The Influence of Terminal Heat Stress on Meiosis Abnormalities in Pollen Mother Cells of Wheat

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Summary To study the effects of high temperature on the meiosis of pollen mother cells (PMCs) and to determine their relationship with grain set, two in situ experiments were conducted between 2010 and 2012 on four wheat cultivars, Kauz, Montana, M6 and Chamran, under normal (normal cultivation) and terminal heat stress (late cultivation) conditions. Due to the delay in cultivation for the cultivars under stress conditions, their flowering stage faced heat stress at the end of the growing season, and therefore, a significant \( p \leq 0.05 \) increase was observed in meiotic abnormalities. Cytogenetic studies discovered that the meiosis in PMCs and the pollen development are strongly influenced by heat. Based on the results, abnormalities such as precocious chromosome migration to the poles, laggar chromosomes, micronuclei, absence of metaphase plate, pyknosis, abnormal cytokines, cytomixis and abnormal tetrad were observed in different cultivars. The sensitive cultivars, M6 and Montana, had the greatest percentages of meiotic abnormalities. The meiotic abnormalities showed a significant negative correlation with the number of kernels per spike under terminal heat stress conditions \( r = -0.54, n = 16, p \leq 0.01 \). In other words, due to increasing meiosis abnormalities under terminal heat stress, the grain set was greatly reduced, especially in the susceptible cultivars. The result of this experiment showed that studying the meiosis in PMCs can be suggested as one of the research necessary to improve commercial heat-tolerant cultivars, and to some extent, as a method for screening tolerant lines in breeding programs.

Key words Cytomixis, Grain setting, Heat, Laggard chromosome, Pyknosis.

Due to the increase in greenhouse gases, the earth’s surface temperature has increased approximately 0.6°C since the late 19th century, and is expected to rise up to 1.4–5.8°C by the end of this century (Houghton et al. 2001). This increase in global temperature will have a severe impact on plant growth (Cao et al. 2008). Field experiment results showed that high temperature reduces wheat grain performance two- to four-fold compared to favorable conditions (Zhong-Hu and Rajaram 1994). The stability of a genotype is assessed through evaluation of genotype changes in non-uniform environments (Rosielle and Hamblin 1981). Thus, experiments to compare performance in different regions and years are performed with the aim of selecting genotypes with consistent and stable performance under normal conditions and conditions with stress (Fernandez 1992). Several indicators based on wheat grain performance for assessing stress tolerance in plants have been provided so far, of which two indicators, stress tolerance index (STI) and stress susceptibility index (SSI), have been identified as more amenable indices (Fernandez 1992, Fischer and Maurer 1978).

Previous studies have shown that reproductive growth of plants is more sensitive to high
temperatures compared to vegetative growth (Sakata et al. 2000, Sato et al. 2002). The meiosis stage as a key step in the development of gametes is very sensitive to high temperature (Ahmad et al. 1984, Cao et al. 2008), and studies show that the most sensitive stages of the plant life cycle are from the onset of meiosis until the conclusion of tetrad break-up and the release of young microspores in anthers (Fuzinatto et al. 2008, Saini and Aspinall 1982). High temperature causes disorder and abnormality in this process, leading to the creation of premature gametes or gametes with unusual ploidy levels, such as aneuploidy and euploidy (Negri and Lemmi 1998, Pecrix et al. 2011), which leads to reduced fertility and ultimately to decreased grain yield. It is reported that in wheat, an increase in temperature by 10°C from the optimal temperature at the start of meiosis stage of pollen mother cells (PMCs) causes abnormal growth of pollen, and thereby the sterility in the male organ (Saini et al. 1984). Although cytogenetic studies in the past decades have reported different meiotic abnormalities in wheat and other different plants, studies on the heat effects on meiosis and related abnormalities have been rarely reported. In previous studies, the following meiotic abnormalities have been reported: the presence of laggard chromosomes in anaphase I and II in wheat (Rezaei et al. 2010), triticale (Falcao et al. 1981, Guerra et al. 2011) and Isoetes sinensis (Heng-Chang et al. 2007); precocious migration of chromosomes in the metaphase stage in wheat (Rezaei et al. 2010) and Brachiaria inter-species hybrid (Fuzinatto et al. 2008); presence of micronuclei in tetrad and produced microspores in triticale (Guerra et al. 2011, Rezaei et al. 2010) and Brachiaria inter-species hybrid (Fuzinatto et al. 2008); non-placement of chromosomes in the equatorial plate of cells in the metaphase stage in Brachiaria brizantha (Daniela et al. 2005); pyknosis in soybean (Bione et al. 2000); occurrence of cytomixis in Bromu ssp. (Sheidai and Fadaci 2005), Isoetes sinensis (Heng-Chang et al. 2007) and species of grasses (Falistocco et al. 1995); and occurrence of abnormal cytokinesis in Rosa sp. (Pecrix et al. 2011), triticale (Rezaei et al. 2010) and Brachiaria inter-species hybrid (Fuzinatto et al. 2008).

