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Hydraulic and Non-hydraulic Root-sourced Signals in Old and Modern Spring Wheat Cultivars in a Semiarid Area

You-Cai Xiong,[1,2] Feng-Min Li,[1,2,*] Bing-Cheng Xu,[1,2] and
Kenneth C. Hodgkinson[3]

[1]*MOE Key Laboratory of Arid Agroecology, School of Life Science, Lanzhou University, Lanzhou 730000, China;* [2]*State Key Laboratory of Soil Erosion and Dryland Farming on Loess Plateau, Institute of Conservation of Soil and Water, CAS and Ministry of Water Resources; Northwest Sci-tech University of Agriculture and Forestry, Yangling, Shaanxi 712100, China;* [3]*CSIRO Sustainable Ecosystems, GPO Box 284, Canberra ACT 2601, Australia*

ABSRACT

Non-hydraulic root-sourced signal (NRS) is so far affirmed to be a unique ''early-warning'' response to soil drying in plants, but little is known about the quantitative effect of this early-warning mechanism on crop production. To evaluate the link of NRS to a drought tolerance profile, a pot-culture study was carried out in a plant growth chamber with eight spring-wheat (*Triticum aestivum* L.) cultivars bred in semiarid China. The NRS was judged to begin when there was a significant lowering of stomatal conductance without any change in leaf relative water content (RWC), and the hydraulic root signal (HRS) was judged to begin when leaf RWC changed significantly. Soil water contents (SWC), at which the NRS and HRS were switched on, differed among the eight cultivars. For ''*Monkhead*'' and ''*Jinby,*'' representing ''old'' cultivars, the NRS and HRS were initiated successively at about 60% FWC (field water capacity) and 45% FWC, respectively. Conversely, ''*Longchun8139-2*'' and ''*Plateau 602*'' (recent cultivars) showed the NRS and HRS occurring between 70% FWC and 35% FWC, a much wider range. The events of the other four non-old cultivars were generally intermediate. This threshold range (TR) of soil FWCs between the onset of NRS and HRS also narrowed over the successive developmental stages from seedling to seed filling. Fewer survival days (SD), lower maintenance rate of grain yield (MRGY), and higher lethal leaf water potentials (LLWP) had been found in old cultivars. Widening TR was significantly correlated with increasing SD and MRGY ($r = 0.8713^{**}$ and 0.7318^{*}, respectively), and with decreased LLWP ($r = 0.8591^{**}$). This survey of different-decade cultivars suggests that advances in grain yield and drought tolerance would be made by targeted selection for a wider TR of root-sourced signals.

Key words: Drought tolerance; Root-sourced signals; Soil water content; Spring wheat (*Triticum aestivum* L.); Stomatal conductance

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*Corresponding author; e-mail: fmli@lzu.edu.cn

INTRODUCTION

Water deficit is probably the most important stress factor determining plant growth and productivity worldwide. As water resources for agronomic uses become more limiting, the development of drought-tolerant lines becomes more important (Kitchen and others 1999; Wesley and others 2002). Overt attempts by physiologists and breeders to collaborate have led, almost without exception, to failures (Boyer and others 1975; Laing and Fischer 1977; Blum and others 1981; Passioura 1983; Rajaram 2001; Trethowan and others 2002). A major factor that has prevented progress in wheat breeding for drought resistance is the complexity of the critical traits available for selection (Richards 1996). Understanding the crops' mechanism of adaptation to drought stress in stress-prone environments will provide opportunities to improve the breeding process (Wesley and others 2002). The most sensitive indicator of plants' overall physiological state is often stomatal behavior (Smith and Hollinger 1991). Stomata respond rapidly to changes in soil drying, allowing plants to balance water loss with carbon uptake during periods of reduced soil moisture (Croker and others 1998).

At present, one of the most widely acknowledged and keenly attended advances is shoot-root communication theory. Since Blackman and Davies (1985) found root-sourced chemical signals formed when soil was drying, many subsequent experiments have elucidated how root-shoot communication might operate (Jensen and others 1989; Ludlow and others 1989; Croker and others 1998; Mingo and others 2003; Dodd 2003; Norman and others 2004). Reduced stomatal conductance (Gs) can occur in plants grown in drying soil in which shoot water status is held constant (Gollan and others 1986; Gowing and others 1990), which is extensively considered to be a non-hydraulic mechanism. This mechanism enables plants to ''sense'' drought in the roots and is expressed as an alteration of growth or Gs in the leaves (Davis and Zhang, 1991; Gowing and others 1990). This is a typical ''early warning'' response of plants to drought (Blum and Johnson 1993). Continuing drought initiates up a hydraulic gradient between the leaf and the drying soil. This hydraulic gradient speeds up the development of leaf water deficit by loss of leaf turgor pressure (Blum and Johnson 1993; Comstock and Jonathan 2002) and lowers stomatal conductance, weakening gas exchange with the atmosphere, and this eventually retards plant growth. It is at the commencement of the hydraulic root-sourced signal (HRS), that retardation of plant growth is furthered.

The non-hydraulic early warning response, like other drought response characteristics (for example osmotic adjustment and leaf rolling), likely varies among species. The thresholds of soil water contents (SWC) at which NRS and HRS are triggered successively can reflect a relatively accurate effect of this early warning on crop productivity. Therefore, to determine the consequences for plant production in different water regimes, it is important to gain a greater understanding of how this early warning system integrates with the whole growth system. Nevertheless, nearly all previous investigations have focused on individual plants and abscisic acid (ABA) levels (Blackman and Davies 1985; Gutschick and Simonneau 2002; Dodd and others 2003). This restriction in methodology has limited the progress in this field. First, the ''split-root'' experiments that were widely used to elucidate the objective occurrence of NRS (Mingo and others 2003; Maurel and others 2004) in individual plants provided qualitative information on the effect of early warning, but the quantitative nature of the process is unknown. A quantitative study should reasonably be based on a comparative analysis between leaf Gs and leaf moisture status. Continuous monitoring of leaf Gs and leaf water status has to be carried out, but repeated destructive sampling in a single plant during long-term drying makes continuous monitoring impossible. Furthermore, although a pivotal role for ABA can be implicated in the control of stomatal aperture, there is an increasing awareness of the relative importance of ABA regulation. The presence of increased concentrations within the leaf are not always necessary to elicit stomatal closure (Blackman and Davies 1985; Wigger and others 2002). Also, other chemical regulators and interactions between them undoubtedly play an important role (Sharp and others 2000; Hansen and Grossmann 2000; Sharp 2002; Desikan and others 2004; Pandey and others 2005). Therefore, the early warning provided by NRS does not only involve variation in ABA concentration but also the effect of all relevant regulators under drought conditions. Based on this understanding, the judgment criteria on NRS and HRS should be mainly focused on the relationship among soil moisture regime, leaf water status, and leaf Gs, and not on ABA concentration in leaves alone.

Crop production is not an individual performance but a population process (Weiner 1990). Non-hydraulic early warning, like plant height and leaf area, exhibits a physiological diversity among

Table 1. The Climatic Conditions of the Experimental Site during the Experiment Period in the Spring of 2003

Year	Annual mean temperature (°C)	Annual mean precipitation (mm)	Precipitation during growing season (mm)	Annual mean evaporation (mm)	Evaporation during growing season (mm)	Relative humidity (%)
1994–2003	9.1	328	158	1365	938	59
2003	10.8	376	229	1113	703	58

Data from Lanzhou meteorological administration, Gansu Province, China.

individual plants. Thus, the assessments of NRS and HRS should be performed on the population level. The record of leaf Gs and leaf water status can reasonably be made in different crop populations grown under the same cultivation conditions because there is genetic stability among the various physiological aspects of different strains.

In this study, we used the term "drought resistance" to define the capacity of a plant to withstand periods of dryness, that is, the ability to survive drought while minimizing reductions in growth, and ultimately fitness (Bettina and Thomas 2003). Our goal was to examine the eco-physiological significance of the non-hydraulic early warning response in the context of drought resistance at the population level. We experimentally quantified the soil water content threshold when NRS and HRS appeared successively under drying conditions for eight different-decade wheat cultivars bred for the semiarid croplands of China. We also quantified their respective drought resistance in terms of survival ability (SD), lethal leaf water potential (LLWP), and maintenance rate of grain yield (MRGY) under both drought conditions and irrigated conditions in pot culture. We chose different-decade cultivars because their genetic background represented a lengthy natural evolution in modern wheat breeding, and they were therefore likely to reflect the ultimate adaptation mechanism for survival under dry conditions. This approach would allow us to link variation in species' drought resistance with patterns of non-hydraulic early warning across gradients of soil water availability.

Genetic variation in non-hydraulic root signaling is essential for exploring genetic control in anti-drought breeding (Blum and Johnson 1993; Khan and others 2001; Passioura 2004). With the view of root signaling, wheat breeding for drought resistance might reveal a certain trend, and following such a trend would be a critical approach in overcoming the obstacles to breeding cultivars that can succeed under arid and semiarid conditions.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Eight spring wheat cultivars were compared from the semiarid dryland agricultural area of Loess Plateau, among which cultivars of "*Monkhead*" (A) and "*Jinbaoyin*" (B) are regarded as "old" cultivars that were used during the period about from 1940s to 1970s; "*Dingxi 24*" (C), "*Plateau 602*" (D), and "*Longchun 8139-2*" (E) are seen as "recent" cultivars dating from the 1980s to 2000; "*021-128*" (F), "*92-46*" (G), and "*Longchun 8275*" (H) are defined as "modern" cultivars, dating from the late 1990s. The experiments were conducted in 2003, at the Experimental Station of Lanzhou University in Yuzhong County, Gansu Province (35°51′ N, 104°07′S, altitude 1620 m). The experimental site was typical of semiarid climate in northwestern China, with 229 mm rainfall, 703 mm evaporation, 14.2°C mean temperature, and 58% relative humidity during the growing season. (Table 1) .All plant cultures were performed in a rain shelter (50 m long × 24 m wide × 5.7 m high).

