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# Evolution mechanism of non-hydraulic root-to-shoot signal during the anti-drought genetic breeding of spring wheat

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## Abstract

The objectives of this study were to: (1) characterize the evolutionary tendency of the non-hydraulic root-sourced signal (NRS) from wheat wild relatives to its modern hexaploid species, and (2) test whether species sensitivity to the NRS was allied with their drought tolerance profiles. The NRS was judged to begin when there was a significant lowering of stomatal conductance without change in leaf relative water content (RWC). The lethal soil water content (LSWC) was operationally characterized as the soil water content (SWC) at the drying lethal point of wheat plants. The threshold of soil water content (TSWC) at which the NRS was triggered, and the LSWC differed amongst six wheat species. For “*MO1*” and “*MO4*” representing ‘diploid’ species, the TSWC and the LSWC were initiated successively at about 51% FWC (field water capacity) and about 30% FWC, respectively. Conversely, “*Plateau 602*” and “*Longchun 8139-2*” (modern hexaploid species) exhibited the TSWC and the LSWC between about 68% FWC and less than 14% FWC, a much wider threshold range (TR). Increasing TSWC was significantly correlated with decreasing LSWC ( $r=0.9464^{**}$ ). The widened TR from the TSWC to the LSWC was also significantly correlated with longer survival days (SD) and higher maintenance ratio of grain yield (MRGY), respectively ( $r=0.9411^{**}$  and  $0.8068^*$ , respectively). Meanwhile, those species having higher TSWC had the least reduction ratio of stomatal conductance under the decreasing soil moisture from  $-0.2$  to  $-1$  MPa. This suggests that advances in yield performance and drought tolerance would be made evolutionally by targeted selection for an earlier onset of NRS.

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**Keywords:** Non-hydraulic root-sourced signal (NRS); Spring wheat; Lethal soil water content (LSWC); Drought tolerance; Evolutionary mechanism; Threshold range

## 1. Introduction

Wild species selected for crossing with cultivated wheats belong to wheat immediate progenitors (Valkoun, 2001), and are an important source for the introduction of economically useful genes (Kawahara, 2002) and then for the enhancement of genetic diversity of wheat (Narasimhamoorthy, 2003). There is a great need to explore all genetic variability that can be used in breeding for stress environments (Khan et al., 2001; Passioura, 2004). Soil drought is probably the most important stress factor determining

plant growth and productivity world-wide. As water resources for agronomic uses become more limiting, the development of drought-tolerant lines becomes increasingly more important (Kitchen et al., 1999; Bruce et al., 2002). Overt attempts by physiologists and breeders to collaborate have led, almost without exception, to failures (Boyer and McPherson, 1975; Laing and Fischer, 1977; Blum et al., 1981; Passioura, 1983; Sinha, 1987; Rajaram, 2001; Trethowan et al., 2002). A major factor that has prevented progress in wheat breeding for drought resistance is the complexity of which is the critical traits to select (Richards, 1996). Understanding the crops’ mechanism of adaptation to drought stress in stress-prone environments will provide opportunities to improve the breeding process (Bruce et al., 2002). The most sensitive indicator of plants’ overall physiological state is often stomatal behaviour (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 et al., 1998).

**Abbreviations:** FWC, field water capacity; LSWC, lethal soil water content; MRGY, maintenance ratio of grain yield; NRS, non-hydraulic root-to-shoot signal; NEWR, non-hydraulic ‘early-warning’ response; RRSC, reduction ratio of stomatal conductance; RWC, relative water content; SWC, soil water content; TR, threshold range; TSWC, the threshold of soil water content

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Currently, one of the most widely acknowledged and keenly attended advances is root-to-shoot communication theory. Since Blackman and Davies (1985) found root-sourced chemical signals formed when soil drying, many subsequent experiments have elucidated how root-shoot communication might operate (Jensen et al., 1989; Ludlow et al., 1989; Gallardo et al., 1994; Blum et al., 1991; Tardieu and Davies, 1993; Croker et al., 1998; Mingo et al., 2003). Reduced stomatal conductance ( $G_s$ ) can occur in plants grown in drying soil in which shoot water status is held constant (Gollan et al., 1986; Gowing et al., 1990), which is extensively believed 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  $G_s$  in the leaves (Davis and Zhang, 1991; Gowing et al., 1990; Gallardo et al., 1994; Li et al., 2000). This is a typical ‘early-warning’ response of plants to drought (Blum and Johnson, 1993). Continuing drought starts 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; Tardieu and Davies, 1993; Comstock and Jonathan, 2002) and lowers stomatal conductance weakening gas exchange with the atmosphere that eventually retards plant growth.

Non-hydraulic root-sourced signal (NRS) is so far affirmed to be a unique non-hydraulic ‘early-warning’ response (NEWR) to soil drying in plants, but little is known about the quantitative effect of this ‘early-warning’ mechanism on crop production. The NEWR likely varies among species, like other drought response characteristics (e.g. osmotic adjustment and leaf rolling). The threshold of soil water contents (SWC) at which NRS was triggered can reflect the relative accurate effect of this ‘early-warning’ on crop productivity. Therefore, more knowledge is needed on how this ‘early-warning system’ integrates with the whole system and what the consequences are for plant production in different water regimes. With respect to the previous study in NRS, however, nearly all attempts are mainly focused on either individual plant level or abscisic acid (ABA) (Blackman and Davies, 1985; Gutschick and Simonneau, 2002; Dodd et al., 2003). This restriction in methodology and knowledge directly prevents the progress in this field. On one hand, all sorts of ‘split-root’ experiments have been widely used to elucidate the objective occurrence of NRS (Mingo et al., 2003) in individual plant, but these studies are not quantitative but qualitative ones about the effect of ‘early-warning’ response on crops adaptation to drought. This quantitative study ought to be dependent only on the comparative analysis between leaf  $G_s$  and leaf moisture status. It will be the first necessary for determination of NRS that a continuous monitor on leaf  $G_s$  and leaf water status has to be made in the same plant. But, one critical technical obstacle is that the repeated destructive sampling has to be performed on the same one plant. That would make it impossible for a continuous monitor on the same plant during the long-term drying treatment. On the other hand, although a pivotal role for ABA can considerably suggest in the control of stomatal aperture, there is an increasing awareness of the relative importance of ABA regulation, which does not always require the presence of increased concentrations within the leaf to elicit stomatal closure (Blackman and Davies,

1985; Wilkinson and Davies, 1997; Wigger et al., 2002), and other chemical regulators and interactions between them also undoubtedly play an important role (Sharp et al., 2000; Hansen and Grossmann, 2000; Sharp, 2002; Desikan et al., 2004). Furthermore, dehydration-induced accumulation of some adaptive materials such as free leucine and isoleucine is achieved independent of ABA (Nambara et al., 1998). Therefore, the NRS is not only referred to as ABA concentration variation, but also as the overall effect of all relevant regulators under drought conditions. Based on this understanding, the judgment criteria on the NRS should be mainly focused on developing the relations between leaf water status and leaf  $G_s$ , rather than limitedly on ABA concentration in leaves or other organs.