Heat stress is one of the factors inducing deformity and dysfunction in meiosis, and has the most impact on grain set and grain yield. According to our knowledge, studies on meiotic abnormalities in wheat affected by heat are very rare if not unavailable. Therefore, this experiment was performed to study the effect of temperature on the meiosis of PMCs and to determine the relationship between meiotic abnormalities and grain set and grain yield of wheat in field experiments.

Materials and methods

Field trials

Four wheat varieties, Kauz, Montana, M6 and Chamran, were studied in this project. The Kauz variety was introduced at the International Maize and Wheat Improvement Center (CIMMYT) as a heat tolerant variety, and the Montana variety has been created in Montana State of the United States and is sensitive to heat. The M6 variety is used as an international variety in studies of heat stress. The Chamran variety was studied as the control commercial variety in moderate and warm area of Iran.

The varieties were evaluated during 2010–2011 and 2011–2012 on the research farm of the Agricultural Faculty in Shahid Chamran University of Ahwaz, Iran under two conditions, normal (normal cultivation) and heat stress (late cultivation). The Ahwaz site is located 20 m above sea level (32°20′N, 40°20′E). The experiment in each condition was arranged in a randomized complete block design with four replications. The cultivation was performed on November 5th (normal planting date) and January 19th (late planting date) so that in late planting, the flowering stage of the varieties was exposed to heat stress at the end of the growing season. The soil at the site was a sandy loam with slight alkalinity (pH=7.72) and an electrical conductivity of 3.1 dSm$^{-1}$. The meteorological information related to temperature and relative humidity of the region during the
The growing season is given in Table 1. Apart from natural heat stress applied due to the delay of planting date, all operations of planting, maintaining and harvesting were performed exactly the same in both normal and stress conditions. The seeds of each variety were planted on a ridge and furrow system with three two-meter ridges considering three lines on each ridge with a density of 350 plants per square meter. After taking samples from the soil and measuring the soil fertilizers, the field received a pre-planting broadcast application of N, P and K fertilizers reaching 250, 100, and 50 kg ha$^{-1}$, respectively, each year. Controlling the weeds during the growing period of the plant was conducted through hand weeding. Also, irrigation was performed continuously based on the soil moisture, determined by measuring the weight loss of dried samples of the field soil at depths of 0–30 and 30–60 cm.

**Experimental traits**

The number of kernels per spike and the grain yield of different varieties were calculated based on the average number of seeds per 10 plants and the grain yield in a square meter of each experimental plot, respectively. The following two indices were calculated based on the grain yield to determine the sensitivity and tolerance of varieties to heat stress:

**Stress Tolerance Index (STI):**

\[
STI = \frac{(X_p \times X_s)}{(Y_p)^2}
\]

**Stress Susceptibility Index (SSI):**

\[
SSI = \frac{1 - (X_s/X_p)}{1 - (Y_s/Y_p)}
\]

Here, $X_p$ and $X_s$ respectively represent the yield of each variety under normal and heat stress conditions, and $Y_p$ and $Y_s$ are respectively the average yield of all varieties under normal and heat stress conditions.

**Cytogenetic studies of PMCs**

For meiotic analysis, young spikes at different phases of the booting stage were collected on both the first and second sowing dates. Sampling of the immature spikes was done in the early morning (7–8 a.m.). The harvested spikes in the farm were placed in a fixative solution of Carnoy (3:1 ethanol:glacial acetic acid) on ice and were immediately transported to the laboratory refrigerator. The immature spikes were fixed within the Carnoy’s solution for 24 h. After fixation, the samples were washed and then transferred into 70% ethanol solution and kept at 10°C.

