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Supplementary Material: *Functional Plant Biology*

# Supplementary Material

## Stomata coordinate with plant hydraulics to regulate transpiration response to vapour pressure deficit in wheat

*S. R. W. M. C. J. K. Ranawana*[A,B,E], *K. H. M. Siddique*[A], *J. A. Palta*[A,C], *K. Stefanova*[A] *and H. Bramley*[A,D]

[A]The UWA Institute of Agriculture, The University of Western Australia, Perth, WA 6001, Australia.

[B]Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla 90000, Sri Lanka.

[C]CSIRO Agriculture, Private Bag No. 5, Wembley, WA 6913, Australia.

[D]Plant Breeding Institute, Faculty of Agriculture and Environment, The University of Sydney, 12656 Newell Highway, Narrabri, NSW 2390, Australia.

[E]Corresponding author. Email: chandijeewani@yahoo.com

The supplementary materials indicate the wheat genotypes selected for this study and their morpho-physiological attributes (Table S1), diurnal light intensity in the growth chamber during plant growth (Table S2), temperature and relative humidity (RH) conditions programmed in the growth chamber to reach the targeted vapour pressure deficit (VPD) treatments (Table S3), mean parameters of the segmented or simple linear regression models for the relationship between transpiration rate ($T_r$) and VPD (Table S4), mean parameters of the segmented or simple linear regression models for the relationship between stomatal conductance ($g_s$) and VPD (Table S5) and the relationship between $g_s$ on abaxial and adaxial leaf surfaces and VPD (Table S6, Fig. S1 and S2) and the correlation between transpiration rate (Tr) and stomatal conductance ($g_s$) in intact plants under well-watered (WW), de-rooted shoots in deionised water (Fig. S3.1) and water-stressed (WS) plants (Fig. S3.2) of the eight wheat genotypes studied.

**Table S1. Wheat genotypes selected for this study and their morpho-physiological attributes**

Genotypes were grown in pairs under identical conditions due to the large number of measurements to be taken simultaneously

| Pair | Genotype | Morpho-physiological attributes | Reference |
|---|---|---|---|
| 01 | LongReach-Envoy | Putative high transpiration efficiency<br>Semi-dwarf | Dr B Jacobs, LongReach Plant Breeders, pers. comm.<br>Seednet (2011) |
| | Excalibur | High $T_r$ (high $g_s$)<br>Drought and heat tolerant | Izanloo *et al.* (2008)<br>Dr D Mullan, InterGrain, pers. comm. |
| 02 | Drysdale | High transpiration efficiency | Richards (2006) |
| | Espada | Glaucous/broad erect leaves | Australian Grain Technologies (2010*a*) |
| 03 | Gladius | Drought tolerant/glaucous<br>Broad erect leaves<br>Heat tolerant | Australian Grain Technologies (2010*b*)<br>Fleury *et al.* (2010) |
| | Mace | Drought tolerant | Australian Grain Technologies (2013) |
| 04 | Glennson 81 | High canopy temperature depression | Amani *et al.* (1996) |
| | Sonora 64 | Low canopy temperature depression | Amani *et al.* (1996) |

**Table S2. Diurnal light intensity in the growth chamber during plant growth**

| Time of day | Photosynthetic photon flux density ($\mu mol\ m^{-2}\ s^{-1}$) |
|---|---|
| 08:00–09:00 | 400 |
| 09:00–10:00 | 600 |
| 10:00–18:00 | 800 |
| 18:00–19:00 | 600 |
| 19:00–20:00 | 400 |
| 20:00–08:00 | 0 |

**Table S3. Temperature and relative humidity (RH) conditions programmed in the growth chamber to reach the targeted vapour pressure deficit (VPD) treatments**

| Day | Growth chamber conditions | | |
|---|---|---|---|
| | Temperature (°C) | RH (%) | Target VPD (kPa) |
| 1 | 22 | 69 | 0.8 |
| | 26 | 61 | 1.3 |
| | 31 | 53 | 2.1 |
| 2 | 31 | 35 | 2.9 |
| | 34 | 30 | 3.7 |
| | 38 | 32 | 4.5 |

**Table S4. Mean (±s.e.) parameters of the segmented or simple linear regression models for the relationship between transpiration rate ($T_r$) and vapour pressure deficit (VPD) in eight wheat genotypes in intact plants under two watering regimes, well-watered (WW) and water-stressed (WS), and de-rooted shoots in deionised water**

