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Investigation of Chromosome Numbers and Plant Characteristics of *Triticum compactum* × *Triticum turanicum* Interspecific Hybrid in F_2 Generation

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ABSTRACT

The objective of this study was to identify the plants with varying chromosome numbers in the F_2 generation, resulting from interspecific hybrids between hexaploid *Triticum compactum* and tetraploid *Triticum turanicum*, and to examine the morphological, physiological and agronomic characteristics of these plants. Therefore, the objective was to assess the potential for developing monosomic lines (particularly pentaploid) for the D-genome of wheat, with a view to their utilization in future breeding programs of wheat, and to ascertain the correlation between the estimated chromosome numbers and the superior phenotypic characteristics of the plants in question. The germination percentage was determined by germinating 230 seeds, which will form the F_2 generation of *Triticum compactum* × *Triticum turanicum* interspecific hybrid, in Petri dishes together with the parents. Thereafter, the plants were transferred to 2 m long rows, 30 cm between rows and 10 cm above rows. The F_2 plants were subjected to evaluation in order to ascertain their morphological, physiological and agronomic characteristics. Furthermore, the nuclear DNA contents of the F_2 plants were determined by flow cytometry, and chromosome numbers were estimated based on the DNA contents of the parents. Finally, the correlations between the estimated chromosome numbers and the measured plant traits were determined. The nuclear DNA contents of F_2 plants exhibited variability, with values ranging between 7870.39 and 11632.1 pg. Additionally, three plants with 35 chromosomes were identified. The F_2 plants showed superior physiological traits compared to the parents, however, they displayed lower values for spike traits that affect yield. The superior traits had by F_2 plants can be observed in subsequent generations, thus providing a valuable genetic resource for breeding programs and certain genomic studies.

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Introduction

The genus *Triticum* comprises species at three distinct ploidy levels: diploid (2n = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42). Some of these species are commercially important (Igrejas et al., 2020). Although Khorasan wheat (*T. turgidum* ssp. *turanicum*) has been present in agricultural systems in the Middle East and Central Asia for centuries, it has been relatively neglected and underutilized among tetraploid wheats. The grain of Khorasan wheat is notably large, with a remarkably high thousand-grain weight (50-60 g) (Grausgruber et al., 2005). Furthermore, the grain is amber in color and exhibits a high degree of transparency. Given its elevated plant height, Khorasan wheat, which demonstrates resilience to lodging and vulnerability to mildew disease, is regarded as a more optimal choice for organic cultivation. This wheat is known worldwide under the trademark Kamut. In 1990, the United States Department of Agriculture registered the QK-77 variety under the name Kamut, which is now used and traded as a trademark. The available data on the physical properties and chemical composition of the grain indicate that Kamut grains contain 20-40% more protein than other wheats and are rich in lipids, amino acids, vitamins, and minerals (Singh, 2007).

Topbaş wheat (*T. aestivum* ssp. *compactum*) is distinguished from other subspecies by its predominant compact spike form (Nilsson-Ehle, 1911). The origin of Topbaş wheat has been a topic of debate, however it is widely accepted that it is the result of a mutation at the C locus in hexaploid bread wheat (*T. aestivum* ssp. *aestivum*). This is because all forms of *T. aestivum* share a common gene pool, and studies have demonstrated that there is no known tetraploid wheat (*T. turgidum* L.) or diploid *Ae.*

tauschii sequence that exhibits a truly compact spike structure (Feldman, 2001). Topbaş wheat varieties are still cultivated commercially, albeit on a limited scale. These attributes contribute to their preference, including resistance to drought and breakage, hard straw, earliness, and competitive yields. The smaller grains of Topbaş wheats are a consequence of their compact spike structure, yet this reduction in size is offset by an increase in seed number (Zwer et al., 1995). Topbaş wheats has reduced thousand-grain weights and protein ratios.

The process of interspecific hybridization has been employed as a means of combining the distinctive characteristics displayed by these two wheat varieties. The majority of crosses between tetraploid and hexaploid wheats were conducted between *T. aestivum* and *T. durum*. In these studies, the objective was to transfer traits such as yellow rust resistance, drought tolerance, and high protein content from durum wheat to bread wheat, while winter hardiness was targeted for transfer from bread wheat to durum wheat (Özkan and Genç, 1998; Ali et al., 2020). The interspecific hybridization of hexaploid and tetraploid wheat species results in the formation of pentaploid F_1 hybrids, having distinctive chromosome structures (AABBD, 2n=35). The pentaploid hybrids obtained from crossing bread wheat and durum wheat have the potential to enhance the genetic background of both parents by transferring relevant traits. The utilization of pentaploid wheat hybrids in breeding initiatives remains limited. However, available data suggests that wheat lines developed from pentaploids will be a useful supplement to commercial plant breeding efforts that target enhancing abiotic stress tolerance, fungal disease resistance, quality criteria, and agronomic features. Pentaploid-derived wheat lines have been demonstrated to have the potential to enhance tolerance to the cereal root lesion nematode (Sheedy et al., 2012, 2015; Thompson et al., 2012). Furthermore, the significance of utilizing pentaploid-derived wheat lines in the improvement of drought, salinity, cold, and high temperature tolerance traits has been underscored (Reynolds et al., 2009; Hassan et al., 2016; Mohamed et al., 2020).