Crop production is not of an individual performance but of a population process (Weiner, 1990). It is evident from the literature that wheat possesses a large genome (16,000 Mb) and its adaptation to drought condition is under the control of multiple genes (Shao et al., 2005). The NEWR, like plant height and leaf area, is to exhibit a physiological diversity among individual plants. The definition of NRS is not so much a physiological conception on the level of individual plant as ecophysiological one on the crop population. Thus, the determination on NRS should be performed on the level of population. The traces towards leaf  $G_s$  and leaf water status can be reasonably conducted on a certain number of individual plants within the same crop population grown in the same cultivated conditions, for the reason that there exists a genetic stability in crop’s ecophysiological aspects.

In this study, we used the term drought resistance as “the capacity of a plant to withstand periods of dryness”, i.e. the ability to survive drought while minimizing reductions in growth, and ultimately fitness (Bettina and Thomas, 2003). Our goal in this study was to examine the eco-physiological significance of the NEWR in the context of drought tolerance profiles on the crop’s population level. We experimentally quantified the threshold of soil water content (TSWC) when the NRS was triggered in drying soil for six wheat species differing in chromosome sets in semi-arid croplands, and also quantified their respective lethal soil water contents (LSWC) when wheat plants were subjected to a continuous drought episode. Furthermore, we were to assess the ecophysiological effects of NEWR on drought resistance such as the survival days (SD), and maintenance ratio of grain yield (MRGY) under drought compared with irrigated conditions in the pot-culture experiment.

We chose different-ploid species on the basis of their broad genetic background as they represented the direction of lengthy natural evolvement in modern wheat breeding in semi-arid areas, and were likely to reflect the ultimate mechanism adapted to drying environment for crops. Genetic variation in non-hydraulic root signaling is essential for exploring the genetic control in anti-drought breeding (Blum and Johnson, 1993; Khan et al., 2001; Passioura, 2004). The experimental materials used in this study were two diploids (*MO1* and *MO4*), two tetraploids (*DM22* and *DM31*) and two modern hexaploids (*Plateau 602* and *Longchun 8139-2*). The A and B genomes of *MO1* and *MO4* (*Triticum monococcum* L.,  $2n = 14$ , AA), *DM22* and *DM31* (*Triticum dicoccum* Schuebl L.,  $4n = 28$ , AABB) were identified as a donor of modern hexaploid bread wheat genome ( $6n = 42$ ,

Table 1  
A brief introduction of six species

Ploids	Species	Genotypes	Germplasm	Plant properties
Diploid	<i>MO1</i>	AA	Wild relatives	<i>T. monococcum</i> L.
	<i>MO4</i>	AA	Wild relatives	<i>T. monococcum</i> L.
Tetraploid	<i>DM22</i>	AABB	Wild relatives	<i>T. dicoccum</i> Schuebl
	<i>DM31</i>	AABB	Wild relatives	<i>T. dicoccum</i> Schuebl
Hexaploid	<i>Plateau 602</i>	AABBDD	Modern wheat	Modern cultivar
	<i>Longchun 8139-2</i>	AABBDD	Modern wheat	Modern cultivar

AABBDD) (Kimber, 1993) (Table 1). With the view of root signal, wheat breeding of drought resistance might follow a certain tendency, which would be the critical approach to solve the current obstacle in breeding theory under arid and semi-arid conditions. This approach may allow us to link variation in species' drought resistance with patterns of the NEWR across gradients of soil water availability.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Four wild relatives of modern hexaploid wheat (two diploids, *MO1* and *MO4*; two tetraploids, *DM22* and *DM31*), and two modern hexaploid wheat species (*Plateau 602* and *Longchun 8139-2*) were compared, representing different genomes in the program of wheat breeding (Table 1). In 2003, the experiments were conducted at the Experimental Station of Lanzhou University in Yuzhong County of Gansu (35°51'N, 104°07'S, altitude 1620 m). Experimental site was typical of semi-arid climate in northwestern China, with 229 mm rainfall, 703 mm evaporation, 14.2 °C mean temperature and 58% relative humidity during the growing season (Data comes from Lanzhou Meteorological Administration, Gansu Province, China). All plant cultures were performed in a rain shelter (50 m long × 24 m wide × 5.7 m high).

Seeds were vernalised at 4 °C for 24 h, germinated in an incubating cabinet. Plants were grown in plastic pots (36 cm diameter × 30 cm high). Pots were filled with 14 kg of sieved topsoil, a sandy loam of 26.6% field water capacity. Plastic film was placed on the soil surfaces to restrain evaporation. Till the trefoil stage extra seedlings were removed but 24 seedlings left for 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 six species in a pot. Each pot was divided into six zones of equal area, and four seedlings of the same cultivar were planted in a zone. The space between plants was also 6 cm. All pots were watered to saturation daily, and supplemented with full-strength Hoagland's solution (Hoagland and Arnon, 1950) (200 ml/pot) every third day to prevent the development of any plant water and nutrition deficit till the commencement of drought treatment.

### 2.2. Soil water content (SWC), stomatal conductance (Gs) and relative water content (RWC)

In order to eliminate the effect of interspecific competition on physiological response of plant under mixed-culture condition, monoculture 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 tillering, 1–7 May), Period 2 (jointing to booting, 24–30 May) and Period 3 (flowering to filling, 16–23 June). Three days prior to the start of treatment at each of three developmental stages, monoculture pots were transferred from rain shelter to the controlled-environment growth chamber. The chamber conditions were: day/night temperature 25 °C/15 °C; day/night relative humidity (RH), 45/60 ± 5%; photon flux density (PFD), 150 μmol m<sup>-2</sup> s<sup>-1</sup> 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 water contents (SWC) were determined gravimetrically every 4 h by weighing pots at the start and end of the photoperiod throughout the whole drying period. The SWCs at each treatment hours were calculated on the basis of following formula:  $SWC = (W_t - W_d - W_e - W_p) / (W_d - FWC) \times 100\%$ , where  $W_t$  is the temporary whole pot weight,  $W_d$  the net weight of dried soil in pot,  $W_e$  the weight of empty pot,  $W_p$  the estimated fresh weight of all plants in the pot, and FWC is the field water capacity, respectively. The estimated fresh weight of all plants in one pot was determined in advance on extra pots in 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 growth chamber (Imad and Robert, 1989). All drying treatments lasted 168 h (7 days). For each test period, drying treatments started at the same time at 6:00 am on the first day. Values of SWC at any time were calculated from regressions of SWC against treatment hours (Fig. 1). In order to facilitate the 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 FWC35 group). In these treatments, FWC80 was considered to be the well-watered check group.