Seeds were vernalized at 4°C for 24 h and germinated in an incubating cabinet. Plants were grown in plastic pots (36 cm diameter × 30 cm high) filled with 14 kg of sieved topsoil, a sandy loam of 26.6% field water capacity (FWC). Plastic film was placed on the soil surface to restrain evaporation. Up until the trefoil stage, extra seedlings were removed, but 24 seedlings were left in each pot.

Two culture methods were compared. Monocultures came from planting 24 seedlings of a cultivar in a pot, in which each seedling was planted 6 cm apart. Mixed-cropping cultures came from planting seedlings of each of the eight cultivars in a pot. Each pot was divided into eight zones of equal area, and three seedlings of the same cultivar were planted in each zone. The space between plants was also 6 cm. All pots were watered to saturation daily and supplemented with full-strength Hoagland's solution

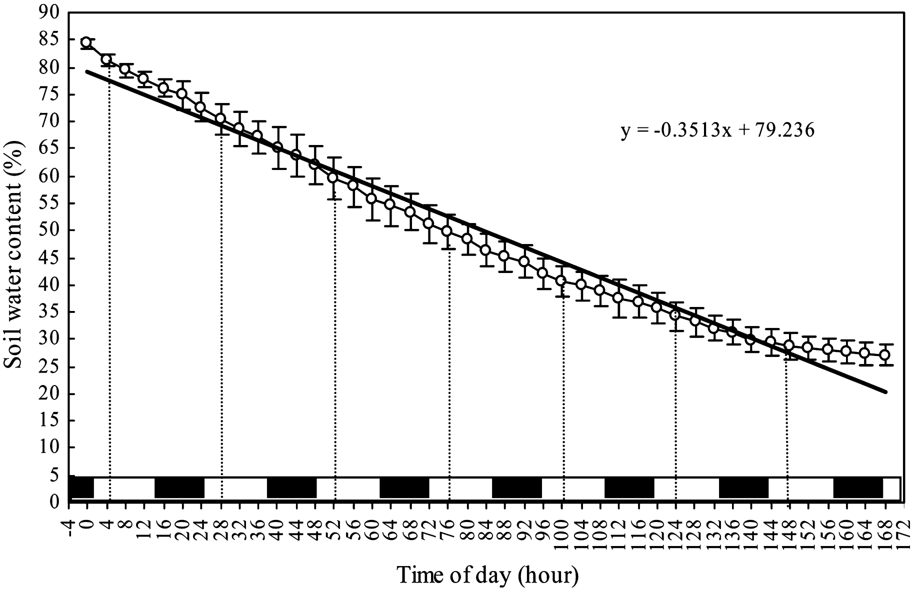

Figure 1. Variation in soil water content as a function of time of day during 168 h after water was withheld from well-watered plants. Shaded bars indicate period when lights are off. Points are the means of 135 observations ± s.e. at the same hours in three test periods (collected from 135 pots used in leaf stomatal conductance [Gs] and relative leaf water content [RWC] measurement).

(200 ml/pot) every third day to prevent the development of any plant water and nutrition deficit until the commencement of drought treatment.

Soil Water Content (SWC), Stomatal Conductance (Gs) and Relative Water Content (RWC)

The mixed-cropping method was used to track leaf Gs and RWC of each cultivar. Measurements were made during each of the three developmental stages, including period 1 (seedling to start of tilling, May 1 to May 7), period 2 (jointing to booting, May 24 to 30), and period 3 (flowering to filling, June 16 to 23). Two days prior to the start of treatment at each of three developmental stages, mixed-cropping pots were transferred from the rain shelter to the controlled-environment growth chamber. The SWC in all pots was maintained at 85% FWC before the start of the experiment. The chamber conditions were as follows: day/night temperature 25°C/15°C; day/night relative humidity (RH), 45/60 ± 5%; photon flux density (PFD), 150 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at plant height supplied by cool-white fluorescent lamps (the Far East Electric, Shanghai, China); light period, 14 h (6:00 am to 8:00 pm).

Soil was sampled from the sieved topsoil before the start of the experiment ($n = 10$). Field water capacity (FWC) was calculated as FWC $= [(SW - DW)/DW]*100\%$, where SW was the saturated weight of soil and DW the dry weight of soil. Soil water content (SWC) was expressed as percentage FWC (FWC%) as determined gravimetrically every 4 h by weighing pots at the start and end of the photoperiod throughout the drying period. The SWC at each treatment hour was calculated on the basis of the following formula: SWC $= (W_t - W_d - W_e - W_p)/(W_d \text{ FWC}) * 100\%$, where W_t is the temporary whole pot weight, W_d the net weight of dried soil in the pot, W_e the weight of the empty pot, W_p the estimated fresh weight of all plants in the pot, and FWC the field water capacity, respectively. The estimated fresh weight of all plants in one pot was determined in advance from extra pots at each test period. Leaf water relations and stomatal conductance were monitored for 3 days prior to the start of the drought treatments to ensure that constant conditions had been achieved after pots were transferred into the growth chamber (Imad and Robert 1989). All drying treatments lasted 168 h (7 days). For each test period, dying treatments started at the same time at 6:00 am of the first day. Values of SWC at any time were calculated from regressions of SWC against treatment hours (Figure 1). To facilitate development of the relationship between soil moisture and leaf growth parameters (RWC and Gs), a variety of SWCs measured in a continuous drying episode were classified into a series of soil water gradients, in which the soil water content was at the levels of 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, and 80% FWC (with a fluctuation range of 2.5% in each group; for example, the soil water content of 35% ± 2.5% was taken as the FWC35 group). In these treatments, FWC80 was considered to be the well-watered check group.

Thirty mixed-cropping pots were used to measure stomatal conductance in three test periods (10 pot replications × 3 test periods). One plant was randomly chosen from each pot for each cultivar, and then one of the youngest fully expanded leaves was selected from each chosen plant. Ten selected leaves were maintained for each cultivar throughout each test period. The total number of selected leaves was 80 for each test period. Stomatal conductance (Gs) was measured constantly on the central part of the abaxial surface of the selected leaves using a steady-state CIRAS-1 Portable Photosynthetic Monitor (PP Systems Company, U.K.). Leaves used for measurement were unshaded and kept equidistant from lights. The foliar region used for measurement was marked with a waterproof pen to ensure that each measurement could be accurately located at the same leaf position. Before the start of the photoperiod on the first day of each test period, watering was simultaneously ceased in all well-watered pots. Throughout the drying episode of 168 h, stomatal conductance was measured every 4 h from 8:00 am to 8:00 pm in the light period on three replicate readings for each leaf in each of 10 pots. Stomatal conductance was sampled in a specific order each day: from the first leaf in the first pot to the tenth leaf in the tenth pot. Once 10 plants of the first wheat cultivar had been sampled this way, the pattern was repeated until all replicates of each cultivar were measured. The cultivar order and treatment sampling order were maintained each day. Replicates were blocked in this way, so that within each block, the set of replicates were measured within about 10 min of one another.

At the time as Gs measurement, 105 pots with the same pretreatment as the Gs measurement were used to measure leaf RWC (35 pot replications * 3 test periods). Leaf RWC was determined during the light period every 4 h from 8:00 am of the first day through each 168 h test period. Ten plants were randomly chosen, and one of the most recently expanded leaves was selected from each plant. Afterwards, two leaf discs (5 mm in diameter) were cut with a cork borer from each leaf selected and weighed immediately for *FW*. The discs were floated in distilled water for 6 h under about 10 μmol m^{-2} s^{-1} PAR (Turner 1981), blotted with bibulous paper, and weighed to obtain *TW*. Dry weight (*DW*) was measured after drying the discs at 70°C in a forced-air oven for 24 h. The relative water content was calculated as $RWC = [(FW - DW)/(TW - DW)] * 100\%$ (Turner 1981). The RWC of each disc was then measured. The total number of observations was 20 in each 4 h sampling cycle for each cultivar.

Comparison of Drought Tolerance among Cultivars

The survival ability of eight wheat cultivars to tolerate continuous natural drying soil was conducted in a rain shelter, which was covered with transparent plastic sheets to protect the pots from any dry-season rains. Two culture methods (mono- and mixed-culture) were used. Soil moisture in the pots was maintained at field capacity until withholding of water at each of three test periods. Water supply was stopped simultaneously, and all eight cultivars dried naturally. When water content of leaves went beyond permanent withering, the point at which the leaves could not recover and survive after re-watering, the days to reach a leaf's RWC at which permanent wilting occurred was determined by repeated measures on the same plants. In our study, plant survival was based on the presence of living, above-ground tissues. For all eight wheat species, distinguishing dead plants from live ones was practical and easy because a wheat plant cannot survive without living leaves.

Drought tolerance was also characterized by measuring lethal leaf water potential (Ψ) (Robert and others 1998). This index of drought tolerance has been operationally defined as the Ψ (lethal Ψ) of the last surviving leaves on a plant subjected to a slow, continuous soil-drying episode (Ludlow and others 1989). Preliminary trials were conducted on extra wheat plants to determine the visible signs of the lethal drought point for each species, by excising leaves at various levels of dehydration to ascertain which would rehydrate and which had died. For all eight wheat species reported here, determining when to sample leaves was fairly clear, as leaves developed extensive necrotic areas as they died. Lethal leaf Ψ determinations were also performed on five replicate plants for each cultivar. Determinations were carried out on strips cut from leaf lamina adjacent and parallel to mid-veins and placed inside the psychrometer chamber with the abaxial sides exposed to the center of the sample cup (Robert and others 1998). Leaf water potential determination was performed on two leaves per cultivar with a WP4 PotentialMeter (Decagon Devices, Inc., Pullman, WA) calibrated daily with a graded series of KCl solutions. This trial was intended to compare the tolerance of the eight wheat cultivars to drought. There were 81 pots, 72 pots in the monoculture group (8 cultivars × 3 replications × 3 developmental stages) 9 in the mixed cropping group (3 replications × 3 developmental stages)—all arranged in a randomized block design. Here,

Table 2. The Relationship between Soil Suction and Soil Water Content

Water treatments	Extreme stress, ES	Severe stress, SS	Intermediate stress, IS	Mild tress, MS	Sufficient water, CK
Soil water content (%FWC)	30	40	50	60	80
Soil suction (KPa)	1535 ± 184.8	1275.5 ± 104.9	906.3 ± 108.1	472.5 ± 93.9	227.5 ± 110.3

Based on the statistical analysis of correlation between soil suction and soil field water capacity (FWCs) in varying soil moisture levels, a model curve equation between them for standard sample soil was developed : $Y = 168.28\ X^{-1.9628}$, Y is soil suction (KPa), and X is soil water content (percentage in FWC); $R^2 = 0.9035^{**}$; $p < 0.01$. *Four drought-stress gradients were developed to describe the characteristics of soil used in the experiment, including 30% FWC (extreme stress), 40% FWC (severe stress, SS), 50% FWC (intermediate stress, IS), and 60% FWC (mild stress, MS).*

foliar dehydration tolerance was compared under monoculture condition at the same time.

