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ECO-PHYSIOLGOICAL ROLE OF ROOT-SOURCED SIGNAL IN THREE GENOTYPES OF SPRING WHEAT CULTIVARS: A CUE OF EVOLUTION

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Abstract

Non-hydraulic root-sourced signal (nHRS) is so far affirmed to be a unique and positive 'early-warning' response of plant to drying soil, but its functional role and potential evolutionary implication is little known in dryland wheat. Three spring wheat cultivars, *Monkhead* (1940-1960s), *Dingxi 24* (1970-1980s) and *Longchun 8139* (1990-present) with different drought sensitivity were chosen as materials for the research. Physiological and agronomic parameters were measured and analyzed in two relatively separated but closely related trials under environment-controlled conditions. The results showed that characteristics of nHRS and its eco-physiological effects varied from cultivars. Threshold ranges (TR) of soil moisture at which nHRS was switched on and off were 60.1-51.4% (% of FWC) in *Monkhead*, 63.8-47.3% in *Dingxi 24* and 66.5-44.8% in *Longchun 8139* respectively, suggesting that earlier onset of nHRS took place in modern cultivars. Leaf abscisic acid (ABA) concentration was significantly greater and increased more rapidly in old cultivars, *Monkhead* and *Dingxi 24* than that of *Longchun 8139* during the operation of nHRS. As a result of nHRS regulation, maintenance rate of grain yield was 43.4%, 60.8% and 79.3%, and water use efficiency was 1.47, 1.65 and 2.25 g/L in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively. In addition, drought susceptibility indices were 0.8858, 0.6037 and 0.3182 for the three cultivars, respectively. This suggests that earlier trigger of nHRS led to lower ABA-led signal intensity and better drought adaptability. It can be argued that the advances in yield performance and drought tolerance might be made by targeted selection for an earlier onset of nHRS. Finally, we attempted developing a conceptual model regarding root-sourced signal weakening and its evolutionary cue in dryland wheat.

Introduction

Drought stress is one of the most important limiting factor determining plant growth and crop productivity worldwide. As water resources for agronomic use has become more limited, the development of drought-tolerant crop lines becomes increasingly important (Donald & Hamblin, 1983; Kitchen *et al*., 1999; Wesley *et al*., 2002; Li *et al*., 2011). Over last few decades, a number of attempts have been made by physiologists and breeders; however most of them led to failure (Boyer *et al*., 1975; Laing & 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 critical traits to select (Richards, 1996; Khan *et al*., 2001). Understanding the crops' mechanism of adaptation to drought stress in stress-prone environments will provide opportunities to improve the breeding process (Khan *et al*., 2001; Wesley *et al*., 2002). The most sensitive indicator of plants' overall physiological state is often the stomatal behavior (Smith & Hollinger, 1991). Stomata rapidly respond to the changes in soil drying, allowing plants to balance water loss with carbon uptake during periods of reduced soil moisture (Croker *et al*., 1998).

Currently, one of the most widely acknowledged and keenly attended advances is shoot-root communication theory. Since Blackman and Davies in 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 & Davis, 1993; Croker *et al*., 1998; Mingo *et al*., 2003; Xiong *et al*., 2006ab and 2007; Li *et al*., 2011). Reduced stomatal conductance (Gs) can occur in plants grown in drying soil in which shoot water status is held constant (Gollan *et al*., 1986; Gowing *et al*., 1990; Fan *et al*., 2008, 2009; Wang *et al*., 2008; Noorka *et al*., 2013), which is considered to be a non-hydraulic regulatory mechanism. Non-hydraulic root-sourced signal (nHRS) enables plants to 'sense' drought in the roots and is expressed as an alteration of growth or Gs in the leaves (Davis & Zhang, 1991; Gowing *et al*., 1990; Blum & Johnson, 1993; Gallardo *et al*., 1994; Li *et al*., 2000; Li *et al*., 2011). 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 & Johnson, 1993; Tardieu & Davies, 1993; Comstock, J.P., 2002; Fan *et al*., 2008), lowers stomatal conductance and weakens gas exchange with the atmosphere that eventually retards plant growth. It is at the commencement of the hydraulic root-sourced signal (HRS), that retardation of plant growth is furthered.