Slope 1 and 2 are the slopes of the first and second phases of the segmented linear regression. P values indicate significant regressions (Slope1 or $Slope_{(linear)} > 0$). The number of data points for each regression was 18-24. NS = not significant at $P > 0.05$

| Genotype | Treatment | Model | Slope 1 or $Slope_{(linear)}$ (mg $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Slope 2 (mg $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Y-Intercept (mg $m^{-2}$ $s^{-1}$) | Break point (kPa) | $R^2$ | *P* |
|---|---|---|---|---|---|---|---|---|
| Gladius | WW | Linear | 13.4 (1.7) | NA | 22.2 (5.1) | NA | 0.73 | 0.0001 |
| | De-rooted | Segmented | 47.8 (14.8) | –18.7 (14.4) | 26.3 (27.8) | 2.7 (0.4) | 0.43 | 0.0009 |
| | WS | Linear | 3.5 (0.6) | NA | 12.7 (1.7) | NA | 0.63 | 0.0001 |
| Mace | WW | Linear | 10.9 (0.9) | NA | 18.0 (2.8) | NA | 0.86 | 0.0001 |
| | De-rooted | Segmented | 48.4 (18.5) | –14.1 (17.8) | 22.5 (34.8) | 2.7 (0.5) | 0.43 | 0.0056 |
| | WS | Linear | 1.2 (0.5) | NA | 11.1 (1.5) | NA | 0.21 | 0.0240 |
| Excalibur | WW | Segmented | 27.0 (2.5) | 7.2 (3.1) | 10.5 (3.5) | 2.1 (0.2) | 0.94 | 0.0001 |
| | De-rooted | Segmented | 69.2 (16.4) | –40.8 (19.6) | 18.6 (23.3) | 2.1 (0.3) | 0.52 | 0.0001 |
| | WS | Linear | 5.5 (0.7) | NA | 17.4 (1.5) | NA | 0.80 | 0.0001 |
| LongReach-Envoy | WW | Segmented | 19.5 (2.2) | 5.7 (2.8) | 7.9 (3.1) | 2.2 (0.3) | 0.92 | 0.0001 |
| | De-rooted | Linear | –6.5 (2.2) | NA | 49.4 (4.9) | NA | 0.36 | 0.0089 |
| | WS | Linear | 2.4 (0.7) | NA | 12.6 (1.7) | NA | 0.33 | 0.0035 |
| Drysdale | WW | Segmented | 31.2 (3.8) | 2.6 (3.1) | 3.9 (6.1) | 2.6 (0.2) | 0.92 | 0.0001 |
| | De-rooted | Segmented | 67.5 (4.8) | –76.9 (8.6) | 6.2 (9.1) | 3.0 (0.1) | 0.95 | 0.0001 |
| | WS | Segmented | 10.8 (1.2) | –2.4 (2.1) | 2.2 (2.3) | 2.9 (0.2) | 0.87 | 0.0001 |
| Espada | WW | Segmented | 23.7 (3.0) | 7.7 (2.3) | 3.3 (4.7) | 2.6 (0.3) | 0.94 | 0.0001 |
| | De-rooted | Segmented | 42.5 (6.2) | –46.1 (11.2) | 25.3 (11.8) | 2.9 (0.1) | 0.78 | 0.0001 |
| | WS | Segmented | 6.5 (1.1) | –1.3 (1.8) | 2.4 (2.0) | 3.1 (0.3) | 0.79 | 0.0001 |
| Glennson 81 | WW | Segmented | 15.9 (1.5) | –2.2 (3.5) | 14.2 (3.0) | 3.4 (0.2) | 0.91 | 0.0001 |
| | De-rooted | Segmented | 46.7 (5.1) | –32.8 (4.1) | 27.1 (8.1) | 2.4 (0.1) | 0.88 | 0.0001 |
| | WS | Linear | 1.1 (0.6) | NA | 15.1 (1.6) | NA | 0.17 | NS |
| Sonora 64 | WW | Segmented | 21.7 (2.0) | –2.9 (13.8) | 13.1 (4.2) | 3.4 (0.7) | 0.90 | 0.0001 |
| | De-rooted | Segmented | 51.2 (10.8) | –57.9 (8.7) | 44.3 (16.9) | 2.5 (0.2) | 0.77 | 0.0001 |
| | WS | Linear | 1.2 (0.6) | NA | 20.4 (1.6) | NA | 0.16 | NS |

