

Test weight, kernel shrivelling, and aneuploidy frequency in triticale

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Abstract Kernel shrivelling is one of the main problems in kernel appearance and test weight that cause yield loss. Aneuploidy frequency and its relationship with kernel shrivelling were determined in four triticale genotypes. Firstly, genotypes were divided into two main groups based on their test weights. Secondly, these two groups were subdivided into two subclasses based on kernel appearance. While euploids rate was 100% in plump kernels of high test weight genotypes, this rate was 92 and 94% respectively in a group of shrivelled kernels. Ploidy levels of $2n = 40$ and $2n = 41$ were observed in aneuploids. A ploidy of $2n = 40$ chromosomes was not observed in the plump kernel group of high test weight genotypes, but in the shrivelled kernel group made up 1%. While there were no aneuploids in the plump kernel group of high test weight genotypes, the aneuploidy rate was 7% in the shrivelled kernel group of same genotypes. On the other hand, the aneuploidy rate was 8 and 13% in the plump and shrivelled kernel groups of low test weight genotypes, respectively.

Keywords triticale; test weight; kernel shrivelling; aneuploidy

INTRODUCTION

Triticale (*X Triticosecale* Wittmack) is the product of a cross between members of the wheat genus (*Triticum* L.) and the rye genus (*Secale* L.) (Gustafson 1976). It has the potential to be a cereal crop on its own. Triticale is grown primarily for animal feed as either a grain or forage crop. Agronomically, triticales are attractive due to a broad spectrum of disease resistance. Kernel shrivelling is a major problem in kernel appearance and test weight, causing yield loss (Shealy & Simmonds 1973). Weimarck (1975) reported that large kernels germinated better than medium and small kernels, and seedlings from large kernels had a higher survival rate than others in the bulk population under field conditions.

The relationship between kernel size and aneuploidy frequency has been investigated in some studies in which materials were classified based on kernel size (Weimarck 1975; Hafiz & Larik 1984). In a different approach, we classified kernels based on shrivelling and plumpness, rather than on kernel size.

The aim of this study was to identify the relationship between aneuploidy and kernel shrivelling, emergence rate, and germination rate.

MATERIALS AND METHODS

Four hexaploid triticale genotypes released by CIMMYT (International Maize and Wheat Improvement Center) were used in this study. Seeds were obtained from plants grown under the same conditions (annual total precipitation 386.1 mm and annual mean temperature 5.5°C) at the experimental site of the Agricultural Faculty of Erzurum (Turkey). They were divided into two groups based on test weight. Two genotypes had a high test weight while the other two had a low test weight. In addition, these two groups were subdivided into two subclasses, plump and shrivelled kernels. Information about genotypes based on kernel size and use in this study is presented in Table 1.

For somatic chromosome counts, 75 seeds from each class were germinated on moist filter paper in

Petri dishes. Root tips of 50 seedlings were pre-treated in chilled water (0–2°C) for 20–24 h, fixed in Carnoy's solution (6:3:1; ethanol:acetic acid:chloroform, respectively), stained by the Feulgen method after hydrolysing for 20 min in 1N HCl at 60°C, and squashed in 45% acetic acid (Tosun 1999; Sağsöz et al. 2002).

To determine germination rate of kernels, 100 seeds were taken from each group, plated in petri dishes, and germinated in a Copenhagen tank at room temperature with four replications. At the end of 8 days, seeds that produced seedlings were counted (Anonymous 1996).

To determine the emergence rate, 400 seeds from each group with four replications (100 seeds for each replication) were sown at a depth of 3 cm into pots (10 seeds per pot) containing a mixture of soil:farm manure:sand (1:1:1) and grown in a greenhouse at room temperature. After 2 weeks, seedlings were counted. Emergence rate was calculated as a proportion of number of seedlings to number of seeds planted.

Data on germination and emergence rates were subjected to analysis of variance (ANOVA) using the completely randomised design with four replicates.

RESULTS AND DISCUSSION

Cytogenetic investigations

Euploidy ($2n = 42$) was 100% in plump kernels of high test weight genotypes (lines #23 and #27), but only 92 and 94%, respectively, in high test weight groups with shrivelled kernels. In low test weight groups, euploidy was 92% in plump kernels, but only 84 and 90% in shrivelled kernels (lines #30 and #32, respectively). Plump kernels had 100% euploid seedlings compared with a mean of 93% from shrivelled kernels of high test weight genotypes, and 92 and 87% euploidy in plump and shrivelled kernels, respectively, in genotypes of the low test weight group. The variation in chromosome numbers in different classes is shown in Table 2.