Non-hydraulic 'early warning' response likely varies among species, as in other drought response characteristics (e.g. osmotic adjustment and leaf rolling). In terms of quantitative assessment on nHRS characteristic, threshold range (TR) of soil water contents (SWC) at which nHRS and HRS are triggered successively is a critical physiological indicator. Our previous studies showed that the width of TR was positively associated with survival days (under the condition of water withholding), lethal leaf water potential and grain yield in dryland wheats (Xiong *et al*., 2006abc, 2007) and oat crops (Gong *et al*., 2010). Early trigger of nHRS played a critical role in activating PM H-ATPase and thereafter improving the ability of osmotic modulation in plant (Gong *et al*., 2010). However, the dynamics of nHRS intensity and its functional role in affecting drought susceptibility index, water use and yield formation were not determined and analyzed in our previous study. The nHRS is so far affirmed to be a unique 'early-warning' response of plant to drying soil, but little is known about the functional role of this 'early-warning' signal and its potential evolutionary implication. It is well known that abscisic acid (ABA) is the major root-sourced signal substance (Donald & Hamblin, 1983; Blackman & Davies, 1985; McAinsh *et al*., 1990; Hartung *et al*., 1998; Li *et al*., 2011). When the root system senses the drought stimuli of soil, ABA will be synthesized massively and transferred from root to leaf via the stem transportation system (Xiong *et al.*, 2006c). Leaf ABA plays a pivotal role in the control of stomatal aperture (Dodd *et al*., 2003; Latif, 2014). Yet, 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 & Davies, 1985; Wilkinson & Davies, 1997 and 2002; Hartung *et al*., 1998; Wigger *et al*., 2002; Xiong *et al*., 2006c; Schachtman & Goodger, 2008; Noorka *et al.*, 2013).

It is presumed that excessive ABA concentration may play a negative role in affecting plant growth, grain yield and water use. Based on this understanding, the judgment criteria on nHRS and HRS should be mainly focused on developing the relations among soil moisture regime, leaf water status and leaf Gs, in which leaf ABA concentration in leaves acts as a critical signal indicator. We therefore propose a hypothesis that during the process of artificial selection, chemical root-sourced signal may tend to be weakened gradually. Earlier trigger of nHRS might be a more positive and active response to soil drying, which would inhibit excessive biosynthesis of ABA, reduce the physiological injury caused by excessive ABA and accordingly lead to better performance in plant growth and yield formation (Ober & Sharp, 2003; Xiong *et al*., 2006c; Gong *et al*., 2010). We experimentally quantified soil water content threshold when nHRS and HRS appeared successively under drying soil for 3 different wheat cultivars bred for semi-arid croplands of China. Also, a few critical physiological parameters including drought susceptibility index, water use efficiency (in agronomic concept), yield formation, leaf ABA concentration and maintenance rate of grain yield were measured under the regulation of nHRS. With the view of root signal, evolvement of drought-resistant trait might follow a certain tendency in dryland wheat, which is likely to provide new insights on current crop breeding theory and application under arid and semiarid conditions.

Materials and methods

Plant material and experimental design: Three spring wheat cultivars including *Monkhead*, *Dingxi 24* and *Longchun 8139* were chosen from the semiarid dryland agricultural area of Loess Plateau, northwest China. *Monkhead* is old cultivar that has been extensively planted during the period from 1940s to 1960s while *Dingxi 24* and *Longchun8139* were released and used as major cultivars in semiarid northwest China from 1970-1990s and 1990-present respectively. *Monkhead* and *Dingxi 24* are the spring wheat cultivars with large root system but relatively high drought sensitivity. *Longchun 8139* is the cultivar with small root system but has strong drought tolerance. Seeds were vernalized at 4°C for one week, and germinated in an incubating cabinet. 18 seedlings were planted in plastic pots (20 cm diameter and 30 cm-depth) containing 10 kg of air-dried, steam sterilized, passed 0.5-mm sieve, sandy - loam soil (with 28.6% field water capacity) and vermiculite (2:1 v/v). Fourteen gram of N6: P20: K10 fertilizer in each pot was thoroughly mixed with the soil, to ensure that nutrient deficiency would not be a limiting factor (Cissé *et al*., 1996). Water was added at 2–3-day intervals to maintain field capacity until plants grew three leaves. Plastic film was placed on the soil surface to restrain evaporation. Two relatively separate but closely related trials in this study were designed as follows:

Trial 1: The assessment on physiological characteristics of root-sourced signal. Leaf Gs, relative water content and ABA concentration were determined and analyzed in the three cultivars. The booting stage was chosen to observe the physiological performance of nHRS since this stage is the most sensitive to drought stress. The chamber conditions were maintained as: day/night temperature 25°C/15°C; day/night relative humidity (RH), 45/60±5%; Photon flux density (PFD), 150 $\mu m^{-2}s^{-1}$ 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 20:00 pm). The data collected for repeatedly measured plants was used to develop relationships between leaf RWC, leaf Gs and soil water content. The nHRS was judged to begin when there was a significant lowering of leaf stomatal conductance without change in leaf RWC (compared with Gs in FWC 80%), and the HRS was judged to begin when there were significant difference for above leaf parameters (Xiong *et al*., 2006ab). This judgment criterion was used to evaluate the threshold of soil water content (TSWC) at which nHRS and HRS started to appear respectively. The threshold range (TR) was the difference in soil water contents between the beginnings of the nHRS and HRS respectively.

Trial 2: The assessment on functional role of root-sourced signal on agronomic traits. According to preliminary experiments, soil moisture was controlled at around 55% (±2.5%) field water capacity (FWC) to ensure that nHRS can insistently operate during the growing season for all three cultivars. Pot-culture experiment was conducted to evaluate the effects of root-sourced signal on plant growth, water use, drought susceptibility index and yield formation in greenhouse. The experimental location was the Experimental Station of Lanzhou University in Yuzhong County of Gansu ((35°56′34.47″N, 104°08′ 49.35″E; altitude of 1620 m). Experimental site was typical of semi-arid climate in northwestern China, with the rainfall of 229 mm, the evaporation of 703mm, mean temperature of 14.2° and relative humidity of 58% during the growing season. All plant cultures were performed in a greenhouse (50 m long × 24 m wide × 5.7 m high). At maturing stage, plant growth parameters (leaf area, plant height, root and shoot biomass, spike-related index, total water consumption, yield) were determined for further analyses.

Determination of soil water content (SWC), leaf Gs and relative water content: Soil water contents were determined gravimetrically every four hours by weighing pots at the start and end of the photoperiod throughout the whole drying period (Xiong *et al*., 2006ab). The SWCs at each treatment hours were calculated on the basis of following formula: SWC = (Wt – Wd –We – Wp) / (Wd•FWC)*100%, where Wt is the temporary whole pot weight, Wd the net weight of dried soil in pot, We the weight of empty pot, Wp the estimated fresh weight of all plants in the pot, and FWC the filed water capacity, respectively. The estimated fresh weight of all plants in one pot was determined in advance on extra pots at each test period. Leaf water relations and stomatal conductance were monitored for 3 d prior to the start of the drought treatments to ensure that constant conditions had been achieved after pots were transferred into growth chamber. All drying treatments lasted 168 h (7 days). Dying treatments started at 6:00 am on the first day. In order to facilitate to develop 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 (Xiong *et al*., 2006ab).

Six pots were used to measure stomatal conductance and leaf RWC for each cultivar, with the total of 18 pots (6 pot replications × 3 cultivars). 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 54 in each test period (3 leaf replications × 6 pots × 3 cultivars). Leaf 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 equidistant from lights, and the foliar region used for measurement was marked using marker pen to ensure that each measurement was able to be accurately located at the same leaf position. Watering was simultaneously ceased in all well-watered pots at 6:00 in the first morning. Throughout the drying episode of 168 h, stomatal conductance was measured every two hours from 8.00 am to 20.00 pm in the light period on 3 replicate readings for each leaf in each of 6 pots (Xiong *et al*., 2006ab).