Cytogenetic studies of PMCs were performed as factorial in a complete randomized block design with four replications. For observing meiosis, several anthers were separated from florets in a spikelet and squashed on a glass slide. After staining by acetocarmine 3%, they were studied using...
an OLYMPUS optical microscope (model BH₂, made in Japan) with 40× magnification. The PMCs of anthers in various stages of meiosis were examined. Ten plants of each genotype (five plants from each planting date) and about 100 PMCs of each plant were investigated for different abnormalities. Photographing of chromosomes was conducted using the OLYMPUS camera (model DP₁₂, made in Japan) with 40× magnification.

**Statistical analysis**

Before performing the ANOVA test, two main assumptions of analysis of variance, including normality of data and homogeneity of variance were checked, and if necessary, data transformation was made for establishment of these assumptions. Data analysis for the number of kernels per spike and grain yield traits was performed based on combined analysis in both normal and heat stress conditions. Cytogenetic data analysis was performed based on factorial in randomly complete block design using the statistical software SAS 9.1 (SAS Institute). For performing comparisons of the mean, Duncan’s test and the least significant difference (LSD) test was used. The calculation of heat tolerance indices was done by Excel software, and the correlation coefficients were calculated by SAS 9.1 software.

**Results**

**Kernel per spike and grain yield**

The results of the analysis of variance for the number of kernels per spike and grain yield traits showed a significant difference ($p \geq 0.01$) between varieties and stress conditions in both years. A significant decrease ($p \geq 0.01$) of grain set and grain yield in different varieties was detected in response to heat stress. The interaction between thermal conditions and variety for kernel per spike in both years was significant ($p \geq 0.01$), but was not significant for grain yield in 2010–2011 (Table 2).

The yield average of the different varieties under favorable and heat stress conditions, and the values of the STI and SSI indices are given in Table 3. The Kauz and Chamran genotypes, in addition to good performance under normal conditions, had the highest yield under heat stress in both years. Moreover, these varieties had the maximum values of STI and the minimum values of SSI in both years. Thus, these varieties were identified as heat tolerant varieties. In contrast, the Montana and M6 varieties were detected as sensitive varieties to heat stress (Table 3).

**Meiotic abnormalities**

Anomalies occurring at the chromosome segregation were the most common abnormalities

| Table 2. Combined analysis of variance for grain yield and kernel per spike. |
|---------------------------|---------------------------|---------------------------|
| Conditions (C) | 1  | 682.65** | 39382812.50** |
| Block (C)     | 6  | 16.82    | 439795.83   |
| Varieties (V) | 3  | 870.50** | 8246179.17** |
| VxC          | 3  | 78.01**  | 266412.50ns |
| Error        | 18 | 16.98    | 345040.28   |
| CV (%)       |    |          |            |

** and *: Significant difference of 1 and 5%, respectively, ns: non-significant.
observed among genotypes, which included precocious chromosome migration to the poles in metaphase I and II as well as laggard chromosomes in anaphase I and II (Fig. 1a, b). The presence of micronuclei in telophase I (Dyads) and telophase II (Tetrads) of meiosis was the second abnormality that occurred frequently among the varieties, especially in the M6 variety (Fig. 1c). Another type of anomaly seen in this study was the lack of metaphase plate within the cell, resulting in the dispersion of chromosomes in the cell cytoplasm (Fig. 1d). No metaphase plate, and thus failure in anaphase occurrence and chromosome migration to the poles, resulted in the creation of two pyknotic nuclei placed in the cell center. This is called pyknosis (Fig. 1e).

Another anomaly frequently observed among the varieties was abnormal cytokinesis, which

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<tr>
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<tbody>
<tr>
<td></td>
<td>$Y_p$ (kg ha$^{-1}$)</td>
<td>$Y_s$ (kg ha$^{-1}$)</td>
</tr>
<tr>
<td>Kauz</td>
<td>7060a</td>
<td>4700a</td>
</tr>
<tr>
<td>Chamran</td>
<td>6740a</td>
<td>4900a</td>
</tr>
<tr>
<td>Montana</td>
<td>5190b</td>
<td>3175b</td>
</tr>
<tr>
<td>M6</td>
<td>5340b</td>
<td>2680b</td>
</tr>
</tbody>
</table>

Means with similar letters in the columns are not significantly different at 5% probability level using Duncan’s multiple range test. $Y_p$ and $Y_s$: yield of each genotype under normal and stress conditions, respectively; STI: stress tolerance index; SSI: stress susceptibility index.
led to the formation of dyad and triad, thus producing microspores with different sizes containing a number of non-uniform triad and non-reduced dyad chromosomes after the end of meiosis II (Fig. 2a, b). Another anomaly abundantly seen in different varieties was a combination of the two-cell cytoplasm, or in other words, the occurrence of cytomixis in zygotene stage (Fig. 2c). Another anomaly seen in the varieties included abnormal tetrad formation (Fig. 2d).