Comparison of Yield Stability among Cultivars under Soil Drought Stress

The relationship between soil water potential and soil water content was used to estimate the extent of drought treatment. Pots were maintained at one of several different soil water levels: 80, 70, 60, 40, and 30 soil moisture percentage. These represented well-watered soil (80%), mild drought stress (60%), intermediate drought stress (50%), severe drought stress (40%), and extreme drought stress (30%) (Table 2). This trial was performed under monoculture conditions. The effect of drought was estimated by comparing grain from two groups of plants. One group was maintained at 80% soil FWC and a second group was held at a soil FWC of about 60% from the jointing stage onward. The pots were weighed each day and when they fell below designated weights equivalent to soil FWCs, set but different quantities of water were added to each pot. After grain was filled, the plants were harvested and spikes for each pot were dried and weighed. Maintenance rate of grain yield was used to judge grain stability as follows: $MRGY = Y_s/Y_{ck}$ (Y_s and Y_{ck} = yield under stress and non-stress conditions, respectively). There were 48 pots (2 groups × 8 cultivars × 3 replications) arranged in a randomized block design.

Judgment about Non-hydraulic Root-sourced Signal (NRS) and Hydraulic Root Signal (HRS)

The data collected for repeatedly measured plants was used to develop relationships between leaf RWC, Gs, and soil water content. As proposed originally by Blackman and Davies (1985), NRS was defined as stomatal conductance decreased significantly without significant decrease in leaf water status in plants subjected to drought stress. Therefore, the NRS was judged to begin when there was a significant lowering of leaf stomatal conductance without change in leaf RWC (compared with Gs in FWC80), and the HRS was judged to begin when there were significant differences for both of the above leaf parameters. This judgment criterion was to evaluate the thresholds of soil water content (TSWC) at which NRS and HRS started to appear, respectively. The threshold range (TR) was the difference in soil water content between the beginning of the NRS and HRS, respectively.

Statistical Analysis

Treatments were arranged in a split, split, split plot, completely randomized design. Cultivars were the main-plot factors, soil water contents (SWC) the subplot factors, and leaf RWCs or Gs the sub-sub-plot factors. Three-way factorial analyses of variance (ANOVA) were used to determine the statistical significance of changes that occurred in leaf RWC and Gs in response to (1) cultivar and (2) SWC level. Means of leaf RWC and Gs were calculated on 30 and 20 replications for each ''cultivar × SWC'' combination, respectively, and compared by LSD (Least Significant Difference) at the 0.05 confidence level. Analysis of variance residuals were used for the calculation of the 5% LSD; this was done under the assumption of homogeneity of variances (Levene test). The beginning of the NRS was judged by there being an insignificant change in leaf RWC but a significant decrease in leaf Gs compared to the leaf RWC and leaf Gs in the FWC80 group. The SWC at the occurrence of NRS was termed the ''upper limit.'' The beginning of the HRS was judged by the occurrence of a significant decrease in leaf RWC compared to leaf RWC in FWC80, and the SWC of HRS occurrence was termed the ''lower limit.'' The threshold range

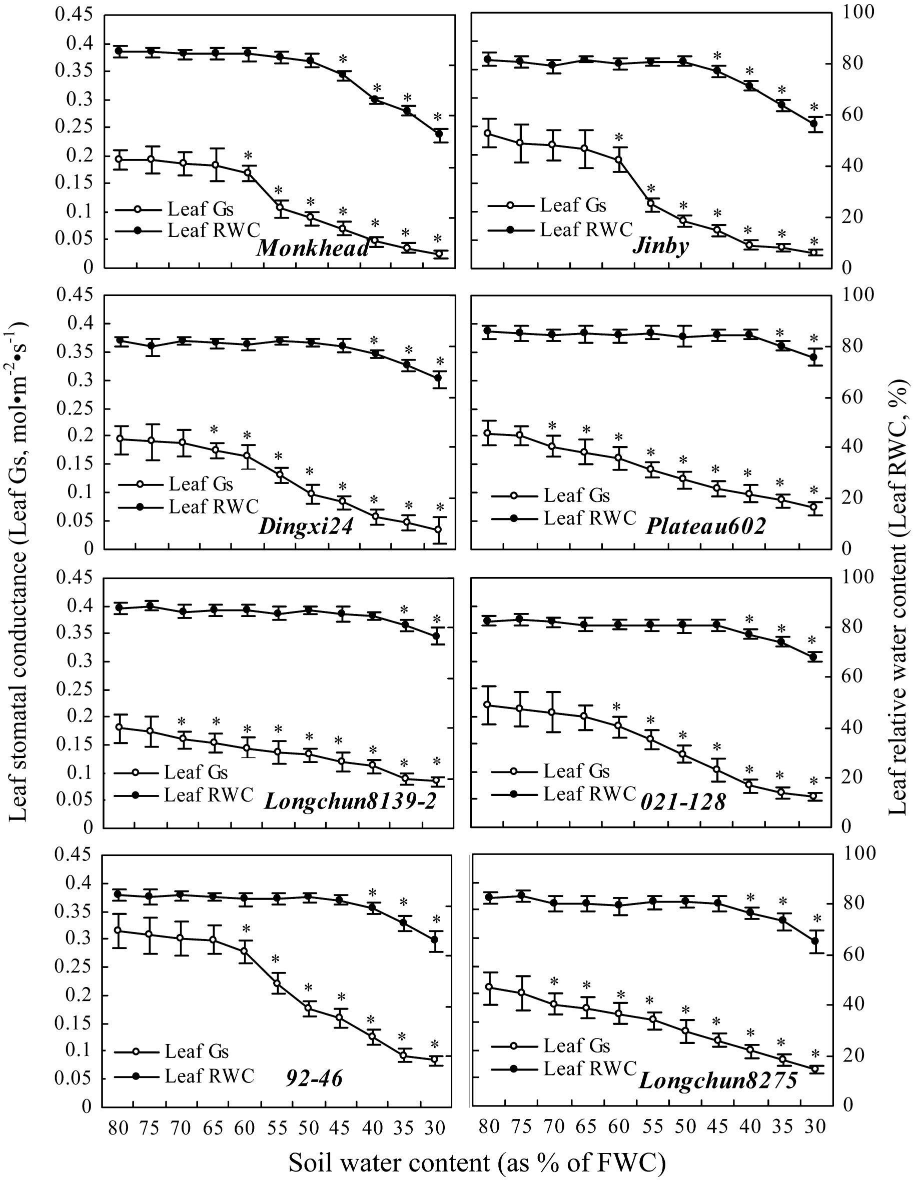

Figure 2. Soil moisture trends in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of eight wheat species used in this study. *Significantly different from the well-watered treatments (FWC80 groups) at $p = 0.05$ ($n = 30$ and 20 for leaf Gs and leaf RWC, respectively). Points are the means of 30 and 20 observations ± s.e. for Gs and leaf RWC, respectively (period 1).

(TR) of operative NRS was the difference between the upper limit and the lower limit. The ultimate means of survival days (SD) and lethal leaf Ψ (LLWP) were calculated on nine replications from the SDs and LLWPs in three test periods for each cultivar (3 replications × 3 test periods = 9 replications). The maintenance rate of grain yield was the percentage of grain yield per pot in three stress groups relative to that in the well-watered group (FWC80 group) (3 replications). Regression and correlation analyses were used to describe relationships between TR and SD, LLWP, and MRGY, respectively. Sub-regression analysis was used to distinguish the three processes in the double "Z" model for old and modern cultivars.