Five monoculture pots were used to measure stomatal conductance and leaf RWC in each cultivar, with the total of 90 pots (5 pot replications × 6 species × 3 test periods). Three plants were randomly chosen from each pot, and then the youngest fully expanded leaf was selected from each of the chosen three plants. The total number of selected leaves was 90 in each test period (3 leaf replications × 5 pots × 6 species). Stomatal conductance (Gs) was measured constantly on the central part of 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

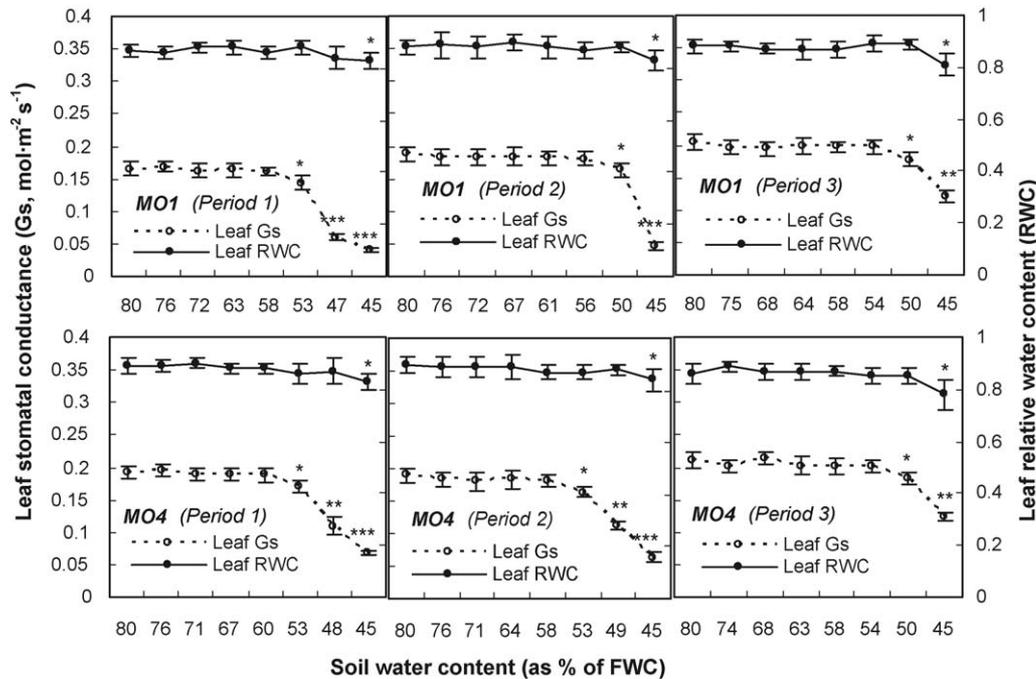


Fig. 1. Soil moisture trend in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of two diploid wheat (*MO1* and *MO4*). \*, \*\* and \*\*\* significantly different from the well-watered treatments (FWC80 groups) at  $p=0.05$ , 0.01 and 0.001, respectively. Points are the means of 45 and 30 observations  $\pm$  S.E. for Gs and leaf RWC, respectively. (Diploid wheat).

equidistant from lights, and the foliar region used for measurement was marked with a waterproof marker pen to ensure that each measurement was accurately located at the same leaf position. During each of the three developmental stages, watering was simultaneously ceased in all well-watered pots at 6:00 am in the first morning. Throughout the drying episode of 168 h, stomatal conductance was measured every 2 h from 8:00 am to 8:00 pm in the light period on three replicate readings for each leaf in each of five pots. Stomatal conductance was sampled in a specific order each day: from the first leaf in first pot to the 10th leaf in the fifth pot. Once 10 plants of first wheat cultivar had been sampled this way, the pattern was repeated until all replicates of each cultivar were measured. Each day the cultivar order and treatment sampling order were maintained. Replicates were blocked in this way, so that within each block, one setoff replicates was always measured within about 10 min of one another, minimizing confounding effects arising from any possible physiological changes throughout the day.

At the same time of Gs measurement leaf RWC was determined in lights period every 4 h from the first 8:00 am throughout each test period of 168 h. From the remaining 21 plants in each same pot as used in Gs measurement, two plants were randomly chosen and the most recently expanded leaf was then selected from each selected plant. Afterwards, three leaf discs (5 mm in diameter) were cut with a cork borer from each selected leaf, and weighed immediately for FW. The discs were floated in distilled water for 6 h under about  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (Turner, 1981), blotted with bibulous paper, and weighed to obtain TW. Dry weight (DW) was measured after drying the discs at  $70^\circ\text{C}$  in a forced-air oven for 24 h. The relative water content was cal-

culated as  $\text{RWC} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100\%$  (Turner, 1981). The total number of harvested leaf discs was six in each 4-h sampling circle, and the RWC of each disc was measured, respectively.

### 2.3. Comparison of drought tolerance among species

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

Lethal soil water content (LSWC) was measured on soil samples extracted from the root zone, 18–25 cm below the soil surface near the center of the pot. Soil water content (SWC) was defined following the previous description. The pots were weighed simultaneously as the plants in pots were dried, which were used to calculate the lethal soil water content (LSWC) for each cultivar. This trial was to compare the tolerance of the six wheat species to drought. There were 63 pots; 54 pots were

in the monoculture group (6 species  $\times$  3 replications  $\times$  3 test periods) and nine pots were in the mixed cropping group (3 replications  $\times$  3 developmental stages)—all arranged in a randomized block design.

#### 2.4. Comparison of grain yield among species under soil drought stress

The relationship between soil water potential and soil water content was used to estimate the extent of drought treatment. In this study, the soil water characteristics could be concluded according to different soil water levels: 80, 70, 60 and 45 soil moisture percentage. These represented well-watered soil (CK, 80% FWC), mild stress (MS, 70% FWC), intermediate stress 2 (IS, 60% FWC), and severe stress (SS, 45% FWC) (Table 2). This trial was performed under monoculture condition. The effect of drought was estimated by comparing the grain yield in two groups of plants. One group maintained at 80% soil FWC and a second group were held at a soil FWC of about 60% from the jointing stage onwards. The pots were weighed each day and when they fell below designed weights equivalent to soil FWCs, set but different quantities of water were added to each pot. After grain was filled, the yield was harvested for each pot. Maintenance ratio of grain yield (MRGY) was used to judge grain stability as follows:  $MRGY = Y_s/Y_{ck}$  ( $Y_s$  and  $Y_{ck}$  = grain yield under stress and non-stress conditions, respectively). There were 60 pots (2 groups  $\times$  6 species  $\times$  5 replications) arranged in a randomized block design.

#### 2.5. Judgment about non-hydraulic root-sourced signal (NRS)

The data collected for repeatedly measured plants was used to develop relationships between leaf RWC, Gs and soil water content. 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). This judgment criterion was to evaluate the threshold of soil water content (TSWC) at which NRS started to appear. The threshold range (TR) was the difference between TSWC and LSWC (TR = TSWC – LSWC).

Table 2

The classification of varying drought stress according to the soil suction and soil water content

Water treatments	Soil water content (%FWC)	Soil suction (kPa)
Severe stress (SS)	45	1012.6 $\pm$ 93.5
Intermediate stress (IS)	60	472.5 $\pm$ 93.9
Mild stress (MS)	70	335.8 $\pm$ 101.4
Sufficient water (CK)	80	227.5 $\pm$ 110.3
Soil bulk density (g/m <sup>3</sup> )	1.22	1.22

Based on the statistical analysis of correlation between soil suction and soil FWCs in varying soil moisture levels, a model curve equation between them for standard sample soil is developed:  $Y = 168.28X^{-1.9628}$ ,  $Y$  is soil suction (kPa) and  $X$  is soil water content (%FWC);  $R^2 = 0.9035^{**}$  ( $p < 0.01$ ). And three drought-stress gradients were developed to describe the characteristics of soil used in experiment, including 45% FWC, severe stress (SS) 60% FWC, intermediate stress (IS) and 70% FWC, mild stress (MS).