At the same time of Gs measurement, leaf RWC was determined in light period every 4 h from the first 8:00 am throughout each test period of 168 h, am d. From the remaining 18 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, 3 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 mol 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% (Xu *et al.*, 2009). The total number of harvested leaf discs was 6 in each 4-h sampling circle, and the RWC of each disc was measured respectively.

Comparison of drought adaptability among cultivars: Drought susceptibility index (DSI) is defined as the below formula according to the concept of Fischer & Maurer (1978):

$$DSI = (1 - Yd / Yp) / D \qquad (1)$$

where Yd is grain yield of the genotypes under moisture stress condition. Yp is grain yield of the genotypes under irrigated condition. D is the extent of drought. Here, the D value is calculated as follows:

$$D = X / Yp \qquad (2)$$

where X is mean yield of all strains under moisture stress condition, and Xp is mean yield of all strains under irrigated condition.

The effects of nHRS on growth and grain yield were determined in well-watered group (CK) and stress group. CK group was maintained at 80% soil FWC and the stress group was held at a soil FWC of about 55% from the jointing stage onwards. The pots were weighed each day and when they fell below designed weights equivalent to soil FWC's, set but different quantities of water were added to each pot. After grain was filled, the plants were harvested, with spikes dried and weighed for each pot. Maintenance rate of grain yield (MRGY) was used to judge grain stability as follows: MRGY = Ys / Yck (Ys and Yck = yield under stress and non-stress conditions, respectively). There were 36 pots (2 groups x 3 cultivars x 6 replications) arranged in a randomized block design.

Statistical analysis: The data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez & Gomez (1984). The significance of differences among means was compared using Duncun's Multiple Range Test (DMRT) (Steel & Torrie, 1997). Regression analyses were used to describe the dynamics of leaf Gs and leaf RWC.

Results

Threshold range of soil water content at which root-sourced signal continuously operates: The characteristics of root-sourced signal varied from wheat cultivars under soil drying. As shown in the Figure 1, leaf Gs and RWC were significantly lowered at different critical points of soil moisture successively. Critical soil moisture at which Gs started to decrease significantly without detectable change in leaf water status was 60.1, 63.8 and 66.5 % of FWC in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively. It reflected an occurrence of nHRS at respective critical point for each cultivar. With further decrease in soil moisture, leaf RWC was observed to decrease significantly at 51.4, 47.3 and 44.8% FWC in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively (Fig. 1). The results showed that *Longchun 8139* as modern cultivar had the widest threshold range of soil moisture (44.8-66.5% FWC) at which nHRS was switched on and off. The threshold range was the narrowest in *Monkhead* (Fig. 1).

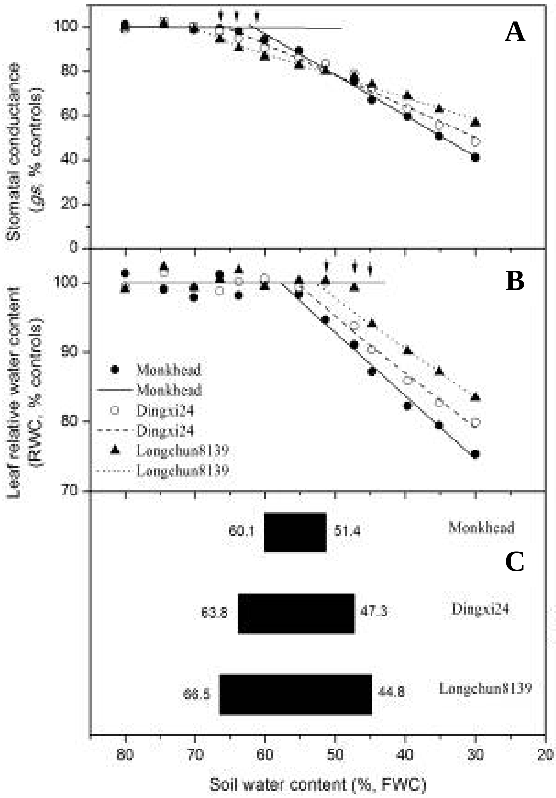

Fig. 1. Dynamics of leaf stomatal conductance (A), relative water content (B) and the threshold range of soil moisture during the operation of root-sourced signal (C) along with soil drying in three spring wheat cultivars.