RESULTS

Stomatal Conductance and Leaf RWC *versus* Soil Water Content (SWC)

Stomatal conductance, averaged for the eight cultivars, was 0.19–0.38 mol $m^{-2}s^{-1}$ and tended to rise with growth stages (Figure 2–4). Soil water content was expressed as percentage of FWC, and FWC80 was taken as the well-watered group. In the FWC75 group, stomatal conductance remained high throughout three test periods for all eight cultivars, without significant changes with respect to those of FWC80. However, with the aggravation of soil drying, drought decreased stomatal conductance

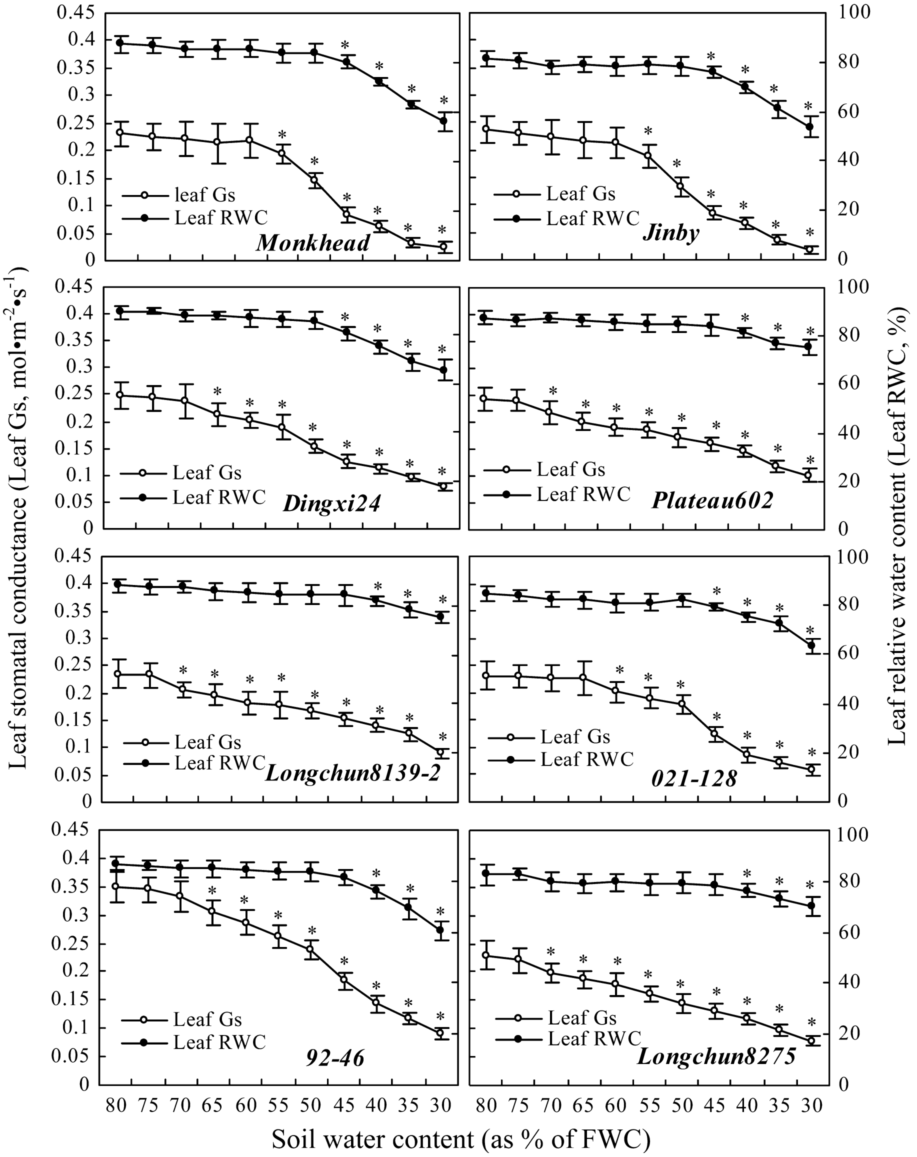

Figure 3. Soil moisture trends in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of eight wheat species used in this study. *Significantly different from the well-watered treatments (FWC80 groups) at $p = 0.05$ ($n = 30$ and 20 for leaf Gs and leaf RWC, respectively). Points are the means of 30 and 20 observations ± s.e. for Gs and leaf RWC, respectively (period 2).

significantly in all cultivars. Stomatal responses of cultivars to drought differed over the three test periods. In the first test period (period 1), *Plateau602* and *Longchun8139-2* (recent cultivars), and *Longchun8275* (a modern cultivar) responded earliest to variation in SWC, having a significant decrease in stomatal conductance in the FWC70 group. *Monkhead* and *Jinby* (old cultivars) and *021-128* and *92-46* (modern cultivars) had the latest response to drought, not showing a significant decrease until SWC dropped to 60% (in FWC60). *Dingxi24* (a recent cultivar) was intermediate, with the onset of significant decrease in FWC65 (Figure 2). Stomatal conductance in periods 2 and 3 was mostly similar to that in period 1. Although the cultivar ranks of responses to decreasing SWC in the latter two periods followed a similar trend to that in period 1, most of the eight cultivars tended to respond later to drought than they did in period 1. There was a tendency for the soil FWCs at which Gs decreased significantly in periods 2 and 3 to be about 5% lower for period 1, except that *Plateau602*, *Longchun8139-2*, and *021-128* remained at constant SWC levels in the latter two periods and *92-46* had a 5% rise in period 2 over that measured in period 1(Figures 2–4). In contrast, Gs declined more rapidly after commencement of its significant decrease in all cultivars than before. However, the extent of Gs decline for old cultivars (*Monkhead* and *Jinby*) was more acute than that for six non-old cultivars (Figures 2–4).

The RWC of leaves always exceeded 80% in the eight cultivars when the soil was well watered. Withholding water did not change the high leaf

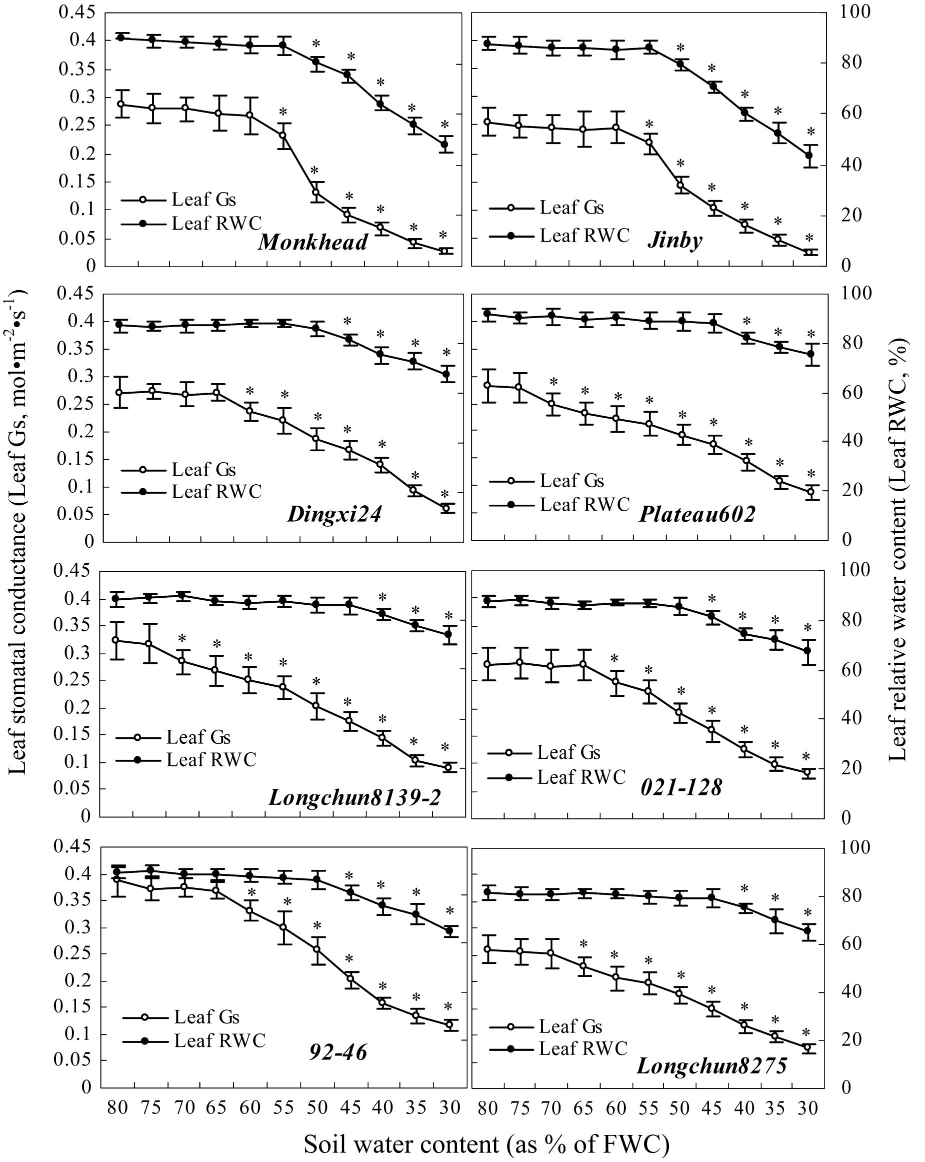

Figure 4. Soil moisture trend in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of eight wheat species used in this study. *Significantly different from the well-watered treatments (FWC80 groups) at $p = 0.05$ ($n = 30$ and 20 for leaf Gs and leaf RWC, respectively). Points are the means of 30 and 20 observations ± s.e. for Gs and leaf RWC, respectively (period 3).

RWC. Leaf RWC stayed high until SWC dropped to less than 55% FWC for all cultivars throughout the three test periods. Plots of leaf RWC *versus* soil moisture content depicted a leaf RWC of 80%–90% in FWC80, FWC75, FWC65, FWC60, and FWC55 (Figures 2–4). In period 1, the beginning of the significant decline in leaf RWC differed among cultivars. The earliest significant decline took place in *Monkhead* and *Jinby*, that is, in the FWC45 group. In contrast, *Plateau602* and *Longchun8139-2* had the latest response of significant decline, that is, in the FWC35 group. The responses of other cultivars were intermediate. Their leaf RWC began to decline significantly in the FWC40 group. Changes in leaf RWC in the latter two periods, as expected, closely paralleled trends in period 1. There was also a tendency for the soil FWCs at which leaf RWC declined significantly in periods 2 and 3 to be about 5% higher than for period 1.

