

*Full Length Research Paper*

# A dwarf wheat mutant is associated with increased drought resistance and altered responses to gravity

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Drought resistance is an important trait for crops. Here we report a new wheat mutant with increased drought resistance and altered gravitropism. During the bombardment transformation of Chinese wheat cultivar 'Jingdong 6', a recessive remarkably dwarf wheat mutant named 's-dwarf' (super dwarf wheat) was created. It was severely dwarf and gibberellic acid insensitive. The relative water loss ratio of the detached leaf and the transpiration rate of s-dwarf seedlings were remarkably lower than Jingdong 6 while the photosynthetic rate of s-dwarf seedlings was significantly higher than Jingdong 6. Most of the s-dwarf seedlings survived in recovering experiment after water loss. The stalk of s-dwarf seedling also showed reduced gravitropism. This is the first report about a new dwarf wheat mutant associated with increased drought resistance and altered stalk gravitropism.

**Key words:** s-Dwarf, wheat, increased drought resistance, gravitropism, relative water loss ratio, transpiration rate, photosynthetic rate.

## INTRODUCTION

Crop drought resistance is a major factor in the stabilization of crop performance in drought prone environments. Drought resistance is now considered by both breeders and molecular biologists as a valid breeding target. However, success in breeding for tolerance has been limited because tolerance to stress is controlled by many genes, and their simultaneous selection is difficult. A physiological approach would be the most attractive way to develop new varieties rapidly. Drought resistance is attained within three major physiological domains: (a) the maintenance of a high plant water status during stress; (b) the maintenance of plant function at low plant water status, and (c) the recovery of plant water status and plant function after stress. Many parameters for quantifying plant water stress response have been used in research and these include leaf water potential, relative water content,

photosynthetic and transpiration rates, relative water loss and plant recovery.

Drought resistance and dwarfism are also important traits for wheat breeding. Some of the gene controlling wheat height, such as Rht1B and Rht1D, had been cloned (Peng et al., 1999). During the bombardment transformation of Chinese wheat cultivar 'Jingdong 6', a recessive remarkably dwarf wheat mutant called 's-dwarf' (super dwarf wheat) was created. It was gibberellin insensitive and also our PCR result showed the drought resistant wheat mutant in our research is not Rht1B or Rht1D mutant (data not shown). It was very interesting to know whether the s-dwarf mutant has high water use efficiency and how it responded to gravity.

## MATERIALS AND METHODS

All the wheat grains used in our experiments were freshly harvested. The grains were sowed in a tray in the growth chamber at 25°C with 16 h light. At the 10th day, when three true leaves appeared in all the seedlings, the 2nd appeared true leaf was used for measuring photosynthetic and transpiration rate and relative water loss ratio.

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**Table 1.** Comparison the survival ratio of the 10-day-old seedlings after dehydration and re-watering.

cultivar	Dehydration		Recovery		
	Total	survived	survival	recovered	recovered
s-dwarf	25	20	0.80*	12	0.48**
Jingdong 6	25	0	0	0	

Each value was expressed as the mean number, taken from a data set consisting of 25 plants, the experiment was repeated three times. The survival and recovered ratio of s-dwarf was significantly different from *Jingdong 6* (T-test, 1% level).

### Photosynthetic and transpiration rates measurement

Photosynthetic and transpiration rates were measured on young fully-expanded 2nd appeared leaves using a portable photosynthesis system (Li-COR LI-6400). Ten repeats were done in this experiment.

### Relative water loss ratio

Immediately after being cut at the base of lamina, the 2nd appeared true leaves from the 10-day-old seedlings were sealed within plastic bags and quickly transferred to the laboratory. Fresh weights were determined every hour in the next 9 hours. The relative lost weight was calculated as relative water loss ratio. Three repeats were done in this experiment.

### Drought tolerance

After given enough water, twenty five 10-day-old seedlings from each line were treated for 8 days without watering. The number of survived seedlings was calculated. After all the seedlings wilted, water was supplied again every day till the 7th day. The number of recovered seedlings was calculated. Three repeats were taken in this experiment.

### Stalk responses of gravitropism

The seeds were sowed in vertical pots. After germination, the pots were placed horizontally for 2 days in the growth chamber. The pictures were taken. Ten seedlings of each variety were measured in this experiment.

