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ARTICLE

Effect of water deficit and elevated temperature on pollen development of drought sensitive and tolerant winter wheat (*Triticum aestivum* L.) genotypes

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ABSTRACT The effects of meiotic water deficit and combined heat and drought stress were studied on microsporogenesis and fertility of wheat. Among normal pollen, 12% of the drought stressed tolerant Plainsman V and 34% of the sensitive Cappelle Desprez pollen were arrested at early stages of gametogenesis. Drought stress manifested in significant reduction of the mean fertility in both sensitive (41%) and tolerant (33%) genotypes. Combined stress applied during meiosis among developmental arrests caused serious morphological anomalies in the sensitive genotype. When plants of the Plainsman V variety were subjected to simultaneous drought and heat, additional 24% significant decrease occurred in the ratio of normal pollen. The fertility of the basal part of the spikes was similar to the control in both genotypes, but the seed set in the middle and on the top of the spikes decreased significantly as a consequence of combined drought and heat stress. **Acta Biol Szeged 52(1):67-71 (2008)**

KEY WORDS

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Water deficit limits global crop productivity more than any other stress. The nature and extent of damage, and the ability of a plant to recover from it, depend on the developmental stage at which a plant encounters the stress. During reproductive development, wich lasts from just before the onset of meiosis in micro- and macrospore mother cells until the end of the grain maturing process, plants respond extremely sensitively both to water deficiency and to drastic increase in the temperature (reviewed by Barnabás et al. 2008). In the case of wheat, drought stress during meiosis results in increased pollen sterility (Saini et al. 1984). The main cause of this is the development of sterile, dysfunctional pollen grains resulting from irregulations during microsporogenesis and microgametogenesis. Microspore mother cells apparently complete meiosis, but further microspore development is arrested at various stages as a consequence of the dislocation of the microspores from their normal peripheral position. In some anthers, abnormal vacuolisation of the tapetum can be seen soon after meiosis. Lalonde et al. (1997) suggests that the tapetal dysfunction leads to the loss of microspore orientation. The developmental anatomy of stress-affected anthers gives some promising clues about the metabolic events that may be linked to the failure of pollen development (Saini 1997). Water deficiency disturbs photosynthetic processes in vegetative plant tissues, particularly in leaves, resulting in a reduction in the water-soluble carbohydrate level in the anthers (Saini 1997). Because of the disturbances in the carbohydrate metabolism the intine is unable to develop normally and insufficient amounts of reserve nutrients are stored in the pollen grains (Dorian et al. 1996; Sheoran and Saini 1996). Without starch to fuel pollen tube growth on the stigma, pollen tubes could not reach the ovule (Clément et al. 1994).

Similarly, heat stress during meiosis detrimentally affects pollen functionality in cereals (Stone 2001). In wheat, two types of abnormal pollen development can be occur due to high-temperature stress. The first is apparently caused by tapetal degradation during meiosis, when the microspores are not able to complete the first mitosis. In the second case, all the microspores complete the first mitotic division, but only a few of them are able to divide further to develop into normal tri-cellular pollen grains. The rest of the microspores remain immature and do not accumulate starch, so the anthers contain a mixture of fertile and sterile pollen grains (Saini et al. 1984).

It is widely known that the simultaneous occurrence of several abiotic stresses, rather than a particular stress condition, has a detrimental impact on field crops. Recent study (Rizhsky et al. 2004) has demonstrated that the molecular and metabolic responses of plants to a combination of two different abiotic stresses cannot be directly extrapolated from the response of plants to each of the different stresses individually.