The dynamics of leaf ABA concentration with the development of soil drying: Leaf ABA level tended to rise gradually under mild and intermediate drought stresses, but turned to decline under severe drought stress in three cultivars. Under well-watered condition, there was not significant difference in leaf ABA concentration among three cultivars. However, with soil drying, average leaf ABA concentration tended to increase rapidly, but the increasing scope was differed from the cultivars. In *Dingxi 24* and *Longchun 8139*, leaf ABA level was increased to 90.6 and 88.3 ng • g-1 FW on the critical point at which nHRS was switched on respectively. In *Monkhead*, the value ABA level was up to 118.1 ng •g-1 FW, being significantly greater than those of other two cultivars. When the soil moisture was decreased to critical point at HRS, the nHRS was switched off. At the point of HRS, leaf ABA concentrations were 231.3, 170.1 and 150.6 ng • g-1 FW in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively. Old cultivar, *Monkhead* experienced a more acute increase in leaf ABA biosynthesis than other two cultivars. Also, the ratio of increase in leaf ABA concentration in *Dingxi 24* was greater than that of *Longchun 8139*. With further drying of soil in pots, wheat plant was dehydrated to temporary wilting point (TWP) and permanent wilting point (PWP) successively (Fig. 2). From the HRS to TWP, both *Monkhead* and *Dingxi 24* were observed to increase ABA accumulation in leaves (254.8 and 178.3 ng • g-1 FW), while *Longchun 8139* turned to decrease acutely (81.3 ng • g-1 FW). At the last stage from TWP to PWP, leaf ABA concentration tended to decrease gradually (Fig. 2).

Leaf ABA concentration was significantly lower in *Longchun 8139* than those of *Dingxi 24* and *Monkhead* in all drought stress groups (Fig. 2). ABA as major physiological indicator of root-sourced signal proved to be stronger in *Monkhead* than that of *Dingxi 24*. Furthermore, *Longchun 8139* had the weakest signal intensity among three cultivars (Fig. 2). Finally, the increase in leaf ABA concentration lasted longer time till the onset of TWP in *Monkhead* and *Dingxi 24* in comparison with *Longchun 8139* (Fig. 2).

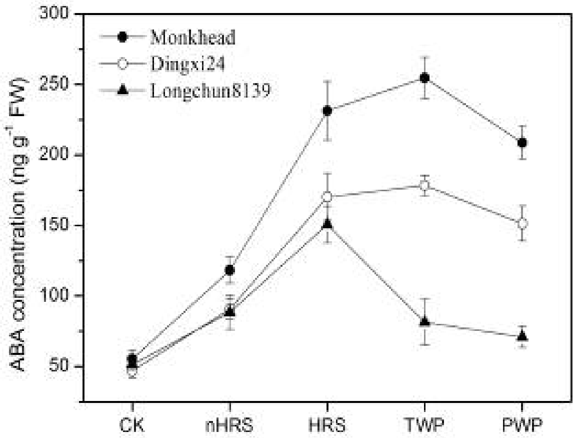

Fig. 2. The dynamics of leaf ABA concentration along with the development of soil drying in three spring wheat cultivars.

The effects of root-sourced signal regulation on plant growth in three spring wheat cultivars: In second trial of plant growth assessment (Trial 2), soil moisture was maintained within the threshold range during the operation of root-sourced signal. To doing so, we can assess the effects of nHRS on plant growth performance. Three major parameters of plant growth in CK and drought stress group were compared among the cultivars (Table 1). Generally, leaf area and plant height were decreased significantly under drought stress, however the extent of decrease varied from cultivars. Leaf areas were 31 and 38.1 cm^2 and plant heights were 75.7 and 91.1 cm in *Longchun 8139* in drought stress group and CK group respectively. This suggested that *Longchun 8139* had the least reduction rate in the two parameters. On the other hand, root-shoot ratios were increased in three cultivars as a result of drought stress treatment. Furthermore, *Longchun 8139* had the least root-shoot ratio, showing a typical trait of small root system (Table 1).