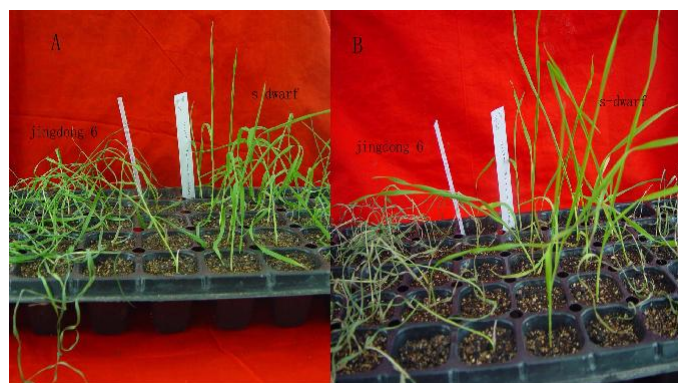
## RESULTS

### Drought resistance

The growth of s-dwarf seedlings in the growth chamber was retarded; the internodes of stalk were much shorter and thicker than *Jingdong 6*, even after 100  $\mu$ M gibberellic acid (GA) spraying. In order to check the difference of adaptation to stress between s-dwarf and *Jingdong 6*, twenty five 10-day-old seedlings of s-dwarf mutant and twenty five *Jingdong 6* seedlings were observed without watering for 8 days. The experiments were repeated 3 times. The results were shown in Figure 1 and Table 1. Twenty seedlings of s-dwarf mutant grew well, while all the 25 *Jingdong 6* seedlings showed

severely wilting phenotype. The survival ratio of the seedlings showed remarkable difference between s-dwarf mutant and *Jingdong 6*. It indicated that s-dwarf mutant seedlings were more tolerant to drought stress.

Recovery of the dehydrated plants was also determined in our experiments. After all the s-dwarf seedlings were wilted without watering for 11 days, water was given to all the wilted seedlings of s-dwarf mutant and *Jingdong 6* for 7 continuous days. The number of recovered seedling is shown in Table 1. From Table 1, it was shown that none of the wilted *Jingdong 6* seedlings can be recovered with re-watering for 7 days; on the contrary, 48 percent of wilted s-dwarf seedlings were recovered after 7 days re-watering (Figure 1). It was concluded that s-dwarf seedlings were more resistant to drought resistance.



**Figure 1.** drought tolerance of s-dwarf and *Jingdong 6* seedlings. A. Phenotype of the 10-day-old seedlings of *Jingdong 6* and s-dwarf without watering for 8 days. B. The phenotype of wilted seedlings of *Jingdong 6* and s-dwarf after 7-days re-watering.

### Photosynthetic and transpiration rates

In order to determine the water use efficiency, ten samples of the s-dwarf seedlings and *Jingdong 6* seedlings were taken for photosynthetic and transpiration rate determination. The results were shown in Table 2.

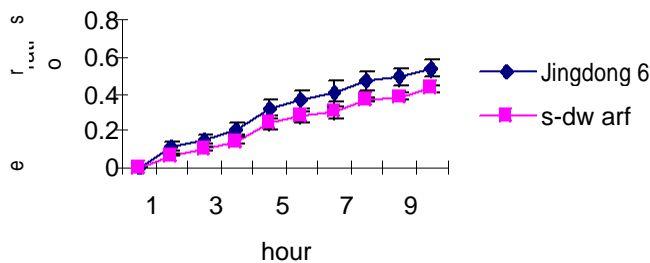
The photosynthetic rate of s-dwarf seedlings was remarkably higher than the *Jingdong 6* seedlings; the transpiration rate of s-dwarf seedlings was remarkably

**Table 2.** Photosynthetic and transpiration rate of the seedlings.

Cultivar	Photosynthetic rate	Transpiration rate	water use efficiency
s-dwarf	11.14	2.78*	4.00**
Jingdong 6	9.39	4.52	2.08

Each value was expressed as the mean photosynthetic rate per leaf, taken from a data set consisting of 10 plants.

lower than Jingdong 6. That means more carbon was fixed in s -dwarf and less water lost via transpiration than Jingdong 6. Water use efficiency of s-dwarf was significantly higher than Jingdong 6.



**Figure 2.** Relative water loss ratio of detached 2<sup>nd</sup> appeared leaf from s-dwarf and Jingdong 6 seedlings. Each value was expressed as the mean number, taken from a data set consisting of 4 plants.