#### 2.6. Statistical analysis

Treatments were arranged in a split, split, split plot, completely randomized design. Species were the main-plot factors, soil water contents (SWC) the subplot factors, and leaf RWCs or Gs the sub-subplot factors. Three-way factorial analyses of variance (ANOVA) were used in order to determine the statistical significance of changes that occurred in leaf RWC and Gs in response to: (i) cultivar, (ii) SWC level. Means of leaf RWC and Gs were calculated on 45 and 30 replications for each 'cultivar  $\times$  SWC' combination, respectively, and compared by least significant difference (LSD) at the 0.05 confidence level. ANOVA 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 not a significant change in leaf RWC but being significant decrease in leaf Gs compared to the leaf RWC and leaf Gs in FWC80 group, respectively. The SWC at the occurrence of NRS was termed the upper limit. The LSWC was termed as the lower limit. The threshold range (TR) was the difference between the upper limit and lower limit. The ultimate means of survival days (SD) was calculated on nine replications from the SDs in three test periods for each cultivar, respectively (3 replications  $\times$  3 test period = 9 replications). The maintenance ratio of grain yield (MRGY) was the percentage of grain yield per pot in the stress groups relative to that in well-watered group (FWC80 group) (3 replications). Regression and correlation analyses were used to describe relationships between TR and SD, and MRGY, respectively.

### 3. Results

#### 3.1. Stomatal conductance (Gs) and leaf relative water content (RWC) versus soil water content (SWC)

In order to determine the changes of leaf Gs versus various soil water regimes, a continuous monitor was performed on the selected wheat leaves within 7 days in each of three test periods. A statistical variance analysis was made among varying soil moisture from 80% field water capacity (FWC) to 45% FWC, and the 80% FWC soil (termed as FWC80, the same below) was taken as the sufficient watering group. The stomatal responses to drought differed among species over all three test periods. For all six wheat species in the sufficient watering group, the leaf Gs ranged from 0.16 to 0.3 mol m<sup>-2</sup> s<sup>-1</sup>. And for each wheat species, Gs tended to rise with growth stage (Table 3). Figs. 1–3 show Gs of six wheat species, grouped by progressively dried SWC. At about 75% FWC, Gs remained relatively stable for all the six species in three test periods, without significant changes with respect to the values of FWC80. However, with the aggravation of soil drying, drought decreased Gs significantly in all species.

In first test period (Period 1), *Plateau 602* and *Longchun 8139-2* (modern hexaploid cultivars) responded earliest to soil drought, having a significant decrease in Gs in about 70% FWC soil (Fig. 3). *MO1* and *MO4* (diploid species) had the latest response to drought, not having a significant decrease in Gs

Table 3  
Leaf stomatal conductance (Gs) and relative water content (RWC) of six wheat crops over three test periods under the well-watered condition

Species name	Leaf Gs ( $\text{mol m}^{-2} \text{s}^{-1}$ )			Leaf relative water content (RWC)		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
<i>MO1</i>	$0.165 \pm 0.011$	$0.189 \pm 0.013$	$0.204 \pm 0.014$	$0.87 \pm 0.022$	$0.88 \pm 0.031$	$0.88 \pm 0.027$
<i>MO4</i>	$0.193 \pm 0.012$	$0.19 \pm 0.011$	$0.21 \pm 0.013$	$0.89 \pm 0.031$	$0.88 \pm 0.032$	$0.86 \pm 0.038$
<i>DM22</i>	$0.202 \pm 0.011$	$0.218 \pm 0.013$	$0.221 \pm 0.015$	$0.88 \pm 0.028$	$0.9 \pm 0.033$	$0.86 \pm 0.039$
<i>DM31</i>	$0.25 \pm 0.013$	$0.224 \pm 0.014$	$0.233 \pm 0.012$	$0.86 \pm 0.024$	$0.89 \pm 0.03$	$0.88 \pm 0.031$
<i>Plateau 602</i>	$0.278 \pm 0.014$	$0.242 \pm 0.016$	$0.27 \pm 0.016$	$0.88 \pm 0.031$	$0.9 \pm 0.028$	$0.9 \pm 0.027$
<i>Longchun 8139-2</i>	$0.282 \pm 0.011$	$0.268 \pm 0.018$	$0.288 \pm 0.017$	$0.89 \pm 0.025$	$0.89 \pm 0.031$	$0.89 \pm 0.031$

until SWC dropped to 53% FWC (Fig. 1). *DM22* and *DM31* (tetraploid species) were intermediate, whose Gs significantly decreased in 61% and 58% FWC (Fig. 2). The variation of Gs in Periods 2 and 3 were mostly similar to that 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 than in Period 1. Based on the results of three test periods, the means of the critical SWCs at which a significant decrease in Gs occurred were calculated for each cultivar. *Plateau 602* and *Longchun 8139-2* had the relatively high critical SWC, with 68.7% and 67.3%, respectively. The critical SWCs in *MO1* and *MO4* were the lowest, 51% and 52% for both of them, respectively. *DM22* and *DM31* were intermediate, with 57.3% and 54.7%, respectively (Figs. 1–3).

On the other hand, Gs would decline more swiftly after the occurrence of significant decrease in Gs in all species. However, the extent of decrease in wild diploid species (*MO1* and *MO4*) was more than other four species (Figs. 1–3). For all species, the extent of inhibition in Gs was linked to the extent of decline in SWC after water withholding in pots. Table 4 demonstrated the

reduction ratio of stomatal conductance (RRSC) of six wheat species as a function of the decrease in soil water potential from  $-0.2$  to  $-1$  MPa (be equivalent to: from  $\geq 80$  to 45% FWC). For *MO1*, the reduction ratio in Gs were the highest, with 67%, compared with the values in FWC80. *Plateau 602* and *Longchun 8139-2* had the lowest reduction ratio, 39% and 38%, respectively. Other two species were in the middle, with 59%, 49% and 56% for *MO4*, *DM22* and *DM31*, respectively (Table 4). It can be argued that *MO1* and *MO4* had the highest stomatal sensitivity, and two modern hexaploid cultivars the lowest stomatal sensitivity.

SWC-course of changes in leaf RWC was monitored in the same time pattern as the measurement of Gs. Leaf RWC always exceeded 80% in the six species when the soil was well-watered (Table 3). Withholding water did not change the high leaf RWC. Leaf RWC stayed high till the SWC dropped to no more than 50% FWC for all species throughout three test periods. Plots of leaf RWC versus soil moisture content depicted a leaf RWC of 80–90% within the soil moistures range from 80 to 45% FWC (Figs. 1–3). Across all the three test periods, a significant decline