**Table S5. Mean (±s.e.) parameters of the segmented or simple linear regression models for the relationship between stomatal conductance ($g_s$) and vapour pressure deficit (VPD) in eight wheat genotypes in intact plants under two watering regimes, well-watered (WW) and water-stressed (WS), and de-rooted shoots in deionised water**

Slope 1 and 2 are the slopes of the first and second phases of the segmented linear regression. *P* values indicate regressions were significant (Slope1 or $Slope_{(linear)}$ > 0). The number of data points for each regression was 15-20. NS = not significant at $P > 0.05$

| Genotype | Treatment | Model | Slope 1 or $Slope_{(linear)}$ (mol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Slope 2 (mol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Y-Intercept (mol $m^{-2}$ $s^{-1}$) | Break point (kPa) | $R^2$ | *P* |
|---|---|---|---|---|---|---|---|---|
| Gladius | WW | Linear | 0.03 (0.06) | NA | 0.73 (0.18) | NA | 0.02 | NS |
| | De-rooted | Linear | –0.37 (0.06) | NA | 1.94 (0.21) | NA | 0.64 | < 0.0001 |
| | WS | Linear | –0.03 (0.01) | NA | 0.32 (0.04) | NA | 0.37 | 0.0061 |
| Mace | WW | Linear | –0.07 (0.05) | NA | 0.90 (0.17) | NA | 0.10 | NS |
| | De-rooted | Linear | –0.32 (0.06) | NA | 1.76 (0.19) | NA | 0.69 | 0.0001 |
| | WS | Linear | –0.05 (0.01) | NA | 0.32 (0.03) | NA | 0.66 | < 0.0001 |
| Excalibur | WW | Linear | –0.15 (0.04) | NA | 1.32 (0.09) | NA | 0.47 | 0.0008 |
| | De-rooted | Linear | –0.36 (0.05) | NA | 1.47 (0.12) | NA | 0.74 | < 0.0001 |
| | WS | Linear | –0.06 (0.02) | NA | 0.55 (0.05) | NA | 0.43 | 0.0079 |
| LongReach-Envoy | WW | Linear | –0.18 (0.02) | NA | 1.15 (0.05) | NA | 0.82 | < 0.0001 |
| | De-rooted | Linear | –0.04 (0.03) | NA | 0.23 (0.07) | NA | 0.12 | NS |
| | WS | Linear | –0.07 (0.01) | NA | 0.35 (0.03) | NA | 0.69 | < 0.0001 |
| Drysdale | WW | Linear | –0.20 (0.03) | NA | 1.33 (0.09) | NA | 0.71 | < 0.0001 |
| | De-rooted | Segmented | –0.02 (0.11) | –0.56 (0.08) | 0.98 (0.21) | 2.7 (0.2) | 0.84 | < 0.0001 |
| | WS | Linear | 0.01 (0.01) | NA | 0.16 (0.03) | NA | 0.03 | NS |
| Espada | WW | Linear | –0.10 (0.03) | NA | 1.13 (0.09) | NA | 0.39 | 0.0032 |
| | De-rooted | Segmented | -0.08 (0.10) | –0.39 (0.07) | 1.02 (0.20) | 2.7 (0.4) | 0.78 | < 0.0001 |
| | WS | Linear | 0.03 (0.01) | NA | 0.14 (0.04) | NA | 0.22 | NS |
| Glennson 81 | WW | Segmented | –0.26 (0.09) | 0.01 (0.05) | 1.43 (0.17) | 2.4 (0.4) | 0.47 | 0.0016 |
| | De-rooted | Linear | –0.46 (0.07) | NA | 2.14 (0.21) | NA | 0.76 | < 0.0001 |
| | WS | Linear | –0.01 (0.01) | NA | 0.21 (0.03) | NA | 0.02 | NS |
| Sonora 64 | WW | Segmented | –0.44 (0.14) | –0.08 (0.07) | 1.69 (0.28) | 2.4 (0.4) | 0.60 | 0.0003 |
| | De-rooted | Linear | –0.36 (0.05) | NA | 1.49 (0.15) | NA | 0.74 | < 0.0001 |
| | WS | Segmented | 0.00 (0.01) | –0.07 (0.02) | 0.14 (0.03) | 3.0 (0.4) | 0.70 | < 0.0001 |