Table 1 Characteristics of genotypes used in this study.

| Genotypes | | Test weight (kg/hl) | 1000 grain weight (g) | |
|-----------|-----------------------|---------------------|-----------------------|------------|
| No. | Pedigree | | Plump | Shrivelled |
| 23 | Hare 7265/Yogui 1 | 73.59 (high) | 40.85 | 25.03 |
| 27 | Stier 22-1 | 73.24 (high) | 38.55 | 24.74 |
| 30 | Drira 2/27082 | 65.53 (low) | 39.65 | 28.45 |
| 32 | Cachirulo-Lince/23043 | 63.45 (low) | 42.10 | 28.15 |

Table 2 Seed groups and mitotic chromosome numbers of triticale genotypes. HTW, high test weight; LTW, low test weight.

| Genotypes | Seed groups | Euploids | | Aneuploids | | | | Total | |
|--------------|-------------|----------|-----|------------|-----|-----------|-----|--------|----|
| | | Number | % | $2n = 40$ | | $2n = 41$ | | Number | % |
| 23 (HTW) | Plump | 50 | 100 | – | – | – | – | – | – |
| | Shrivelled | 46 | 92 | – | – | 4 | 8 | 4 | 8 |
| 27 (HTW) | Plump | 50 | 100 | – | – | – | – | – | – |
| | Shrivelled | 47 | 94 | 1 | 2 | 2 | 4 | 3 | 6 |
| Mean | Plump | 50.0 | 100 | – | – | – | – | – | – |
| | Shrivelled | 46.5 | 93 | 0.5 | 1 | 3 | 6 | 3.5 | 7 |
| 30 (LTW) | Plump | 46 | 92 | 1 | 2 | 3 | 6 | 4 | 8 |
| | Shrivelled | 42 | 84 | 2 | 4 | 6 | 12 | 8 | 16 |
| 32 (LTW) | Plump | 46 | 92 | – | – | 4 | 8 | 4 | 8 |
| | Shrivelled | 45 | 90 | 1 | 2 | 4 | 8 | 5 | 10 |
| Mean | Plump | 46.0 | 92 | 0.5 | 1 | 3.5 | 7 | 4 | 8 |
| | Shrivelled | 43.5 | 87 | 1.5 | 3 | 5 | 10 | 6.5 | 13 |
| General mean | Plump | 48 | 96 | 0.25 | 0.5 | 1.75 | 3.5 | 2.0 | 4 |
| | Shrivelled | 45 | 90 | 1.00 | 2.0 | 4.00 | 8.0 | 5.0 | 10 |

Ploidy levels of $2n = 40$ and $2n = 41$ were observed in aneuploids. According to the average number of chromosomes in aneuploids, the occurrence of $2n = 40$ (2.5%) was found to be lower than $2n = 41$ (11.5%). A ploidy of $2n = 40$ was not observed in the plump kernel group of high test weight genotypes, but made up 1% of the shrivelled kernel group. In low test weight lines, a ploidy of $2n = 40$ had a 1% occurrence in the plump kernel group, but 3% in the shrivelled kernel group (Table 2). A ploidy of $2n = 41$ occurred in 0 and 6% of plump and shrivelled kernels, respectively, in high test weight genotypes, while these values increased to 7 and 10%, respectively, in low test weight genotypes. The mean occurrence of $2n = 41$ seedling was 3.5% in plump kernels and 8.0% in shrivelled kernels (Table 2).

Overall, plump kernels of high test weight genotypes had no aneuploids, but aneuploidy was 7% in shrivelled kernels of the same genotypes. Plump kernels of low test weight genotypes showed 8% aneuploidy, with a significant increase to 13% in shrivelled kernels of the same genotypes. Over all four genotypes, mean aneuploidy was 4% in plump kernels and 10% in shrivelled kernels.

According to our results, the aneuploidy percentage was higher in low test weight genotypes as well as in shrivelled kernel groups of the four genotypes tested. Shrivelling of triticale kernels can be caused by various cytological and physiological factors. The first important cytological factor could be aneuploidy. The meiotic instability of triticale results in varying degrees of aneuploidy in the progeny of euploid plants (Scoles & Kaltsikes 1974). Similarly, Merker (1971) found a positive correlation of 0.75 between meiotic instability (as measured by univalents at metaphase I) and degree of aneuploidy, except for one line. Guedes-Pinto et al. (1984), who divided kernels into five groups based on size, although not based on plumpness of kernels, reported that aneuploidy generally increased as kernel size decreased in all hexaploid triticale. Similar results were also reported by Weimarck (1975) in octoploid triticale.