The Threshold of Soil Water Content (TSWC) at which the Non-hydraulic Root-sourced Signal (NRS) Appeared

Stomatal conductance, Gs, generally decreased for plants in successively lower soil FWCs, often without a significant change in leaf RWC compared to the values of FWC80 (Figure 2). Plots of leaf RWC and Gs *versus* soil FWCs depicted the occurrence of NRS for eight cultivars and three test periods (Figures 2–4). The critical value of SWC at

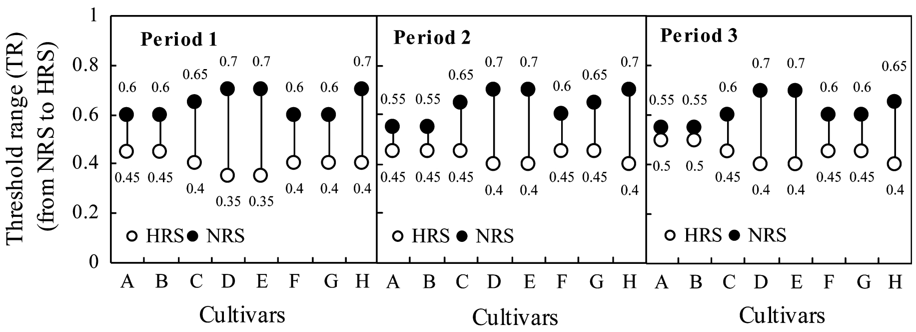

Figure 5. Soil field water capacity (FWC) thresholds for non-hydraulic root-sourced signal (NRS) appearance and hydraulic root signal (HRS) appearance among eight cultivars. Based on Figure 2–4, all the upper limits and the lower limits of the threshold range (TR) are shown here. Filled circle and open circles indicated the upper limit and the lower limits, respectively. The linear lengths from filled circle to open circle in the graph indicated the TR. Generally, *Monkhead* and *Jinby* had a relatively narrow threshold range with respect to six non-old cultivars, and the threshold range tends to become narrower from period 1 to period 3. The cultivars from A to H represent *Monkhead*, *Jinby*, *Dingxi24*, *Plateau602*, *Longchun8139-2*, *021-128*, *92-46*, and *Longchun8275*, respectively.

which the NRS began to appear was taken as the threshold of soil water content (TSWC) of NRS. The TSWCs of NRS differed among cultivars (Figure 2); the TSWCs for old cultivars (*Monkhead* and *Jinby*) were less than 60%, for the recent cultivar *Dingxi24* and modern cultivars *021-128* and *92-46* they were between 60% and 70%, and for the modern cultivar *Longchun8275* and recent cultivars *Plateau602* and *Longchun8139-2* they were above 70%. There was also a tendency for TSWC in periods 2 and 3 to be about 5% lower than in period 1 (Figure 5). A general ranking on TSWC for NRS was that two old cultivars were higher than six other recent or modern ones (non-old cultivars; the same below), suggesting that the onset of NRS in the former was earlier than in the latter.

At lower soil FWCs, leaf RWC did not significantly change, suggesting that the NRS was operative, depressing Gs. From Figures 2, 3, and 4 it can be seen that the NRS was maintained over lower soil FWCs for the eight cultivars until the hydraulic root signal was triggered.

The TSWC at which the Non-hydraulic Root-sourced Signal (NRS) and Hydraulic Root Signal (HRS) Appeared Successively

According to traditional theory about the plant–water relation, the occurrence of a significant decrease in leaf water status was referred to as the appearance of HRS. As described above, the HRS was judged to appear among eight cultivars in three test periods (Figures 2–4). The critical value of SWC at which HRS occurred was taken as another threshold of SWC (TSWC). Means of TSWC for each cultivar were calculated from TSWCs in the three test periods. There were interspecific differences in the TSWC of HRS that in the old cultivars were higher than that in non-old cultivars (Figures 2–5). Therefore, the HRS in old cultivars generally appeared earlier than that in non-old ones, but there was no clear trend for the HRS occurrence among six non-old cultivars.

The threshold range (TR) was the difference in TSWCs between HRS and NRS (that is, TR = *the upper limit – the lower limit;* the upper limit of the TR was TSWC of the NRS, and the lower limit was TSWC of the HRS). From period 1, the TR for cultivars was developed. Typically, old cultivars had the narrowest range (45%–60%). Non-old cultivars had the widest range, especially *Plateau602* and *Longchun8139-2* (range: 35%–70%). Furthermore, at each successive growth stage, the TR narrowed (Figure 5). With respect to the respective means of the upper limits and lower limits in the three test periods, *Plateau602* and *Longchun8139-2* had the highest upper limits (both are 0.7) and the lowest lower limit (both are 0.383). In contrast, *Monkhead* and *Jinby* had the lowest upper limits and highest lower limits (0.567 and 0.467, respectively, for both). Therefore, the TRs of the eight cultivars were generated more precisely as follows: *Plateau602* (0.317), *Longchun8139-2* (0.317), *Longchun8275* (0.283), *Dingxi24* (0.2), *92-46* (0.184), *021-128* (0.167), *Monkhead* (0.1), and *Jinby* (0.1) (Table 3). It can be concluded that modern wheat breeding has evolved in the direction of a wide TR of soil FWCs between a high upper limit and a low lower limit, over which NRS occurs.

Table 3. Maintenance Rate of Grain Yield (MRGY), Lethal Leaf Ψ (LLWP), and Average Soil Threshold Range (TR) from NRS Appearance to HRS Appearance in Whole Growth Stage among Eight Wheat Cultivars

Decades	Old		Recent				Modern	
Cultivar	*Monk-head*	*Jinby*	*Dingxi 24*	*Plateau-602*	*Longchun-8139-2*	*021–128*	*92–46*	*Longchun-8275*
MRGY	0.551 ± 0.02^{a,b}	0.508± 0.03^{a}	0.532± 0.02^{a}	0.681± 0.03^{c}	0.687± 0.04^{c}	0.573± 0.02^{b}	0.649± 0.03^{c}	0.651± 0.04^{c}
Lethal leaf Ψ	−3.05± 0.08^{a}	−2.77± 0.14^{a}	−3.38± 0.14^{b}	−4.03± 0.54^{c}	−3.98± 0.11^{c}	−3.52± 0.09^{b}	−3.69± 0.12^{b}	−4.14± 0.15^{c}
Means of the upper limits	0.567	0.567	0.633	0.7	0.7	0.6	0.617	0.683
Mean of the lower limits	0.467	0.467	0.433	0.383	0.383	0.433	0.433	0.4
Threshold range (TR)	0.1	0.1	0.2	0.317	0.317	0.167	0.184	0.283

Maintenance rate of grain yield (MRGY) is the grain yield per pot in the stress group relative to that in the well-watered group (n = 3). The means of the upper limits and the lower limits were calculated from three test periods for each of eight cultivars (n = 3), and the threshold range (TR) was the difference between the upper limit and the lower limit. Foliar dehydration tolerance was characterized as lethal leaf Ψ, which was the mean of three test periods for each cultivar (n = 9). Similar letters following LLWP and MRGY values indicate statistically similar averages (p < 0.05).

Comparison of Drought Tolerance and Yield Stability among Cultivars

Cultivars, irrespective of culture method, differed in survival days (SD), an index of drought tolerance. Old *Monkhead* and *Jinby* died first with about 8 SD on average after watering ceased. Hence, their drought tolerance appeared to be the poorest under drought stress (Table 4). In contrast, the recent cultivars *Plateau602* and *Longchun8139-2* and the modern cultivar *Longchun8275* survived longer (13 or 14 days after watering ceased). Other cultivars were intermediate with survival times of 10–13 days. The SD index lengthened with development of each growth stage, and SD was also slightly but not significantly longer in the mixed cropping group than in the monoculture group at each growth stage. The SD means of the eight cultivars in mixed cropping culture were about 10.3, 14.2, and 14.1 days, respectively, in the three test periods and 9.3, 11.2, and 13.2 in monoculture. Thus the general sequence of the eight cultivars was as follows: (*Plateau602* and *Longchun8139-2*) > (*Dingxi24*, *021-128*, *92-46*, and *Longchun8275*) > (*Monkhead* and *Jinby*) (Table 4).

Lethal leaf water potential (Ψ) is used to classify the relative foliar dehydration tolerance among cultivars, as Ψ has traditionally been used as the best comparative thermodynamically sound measure among species (Kramer and Boyer. 1995). Table 3 ranks the eight spring wheat cultivars in terms of LLWP. *Monkhead* and *Jinby* had the highest LLWP (−3.05 and −2.77 MPa, respectively), suggesting that they were most sensitive to foliar dehydration. *Dingxi24*, *021-128*, and *92-46* had relatively high LLWP (−3.38 MPa, −3.52 MPa, and −3.69 MPa, respectively). *Plateau602*, *Longchun8139-2*, and *Longchun8275* withstood the most dehydration, with leaves not drying until leaf Ψ dropped to an average of −3.98 MPa or below (Table 3). *Plateau602* and *Longchun8139-2*, having the lowest LLWP, also had relatively longer SD. *Monkhead* and *Jinby*, having the highest LLWP, had the shortest SD (Tables 3 and 4).

The MRGY was the percentage of grain yield in stress group relative to that of 80% FWC group (CK group). It also was closely related to the developmental pattern of a cultivar. *Monkhead*, *Jinby*, and *Dingxi24* tended to be more adversely affected by drought stress than other cultivars, with MRGY measurements of 0.551, 0.508, and 0.532, respectively. *Plateau602* and *Longchun8139-2* were the least depressed, with MRGY measures of 0.681 and 0.687, respectively. Other non-old cultivars were intermediate. A general ranking in MRGY was as follows: (*Plateau602*, *Longchun8139-2*, *Longchun8275*, *92-46*) > (021-*128*, *Monkhead*) > (*Dingxi24*, *Jinby*) (Table 3).