**Table S6. Mean (±s.e.) parameters of the segmented or simple linear regression models for the relationship between stomatal conductance ($g_s$) on the abaxial and adaxial leaf surfaces and atmospheric vapour pressure deficit (VPD) in eight wheat genotypes in intact plants under two watering regimes, well-watered (WW) and water-stressed (WS), and de-rooted shoots in deionised water**

Slope 1 and 2 are the slopes of the first and second phases of the segmented linear regression. NS = not significant at $P > 0.05$

| Treatment | $g_s$ (Abaxial) | | | $g_s$ (Adaxial) | | |
|---|---|---|---|---|---|---|
| | Model | Slope 1 or $Slope_{(linear)}$ (mmol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Slope 2 (mmol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Model | Slope 1 or $Slope_{(linear)}$ (mmol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) | Slope 2 (mmol $m^{-2}$ $s^{-1}$ $kPa^{-1}$) |
| **Well-watered** | | | | | | |
| Gladius | Linear (NS) | –0.1 (22.9) | | Linear (NS) | 33.6 (46.2) | |
| Mace | Linear (NS) | 4.7 (22.9) | | Linear (NS) | –79.1 (39.4) | |
| Excalibur | Linear | –77.3 (19.3) | | Linear | –76.0 (21.5) | |
| Longreach-Envoy | Linear | –91.5 (16.7) | | Linear | –87.9 (25.7) | |
| Drysdale | Linear | –23.9 (10.7) | | Linear | –177.3 (30.6) | |
| Espada | Linear (NS) | –19.4 (25.0) | | Linear | –84.0 (25.9) | |
| Glennson 81 | Linear (NS) | 1.7 (11.2) | | Segmented | –270.1 (67.5) | 70.4 (81.9) |
| Sonora 64 | Linear (NS) | –0.3 (8.9) | | Segmented | –421.6 (117.6) | –70.2 (59.9) |
| **Water-stressed** | | | | | | |
| Gladius | Segmented | 13.3 (3.9) | –12.1 (29.2) | Linear | –38.6 (11.1) | |
| Mace | Linear | 9.5 (3.4) | | Linear | –55.3 (8.3) | |
| Excalibur | Linear (NS) | –7.7 (12.5) | | Segmented | 9.4 (31.4) | –78.9 (16.6) |
| Longreach-Envoy | Linear | 9.6 (1.2) | | Linear | –81.5 (11.4) | |
| Drysdale | Linear | 7.3 (1.7) | | Linear (NS) | 1.0 (10.1) | |
| Espada | Linear | 6.6 (1.2) | | Linear (NS) | 22.9 (13.9) | |
| Glennson 81 | Linear | 8.1 (2.7) | | Linear (NS) | –15.9 (12.1) | |
| Sonora 64 | Linear | 3.1 (0.7) | | Linear | –34.4 (6.8) | |
| **De-rooted** | | | | | | |
| Gladius | Linear | –153.6 (28.1) | | Linear | –213.4 (47.0) | |
| Mace | Linear | –142.5 (32.9) | | Linear | –180.2 (41.4) | |
| Excalibur | Linear | –189.3 (45.1) | | Segmented | –7.1 (195.1) | –255.2 (34.4) |
| Longreach-Envoy | Linear (NS) | 1.6 (8.1) | | Linear (NS) | –37.6 (23.2) | |
| Drysdale | Linear | –103.4 (24.2) | | Segmented | –3.1 (59.1) | –354.4 (43.0) |
| Espada | Linear | –65.0 (12.4) | | Segmented | –42.6 (51.1) | –323.3 (63.7) |
| Glennson 81 | Linear | –200.6 (34.2) | | Linear | –263.8 (38.1) | |
| Sonora 64 | Linear | –87.9 (22.9) | | Linear | –271.5 (39.7) | |