The breeding of pentaploid wheat is hindered by several factors, including low pollen compatibility, poor seed set, inadequate vegetative development, and infertility issues in F_1 hybrids. Nevertheless, the majority of these challenges have been surmounted through the meticulous selection of parents and the utilization of genotypes with elevated ploidy levels as maternal parents (Padmanaban et al., 2017). The goal of studying the production of hexaploid × tetraploid wheat hybrids has been to improve elite bread and durum wheat lines for several commercially desirable characteristics (Martin et al., 2011, 2013; Han et al., 2014, 2016; Kalous et al., 2015). The dominance of heterozygous loci in the A and B genomes, along with the retention of the D genome, has resulted in pentaploid wheat hybrids with high genetic variation. There is still much to learn about the efficient screening, selection, and use of populations obtained from pentaploid wheat hybrids in commercial breeding operations, even if there is a rich supply of genetic variation. Although pentaploid wheat with 35 chromosomes can be obtained in the first generation (F_1) after hexaploid × tetraploid hybridization, it has been reported that chromosome numbers vary between 28 and 42 in the F_2 generation resulting from the selfing of the F_1 generation, with the majority of plants creating 33 to 40 chromosomes. Among the plants with varying chromosome numbers, those with 35 chromosomes were found to have superior fertility and more favorable agricultural characteristics (Kihera, 1982; Belea, 1992; Özkan and Genç, 1998; Mohamed et al., 2020). To select these plants from the F_2 generation, cytological observations or molecular studies utilizing specific markers to differentiate the D genome set were required. Some studies have reported that in the presence of the D genome, the resistance of wheat species to some diseases and environmental stress conditions, grain yield and some yield traits increased, and the rheological properties of the dough improved (Kalous et al., 2015; Camerlengo et al., 2022; Gruet et al., 2024).

The enhancement of molecular and cytological techniques such as flow cytometry utilized for the screening of recombinant progeny will facilitate the optimization of the selection process and assist breeders in accelerating the production of pentaploid organisms. The potential of interploidy hybridization as a tool for developing wheat genotypes capable of adapting to changing climatic conditions is a promising avenue for further investigation. To enable sustainable and increasing global wheat production in the future, it is crucial to start additional research on incorporating such features from bread wheat to durum wheat or from durum wheat to bread wheat through pentaploid wheat hybrids. The objective of this study was to ascertain the morphological traits and chromosome numbers of F_2 plants derived from interspecific crosses between Khorasan wheat, regarded as a functional food due to its grain quality and nutritional value, and Topbaş wheat, which shows competitive yield, drought resistance, and lodging tolerance. Additionally, the study aimed to elucidate the correlation between plant characteristics and chromosome numbers.

Materials and Methods

The tetraploid parents utilized in the research are Khorasan wheat, catalogued as 2483, belonging to the *T. turanicum* species, which were donated to the United States Department of Agriculture (USDA) gene bank by Belgium in 1950. The hexaploid parent is Vardenik-9 of the *T. compactum* species, namely the Topbaş wheat variety, which was developed through combination breeding in Armenia. The variety was donated to the USDA gene bank in 1972, and its pedigree is recorded as "Artashati 42/landrace Erinatseum." The seeds of the parents utilized in the study were obtained from the USDA gene bank.

Following the hybridization of the parents, 40 seeds were obtained and pentaploid F_1 plants were cultivated by sowing the seeds. A total of 468 seeds were obtained from the F_1 plants, and 240 of them were sown to cultivate F_2 plants, while the remainder were retained as genetic stock.

Parental and F_2 seeds were sown in Petri dishes. Before sowing, the seeds were soaked in 70% alcohol for 1 min, 2% sodium hypochlorite (NaOCl) solution for 3 min, rinsed 5 times with sterile distilled water and then placed on filter papers with 20 seeds in each petri dish, with parents in 3 replicates and F_2 seeds in 12 replicates. The

germination temperature was set at 20°C day/15°C night. Vernalization was started 14 days after germination. Plants in petri dishes were maintained in a Nucleon growth cabinet at 20/15°C, 60-70% humidity, 450 µmol m-2 s-1 photosynthetic flux density and 16/8 hours of illumination until vernalization. For vernalization, the temperature was set at 4°C with the same other growth chamber conditions and kept under these conditions for 5 weeks. At the end of the vernalization period, the plants were transplanted into 2 m long rows in high tunnels at Eskişehir Osmangazi University Faculty of Agriculture campus with 30 cm between rows and 10 cm above rows. The standard cultivation procedures (fertilization, irrigation, etc.) used in wheat cultivation were applied.