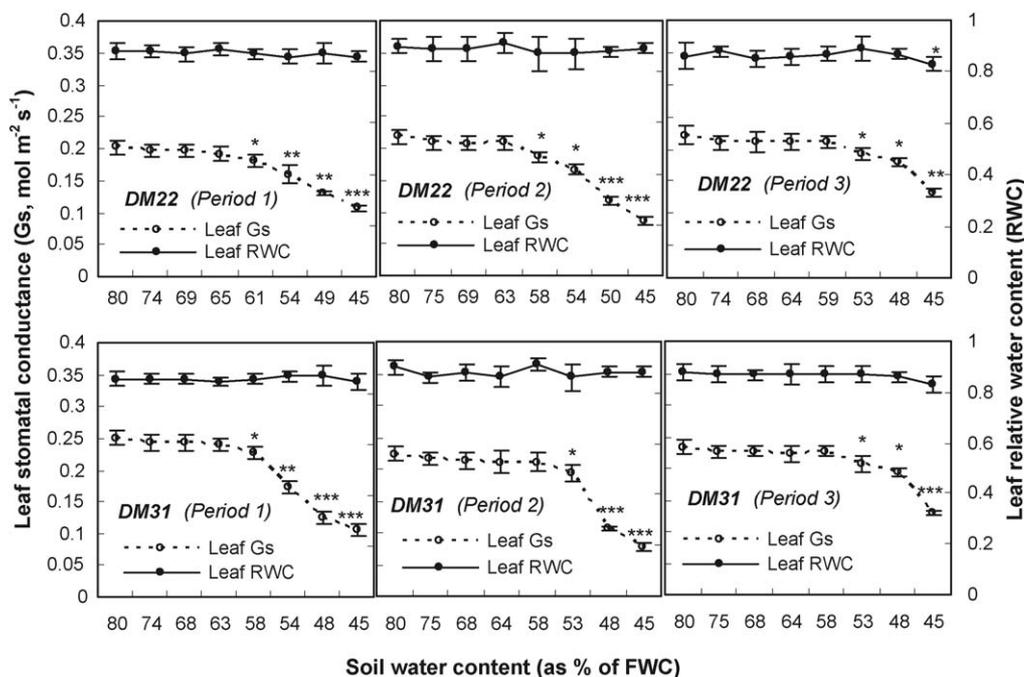


Fig. 2. Soil moisture trend in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of two tetraploid wheat (*DM22* and *DM31*). \*, \*\* and \*\*\* significantly different from the well-watered treatments (FWC80 groups) at  $p=0.05$ ,  $0.01$  and  $0.001$ , respectively. Points are the means of 45 and 30 observations  $\pm$  S.E. for Gs and leaf RWC, respectively. (Tetraploid wheat).

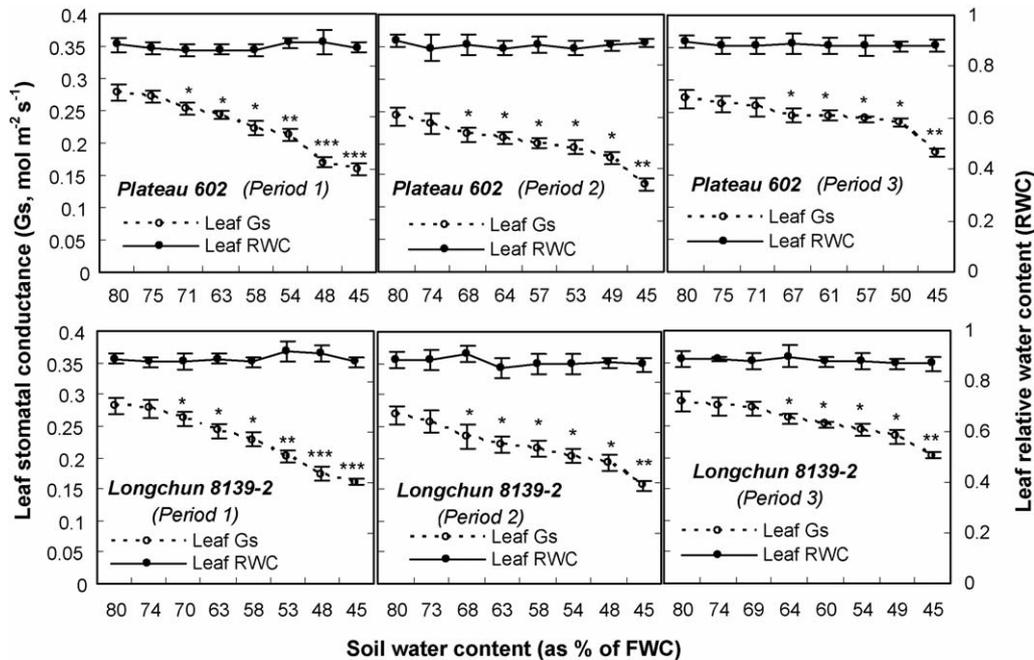


Fig. 3. Soil moisture trend in leaf stomatal conductance (leaf Gs, indicated by open circles) and leaf relative water content (leaf RWC, indicated by filled circles) of two modern hexaploid species (*Plateau 602* and *Longchun 8139-2*). \*, \*\* and \*\*\* significantly different from the well-watered treatments (FWC80 groups) at  $p=0.05$ ,  $0.01$  and  $0.001$ , respectively. Points are the means of 45 and 30 observations  $\pm$  S.E. for Gs and leaf RWC, respectively. (Hexaploid wheat).

in leaf RWC occurred only in *MO1* and *MO4*, till the SWC dropped to about 45% FWC, and but no significant decline can be found in leaf RWC for *Plateau 602* and *Longchun 8139-2* in all the SWC levels (Figs. 1 and 3). For *DM22* and *DM31*, a significant decline occurred in 45% FWC only in Period 3, and no obvious changes were found in the former two periods (Fig. 2).

### 3.2. The threshold of soil water content (TSWC) at which the non-hydraulic root-sourced signal (NRS) appeared

As proposed originally by Blackman and Davies (1985), the NRS was defined as stomatal conductance decreased signifi-

cantly without significant decrease in leaf water status in plants subjected to drought stress. As described above, Figs. 1–3 show the variation in stomatal conductance and leaf RWC at varying soil water content. Stomatal conductance, Gs, generally decreased for plants in successively lower soil FWCs often without a significant change in leaf RWC (Figs. 1–3). That suggested that the NRS began to appear, according to the root-to-shoot communication theory. The critical value of soil moisture at which the NRS was triggered was taken as the threshold of soil water content (TSWC). It can be evident from Fig. 4A that the same-ploid wheat cultivars had the similar TSWCs, but the different-ploid cultivars differed in their TSWCs. Meanwhile, the TSWCs tended to decrease from Period 1 to Period

Table 4  
Comparison of drought tolerance parameters and stomatal sensitivity among six wheat species

Diploids	Species name	Drought tolerance parameters			
		Mixed-cropping survival days	Monoculture survival days	MRGY	RRSC
Diploid	<i>MO1</i>	5.7 $\pm$ 0.6 a	6.3 $\pm$ 0.6 a	0.47 $\pm$ 0.11 a	0.67 $\pm$ 0.11 a
	<i>MO4</i>	6.3 $\pm$ 1.2 a	6.7 $\pm$ 0.6 a	0.48 $\pm$ 0.13 a	0.59 $\pm$ 0.12 b
Tetraploid	<i>DM22</i>	8.3 $\pm$ 0.6 b	8.3 $\pm$ 0.6 b	0.5 $\pm$ 0.08 a	0.49 $\pm$ 0.1 c
	<i>DM31</i>	9.3 $\pm$ 0.6 c	11.3 $\pm$ 1.5 c	0.43 $\pm$ 0.14 a	0.56 $\pm$ 0.11 b
Hexaploid	<i>Plateau 602</i>	13.3 $\pm$ 1.5 d	14.3 $\pm$ 0.6 d	0.66 $\pm$ 0.07 c	0.39 $\pm$ 0.07 d
	<i>Longchun 8139-2</i>	12.7 $\pm$ 1.2 d	13.3 $\pm$ 1.5 d	0.74 $\pm$ 0.08 d	0.38 $\pm$ 0.08 d

Notes: Drying experiments were conducted at each of the three different development stages under two culture methods; the days to reach a leaf RWC at which permanent wilting occurred was determined by repeated measures on the plants ( $n=9$ ). Maintenance ratio of grain yield (MRGY) is the grain yield per pot in stress group relative to that in well-watered group ( $n=3$ ). Reduction ratio of stomatal conductance (RRSC) of six wheat species was calculated as a function of the decrease in soil water potential from  $-0.2$  to  $-1$  MPa, be equivalent to: from  $\geq 80$  to 45% FWC ( $n=9$ ). Each data was the mean  $\pm$  S.E. All analysis was done among species within the same column, and numbers followed by the same letter are statistically similar, Duncan's Multiple Range Test ( $p < 0.05$ ).