Quantifying the meiotic abnormalities

The results of the analysis of variance indicated that meiotic abnormalities are significantly ($p \leq 0.01$) influenced by genotype and environmental conditions. The interaction between genotype and environment was not significant, resulting in the same reaction for a variety in different

Table 4. Analysis of variance of meiotic abnormalities in wheat.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>1.29**</td>
</tr>
<tr>
<td>Conditions (C)</td>
<td>1</td>
<td>1.09*</td>
</tr>
<tr>
<td>Varieties (V)</td>
<td>3</td>
<td>0.74*</td>
</tr>
<tr>
<td>VxC</td>
<td>3</td>
<td>0.06**</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.21</td>
</tr>
</tbody>
</table>

CV (%) 32

** and *: Significantly different at 1 and 5%, respectively, ns: Non-significant. Description: Despite great effort to select anthers in similar developmental stages for different varieties for each repeat, little dissimilarity of selected anthers led to heterogenic increase and increased CV of the experiment.

Table 5. Means of genotypes for meiotic abnormalities under normal and heat stress conditions.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Meiotic abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Kauz</td>
<td>3.3a</td>
</tr>
<tr>
<td>Chamran</td>
<td>4.8a</td>
</tr>
<tr>
<td>Montana</td>
<td>8.8a</td>
</tr>
<tr>
<td>M6</td>
<td>11a</td>
</tr>
</tbody>
</table>

Mean 6.9B 16.8A

The small and capital letters show comparisons in columns and rows, respectively, at 5% probability level using LSD test.
The average of genotypes for meiotic abnormalities is presented in Table 5. The results showed that the varieties were significantly different from each other regarding the percentage of these anomalies; the M6 and Montana varieties had the most quantity of abnormalities with respective means of 21.1 and 12%, and had a significant difference ($p \leq 0.05$) with other varieties. It should be noted that the varieties had the lowest amount of anomaly under normal conditions. In other expression, late cultivation had led to coincidence of the pollen developmental stages with heat at the end of the season and motivated a significant increase of meiotic abnormalities in various varieties.

**Correlation of meiotic abnormalities with the number of kernels per spike and grain yield**

The results of this study on the correlation between meiotic abnormalities in normal and heat stress conditions and the number of kernels per spike and grain yield showed that the meiotic abnormality quantities have a significant negative correlation ($r = -0.54$, $n = 16$, $p \leq 0.01$) with the number of kernels per spike under heat stress. However, this correlation was insignificant ($r = -0.38$, $n = 16$) in normal conditions. In association with grain yield, although there were insignificant negative correlations in both normal ($r = -0.29$, $n = 16$) and heat stress conditions ($r = -0.41$, $n = 16$) between this trait and meiotic abnormalities, the correlation was stronger under heat stress conditions.

**Discussion**

The optimal temperature as well as the threshold maximum temperature of wheat at the flowering stage have been reported as 18–24 and 30°C, respectively (Russell and Wilson 1994, Saini and Aspinall 1982). Since in the current study, the flowering phase in the delayed planting date coincided with high temperature (> 30°C), a significant decrease in grain set and grain yield occurred.

This project showed that meiosis is strongly influenced by genetic as well as environmental conditions. Hereof, other reports have confirmed the impact of genetic (Falcao *et al.* 1981, Nirmala and Rao 1996) and environmental (Bione *et al.* 2000, Guerra *et al.* 2011) factors on meiosis. Among environmental factors, high temperature is one of the most important factors that influence meiotic behavior and cause abnormalities in meiosis (Ahmad *et al.* 1984, Saini and Aspinall 1982).