Table 1. The changes in plant growth and root-shoot ratio in three spring wheat cultivars under the regulation of root-sourced signal.

	Monkhead		Dingxi24		Longchun8139	
	Drought	**CK**	**Drought**	**CK**	**Drought**	**CK**
Leaf area (cm^2)	18.3 ± 2.5	26.7 ± 1.8	18.9 ± 2.1	26.9 ± 3.2	31.0 ± 2.8	38.1 ± 1.9
Plant height (cm)	81.4 ± 7.1	103.8 ± 6.8	62.2 ± 6.1	99.3 ± 10.5	75.7 ± 7.2	91.1 ± 6.9
Root-leaf ratio	0.88 ± 0.12	0.81 ± 0.03	0.72 ± 0.12	0.63 ± 0.07	0.43 ± 0.04	0.37 ± 0.16

Table 2. Yield formation, water use and drought susceptibility under the regulation of root-sourced signal in three spring wheat cultivars.

	Monkhead		Dingxi24		Longchun8139	
	Drought	**CK**	**Drought**	**CK**	**Drought**	**CK**
Spike length (cm)	8.18 ± 1.3	13.13 ± 1.3	7.5 ± 0.9	8.45 ±0.7	8.91 ± 0.9	9.54 ± 0.7
Spike weight (g)	1.42 ± 0.59	1.57 ± 0.42	1.31 ± 0.49	1.67 ± 0.52	2.6 ± 0.63	3.02 ± 0.66
Fertile spikelet number	11.2 ± 1.8	13.1 ± 1.3	11.4 ± 2.0	14.3 ± 2.1	12.4 ± 2.0	14.1 ± 1.5
Infertile spikelet number	2.9 ± 1.7	0.9 ± 0.9	3.1 ± 1.3	1.5 ± 1.3	2.7 ± 1.6	1.5 ± 0.9
Kernel number per spike	27.2 ± 9.8	30.9 ± 5.5	30.9 ± 10.8	43.6 ± 13.5	39.3 ± 7.9	49.9 ± 7.3
Kernel weight per spike (g)	1.14 ± 0.5	1.2 ± 0.36	1.01 ± 0.41	1.18 ± 0.41	2.01 ± 0.54	2.33 ± 0.59
1000-grain weight (g)	42.09 ± 2.4	35.93 ± 3.1	32.55 ± 3.1	27.19 ± 1.9	51.13 ± 3.5	50.37 ± 4.1
Total water consumption (L)	23.62 ± 1.2	50.31 ± 2.1	21.92 ± 1.2	49.34 ± 2.1	20.53 ± 1.1	39.52 ± 1.5
Yield per pot (g)	34.76 ± 3.2	80.06 ± 5.8	36.12 ± 4.1	59.45 ± 5.1	46.23 ± 3.2	58.28 ± 4.7
Average water use efficiency (g/L)	1.47	1.59	1.65	1.21	2.25	1.47
Maintenance rate of grain yield (%)	43.4		60.8		79.3	
Drought susceptibility	0.8858		0.6037		0.3182	
Significant level (p value)	**p<0.05**		**p<0.01**		**p<0.05**	

Characteristics of yield formation, water use and drought susceptibility under the regulation of root-sourced signal: The data of grain yield and water use were harvested from Trial 2. Average grain yield per pot was significantly lowered under drought stress, at which soil moisture was controlled to induce continuous occurrence of nHRS and accordingly exert physiological influence at plant individual level. Absolute grain yield per pot of *Monkhead* was 80.06 g under well-watered condition, a highest value among three cultivars; whilst its yield was largely decreased to 34.76 g under the regulation of nHRS (stress group), i.e. the lowest value among the cultivars. Also, the maintenance rates of grain yield under drought stress were 43.4, 60.8 and 79.3 in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively. This indicated that *Longchun 8139* as modern cultivar had the best drought tolerance.

