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Which One is Responsible for Apical Sterility in Wheat Under Water-Stress Conditions, Ovule or Pollen Aborting

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Abstract: This study was conducted to determine the contribution of pollen and ovule sterility on the floret sterility at the apex of spikes in a sensitive variety when the plants are encountered to water-stress at the meiotic stage. The differences between control and water-stress treatments were highly significant ($p<0.001$) for seed set, pollen viability and pollen fertility indicating that water-stress had severely influenced the fertility of florets at the apex of spike. The results obtained from reciprocal crosses between plants imposed to water-stress and well-watered plants demonstrated that ovule development was negatively affected by water-stress but less severely than anther and pollen development. Both the fertility and viability of pollen grains at the apex of spikes were reduced due to water-stress to a greater extent that those in the middle. Pollen fertility was affected more seriously than pollen viability.

Key words: Seed set, spikelet, developmental sterility, floret, vegetative nucleus, sperm nucleus

INTRODUCTION

Reduced seed-set in wheat (*Triticum aestivum*) has become a common problem in many parts of the world particularly in the regions with inadequate water reserves for irrigation (Rawson, 1996). Environmental stresses, particularly drought and extreme high temperatures often result in reduced seed-set in some cultivars of wheat (Rawson, 1996). Apical sterility or tip-sterility occurs in some wheat cultivars when the terminal spikelets fail to set seed following exposure to water-stress (Mohammady-D *et al*., 2003).

Several researchers have reported that water-stress at the meiotic stage of spike development (41 Zadoks' scale) is an important factor increasing floret sterility as a consequence of pollen sterility (Briggs *et al*., 1999; Dorion *et al*., 1996; Kainth, 1994; Saini and Aspinall, 1981). Bingham (1966) made reciprocal crosses between moisture stressed and well-watered plants of winter wheat varieties. The results indicated that the reduction in seed-set was caused by pollen sterility induced by water-stress, while female fertility was not affected. They came to this conclusion based on differences between seed set of reciprocal cross and did not study the fertility and viability of pollen grains directly. On the other hand, Kainth (1994) reported that the apical spikelets of water-stressed plants of Y82187 failed to set seed when pollinated with pollen from well-watered plants indicating water-stress influenced ovule fertility. The majority of studies carried out on wheat sterility have not focused directly on the effect of water-stress at the meiotic stage on floret fertility in apical spikelets. For instance, in the studies conducted by Briggs *et al*. (1999) and Dorion *et al*. (1996), the water-stress treatments continued until the end of anthesis. In this situation, water-stress at the anthesis stage causes floret sterility because of a deficiency of moisture for pollen germination on the stigma rather than failure in pollen development. On the other hand, if plants subjected to water-stress from the meiotic stage until the onset of anthesis, followed by normal watering during anthesis, produced sterile pollen it can be a sign of a failure in the pollen development processes. Since even under non-limited conditions a few basal florets are sterile, recording the reduction of seed-set in whole spikes might confound the developmental sterility, which happens in the late spikelets at the base of spikes, with the effect of water-stress on pollen and ovule fertility in normally developed spikelets. This study was therefore conducted to investigate the contribution of pollen and/or ovule sterility to the sterility of spikelets (known as apical sterility) under a water-stress condition imposed at the meiotic stage of spike development only.

MATERIALS AND METHODS

Experiment 1: Eighty five plants of the variety Y82187 were grown singly in 10.6 cm pots containing John Innes Compost No. 2 in a greenhouse where the minimum

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temperature was maintained at 16°C and the maximum varied from 22 to 29°C. The pots were watered once daily to field capacity until the plants reached the meiotic stage of spike development (39-41 Zadoks' scale). Sixty plants at the same stage of development were selected and divided equally into two blocks. One was watered normally and the other was subjected to water-stress by withholding water from the plants for five days. Previous experiments indicated that this treatment induced apical sterility in the variety Y82187 (Mohammady-D *et al*., 2003). After 5 days the plants were watered normally to avoid the failure of seed-set and the grain filling process in spikelets lower down the spikes. Reciprocal crosses were made between well-watered and water-stressed plants. In order to estimate the effect of emasculation and artificial pollination on seed-set, the main spikes of 10 well-watered plants were emasculated and pollinated with pollen from other well watered plants. At harvest, the seed-set was recorded as number of seed per floret in the top 6 spikelets of both water-stressed×well-watered and well-watered×water-stressed crosses. These results were compared with the seed-set of the control (well-watered×well-watered) crosses. In addition, the seed-set produced by self-pollination of 10 well watered and 10 water-stressed plants was calculated as the number of seeds/number of florets for the 6 apical spikelets to assess the general effect of water-stress on seed-set at the spike apex.

Experiment 2: This experiment was conducted to study the effect of water-stress on the viability and fertility of pollen grains at the apex and middle of spikes. Forty plants of the variety Y82187 were grown as described in experiment one and 20 of them were exposed to water-stress at the meiotic stage of spike development (39-41 Zadoks' scale). Pollen viability and fertility were evaluated in both well-watered and water-stressed plants as described below. In addition, the seed-set produced by self-pollination of some control and treated plants were again recorded in order to evaluate the effect of water-stress on seed-set.