The occurrence of simultaneous drought and heat stress during the early generative stages e.g. meiosis of cereal ontogeny used to be rare, so it attracted little attention. However, in the light of the increasingly frequent occurrence of early

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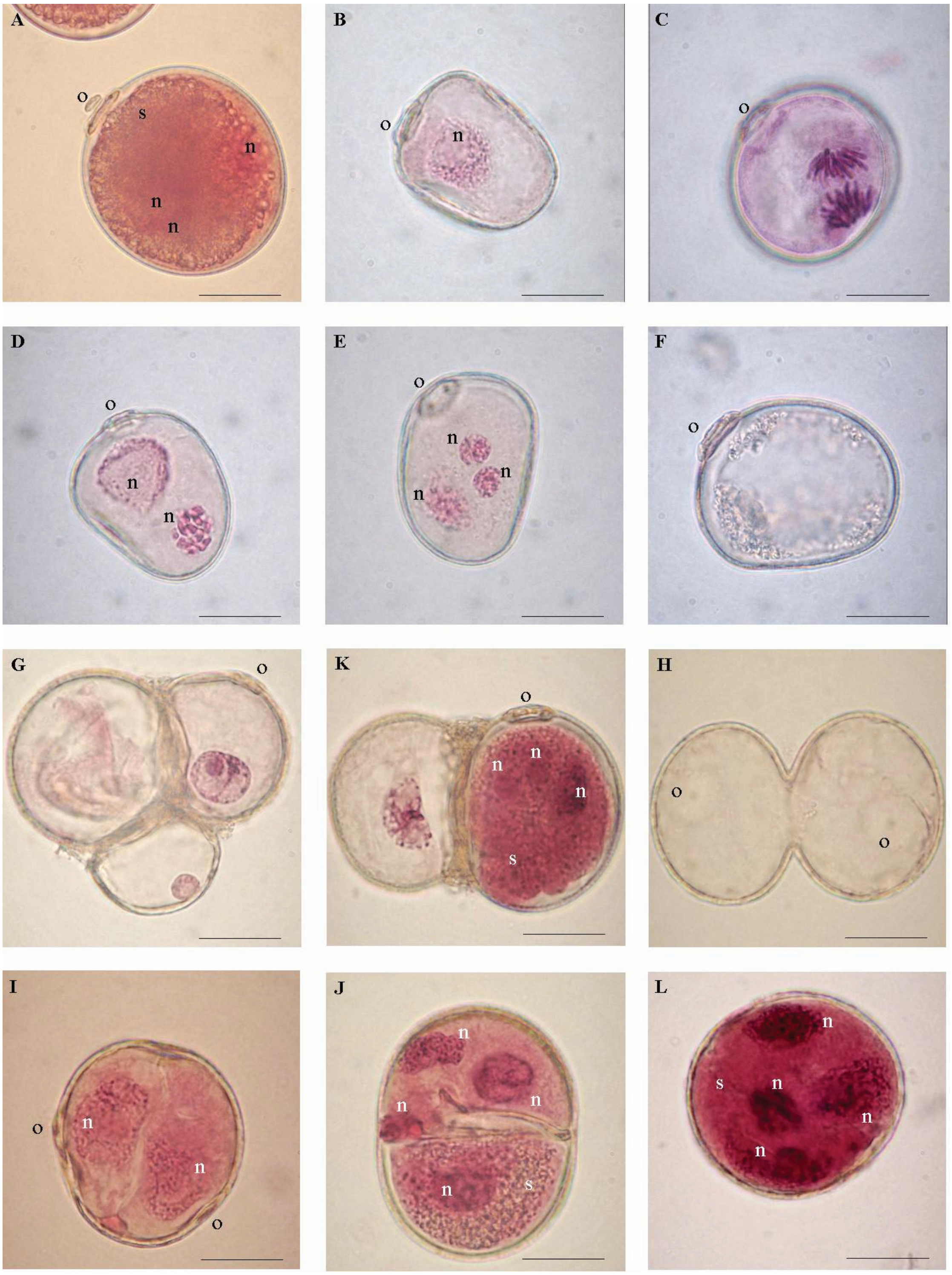

Figure 1. Morphological diversity of the pollen grains observed at the time of flowering, caused by drought and combined drought and heat stress. A: normal trinucleate pollen with a high amount of accumulated starch reserves, B-D microspores and a pollen grain at early stages of pollen development, find in anthers after both treatments, B: uninucleate microspore, C: microspore during the first mitotic division, D: binucleate pollen, D: trinucleate pollen, F: sterile pollen, G-L: abnormal pollen development, G: non-separated tetrad, H: an aborted and a normal pollen grain stuck together, I: two sterile pollen grains within the same pollen wall, J: uninucleate microspore 'twins', K: asymmetric pollen 'twins', L: multinucleate pollen, n: nucleus, o: operculum, s: starch granules; Bar represents 20 μm.