Table 4. Comparative Survival Period (days) for Spring-Wheat Cultivars when Watering was Stopped during Each Growth Stage, and Lengths of Growth Cycles (days)

Cultivars	Growth cycle	Anthesis	Period 1	Period 2	Period 3	Average	Ranking
Monoculture survival days							
OLD							
Monkhead	134	98	6.7 ± 0.6^{a}	7.7 ± 0.6^{a}	10.3 ± 1.5^{a}	8.2	7
Jinby	140	103	6.3 ± 1.5^{a}	6.3 ± 1.5^{b}	10 ± 1.7^{a}	7.6	8
RECENT							
Dingxi 24	125	95	$10.3 \pm 1.2^{b,d}$	$11.3 \pm 1.2^{c,e,f}$	13.3 ± 0.6^{b}	11.7	4
Plateau 602	110	87	11 ± 0.6^{c}	15.3 ± 0.5^{d}	15.7 ± 1.2^{c}	14	1
Longchun 8139-2	105	85	11.7 ± 1.5^{c}	15.3 ± 1.5^{d}	14.3 ± 0.6^{d}	13.8	2
MODERN							
021–128	115	90	9.7 ± 1.7^{b}	11 ± 1.7^{c}	13.3 ± 0.6^{b}	11.3	5
92–46	115	90	10 ± 1.2^{d}	10.7 ± 1.2^{e}	13.3 ± 1.2^{b}	11.8	3
Longchun 8275	120	92	8.3 ± 0.6^{e}	11.7 ± 0.6^{f}	15.3 ± 0.6^{c}	11.2	6
Average days	120.5	92.5	120.5	11.2	13.2	11.2	
Mixed-cropping culture survival days							
OLD							
Monkhead	134	98	7.3 ± 0.6^{a}	10.7 ± 0.6^{a}	12.7 ± 1.2^{a}	10.2	7
Jinby	140	103	6.3 ± 1.2^{b}	10 ± 1.7^{a}	10.7 ± 1.5^{b}	9	8
RECENT							
Dingxi 24	125	95	11.3 ± 1.2^{c}	13.3 ± 1.5^{b}	15.3 ± 0.6^{c}	13.3	4
Plateau 602	110	87	13.3 ± 0.6^{d}	$16.7 \pm 1.5^{c,d,f}$	17.3 ± 0.6^{d}	15.8	1
Longchun 8139-2	105	85	14.3 ± 1.5^{e}	$16.3 \pm 1.2^{c,d}$	$14.7 \pm 2.1^{c,e}$	15.1	2
MODERN							
021–128	115	90	$9.7 \pm 1.2^{f,g}$	15.7 ± 0.6^{d}	14.7 ± 1.2^{e}	13.3	4
92–46	115	90	9.7 ± 0.6^{f}	13.3 ± 1.5^{b}	12.3 ± 1.5^{a}	11.8	6
Longchun 8275	120	92	$10.3 \pm 1.5^{c,g}$	17.3 ± 0.6^{f}	15.3 ± 0.6^{c}	14.3	3
Average days	120.5	92.5	10.3	14.17	14.13	12.86	

As each of the three different development stages was reached, water was not applied in turn to a third of the pots when either tillering, jointing, or flowering began. All ANOVAs were done among eight different cultivars within the same column over three growing periods. Numbers followed by the same letter are statistically similar (n = 3, p < 0.05). Period 1 refers to the time from seedling to tillering; period 2, from jointing to booting; period 3, from flowering to filling (the same in subsequent tables).

The Links of Threshold Range of NRS to SD, MRGY, and LLWP

Data were evaluated with General Linear Models Procedure (SAS, Cary, NC) and means separated by Duncan's Multiple Range Test ($p = 0.05$). There was a significant linear correlation between TR and SD ($r = 0.8713^{**}$, $p < 0.01$), and between TR and MRGY ($r = 0.7381^{*}$, $p < 0.05$) (Figure 6). The TR was significantly correlated with LLWP ($r = 0.8591^{**}$, $p < 0.01$) (Figure 7).

Stomatal Sensitivity and Foliar Water Maintenance between Old Cultivars and Non-old Cultivars

All eight cultivars were divided into two groups, including two old cultivars and six non-old cultivars, respectively. In terms of varying SWC levels from FWC80 to FWC30, the means of Gs were calculated from Gs of all cultivars in each group in three test periods. Leaf RWC was also averaged according to the way of Gs analysis. Based on the means of the upper limits and lower limits of the TR in Table 3 and Figures 2–5, the dynamic variations of Gs could be classified into three clear-cut processes. Process 1 was from FWC80 to the appearance of NRS, Process 2 was from the appearance of NRS to the appearance of HRS, and Process 3 was from the appearance of HRS to lower soil moisture level. For the old cultivar group, the three processes consisted of FWC80-60 (Process 1), FWC55-45 (Process 2), and FWC40-30 (Process 3). For the non-old-cultivar group, they contained FWC80-65 (Process 1), FWC60-40 (Process 2), and FWC35-30 (Process 3). Therefore, the sub-section regression analyses were used to describe the variation of Gs *versus* SWC level in these two groups (Figure 8).The linear slopes from Process 1 to Process 3 were 0.001, 0.0096, and 0.0035 in the old-cultivar group and 0.0015, 0.0053, and 0.0035 in the non-old-cultivar group. The Gs value decreased most rapidly in

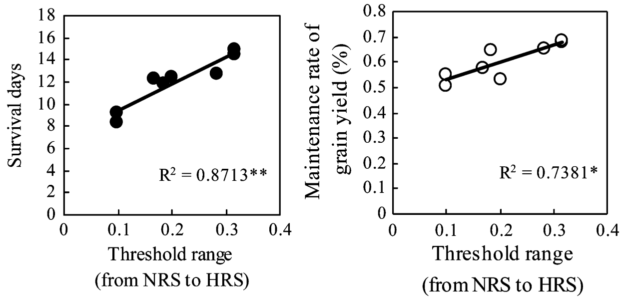

Figure 6. Relationships between threshold ranges (TR), survival days (SD), and maintenance rate of grain yield (MRGY). Filled circles indicate the means of SD on the nine replicates in three test periods for each cultivar. Open circles indicate the means of MRGY on the three replicates at harvest stage for each cultivar. *Significance of the correlation at $p < 0.05$ and **Significance of the correlation at $p < 0.01$, respectively.

Process 2 and most slowly in Process 1. The event of Gs changes was Z-shaped in plants subjected to a continuous drying treatment. At the same time, with respect to the two groups, the decline of Gs value for the old-cultivar group was slower in Process 1 but faster in Process 2 than for the non-old-cultivar group. In Process 3, the variations in Gs were similar in the two groups (Figure 8).

Regression analyses were employed to describe the decline extent of leaf RWC. The changes of leaf RWC in varying soil levels also differed in the two groups. The linear slopes of leaf RWC decline were −0.0308 in the old-cultivar group and −0.0138 in the non-old-cultivar group. Leaf RWC in the former declined earlier (in about 45% FWC, see Figure 4) and more rapidy than in the latter (Figure 9).

DISCUSSION

The start and closure of non-hydraulic root-sourced signaling that reduces the aperture of stomata, as indicated by reduction in Gs values, began at different soil FWCs according to cultivar. Signaling in old cultivars (*Monkhead* and *Jinby*) was in the narrow range of about 56%–46%. Recent cultivars (*Plateau602* and *Longchun8139-2*) were in the widest range of about 70%–38%. Of intermediate range (about 68%–40%) were the recent cultivar *Dingxi24* and modern cultivars *021-128*, *92-46*, and *Longchun8275*. As plants developed, the threshold range tended to narrow. These results suggest that modern breeding has altered the non-hydraulic root-sourced signaling systems so that the water tension cue(s) from drying soil to trigger the non-hydraulic signal is sensed earlier and the signal is sensed longer, than in old cultivars.

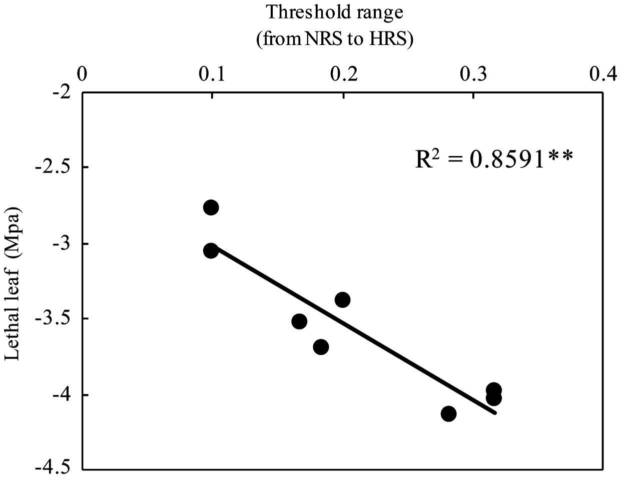

Figure 7. Relationship between threshold range (TR) and lethal leaf Ψ (LLWP). Data are the means of nine replicates in three test periods. **Significance of the correlation at $p < 0.01$.

Stomatal behavior is often the most sensitive indicator (Smith and Hollinger 1991), and the magnitude of Gs declines appears to be a function of the magnitude of soil moisture declines (Croker and others 1998). The Z-model of stomatal sensitivity exhibited the differentiated adaptation of wheat species to drought (Figure 8). This model revealed that a dynamic trend of stomatal behavior in plants imposed a continuous drought stress, including three dissimilar but correlative processes. These three processes began and ceased with marked changes in slope of lines, as depicted in Figure 8. As soil began to dry at the initial stage of drought, Gs changed little until NRS was triggered, this being Process 1. At the end of this process, modern cultivars detect drought earlier and more quickly switch on the NRS than old cultivars. However, in Process 2, ranging from the onset of NRS to the onset of HRS, old cultivars tended to have a higher reduction ratio in Gs than modern lines. Furthermore, in the third process after the HRS began to be initiated (of course, the NRS material was objectively functioning here in a greater role than in the former process), Gs values would follow a similar and relatively slow decrease tendency in all cultivars.