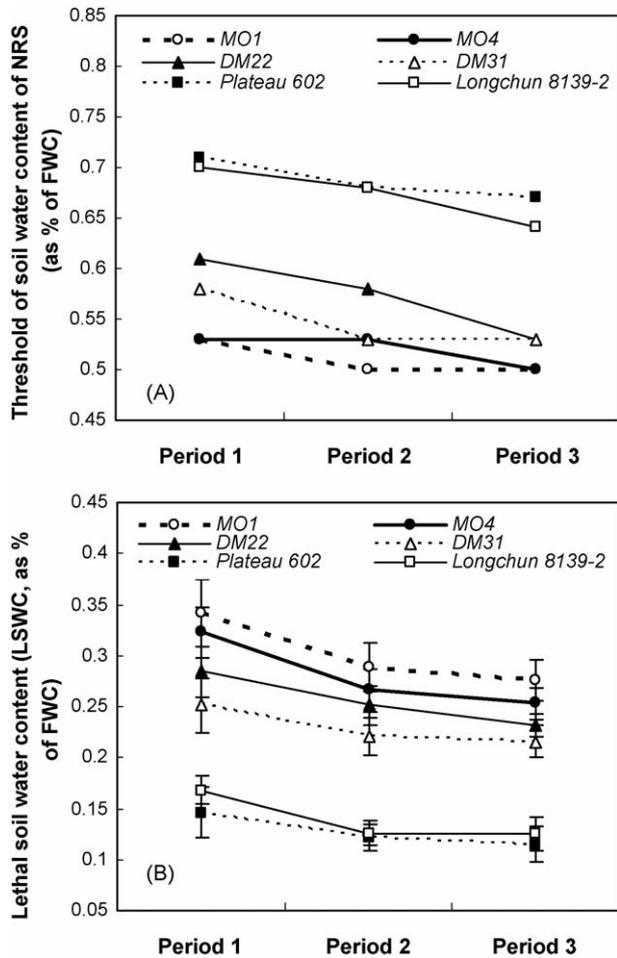


Fig. 4. The thresholds of soil water content (TSWC) at which the non-hydraulic root signal was triggered (A), and lethal soil water content (LSWC, B) for six wheat species among three test periods. Based on Figs. 1–3, the non-hydraulic ‘early-warning’ response (NEWWR) was operationally characterized by the appearance of NRS, expressed using the TSWC. The TSWC in two diploid species (*MO1* and *MO4*) were the lowest, and the modern hexaploid species, *Plateau 602* and *Longchun 8139-2*, was the highest over three test periods. The LSWC is the critical value of soil water content at which wheat plants were dried. The number above the bars indicated the means of LSWC in three test periods ( $n=9$ ).

3. There existed a tendency for the TSWCs to be about 5% lower in Periods 2 and 3 than in Period 1 (Fig. 4A). In this study, the results in three test periods were viewed as three statistical replicates. Based on the SWC-course of changes in leaf Gs and leaf RWC, the TSWC for each cultivar was achieved. The TSWC differed amongst species (Figs. 4A and 5); the TSWCs for diploid species (*MO1* and *MO4*) were 51% and 52%, respectively, for tetraploid *DM22* and *DM31* were 57.3% and 54.7%, and hexaploid cultivars *Plateau 602* and *Longchun 8139-2* were 68.7% and 67.3%, respectively (Fig. 5). Therefore, the onset of NRS was earlier in *Plateau 602* and *Longchun 8139-2* than *DM22* and *DM31*, and in *MO1* and *MO4* was the latest.

From Figs. 1–3, it can be seen that the NRS was maintained over lower soil FWCs for the six species, suggesting that the NRS would be continuously operative, depressing Gs. This non-hydraulic ‘early-warning’ response (NEWWR) would exert an

important function in regulate the adaptation of crops to drying soil.

### 3.3. Lethal soil water content (LSWC) and the threshold range (TR) from TSWC to LSWC

LSWC is used to classify the relative dehydration tolerance among species. Fig. 4B ranks the six spring wheat species in terms of LSWC. Mean LSWC across three test periods accordingly varied among species. The highest LSWC occurred in *MO1* and *MO4*, 30.2% and 28.1%, respectively. *DM22* and *DM31* had relatively high LSWC, 25.6% and 22.9%, respectively. *Plateau 602* and *Longchun 8139-2* withstood the most dehydration, with plants not drying until SWC dropped to 12.8% and 14%, respectively, having the lowest LSWC (Fig. 5A). With respect to test periods, the mean LSWCs tended to decrease from Period 1 to Period 3 for all the six wheat cultivars (Fig. 4B).

In order to investigate the link of NRS to LSWC and other drought-tolerant parameters, we used the threshold range to determine the quantitative effect of NRS. At lower soil FWCs, leaf RWC did not significantly change, suggesting that the NRS was operative. As previously described, the threshold range (TR) was the difference of TSWC and LSWC (i.e.  $TR = TSWC - LSWC$ ). The TR for species was displayed in Fig. 5. Typically, diploid species had the narrowest range (20.8% and 23.9% for *MO1* and *MO4*, respectively), followed by *DM22* (31.7%) and *DM31* (31.9%), and the widest TR being *Plateau 602* (55.9%) and *Longchun 8139-2* (53.3%) (Fig. 5A). It can be concluded that modern wheat breeding evolved in the direction of a wide TR of soil FWCs between TSWC and LSWC.

### 3.4. Comparison of drought tolerance and yield stability amongst species

Survival comparison was made among six wheat species during three test periods. Table 4 exhibited the mean survival days of three test periods. Species, irrespective of culture method, differed in survival days (SD) (an index of drought tolerance). Diploid *MO1* and *MO4* dried first with about 6 days of average survival days (SD) after watering ceased. Hence, their drought tolerance appeared to be the poorest under drought stress (Table 4). In contrast, modern hexaploid *Plateau 602* and *Longchun 8139-2* survived longer (about 13 days after ceasing of water supply). *DM22* and *DM31* were intermediate with survival times of 8–11 days. SD also was slightly but not significantly longer in the mixed cropping group than in the monoculture group in each growth stage. Therefore, the general sequence of the six species was as follows: (*Plateau 602* and *Longchun 8139-2*) > (*DM22* and *DM31*) > (*MO1* and *MO4*) (Table 4).