Table 1. The percentage of normal pollen grains in the anthers subjected to drought and combined drought and heat.

Treatment	Genotype	Normal pollen (%)	
		Control	Treated
Drought	Plainsman V	99.0 ± 1.1	86.7 ± 9.8
	Cappelle Desprez	97.4 ± 4.9	63.5 ± 16.2
Heat & Drought	Plainsman V	99.2 ± 1.2	63.5 ± 19.8
	Cappelle Desprez	98.0 ± 4.3	55.7 ± 22.4

season temperature extremes, in our work the effect of water deficit per se and the joint effect of heat stress plus water deficit on pollen development and fertility were studied in both drought sensitive and tolerant winter wheat genotypes.

Materials and methods

Plant material

Drought tolerant Plainsman V and drought sensitive Cappelle Desprez winter wheat genotypes were used in the experiment. After germination 60-60 seedlings of both genotypes were vernalized for 7 weeks at 2°C, then transplanted into soil (2 kg/pot) and grown in the phytotron using the T1 spring climatic programme (Tischner et al. 1997) until the beginning of meiosis. During this period the initial day/night temperature (12.5/5.5°C) was increased to 19/14°C. Afterwards 30-30 plants were grown under control conditions until anthesis and 30-30 plants were subjected to stress treatments.

Stress treatments

Stressors applied for 5 days during meiosis involved water withholding *per se* in one hand and heat stress at 34/24°C combined with water withholding in the other for 5 days. Plants were subjected to stresses 3 days before meiosis. Prior to and immediately following the combined treatment the plants were kept at a transfer temperature of 28°C during the dawn and dusk phases of the plant growth programme, when illumination was set to 1/3 of the total light intensity. The relative humidity of the air circulating in the chambers was the same as in the control (65/75%) in both treatments. After the treatments 20-20 plants were returned to the control environment with a temperature of 23/14°C and daily water supplies of 150 ml, as for control plants, and were grown to full maturity, with a final daily max/min temperature of 26/17°C.

Cytological observations

At the time of anthesis 8-8 anthers of 10 main spikes were examined for determination of pollen development for each treatment. Fixation of anthers was carried out in Carnoy's fixative (96% ethanol 3 parts, glacial acetic acid 1 part). Squashed anther preparations were stained with 3% acetocarmine using a routine technique. Cytological observations were made using an Olympus BX51 light microscope. All the data were pooled means from the eight replicates.

Determination of fertility

At full maturity the fertility ratio of main spikes was determined. When determining the percentage fertility, three grains characteristic of both genotypes were examined per spikelet. As the nutrient supplies to various parts of the wheat spike are not uniform, the spikes were divided into three sections when determining fertility. All the data were pooled means from the twenty replicates. The data were statistically evaluated by the Student's *t*-test using SPSS for Windows, version 10.0.

Results

Effect of drought and the combination of drought and heat on pollen development.

As a consequence of water withholding *per se*, among normal trinucleate pollen grains accumulating high amount of starch reserves, abnormal, starch deficient trinucleate pollen, pollen arrested at early stages of gametogenesis as well as completely sterile microspores (Fig. 1B-F) were present in the anthers of both genotypes. A significant ($P<0.005$) increase in the ratio of abnormal pollen grains could be observed in both, drought tolerant Plainsman V (12%) and drought sensitive Cappelle Desprez (34%) genotypes compared to the controls (Table 1). Compared to Cappelle Desprez, Plainsman V produced a significantly higher amount of normal male gametophytes.