As soil began to dry during the initial stages of drought, the rapid and early responses of roots are critical to the survival of the plant (Ober and Sharp 2003). This response may be triggered by turgor-sensitive stretch-activated membrane channels or by other osmo-sensing elements (Lew 1996). The earlier the response of plants to drought, the better osmotic adjustment they will have and the better their drought tolerance will be. With respect to the

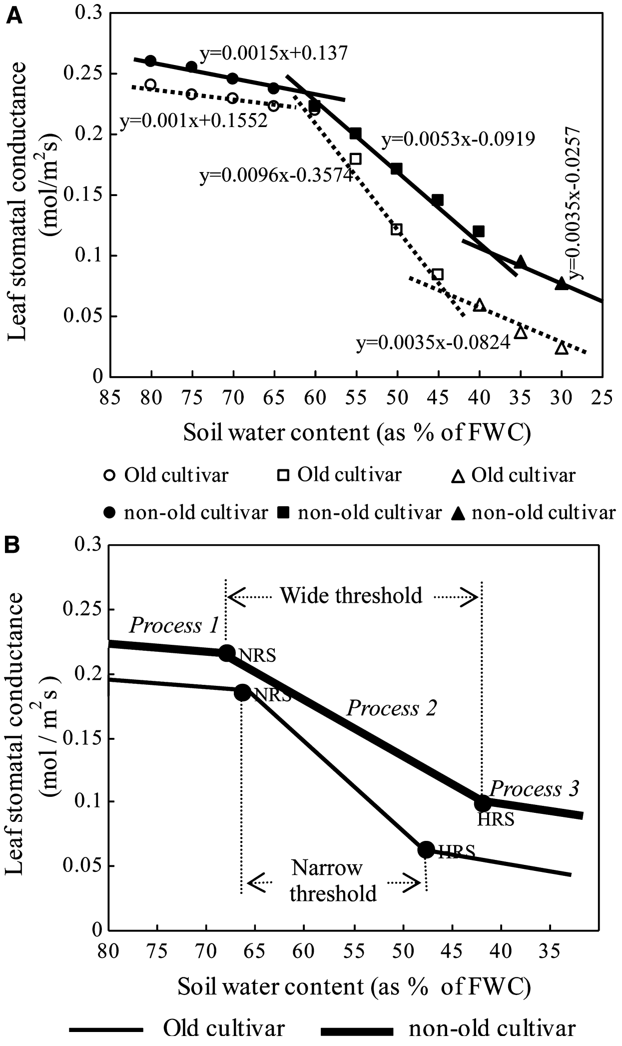

Figure 8. The "double Z" model hypothesis of root-sourced signal development from modern wheat breeding. **A**. A graph generated from actual data of Gs in three test periods for two old cultivars (termed "old cultivar" group) and six recent or modern cultivars (termed "non-old cultivar" group, and further resumptively termed "modern cultivar" group in the following discussion for convenient description). **B.** A stylized plot generated from **A**. In **A,** filled circles and open circles are the means of 810 and 270 replicates of three test periods for two cultivar groups (30 replicates × 3 periods × 6 cultivars for the non-old cultivar group, and 30 replicates × 3 periods × 2 cultivars) at varying soil moisture levels. Based on the plots of means of Gs *versus* soil FWCs, a sub-section linear regression analysis was made to assess the three processes (see **A**), including Process 1 (from FWC80 to the onset of NRS), Process 2 (from the onset of NRS to the onset of HRS), and Process 3 (from the onset of HRS to FWC30).

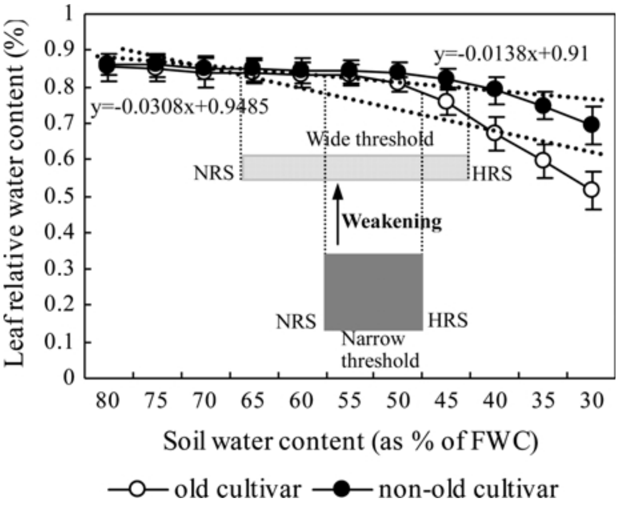

Figure 9. A possible weakening model of non-hydraulic root signal for leaf water maintenance between the old and modern wheat cultivars. Filled circles and open circles are the means of 540 and 180 replicates of three test periods for two cultivar groups (20 replicates × 3 periods × 6 cultivars for non-old cultivar group, and 20 replicates × 3 periods × 2 cultivars), respectively, in varying soil moisture levels. The cultivar grouping in terms of breeding decades is the same as that in Figure 8. The weakening effect of the early-warning NRS on water conservation can be demonstrated in this study. Over the history of wheat-breeding evolution, the threshold range of early-warning NRS originally was narrow but strong in old wheat cultivars. Soil drought tended to initiate excessive accumulation of NRS materials in a short time, producing strong and temporary signals. This certainly would cause a great reduction in water maintenance, and then inhibited plant growth. To some extent, there existed a negative correlation between plant growth and drought-induced NRS. In contrast to the events of old cultivars, modern wheat had a wide threshold range of NRS and a relatively low stomatal sensitivity. Modern wheats displayed better water conservancy, plant growth, and survivability, because they can respond to soil drought stress more swiftly than their wild relatives, producing appropriate signal materials. In this case, during the breeding decades, the effect of NRS on shoot growth and water maintenance had become unpredictably weaker and weaker. This occurred unintentionally in breeding practice.

TR of the early warning response, modern cultivars with a high upper limit of TR had, accordingly, a low lower limit of tolerance. The wide TR of soil FWCs in the non-hydraulic early warning response was a critical trait for plant adaptation to the drought conditions.

The chief purpose of this study was to characterize and compare the NRS and HRS sensitivity of the eight wheat cultivars to soil drying, and then to explore the eco-physiological significance of that sensitivity. The triggered timing of the NRS and HRS appeared to determine plant's drought tolerance. Perhaps the best gauge of NRS and HRS to soil drying is the slopes of TR/SD, LLWP, and MRGY regressions. Slopes were significantly correlated with TR; correlation analysis yielded the correlation coefficient of 0.8713** with SD, 0.8591** with a LLWP and 0.7318* with MRGY, respectively. Those cultivars with a wider TR generally had longer SD, lower LLWP, and higher MRGY (Figures 6 and 7). The measures SD, LLWP, and MRGY were three critical drought-tolerant physiological parameters. Foliar dehydration tolerance is expressed in terms of LLWP: the extent of water loss a plant can withstand before most of its foliage dies (Sinclair and Ludlow 1986; Chapman and Augé 1994). From Process 2 in the Z-model (Figure 8), old cultivars having swifter Gs declines had less dehydration tolerance (higher LLWP; see Table 3). Stomatal sensitivity to non-hydraulic root signals may be mechanistically linked to drought tolerance (Croker and others 1998). Increasing LLWP was significantly correlated with increasing capacity for osmotic adjustment (Augé and others 1998). Morgan and co-workers (Morgan 1977, 1983 and 1995; Morgen and others 1986) in Australia have shown that wheat lines selected for high osmotic adjustment significantly out-yielded those not adjustable actively under drought conditions. Modern cultivars with low LLWP possessed stronger osmotic adjustment and then had longer survival times than old cultivars. And their MRGY were less affected by the drought stress in soil and displayed stronger drought tolerance. Survival was closely correlated with plant growth, including leaf-area changes in dry conditions relative to irrigated conditions (Engelbrecht and Kursar 2003). Longer SD appeared to be closely related to higher MRGY.

Non-synchronization of the growth cycle would not be in contradiction to the results of the drying experiment. Old cultivars with the longest growth cycle had the shortest SD in all three test periods. Especially in period 3, old cultivars were at the peak of growth and development, and yet they had the weakest survival ability compared to modern cultivars (Table 4). Interestingly, the survival times in the mixed cropping group were slightly longer than in the monoculture group throughout development. It might be explained that some reduction in competition among plants may have occurred in the mixed-cropping group. At severe drought levels, inter-specific competition may be lower than intra-specific competition because of differences between cultivars in root structure and function.

Chemical signal materials originated from the root— that is,, NRS materials—have been extensively studied in a large number of plant species (Wilkinson and others 2002; Passioura, 2002; Eric and Robert 2003; Maurel and others 2004). It has long been apparent that the NRS materials, including ABA (Comstock and Mencuccini, 1998), calcium ions (McAinsh and others 1990), apoplastic pH (Patonnier and others 1999), cytokinins (Stoll and others 2000), and nitric oxide (Desikan and others 2004), played an important role in the responses of plants to soil drying. But whether this role was beneficial or detrimental to plant's defense response to drought was directly related to the amount of root-sourced NRS materials induced by soil drought stress. Old wheat cultivars, having a narrow TR, have high non-hydraulic stomatal sensitivity (Figure 8). It can be argued that, although modern cultivars generally responded inactively to drought stress, they were able to synthesize rapidly and massively NRS materials in the root system. This signaling was transported to the shoot swiftly once the urgent early-warning reaction of NRS was triggered. In so doing, they displayed worse shoot water maintenance, and consequently, had worse plant growth and drought tolerance (Figure 5). This idea was supported by the different water-conservation abilities between old and modern cultivars (Figure 9). Old cultivars were generally unresponsive to soil water deficiency and were prone to unnecessary loss of water as soil dries, resulting in early wilting of leaves, early death, and high reduction in grain yield. It can therefore be concluded that there was a negative effect between strong root signaling and crop production. In other words, there was a sort of signal redundancy similar to the growth redundancy proposed by Donald and Hamblin (1983) in old cultivars, possibly because the NRS materials produced were excessive in view of crop production. On the other hand, the case of modern cultivars was opposite that of old cultivars. They were able to make a quick and timely response to mild drought stress; that is, it is likely that they synthesized a moderate amount of NRS material. Recently, ''crosstalk,'' a new concept of the response of plants to abiotic attack, was put forward to describe the complexity and multi-pathway of signaling (Taylor and others 2004). The overall effect of the early-warning NRS was also expressed through NRS materials or pathways. Throughout the anti-drought breeding history of wheat, the intensity of early-warning NRS has borne a weakening process, because the effect of NRS function was being ''diluted'' as the TR was being widened.