The maintenance ratio of grain yield (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. Four wild species tended to be more adversely affected by drought stress than modern hexaploid cultivars, with the MRGY ranging from 43 to 50%, and no obvious

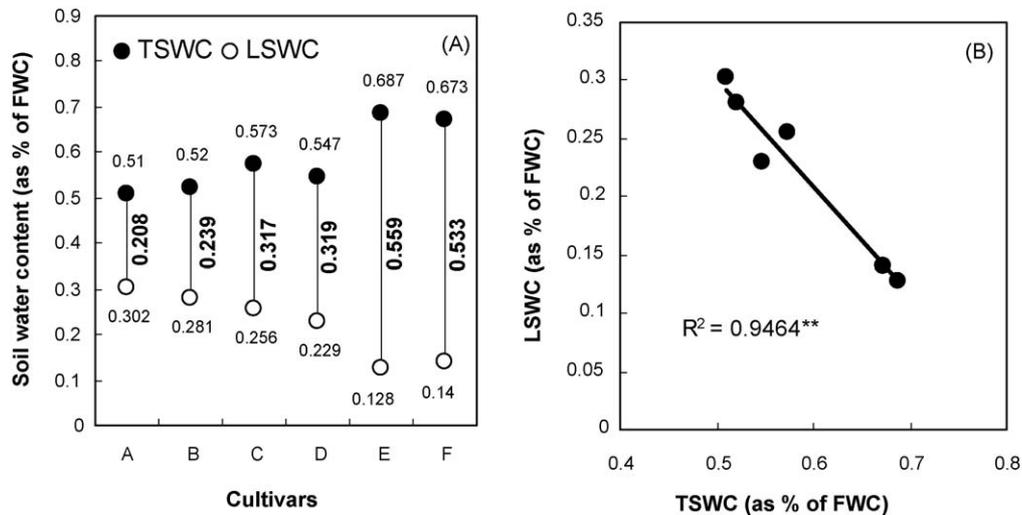


Fig. 5. The thresholds of soil water content (TSWC) at the onset of NRS and lethal soil water contents (LSWC) of six species (A), and the relationship between both of them (B). For each cultivar, the filled circles indicates the average TSWC, which is calculated from the means of the TSWCs in three test periods ( $n=3$ ). The open circles indicate the average LSWC of the values of three test periods for each cultivar ( $n=3$  replicates  $\times$  3 test periods = 9). The linear lengths from filled circle to open circle in figure demonstrate the threshold range (TR) between NRS and LSWC, which are indicated by vertical bold numbers for each cultivar. \*\* indicates significance of the correlation at  $p < 0.01$ . Generally, *MO1* and *MO4* had relative narrow threshold range with respect to four species. The species from A to F represented *MO1*, *MO4*, *DM22*, *DM31*, *Plateau 602* and *Longchun 8139-2*, respectively.

difference was found between diploids and tetraploids. *Plateau 602* and *Longchun 8139-2* were the least depressed, having MRGY of 66% and 74% (Table 4).

### 3.5. The links of threshold rang (TR) to SD and MRGY

Data were evaluated with General Linear Models Procedure (SAS, Cary, NC) and means separated by Duncan's Multiple Range Test ( $p=0.05$ ). Among species, the TSWC was significantly correlated with the LSWC ( $r=0.9464^{**}$ ,  $p < 0.01$ ) (Fig. 5B). There existed a linear significant correlation between TR and SD ( $r=0.9411^{**}$ ,  $p < 0.01$ ) (Fig. 6A), and TR and MRGY ( $r=0.8608^*$ ,  $p < 0.05$ ) (Fig. 6B).

## 4. Discussion

### 4.1. Non-hydraulic 'early-warning' response (NEWWR) and the adaptation of crops under drought stress

The NRS was extensively known as a unique and positive NEWWR under drought stress in plants. The onset of NRS that reduces the aperture of stomata, as indicated by reduction in  $G_s$  values, began at different soil FWCs according to cultivar. Signaling in diploid species (*MO1* and *MO4*) was the latest to emerge, about 51% FWC. Modern hexaploid cultivars (*Plateau 602* and *Longchun 8139-2*) were in the earliest response of about 68.7% and 67.3% FWC, respectively. Of intermediate

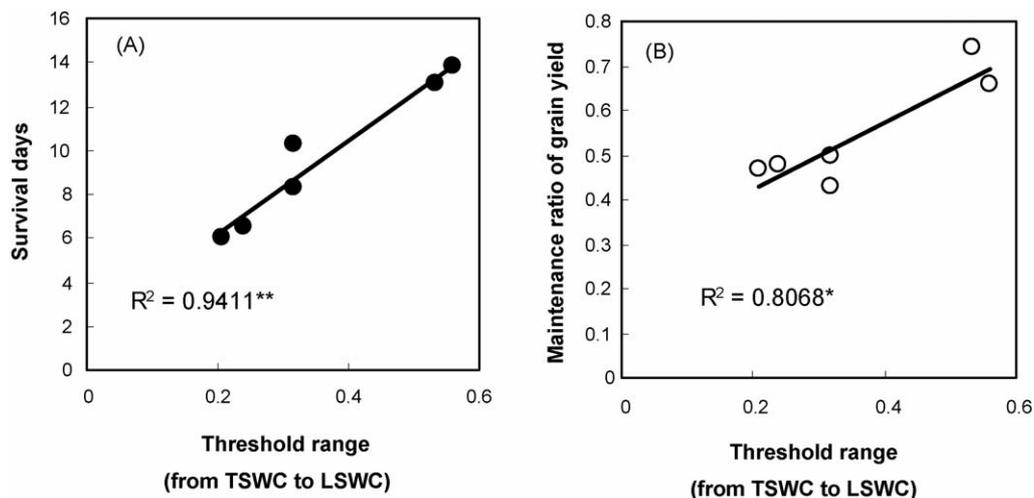


Fig. 6. Relationships between the threshold ranges (TR), survival days (SD) and maintenance ratio of grain yield (MRGY) across six wheat species. The TR refers to the difference between TSWC and LSWC (see Fig. 5 legend for explanation). Filled circle indicated the means of SD on the nine replicates in three test periods for each species. Open circle indicated the means of MRGY on the five replicates at harvest stage for each species. (A) and (B) indicate the correlation coefficient between SD and TR, and between TR and MRGY, respectively. \* and \*\* indicate significance of the correlation at  $p < 0.05$  and 0.01, respectively.

state (57.3% and 54.7%) were tetraploid *DM22* and *DM31* (Fig. 5A).

The chief purpose of this study was to characterize and compare the NEWR sensitivity of the six wheat species to soil drying, and then to explore their eco-physiological significance. The triggered timing of the NRS and lethal point appeared to determine plant's drought tolerance. The LSWC was an important parameter closely related to drought tolerance. Those species with high TSWC tended to have low LSWC, and the correlation coefficient between TSWC and LSWC was 0.9464<sup>\*\*</sup>. Therefore, the threshold range (TR, i.e. TSWC – LSWC) can be used to assess the ecophysiological significance of NEWR in relation to drought tolerance and yield performance. Perhaps the best gauge of TR to soil drying is the slopes of TR/SD and MRGY regressions. Slopes were significantly correlated with TR; correlation analysis yielded the correlation coefficient of 0.9411<sup>\*\*</sup> with SD, and 0.8068<sup>\*</sup> with MRGY, respectively. Those species with wider TR generally had longer SD and higher MRGY (Table 4; Figs. 5 and 6). SD and MRGY were two critical drought-tolerant physiological parameters. Plant's dehydration tolerance is the extent of water loss a plant can withstand before most of its leaves dies (Sinclair and Ludlow, 1986; Chapman and Augé, 1994). The survival was closely correlated with plant growth in dry relative to irrigated conditions (Engelbrecht and Kursar, 2003). Longer SD appeared to be closely related with higher MRGY.