In addition, total water consumption amounts of *Monkhead* and *Dingxi 24* were 50.31 and 49.34 liters during growing season, which was significantly greater than that of *Longchun 8139* (39.52 liters) (Table 2) in the CK group. Interestingly, total water consumption was almost remained at similar level, ranging from 20.5 to 23.6 liters under the regulation of root-sourced signal in three cultivars. This suggested that nHRS might play a critical role in stabilizing water consumption. On the other hand, average water use efficiencies were 1.47 and 1.59 g/L in *Monkhead*, 1.65/1.21 g/L in *Dingxi 24*, and 2.25/1.47 g/L in *Longchun 8139* in drought stress group and CK group respectively. In other words, it was only the WUE of *Longchun 8139* was increased under the operation of nHRS. Furthermore, according to the formula of Fischer & Maurer (1978), there was a great difference in drought susceptibility among three cultivars. Drought susceptibility indexes were 0.8858, 0.6037 and 0.3182 in *Monkhead*, *Dingxi 24* and *Longchun 8139* respectively. This result was consistent with the ranking of drought tolerance comparison as showed in the above (Table 2).

The relevant parameters regarding spike composition accounted for the difference in final yield formation under drought stress among the cultivars. Spike lengths of *Longchun 8139* were 8.91 and 9.54 cm in stress group and CK group respectively, indicating a similar trend as that of Dingxi24. *Monkhead* was observed to have the greatest reduction in spike length (Table 2). While Dingxi24 had the maximum reduction in the spike weight, its kernel number per spike and kernel weight per spike were 30.9/43.6 and 1.01/1.18 g under drought stress and well-watered conditions respectively, indicating a relatively favorable compensation in comparison with *Monkhead*. Totally, *Longchun 8139* showed an obvious advantage at the spike level compared to other two cultivars. Interestingly, there were not significant differences in either infertile or fertile spikelet number among three cultivars, indicating the spikelet number might be a stable physiological indicator under the regulation of root-sourced signal in the evolution history of dryland wheats. Also, 1000-grain weight was observed to increase under drought stress in all cultivars, showing that dryland wheat crop tended to improve the population fitness via increasing seed size.

The link between root-sourced signal characteristics and yield formation/drought tolerance: Earlier trigger of nHRS prolonged the timing of its operation and accordingly led to later occurrence of HRS in three wheat cultivars. It can be seen that *Longchun 8139* responded to mild drought stress at earlier critical point than that of either *Monkhead* or *Dingxi 24*. This directly led to lower speed of the increase in leaf ABA accumulation for the former than the latter (Fig. 2). This phenomenon can account for the threshold range of soil water content at which nHRS operated insistently (Fig. 1). Wider threshold range was responsible for better maintenance of growth and yield in *Longchun 8139* (Tables 1-2). Earlier stomatal closure in *Longchun 8139* improved WUE and drought tolerance consequently (Table 2).

Discussion

ABA is a major factor affecting stomatal closure, water use and plant growth (McAinsh *et al*., 1990; Wilkinson & Davies, 1997; Sharp *et al*., 2000 and 2002; Schachtman & Goodger, 2008; Noorka *et al.*, 2013; Latif, 2014). Under drought stress, non-hydraulic early-warning signal is dominated by ABA. ABA-based nHRS plays a critical role in regulating growth, grain filling, water use and drought adaptability in crops. However, ABA had dual roles in this aspect. Excessive ABA biosynthesis will restrain growth and cause physiological injury (Xiong *et al.*, 2006c; Xiong *et al*., 2007; Latif, 2014). Moderate level of ABA in leaf might be a positive and active response to drought stress. In our study, rapid and excessive biosynthesis of non-hydraulic root-sourced ABA can be seen in old cultivars, *Monkhead* and *Dingxi 24*. This directly led to much higher level of average ABA concentration in two cultivars than that of *Longchun 8139*. Massive and rapid accumulation of ABA was logically associated with later trigger of nHRS in old cultivar. On the contrary, the onset of nHRS in modern cultivar, *Longchun 8139* was earlier, which might be closely related to the early activation of PM-ATPase and electrophysiological response (Eric & Robert, 2003; Gong *et al*., 2010).