In this experiment, the incidence of abnormalities in chromosome segregation led to precocious migration of chromosomes to the poles and the formation of laggard chromosomes in different varieties. According to Bodanese-Zanettini *et al.* (1983), changes in weather conditions, especially temperature increase from delayed planting, cause a series of univalents and some precocious migration of such univalents at metaphase stage, as well as lagging of chromosomes at anaphase stage. The occurrence of chiasmata causes the maintenance of bivalents, which permit normal separation of chromosomes. This process ensures the fertility of pollen, but non-formation of the dyad leads to precocious movement of univalents to the poles (Bione *et al.* 2000). Micronuclei in telophase I and II resulting in microspores were other forms of anomalies that were seen abundantly in the studied genotypes. The origin of the micronuclei is the precocious ascension in metaphase stage and laggard chromosomes in anaphase stage (Rezaei *et al.* 2010). The presence of micronuclei was first identified by Ahmad *et al.* (1977) in inter-species hybrids of *Glycine max* x *Glycine soja*.

Another abnormality observed in this experiment was the absence of metaphase plate, so chromosomes were not able to congregate in the metaphase plate, thereby scattering in the cytoplasm. The absence or defect of such spindle fibers, as well as faultiness of the kinetochore, which connects the spindle fibers to the centromere, could result in the occurrence of this anomaly.
The occurrence of a mutation in maize and failure to form spindle fibers, causing inability of bivalent congregation at metaphase, was reported by Taschetto and Pagliarini (1993). In the following stage, the scattered bivalents connected to each other and formed two pyknotic nuclei placed in the middle of the cell, which led to non-occurring of the first cytokinesis. Pyknosis is an abnormality generally caused by chromosome stickiness (Daniela et al. 2005). Daniela et al. (2005) reported this type of abnormality in Brachiaria brizantha. The high percentage of abnormal cytokinesis in the studied varieties can be related to the misorientation of spindles in meiosis II (Pecrix et al. 2011). Abnormal cytokinesis is considered as one of the reasons for the formation of diploid pollen grains. The formation of fused, parallel and tripolar spindles in meiosis II has been identified in species such as Solanum sp. (Peloquin et al. 1999), Lotus tenuis (Negri and Lemmi 1998) and Medicago sativa (Tavoletti et al. 1991).

Another type of abnormality seen in the varieties was cytomixis. Cytomixis leads to increased or decreased chromosome content and the creation of aneuploid conditions in the cells. These cells eventually lead to the formation of aneuploid gametes or pollens. Another result of cytomixis is complete loss of chromatin material, which results in the formation of abnormal tetrads and sterile pollens. Chromatin material transfer between adjacent cells occurs by connecting the cytoplasm of the two cells through a channel called Plasmodesmata. Also, chromosomes can be transmitted through the decomposition of the cytoplasm wall of adjacent cells and the composition of cytoplasm and their chromatin material (Falistocco et al. 1995). Cytomixis was first reported by Gates (1911) in Oenothera gigas.

The results indicated a reduction in grain set with increased meiotic abnormalities under heat stress conditions. The insignificant correlation between meiotic abnormalities and the number of kernels per spike under normal condition is due to a very low percentage of such abnormalities in favorable conditions. Due to a significant increase in abnormalities in heat stress conditions, the grain set, especially in susceptible varieties, declined sharply. In this regard, Saini and Aspinall (1982) reported that the occurrence of heat stress (>30°C) during the male organ development, from the meiosis beginning in the PMCs to the formation of young microspores and their early development will lead to reduced grain set in wheat. Therefore, it can be stated that the heat stress causes the production of premature pollens by the creation of impairment in meiosis, which reduces the fertility and thereby the grain set greatly.

Conclusion

The results of this experiment showed that the pollen development process and meiosis of PMCs are one of the most sensitive stages of the plant life cycle and are extremely affected by genetics as well as environmental factors. In this experiment, the plants grown in normal conditions were benefiting optimal temperature conditions, and thus showed the lowest abnormality rate in meiosis. However, with the delay in sowing and thereby the coincidence of the flowering stage of the varieties with high temperatures at the end of the growing season, a significant increase was observed in these abnormalities. Since the meiotic abnormalities had a significant negative correlation with the number of kernels per spike under heat stress, it indicates that the heat stress greatly reduces the grain set, especially in the susceptible varieties, by increasing meiotic abnormalities. To summarize, the study of meiosis in PMCs can be suggested as a necessary tool to improve tolerance to heat stress in commercial varieties, and partly as a method for screening tolerant lines against heat stress in breeding programs.
Acknowledgments

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