Non-hydraulic root signals caused early drought adaptation at mild water stress by reducing leaf growth and stomatal conductance and hastening of heading and flowering; osmotic adjustment sustained turgor maintenance and hence the yield-forming processes during moderate and severe water stress (Ali et al., 1999). In our study, the LSWC was correlated with the SD; those plants whose foliage died at lower SWC took longer to dry down to those SWC. This result was consistent with that of Augé et al. (1998). Possibly, earlier onset of the NRS in root system would provide the shoot with more efficient adaptive mechanism, involving better osmotic adjustment and antioxidant potential, etc. Therefore, those species with higher TSWC tended to have lower LSWC. As soil begins to dry during the initial stages of drought, the responses of roots are critical to the survival of the plant. An early response to the imposition of low water potential is  $E_m$  hyperpolarization, involving activation of the plasma membrane  $H^+$ -ATPase (Ober and Sharp, 2003). This response may be triggered by turgor-sensitive stretch-activated membrane channels or by other osmo-sensing-elements (Lew, 1996a, 1996b). During long-term exposure to low water potential, there exists a steady-state change in resting potential. This response may be part of the primary signals that induce other processes necessary for growth maintenance (Ober and Sharp, 2003). The earlier respond for plants to drought, the better osmotic adjustment they will have and in turn the better drought tolerance in them. With respect to the TR of 'early-warning' response, modern species with a high TSWC had accordingly a low LSWC. The wide TR of soil FWCs in non-hydraulic 'early-warning' response (NEWR) was a critical trait for plants to adapt to the droughted conditions. In this study, yield performance of six wheat species under long-term mild drought stress was a result of differentiate adaptation to drought in the NEWR. The

NEWR played an important role in improving drought tolerance and yield performance in crops cultivated in drought soil.

#### 4.2. Stomatal sensitivity and drought tolerance

Stomatal behaviour is often the most sensitive indicator (Smith and Hollinger, 1991), and the magnitude of  $G_s$  declines appears to be a function of the magnitude of soil moisture declines (Crocker et al., 1998). Stomatal conductance sensitivity to the NRS was characterized as the decline in  $G_s$  per unit decline in soil water potential. Old species having higher stomatal sensitivity had the less drought tolerance (less SD and lower MRGY, see Table 4). Stomatal sensitivity to the NRS may be mechanistically linked to drought tolerance (Crocker et al., 1998).

Increasing drought tolerance was significantly correlated with increasing capacity for osmotic adjustment. Morgan and co-workers (Morgan, 1977, 1983, 1995; Morgan et al., 1986) in Australia have shown that wheat lines selected for high osmotic adjustment significantly out-yielded those not adjustable actively under drought conditions. Modern species with low stomatal sensitivity possessed stronger osmotic adjustment and then had longer survival times than old species. And their MRGY were less affected by the drought stress in soil, thus showing stronger drought tolerance. Also, the species having the highest stomatal sensitivity also had the least dehydration tolerance (Table 4). This result was consistent with that of Crocker et al. (1998).

#### 4.3. Ecophysiological significance of the NEWR

Chemical signal materials originated from root, i.e. NRS materials, had been extensively studied in a large number of plant species (Wilkinson and Davies, 2002; Passioura, 2002; Eric and Robert, 2003). It has long been apparent that the NRS materials including ABA (Comstock and Mencuccini, 1998), calcium ions (McAinsh et al., 1990), apoplastic pH (Patonnier et al., 1999), cytokinins (Stoll et al., 2000), and nitric oxide (Desikan et al., 2004) played an important role in 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 accumulation of root-sourced NRS materials induced by soil drought stress. Wild wheat species, having a narrow TR, were found to have a high non-hydraulic stomatal sensitivity (Table 4; Fig. 5A). It can be argued that although wild species generally responded inactively to drought stress, they were to rapidly and massively synthesize NRS materials in root system. This signaling was transported to shoot swiftly once their urgent early-warning reaction of NRS was triggered. That increased the accumulation of NRS materials, which cannot be decomposed or sequestered in time in shoot. In so doing, the NRS effect tended to be excessive, and in turn to inhibit the shoot growth and gas exchange. Consequently, the wild species exhibited a worse plant growth and weaker drought tolerance (Table 4). This idea was supported by the differentiate LSWC between wild and modern species (Fig. 5A). Wild species were generally unresponsive to soil water deficiency and are 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 believed that there existed a negative effect between strong root signaling and crops production.

In another word, there existed a sort of signal redundancy similar to growth redundancy proposed by Donald and Hamblin (1983) in wild species, possibly because the NRS materials produced were excessive in view of crop production. However, the case of modern species was opposite to that of old species. They were able to make a quick and timely response to mild drought stress, i.e. it is more possible that they synthesized the moderate and suitable amount of NRS materials. Recently, ‘crosstalk’, a new conception about the response of plant to abiotic attack, was put forward to describe the complexity and multi-pathway of signaling (Taylor et al., 2004). The overall effect of the early-warning NRS was also expressed through NRS materials or pathways. Over the anti-drought breeding history of wheat, the intensity of early-warning NRS was bearing a weakening process, because the effect of NRS function was being ‘diluted’ with the TR being widened.

#### 4.4. The NEWR and anti-drought breeding of wheat crops in semi-arid area

Wheat is a staple food for more than 35% of the world population and is also the second grain crop in China. Its production status is directly related to social stability, the national economy, the people’s livelihood and sustainable development (Jing and Chang, 2003; Dai and Li, 2004). Our study revealed that those species having higher TSWC also had the lower LSWC. It suggested 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 in turn the signal of modern species is lasted longer but weaker, than in wild species.

Modern anti-drought breeding in wheats had widened the operation of this ‘early-warning’ process. 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 (Bruce et al., 2002). In a sense, the current breeding of drought-tolerance species has been led astray (Passioura, 1983). In the process of modern plant breeding, selecting species for wider thresholds in the NRS trait has occurred unwittingly. It is time to begin breeding purposefully for higher drought tolerance in wheat for semi-arid environments; this should be based on selection for a wider appearance of non-hydraulic ‘early-warning’ response in drying soil. On the other hand, it is possible, then, that examination of a larger group of species with perhaps a broader range of species NRS versus soil water moisture would reveal that non-hydraulic ‘early-warning’ response is in fact linked with drought tolerance and yield performance.

## 5. Conclusion

Low water availability is one of the major causes for crop yield reductions affecting the majority of the farmed regions

around the world. In semi-arid areas, wheat genetic breeding would follow a certain direction. With the breeding decades preceding, the ability of drought tolerance tends to be strengthened. Modern species would gradually evolve in wide ‘early-warning’ TR of ‘high TSWC, low LSWC’ in the long-term evolution from wild species to modern ones. Under soil drying, the earlier crops respond to water deficit in soil, the later water status in shoot decrease. Wider TR from TSWC to LSWC is an important indicator of better drought tolerance, i.e. better osmotic adjustment or antioxidant defense. This result would provide breeders with a possible breeding direction to improve grain yield under drought stress.

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