This study provides a new insight on eco-physiological effects of root-sourced signal and its potential mechanism affecting growth, yield and water use. We therefore proposed a conceptual model on how root-sourced signal exerts its physiological function under drought stress in dryland wheats. The model is to some extent considered as potential evolutionary direction along with the long-term breeding program under natural and artificial selection pressures (Fig. 3). In this study, *Monkhead* and *Dingxi 24* are drought-sensitive genotypes while *Longchun 8139* is drought-tolerant one (Tables 1-2). Drought-sensitive genotype had a narrow threshold range of soil moisture at which nHRS operated insistently, since its nHRS was switched on later than that of drought-tolerant genotype (Fig. 3). Wider threshold range is always mechanically linked with earlier onset of nHRS (Fig. 3). Particularly, the genotype with narrow threshold range tends to have higher level of ABA biosynthesis in leaves. Definitely, the speed of increase in leave ABA concentration was greater in drought-sensitive genotype than that of drought-tolerant one (Figs. 2-3). In other words, the intensity of root-sourced chemical signal is greater in the former than the latter, but lasts for shorter time. Our previous studies showed that earlier trigger of nHRS led to lower temporary wilting point (TWP) and permanent wilting point (PWP) in spring wheat (Fan *et al*., 2008 and 2009). This outcome resulted from anti-oxidant enzymes and reactive oxygen species (ROS) (Wang *et al*., 2008).

We therefore summarize this phenomenon into a conceptual model of root-sourced signal weakening as shown in Fig. 3. ABA-led root-sourced chemical signals tend to be weakened but prolonged to operate for wider threshold of soil moisture under the pressure of artificial selection. This is a typical early-warning response of plant to drying soil. Earlier onset of nHRS postpones the emergence of HRS, which leads to better dehydration-tolerant ability. Following the critical point of HRS, soil in pots will be further drying to severe stress level. According to our observation, TWP and PWP will take place successively at lower critical points of soil moisture (Fig. 3). During the long-term domestication process of dryland wheat, artificial selection improves drought tolerance and water use efficiency in dryland wheat under drought stress (Fig. 3).

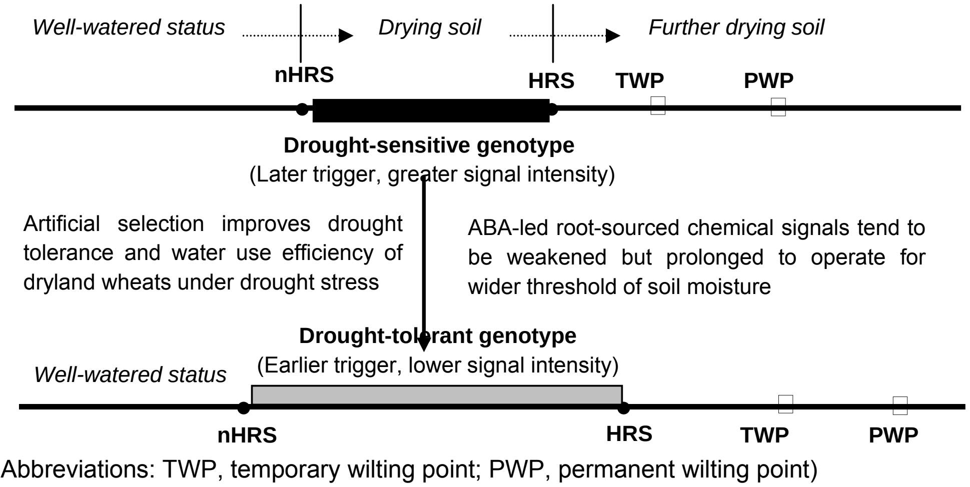

Fig. 3. A possible hypothesis of nHRS weakening regarding its eco-physiological effects and its mechanism in dryland wheat.

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