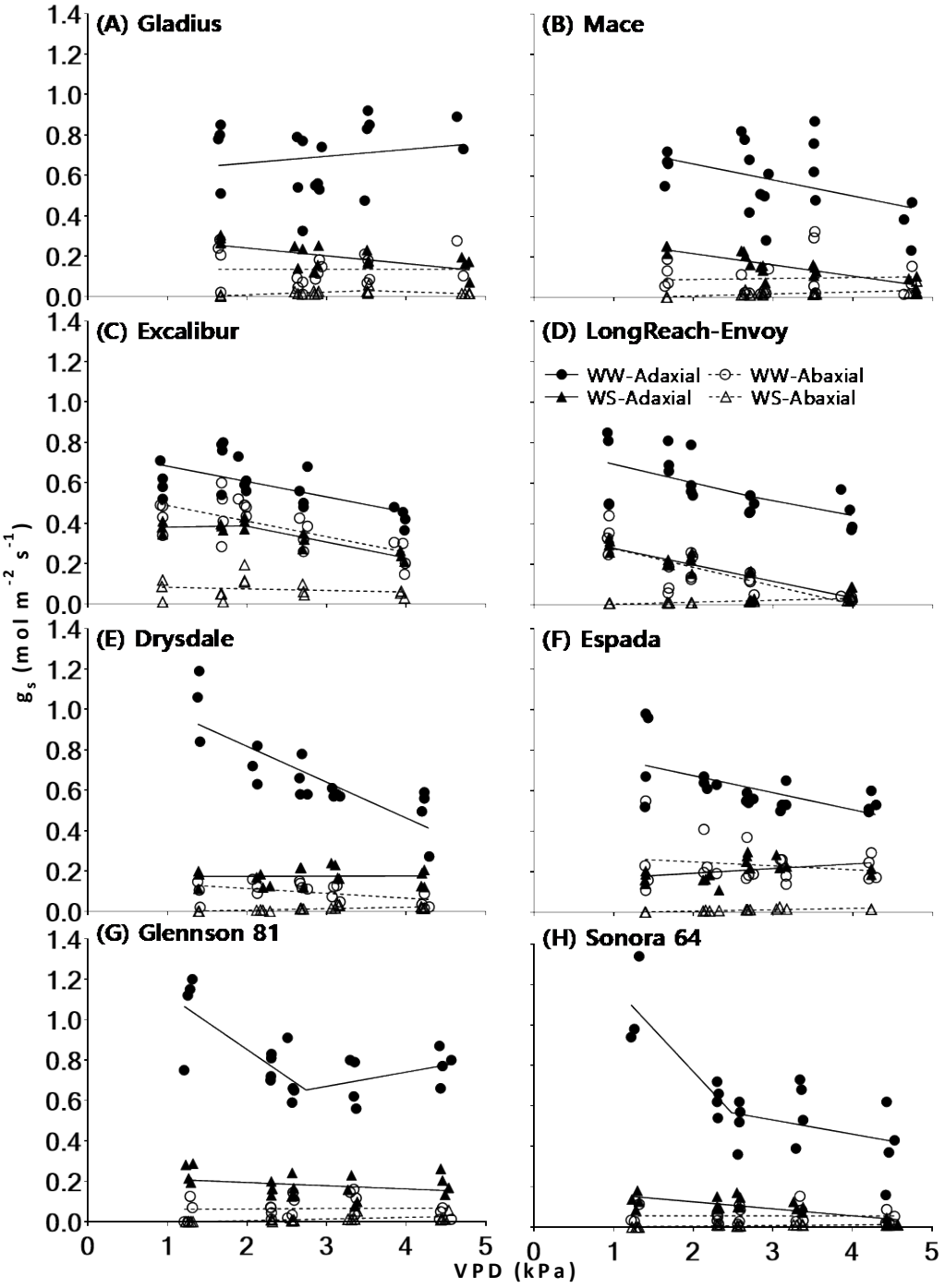


**Fig. S1.** Relationship between stomatal conductance ($g_s$) on the abaxial and adaxial leaf surfaces and atmospheric vapour pressure deficit (VPD) in eight wheat genotypes in intact plants under two watering regimes, well-watered (WW) and water-stressed (WS). Each data point represents an individual plant (replicate). Solid (adaxial) and dashed (abaxial) lines are

the best-fit regressions based on an extra sum of squares *F* test. The regression parameters for each genotype are given in Table S6.

