970.05 cm) and UAS 446 (G1) (955.68 cm). Conversely, GDP 40 (G6) exhibited comparatively shorter roots under both conditions, though a notable increase was observed following inoculation (445.37 to 519.93 cm). The pronounced enhancement in root length under Actinobacteria treatment highlights microbial-mediated stimulation of root elongation, which is critical for accessing deeper soil moisture and improving drought resilience in wheat.

Actinobacteria markedly improved all four root traits surface area, diameter, volume and length demonstrating strong positive effects on below-ground drought adaptation. Genotypes UASBW 13039, UASBW 12982, UAS-334 and UASBW 12380 exhibited highly responsive root systems. The enhanced root morphology likely contributed to improved water uptake and greater biochemical stability observed in microbe treated plants. The combined biochemical and root trait responses confirm that Actinobacteria significantly enhance drought tolerance in wheat by improving osmotic regulation, antioxidative metabolism and root system architecture. The most responsive genotypes UASBW 12982, UASBW 13039, UAS-334 and UASBW 12380 demonstrate superior adaptability under moisture stress and are promising candidates for microbial-assisted drought-resilient wheat improvement (Fig 3).

CONCLUSION

The present study demonstrates that Actinobacteria inoculation significantly enhances drought tolerance in wheat by modulating both biochemical and root architectural traits. Microbial treatment consistently increased proline accumulation and total soluble sugars, reflecting improved osmotic adjustment under moisture stress. Antioxidant enzyme activities, including superoxide dismutase (SOD) and peroxidase (POD), were also elevated in treated plants, indicating strengthened ROS detoxification and reduced oxidative damage. Concurrently, Actinobacteria improved root system architecture, enhancing root surface area, length, volume and diameter, which facilitated greater water and nutrient uptake under stress. Genotype-specific responses were evident, with UASBW 12982 (G14), UASBW 13039 (G15), UAS-334 (G11) and UASBW 12380 (G16) emerging as the most responsive, exhibiting superior biochemical resilience and root adaptability. In contrast, certain genotypes displayed limited responsiveness, highlighting the importance of genetic variability in determining microbial efficacy. The integration of biochemical and root trait analyses provides a holistic understanding of drought adaptation mechanisms mediated by beneficial microbes. Overall, the findings confirm that Actinobacteria inoculation is a promising, sustainable strategy to enhance wheat performance under water-limited conditions. The identified responsive genotypes offer valuable candidates for microbial-assisted breeding programs aimed at developing drought-resilient wheat varieties. Incorporating such microbial interventions with conventional breeding strategies can contribute to sustainable wheat production in semi-arid and drought-prone agroecosystems, supporting food security in the context of climate change.

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