Previous breeding in wheat had narrowed the operation of this early-warning process but this has since been widened. Modern wheat breeding has selected cultivars with better drought tolerance ability and yield stability (Table 3 and 4), as shown in this study. In addition, improved soil water management has significantly increased the yield of grain for water used in the semiarid environments of China and elsewhere. Over the years, crop breeders have aimed to generate hybrids with higher grain yield potential, better grain yield stability, and improved grain traits for end-users (Duvick 1997). However, the variations in harvestable yield have also markedly increased (Wesley and others 2002). In a sense, the current breeding of drought-tolerant cultivars has been led astray (Passioura 1983), because in the process of modern plant breeding, selecting cultivars for wider thresholds in the NRS trait has occurred unwittingly. The drought tolerance of some recent cultivars appears to be stronger than that of modern cultivars (Table 3 and 4). It is time to begin breeding purposefully for higher drought tolerance in wheat for semiarid environments; this should be based on selection for a wider appearance of a non-hydraulic early-warning response in drying soil.

Low water availability is one of the major causes for crop yield reductions affecting the majority of the farmed regions around the world. In semiarid areas, wheat genetic breeding will follow a certain direction. From the work of previous breeding decades, drought tolerance tends to be strengthened. Modern cultivars gradually evolve in wide early-warning TR of ''high higher limit, low lower limit'' in the long-term evolution from old cultivars to modern ones.

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REFERENCES

Bettina MJE, Thomas AK. 2003. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. Oecologia 136:383–393.

Blackman PG, Davis WJ. 1985. Root-to-shoot communication in maize plants of the effects of soil drying. J Exp Bot 36:39–48.

Blum A, Gozlan G, Mayer J. 1981. The manifestation of dehydration avoidance in wheat breeding germplasm. Crop Sci 21:495–499.

Blum A, Johnson JW. 1993. Wheat cultivars respond differently to a drying topsoil and a possible non-hydraulic root signal. J Exp Bot 44:1149–1153.

Boyer JB, MePherson HG. 1975. Physiology of water deficits in cereal crops. Adv Agron 27:1–23.

Chapman DS, Augé RM. 1994. Physiological mechanisms of drought resistance of four native ornamental perennials. J Am Soc Hortic Sci 119:299–306.

Comstock, Jonathan P. 2002. Hydraulic and chemical signaling in the control of stomatal conductance and transpiration. J Exp Bot 53:195–200.

Comstock J, Mencuccini M. 1998. Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T. & G.), a desert subshrub. Plant Cell Environ 25:1029–1038.

Croker JL, Witte WT, Augé RM. 1998. Stomatal sensitivity of six temperature, deciduous tree species to non-hydraulic root-to-shoot signaling of partial soil drying. J Exp Bot 49:761–774.

Davis WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Physiol Plant Mol Biol 42:55–76.

Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ. 2004. ABA, hydrogen peroxide and nitric oxide signaling in stomatal guard cells. J Exp Bot 55:205–212.

Dodd C, Tan LP, He J. 2003. Do increase in xylem sap pH and/or ABA concentration mediate stomatal closure following nitrate deprivation? J Exp Bot 54:1281–1288.

Dodd IC. 2003. Hormonal interactions and stomatal responses. J Plant Growth Regul 22:32–46.

Donald CM, Hamblin J. 1983. The convergent evolution of annual seed crops in agriculture. Adv Agron 36:97–143.

Duvick DN. 1997. What is yield? In: Edmeades GO, Bänziger M, Mickelson HR, Peña-Valdivia CB editors. Developing Drought and Low-N Tolerant Maize Mexico: CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo [International Maize and Wheat Improvement Center], El Batan. pp 332–335.

Engelbrecht BMJ, Kursar TA. 2003. Comparative drought-resistance of seedling of 28 species of co-occurring tropical woody plants. Oecologia 136:383–393.

Eric SO, Robert ES. 2003. Electrophysiological responses of maize roots to low potentials: relationship to growth and ABA accumulation. J Exp Bot 54:813–824.

Gollan T, Passioura JB, Munns R. 1986. Soil water status affects stomatal conductance as an indicator of fully turgid wheat and sunflower leaves. Aust J Plant Physiol 13:459–464.

Gowing DJ, Davies WJ, Jones HG. 1990. A positive root-induced signal as an indicator of soil drying in apple *Malus * domestica* Borkh. J Exp Bot 41:1535–1540.

Gutschick VP, Simonneau T. 2002. Modeling stomatal conductance of field grown sunflower under varying soil water content and leaf environment: comparison of three models of stomatal response to leaf environment and coupling with an abscisic acid-based model of stomatal response to soil drying. Plant Cell Environ 25:1423–1434.

Hansen H, Grossmann K. 2000. Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiol 124:1437–1448.

Jensen CR, Henson IE, Turner NC. 1989. Leaf gas exchange and water relations of lupines during drought-induced stomatal closure. Aust J Plant Physiol 16:415–428.

Khan AJ, Hassan S, Tariq M, Khan T. 2001. Haploidy breeding and mutagenesis for drought tolerance in wheat. Euphytica 120:409–414.

Kitchen NR, Sudduth KA, Drummond ST. 1999. Soil electrical conductivity as a crop productivity measure for claypan soils. J Product Agric 12:607–617.

Laing DR, Fischer RA. 1977. Adaptation of semidwarf wheat cultivars to rainfed conditions. Euphytica 26:129–139.

Lew RR. 1996. Pressure regulation of the electrical properties of growing *Arabidopsis thaliana* L. root hairs. Plant Physiol 112:1089–1100.

Ludlow MM, Sommer KJ, Flower DJ. 1989. Influence of root signals resulting from soil dehydration and high soil strength on growth of crop plants. Curr Topic Plant Biochem Physiol 8:81–89.

Maurel M, Robin C, Simonneau T, Loustau D, Dreyer E, Desprez-Loustau ML. 2004. Stomatal conductance and root-to-shoot signaling in chestnut saplings exposed to *Phytophthora cinnamomi* or partial soil drying. Funct Plant Biol 31:41–51.

McAinsh MR, Brownlee C, Hetherington AM. 1990. Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature 25:186–188.

Mingo DM, Bacon MA, Davies WJ. 2003. Non-hydraulic regulation of fruit growth in tomato plants (*Lycopersicon esculentum* cv. *Solairo*) growing in drying soil. J Exp Bot 54:1205–1212.

Morgan JM. 1977. Difference in osmoregulation between wheat genotypes. Nature (Lond) 270:234–235.

Morgan JM. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Aust J Agric Res 34:607–614.

Morgan JM. 1995. Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. Field Crop Res 40:143–152.

Morgan JM, Hare RA, Fletcher RJ. 1986. Genetic variation in osmoregulation in bread and durum wheats and its relationship to grain yields in a range of field environments. Aust J Agric Res 37:449–457.

Norman JMV, Frederick RL, Sieburth LE. 2004. BYPASS1 negatively regulates a root-derived signal that controls plant architecture. Curr Biol 14:1739–1746.

Ober ES, Sharp RE. 2003. Electophysiological responses of maize roots to low water potentials: relationship to growth and ABA accumulation. J Exp Bot 54:813–824.

Pandey S, Perfus-Barbeoch L, Taylor JP, Zhao Z. 2005. Plant cell signaling: in vivo and –omics approaches. J Plant Growth Regul DOI: 10.1007/s00344-005-0039-5.

Passioura JB. 2004. Increasing Crop Productivity When Water is Scarce—From Breeding to Field Management. Proceeding of the 4th International Crop Science Congress, 1–17.

Passioura JB. 2002. Soil conditions and plant growth. Plant Cell Environ 25:311–318.

Passioura JB. 1983. Roots and drought resistance. Agric Water Manag 7:265–280.

Passioura JB. 1976. Physiology of grain yield on wheat growing on stored water. J Plant Physiol 3:559–565.

Patonnier MP, Peltier JP, Marigo G. 1999. Drought-induced increase in xylem malate and mannitol concentrations and closure of *Fraxinus excelsior* L. *stomata*. J Exp Bot 25:1223–1229.

Rajaram S. 2001. Prospects and promise of wheat breeding in the 21st century. Euphytica 119:3–15.

Richards RA. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regul 20:157–166.

Sharp RE. 2002. Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant Cell Environ 25:211–222.

Sharp RE, LeNoble ME, Else MA, Thorne ET, Gherardi F. 2000. Endogenous ABA maintains shoot growth in tomato independently of effects on the plant's water balance: evidence for an interaction with ethylene. J Exp Bot 51:1575–1584.

Sinclair TR, Ludlow MM. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust J Plant Physiol 13:329–341.

Smith WK, Hollinger DY. 1991. Measuring stomatal behavior In: Lassoie JP, Hinckley TM editors. Techniques and Approaches in Forest Tree Ecophysiology Boca Raton, FL, USA: CRC Press. pp 81–95.

Stoll M, Loveys B, Dry P. 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. J Exp Bot 51:1627–1634.

Taylor JE, Hatcher PE, Paul ND. 2004. Crosstalk between plant responses to pathogens and herbivores: a view from the outside in. J Exp Bot 55:159–168.

Trethowan RM, Ginkel MV, Rajaram S. 2002. Progress in breeding wheat for yield and adaptation in global drought affected environments. Crop Sci 42:1441–1446.

Weiner J. 1990. Plant population ecology in agriculture In: Ronald-Carrol C editor. Agroecology New York, USA: McGraw-Hill. pp 235–262.

Wesley B, Bruce, Gregory O, Edmeades, Barker TC. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. J Exp Bot 53:13–25.

Wigger J, Phillips J, Peisker M, Artaenko O, Conrad UFU. 2002. Prevention of stomatal closure by immunomodulation of endogenous abscisic acid and its reversion by abscisic acid treatment: physiological behavior and morphological features of tobacco stomata. Planta 215:413–423.

Wilkinson S, Davies WJ. 2002. ABA-based chemical signaling: the co-ordination of responses to stress in plants. Plant Cell Environ 25:195–210.