Combined stress applied during the meiosis of microspore mother cells among above-mentioned developmental arrests caused serious morphological abnormalities concerning equal microspore divisions, non-separated tetrads (Fig. 1G), pollen grains stuck together (Fig. 1H), microspores developing within the same wall (Fig. 1I-K), and multinucleate pollen grains (Fig. 1L) exclusively in the sensitive genotype. These abnormal morphotypes were observed in shrivelled anthers characteristic for the flowers located in the upper third of the spikes. When plants of the tolerant variety were subjected to simultaneous drought and heat, an additional 24% significant decrease in the ratio of normal pollen occurred (Table 1). Combined stress did not have further significantly negative impact on the normal pollen percentage of the sensitive variety, if compared to the drought stress.

Effect of drought and combined heat and drought stress on fertility

Drought stress during meiosis manifested in significant ($P<0.005$) reduction in mean fertility in both sensitive (41%) and tolerant (33%) genotypes (Table 2). Concerning the position of the affected anthers on the spike, there was a clear

Table 2. Changes in the fertility ratios of the genotypes.

Treatment	Genotype	Fertility (%)							
		Mean		Spikelet position					
				Basis		Middle		Top	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
Drought	Plainsman V	74 ± 5	67 ± 7	78 ± 7	63 ± 8	90 ± 8	86 ± 11	54 ± 9	53 ± 12
	Cappelle Desprez	69 ± 9	59 ± 11	70 ± 13	65 ± 14	85 ± 11	72 ± 16	53 ± 14	39 ± 18
Heat & Drought	Plainsman V	70 ± 8	58 ± 13	71 ± 15	72 ± 18	89 ± 9	72 ± 18	50 ± 8	28 ± 17
	Cappelle Desprez	69 ± 9	72 ± 18	70 ± 13	72 ± 18	85 ± 11	28 ± 17	53 ± 14	38 ± 19

difference between the genotypes. In Plainsman V the fertility ratio of the basal part of the spike was significantly (P<0.005) lower than the control, although the fertility of the rest of the spike did not differ from the control. In contrast, in the case of Cappelle Desprez the basal part remained unaffected, but the fertility of the upper parts decreased significantly.

When plants were subjected to water withholding and elevated temperature simultaneously, the mean fertility of sensitive genotype did not decrease further if compared to the water deficit per se (Table 2). In the tolerant genotype the mean fertility decreased significantly (P<0.005) by 9% if compared to the fertility ratio of water stressed plants. There was no difference in the fertility of the two genotypes. Concerning the location of aborted kernels both genotypes responded similarly to the combined stress. The fertility of the basal parts of the spikes was similar to the control, but the seed set in the middle and on the top of the spikes decreased significantly (P<0.005).

Conclusions

A complex phenomenon of pollen development depends on a series of coordinated metabolic and structural changes. A dysfunction in a major metabolic pathway, such as sugar metabolism, or a dysfunction of certain cell layers could adversely affect the development of pollen grains. Morphology of microspores and pollen grains observed in the anthers subjected to water deficit during meiosis respond to the observations of Saini et al. (1984). Similarly to the observations made earlier (Dorion et al. 1996, Saini and Aspinall 1981) our results show that a short period of water withholding imposed during meiosis significantly reduced the fertility of both wheat varieties, although the sensitive genotype encountered more sever reduction. When plants were subjected to drought and heat simultaneously, in their anthers among microspores arrested at early stages of development and pollen types described by Saini et al. (1984) abnormal, earlier not described pollen forms were observed. Non-separated tetrads and pollen 'twins' developing within one wall indicate that as a consequence of combined stress, some of the microspore mother cells could not complete meiosis. Pollen grains stuck together presumably by a pollenkitt-like material suggest the malfunction of the tapetum. Our cytological analysis reveals, that the stress-induced reduction in fertility was a consequence of disturbances occurred during pollen development. From the occurrence of multinucleate pollen grains within the anthers of the plants subjected to a combination of osmotic and high temperature stresses it may be concluded that the switch of microgametogenesis to androgenesis is not an exclusive failure of in vitro androgenetic conditions, but it can be triggered *in planta* as well. Fertility data show that whereas there was a difference between genotypes when water withholding was a stressor alone, in the case of combined stress the responses were equalized. It confirms the observations of Rihzsky et al. (2004), who found that a combination of drought and heat stress alters plant metabolism in a novel manner compared with single stresses (Rizhsky et al. 2004).

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