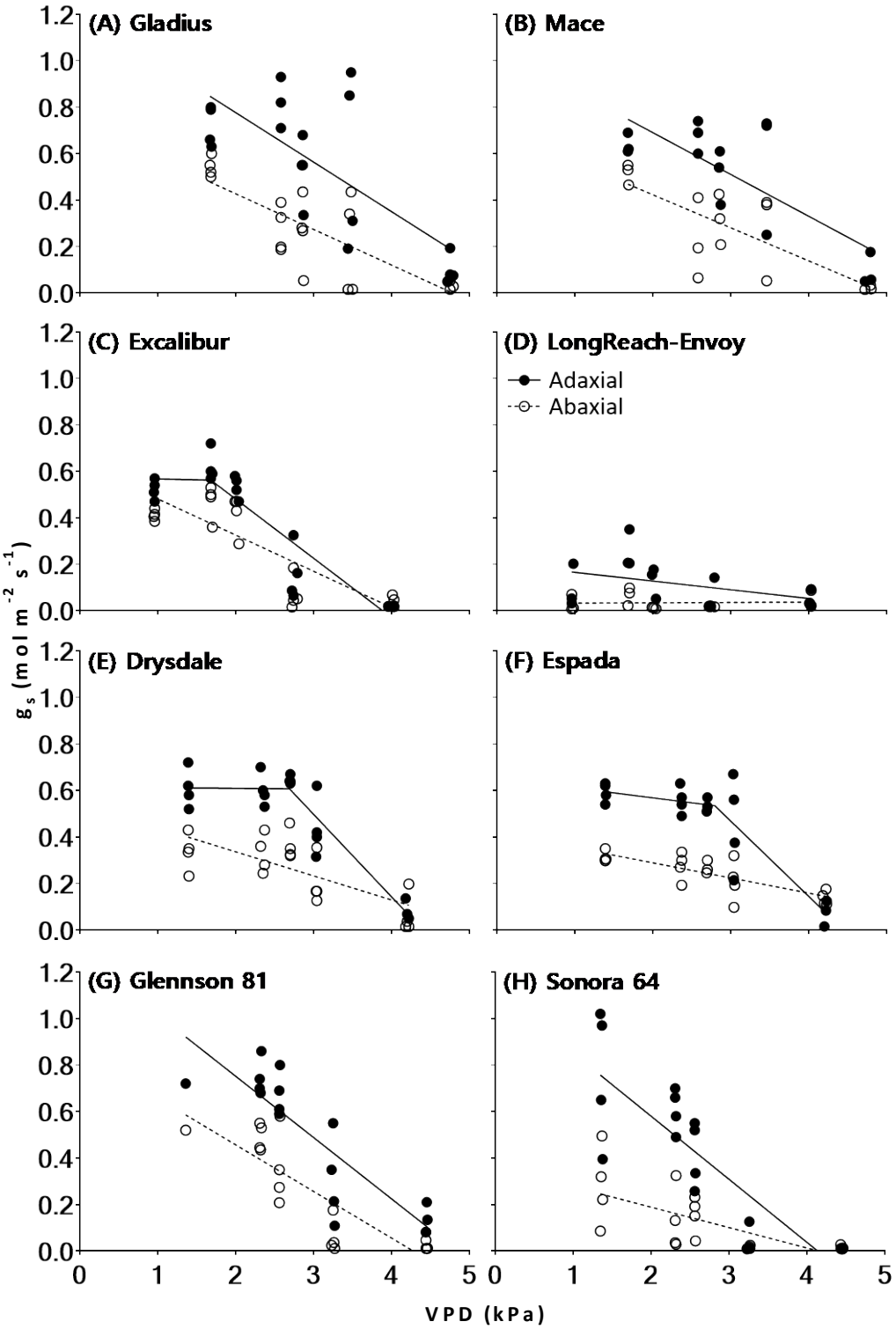

**Fig. S2.** Relationship between stomatal conductance ($g_s$) on the abaxial and adaxial leaf surfaces and atmospheric vapour pressure deficit (VPD) in de-rooted shoots in deionised water in eight wheat genotypes. Each data point represents an individual shoot (replicate). Solid (adaxial) and dashed (abaxial) lines are the best-fit regressions based on an extra sum of squares *F* test. The regression parameters for each genotype are given in Table S6.