Pollen viability was evaluated by testing for β-galactosidase activity following the method of (Trognitz, 1991). In this method, β-galactosidase reacts with 5-bromo-4-chloro-3 indol β-galactopyronoside and produce blue colour by which the activity of enzyme can be assessed as an indicator of pollen viability. In the water-stressed plants, some apical spikelets produced small shrivelled anthers that contained non-viable pollen. Thus one anther from florets of two subsequent spikelets which contained at least one non shrivelled anther were bulked and used for the viability test. Pollen viability was measured on 6 plants in each treatment. Selection was restricted to 6 top spikelets to indicate the pollen viability at the apex of spikes. Normal anthers from the middle spikelets of water-stressed plants and both middle and apical spikelets of control plants were used. Then the percentage of viable pollen (stained pollen) in well-watered conditions was compared with that in water-stress conditions. With regard to pollen grain fertility, fertile pollen grains should contain two sickle shaped sperm nuclei, a vegetative nucleus and dark stained cytoplasm. Fertile pollen grains of stressed plants were identified according to Kihara (1982) using Aceto-carmine staining method.

Statistical methods: For all of the characters a one way ANOVA was performed to evaluate differences between the treatments and Tukey's test was used to compare the means. Appropriate transformation of data was made when the distribution did not fit the normal distribution.

RESULTS AND DISCUSSION

Seed-set: Table 1 indicates the effect of water-stress on seed-set. The differences between control and water-stress treatments were highly significant ($p<0.001$) indicating that water-stress had severely influenced the fertility of florets at the apex of spike. Comparison between control × control and control self-fertilised plants indicated the effects of artificial emasculation and pollination on seed-set. This is probably due to damage to anthers and stigma during manual emasculation and pollination.

Since the mean percentages of seed-set in both control × stress and stress × control were less than control × control, both male and female reproductive organs have been affected by water-stress. In crosses, where well watered plants were used as the male, the percentage of seed-set was significantly higher than crosses with water-stressed plants as the male indicating that water-stress affected pollen grain development more severely than ovule development. This result is in agreement with findings of Bingham (1966). With respect to effect of water-stress on reproductive organs, two different results have been reported. Kainth (1994) reported failure of seed-set in water-stressed plants of line Y82187 when pollinated with pollen from well-watered plants indicating an effect of water-stress on ovule development, while Saini and Aspinall (1981) using reciprocal crosses between moisture stressed and well-

Table 1: Means and standard errors of seed-set at the six top spikelets in crossed and self-fertilised plants of Y82187 under control and water-stress conditions

Treatment	Seed-set (%)
Cross pollination (♀×♂)	
Control ×Stress	2.5±1.70[a]
Stress × Control	20.0±3.09[b]
Control × Control	41.7±3.50[c]
Self-pollination	
Control	80.8±2.70[d]
Stress	7.5±2.60[a]

*: Means followed by a common letter are not significantly different

Table 2: The percentage of normal pollen and seed-set in both well-watered and water-stressed plants at the apex and middle of spikes

Treatment	Fertile pollen (%)[AC]	Viable pollen (%)[β-Ga]	Seed-set (%)
Control			
Apex	80.1±1.5[a]	81.7±2.0[a]	81.9±3.3[a]
Middle	89.8±0.7[b]	93.7±0.6[b]	97.2±1.8[b]
Stress			
Apex	21.5±5.4[c]	24.8±1.4[c]	11.1±3.5[c]
Middle	81.8±1.8[ab]	84.8±1.0[b]	94.4±2.8[ab]

*: Means in each column followed by similar letters are not significantly different at $p<0.01$, AC: Aceto-carmine method, β-Ga: β-galactosidase test

watered plants reported that seed-set was reduced as a consequence of pollen sterility but ovule fertility was unaffected. To sum up, the present results clearly confirmed that water stress affects both ovule and pollen grain development with a much more severe effect on pollen grain fertility (see also next section).

Effect of water-stress on pollen grain viability and fertility: The mean pollen fertility (aceto-carmine method) in anthers from the apex and the middle of spikes from stressed plants was 21.5 and 81.8%, respectively (Table 2). These values were considerably below the control levels of 80.1 and 89.8, respectively. However, the difference between control and stressed plants at the middle of spike was significant only at 5% level of probability ($p = 0.03$). A similar trend was observed using β-galactosidase test (Table 2). In all cases, the percentage of stained pollen grains with β-galactosidase was higher than the percentage of normal pollen identified using the Aceto-carmine method. This is possibly due to failure of some stained pollen grains to produce sickle shaped sperm nuclei during the pollen grain developmental process.

The higher values for pollen grain viability and fertility in the middle of spike is due to the fact that at the start of the drought treatment meiosis takes place in the middle spikelets when water-stress is low and spikelets with fully formed anthers will produce higher ratios of normal pollen grains. As the water-stress becomes greater each day the development of microspores may be aborted resulting in empty pollen grains at the apex of the spikes.

It is expected that hexaploid wheat produces 100% fertile pollen (Kihara, 1982) under non-limited conditions. Despite this idea, many researchers reported less than 100% fertile pollen grains for wheat (Briggs *et al*., 1999; Saini and Aspinall, 1981; Welsh and Klatt, 1971) differing from 76 to 95% depending on the variety. In the present experiment, control plants produced about 90 and 80% fertile pollen at the middle and the tip of spike, respectively. These reductions can be due to unavoidable small changes in temperature or water supply during sunny days. This result also implied that unfavourable conditions affect the apical spikelets more than the middle spikelets.

CONCLUSIONS

The literature is not clear about the mechanism of effect of water-stress on pollen grain fertility. Nonetheless, a major hypothesis has been revealed by Lalonde *et al*. (1997). They reported that abnormal degeneration of the tapetum in water-stressed anthers coupled with a loss of orientation of the reproductive cells and lack of starch are parts of events leading to abortion of microspores. The present study carried out specifically on apical sterility demonstrated that ovule development was affected by water-stress but less severely than pollen development. This study also revealed that effect of water-stress on fertility is more severe at the apex of spikes than on the middle and pollen fertility is affected by water-stress more seriously than pollen viability.

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