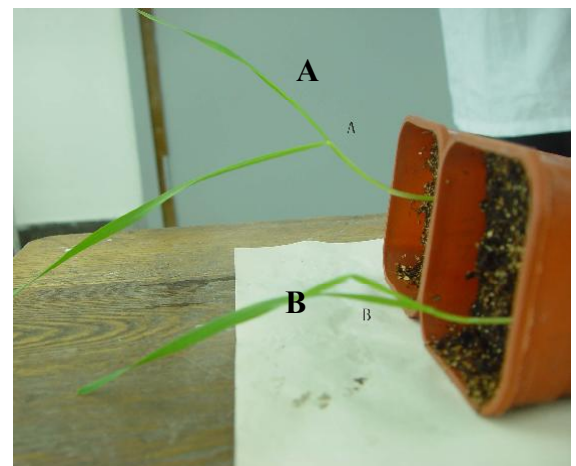
### Relative water loss ratio of the detached leaf

Since the transpiration rate of s-dwarf was much lower, it was worthy to compare the relative water loss ratio between s-dwarf seedlings and Jingdong 6 seedlings. The relative water loss ratio of the detached 2<sup>nd</sup> appeared leaf was determined. The result is shown in Figure 2. We observed that relative water loss ratio of s-dwarf seedlings was much lower than Jingdong 6. The difference became more and more obvious when the detached leaf was left in the air longer. It was consistent with the transpiration rate results. Combined with the result of transpiration rate and relative water loss ratio, it can partially explain why the seedlings of s-dwarf were much more tolerant to drought stress than Jingdong 6.

### Stalk responses of gravitropism

According to the current research, most dwarf crop varieties are related with gibberellin signaling pathway or polar auxin transport. Fu et al (2003) had established the relationship between DELLA protein and polar auxin transport. It might be possible that gibberellin signaling pathway is also involved in plant gravitropism. Since s-

dwarf seedlings were gibberellin insensitive, it was worthy of detecting its gravitropism. In our experiments, the stalk responses of gravitropism of Jingdong 6 and s-dwarf were different. The result is shown in Figure 3. The seedlings of Jingdong 6 had better response to gravitropism than s-dwarf seedlings. From Figure 3, it is very obvious that Jingdong 6 seedlings grew nearly vertical, while s-dwarf seedlings grew still nearly horizontally.



**Figure 3.** Shoot responses of gravitropism. A. The Jingdong 6 stalk response of gravitropism. B. The s-dwarf stalk responses of gravitropism.

## DISCUSSION

The shorter stature and sturdy stalks of the dwarf varieties of wheat and rice renders them resistant to flatterning by wind, rain, or high densities and more effective in converting fertilizer input into higher yield. Recently, the Rht- B1b and Rht-D1b genes were isolated from wheat (Peng et al., 1999). They are orthologous to Arabidopsis GAI gene and maize D8, a de-repressible modulator of gibberellic acid (GA) response (Peng et al., 1997). There are also other pathways causing dwarf varieties, such as modulation of polar auxin transport (Multani et al., 2003), and brassinosteroid signaling pathway (Chono et al., 2003). As we know, some dwarf varieties of wheat has higher yield than the taller ones.

We do not know how the dwarf varieties respond to abiotic stress. In our experiments, s-dwarf seedlings showed excellent drought resistance, and some of its physiological traits were examined. It is also important to point out that the yield of s-dwarf variety is not as higher as Rht-B1b and RhtD-1b semi-dwarfing mutants although s-dwarf had higher photosynthetic rate, its yield is much lower than Jingdong 6. The grain of s-dwarf is much smaller than Jingdong 6. On the other hand, the s-dwarf is more tolerant to drought stress, which is consistent with the lower transpiration rate and lower relative water loss ratio of the detached leaves.

It was very interesting to point out that s -dwarf seedlings showed altered gravitropism in our experiments. It was well known that auxin is involved in gravitropism (Moore, 2002). The height reduction in Maize brachytic2 mutants with compact lower stalk internodes results from loss of a P-glycoprotein that modulates polar auxin transport in maize stalk (Multani et al., 2003). Fu had proved that polar auxin transport was involved in gibberellin signaling pathway (Fu et al., 2003). It might be quite possible that s-dwarf mutant is related with auxin polar transport.

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