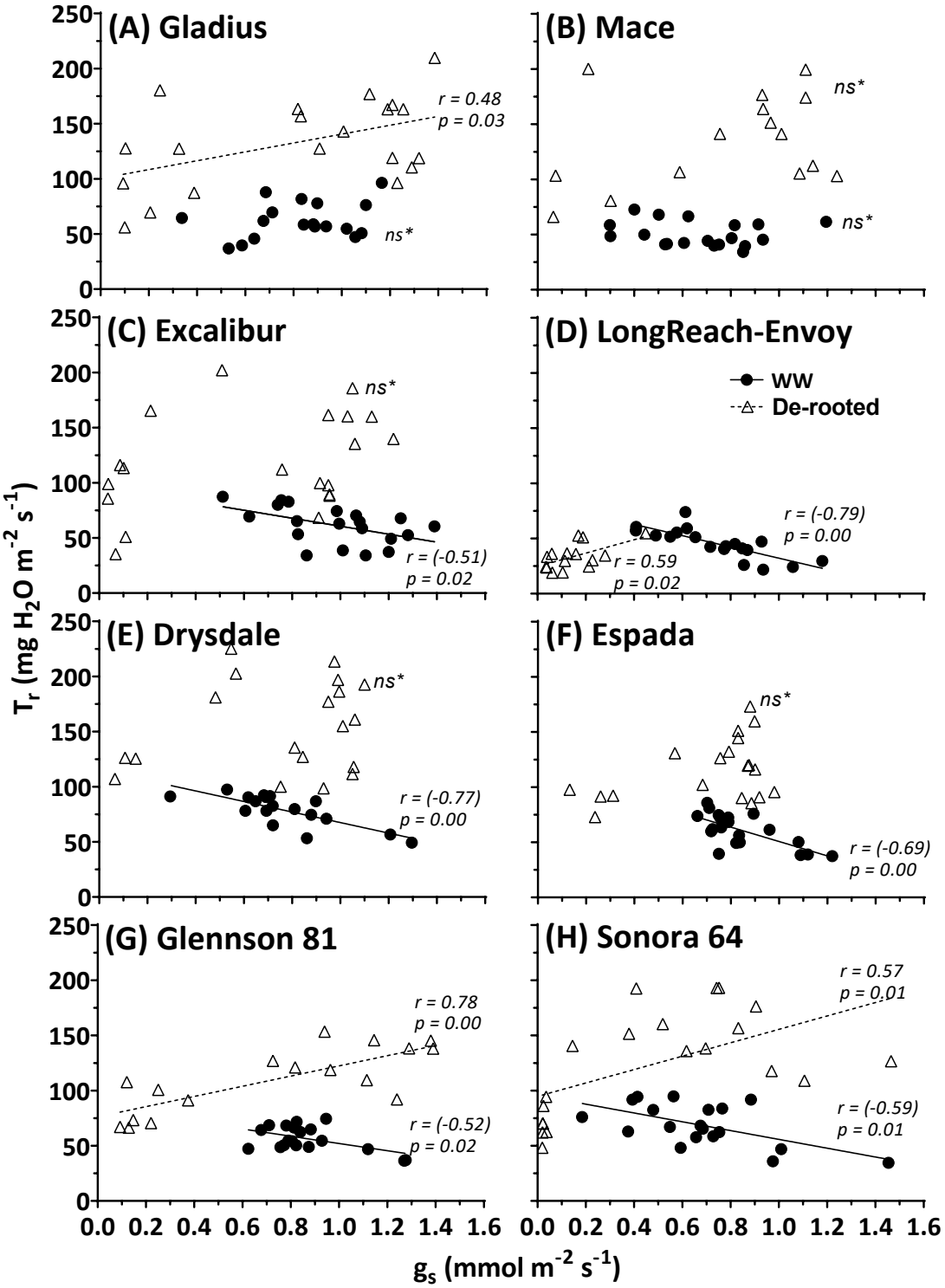


**Fig. S3.1.** Correlation between transpiration rate ($T_r$) and stomatal conductance ($g_s$) of eight wheat genotypes in intact plants under well-watered (WW) and de-rooted shoots in deionised water. Each data point represents an individual plant/shoot (replicate). Pearson correlation coefficient (r) and p value of the simple linear regression are indicated. ns* Not significant ($P > 0.05$).