In this study, we observed hypoploidy in aneuploids, but no hyperploidy was observed. Various researchers also have found similar supportive results (Guedes-Pinto et al. 1984). This phenomenon can be explained either by meiotic irregularities resulting in chromosome loss or by hyperploid gametes being less functional than hypoploid gametes (Hossain 1979). Oudjehih & Boukaboub (2000) emphasised that the importance

of aneuploidy depends on seed morphological characteristics: plants from unshrivelled small seeds were more affected (40%) than those from large normal seeds (5%) or large shrivelled seeds (15%). Both the magnitude and nature of mitosis and meiosis are related to seed morphology, with plants from small seeds or large shrivelled seeds being the most affected.

Aneuploidy of triticale varies with state of kernel shrivelledness as well as kernel size (higher aneuploidy percentage in small kernel size) (Guedes-Pinto et al. 1984). In our study, we found clear differences in aneuploidy between plump (4%) and shrivelled (10%) kernel groups as measured by overall means.

To reduce aneuploid frequency in seeds of triticale, elimination of shrivelled and smaller kernels could be advantageous. However, it should be considered that aneuploidy frequency changes depending upon genotype, and kernel shrivelling might show variability depending upon climate and soil conditions where plants grow. On the other hand, smaller and shrivelled kernels are a preferential material to be used for screening aneuploid plants, for example, in cytological studies or for obtaining a monosomic series in triticale (Guedes-Pinto et al. 1984).

Germination and emergence percentage of kernels

Table 3 summarises the germination and emergence percentage of genotypes in each seed group. The differences among genotypes were significant for both germination and emergence rate. These differences were especially salient for emergence rate between high and low test weight genotypes. According to Duncan's multiple range test, high test weight genotypes formed the same group, low test weight genotypes being another group. When both test weight and plumpness of seeds were taken into account, germination percentage was higher than percentage of emergence. However, emergence percentage varied with test weight and was found to be greater in high test weight genotypes (84.0%) than in low test weight genotypes (72.8%). Similarly, the emergence percentage of plump kernels was higher than for shrivelled kernels. Emergence rates were found to be 92.5% for plump and 75.5% for shrivelled kernels in the high test weight group, and 80.5 and 65.0%, respectively, in the low test weight group.

Seeds planted at a certain depth must have sufficient nutrients in their endosperm to reach and

Table 3 Germination and emergence percentage of triticale genotypes. Within columns, means sharing the same letter do not differ significantly. HTW, high test weight; LTW, low test weight.

| Genotypes | Seed groups | Germination (%) | Mean of genotypes | Emergence (%) | Mean of genotypes |
|-----------|-------------|-----------------|-------------------|---------------|-------------------|
| 23 (HTW) | Plump | 100a | 97.5a | 91a | 84.5a |
| | Shrivelled | 95bc | | 78bc | |
| 27 (HTW) | Plump | 98ab | 96.5ab | 94a | 83.5a |
| | Shrivelled | 95bc | | 73cd | |
| Mean | Plump | 99.0 | 97.0 | 92.5 | 84.0 |
| | Shrivelled | 95.0 | | 75.5 | |
| 30 (LTW) | Plump | 96bc | 94.5b | 79bc | 70.5b |
| | Shrivelled | 93c | | 62e | |
| 32 (LTW) | Plump | 95bc | 94.5b | 82b | 75.0b |
| | Shrivelled | 94c | | 68de | |
| Mean | Plump | 95.5 | 94.5 | 80.5 | 72.8 |
| | Shrivelled | 93.5 | | 65.0 | |

crack the soil surface. If sufficient nutritive materials are not stored in small or shrivelled kernels, seeds usually die or are not able to reach the soil surface, even if they germinate. Therefore, the emergence rate of shrivelled kernels is low. Other studies that focused on kernel size concluded that small seeds have germination rates lower than large seeds (Weimarck 1975; Guedes-Pinto et al. 1984), and this has become generally accepted. Tosun (1995) also found positive and significant relationships between 1000 kernel weight and germination and 1000 kernel weight and emergence rate of seeds.

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