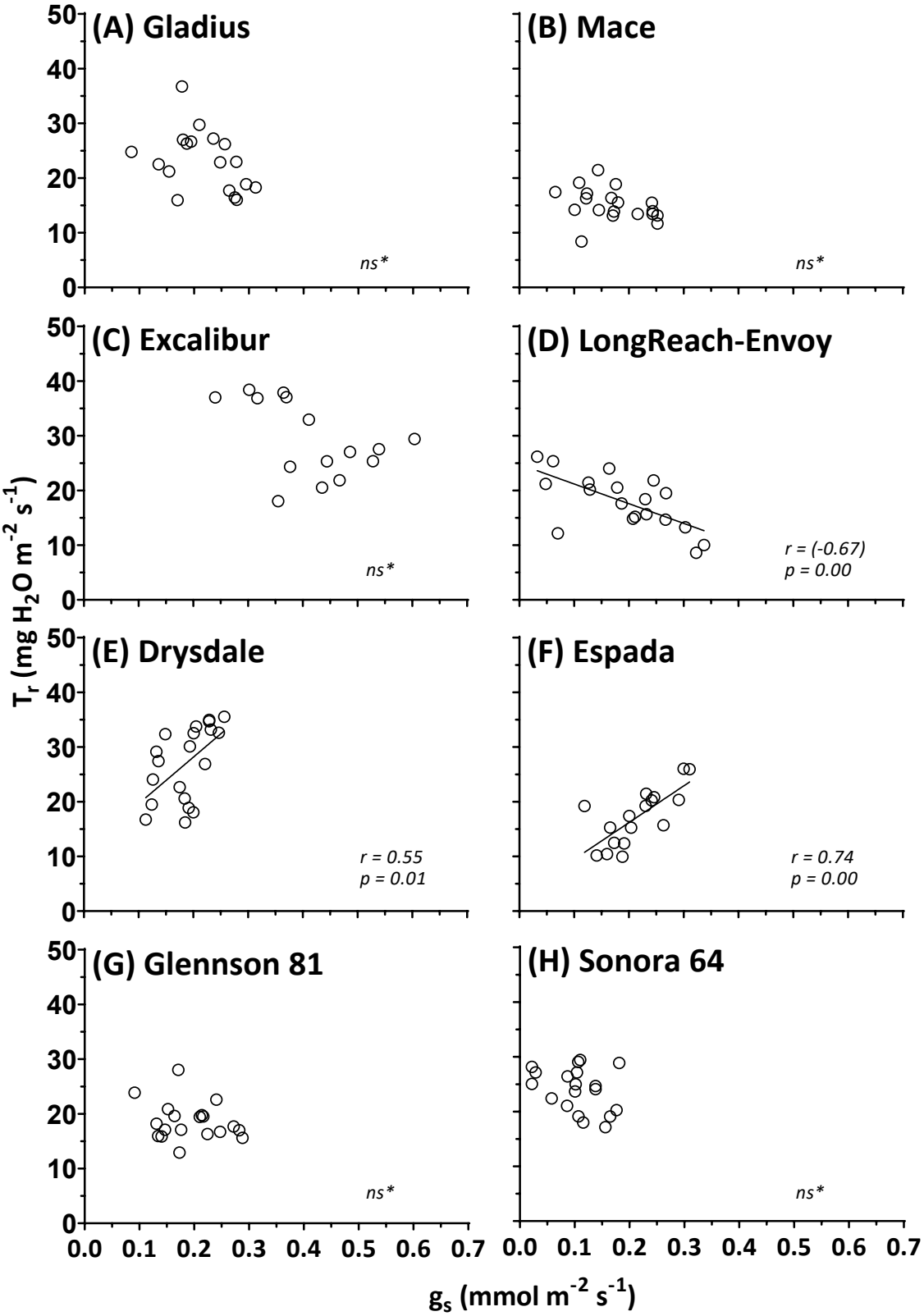


**Fig. S3.2.** Relationship between transpiration rate ($T_r$) and stomatal conductance ($g_s$) of eight wheat genotypes in intact plants under water-stressed (WS) conditions. Each data point represents an individual plant (replicate). Pearson correlation coefficient (r) and p value of the simple linear regression are indicated. ns* Not significant ($p > 0.05$).

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