Seeds with a rootlet length of two millimeters were considered to have germinated. The number of germinated seeds was counted at the end of 10 days, and the germination percentage was determined by dividing the number of germinated seeds by the total number of seeds (ISTA, 2003).

The chlorophyll content of the plant leaves was quantified using a chlorophyll meter (Spectrum Field Scout CM 1000) and the canopy temperature was measured with a portable infrared thermometer following the heading stage (Jackson et al., 1996; Wenkel et al., 2003). To ascertain the number of stomata, a transparent nail polish was applied to the flag leaves of the plants and allowed to dry. Once the gloss had dried, it was meticulously removed from the surface of the leaf and placed on a slide. Subsequently, the number of stomata falling within the microscope area with 4x100 magnification was counted, and the average was calculated and defined as the number.

Discs with an approximate diameter of 2 cm were excised from the flag leaves of the plants and weighed to determine their respective fresh weights (FW) in mg. The leaves were maintained in Petri dishes for four hours to permit complete soaking in distilled water and turgor establishment. The turgor leaf discs were promptly wiped with a paper towel to remove any residual water and reweighed to determine their turgor weights (TW) (mg). Subsequently, the leaf discs were subjected to a 24-hour drying process at 70°C, after which their dry weights (DW) were determined. The relative water content (RWC) of the leaves was calculated in accordance with the following formula, as proposed by Cseuz et al. (2002):

$$RWC\ (\%) = [FW - DW] / [TW - DW] \times 100$$

The width (FLW) and length (FLL) of the flag leaf were measured on the main stems of the plants, and the flag leaf area (FLA) was calculated according to the formula (equation 1, Spagnoletti Zeuli & Qualset, 1990):

$$FLA = FLL \times FLW \times 0.75$$

The plant height in cm was determined by measuring the distance from the soil level to the tip of the top spikelet, excluding the awns from the plant.

Additionally, the following characteristics were quantified: spike length (cm) and the number of spikelets per spike, spike weight (g) and the number of grains per spike, as well as the grain weight per spike (g). The spike harvest index (%) was calculated as a percentage by dividing the values of spike and grain weight per spike. The spike density index was calculated by dividing the number of spikelets per spike by the length of the spike. Additionally, the thousand-grain weight (g), which is a crucial physical quality criterion, was determined.

Nuclear DNA analysis was conducted using fresh leaf samples via a PARTEC brand flow cytometer in the Biotechnology Laboratory of the Ankara Central Research Institute. The standard used in the analyses was *Hordeum vulgare* with a nuclear DNA content of 10.7 pg^2C^{-1}. The analyses were conducted using ready-made kits from PARTEC (CyStain PI absolute P), and the manufacturer's protocol was followed (https://eu.sysmex-flowcytometry.com/reagents/cystain-ploidy-dna-analysis/1424/cystain-uv-precise-p). The absolute nuclear DNA content of a sample was calculated in pictograms (pg) using the values of the fluorescence intensities of the G1 peaks of the sample and the selected standard, based on the following formula:

$$S_{DNA}C = FISP/FISTD \times STD_{DNA}$$

$S_{DNA}C$: Sample DNA Content
FISP : Fluorescence Intensity of Sample Plant
FISTD : Fluorescence Intensity of Standard Plant
STD_{DNA} : DNA Content of Standard Plant

Following the identification of the nuclear DNA contents as previously described, an attempt was made to estimate the chromosome numbers of the F_2 plants using the following formula, taking into account the calculated values of the tetraploid parent with 28 chromosomes and the hexaploid parent with 42 chromosomes.

$$DVSC = (DCTP + DCHP) / (CNTP + CNHP)$$

DVSC: DNA value of a single chromosome
DCTP: DNA content of tetraploid parent
DCHP: DNA content of hexaploid parent
CNTP: Chromosome number of tetraploid parent
CNHP: Chromosome number of hexaploid parent

Once the DNA value of the single chromosome had been determined, the chromosome number of the F_2 plants was calculated using the following formula.

$$CNF_2 = DCF_2 / VSC$$

CNF_2 : Chromosome number of F_2 plant
DCF_2 : DNA content of F_2 plant
DVSC : DNA value of a single chromosome

Hayashi et al. (2009) compared the chromosome numbers calculated using this formula with the chromosome numbers counted from the root tips and found that the chromosome number could be estimated with 95% accuracy.

Descriptive statistics, including mean, standard error, minimum, and maximum values, were calculated using the IBM SPSS 20 statistical program. Additionally, correlations between the characteristics via factor analysis were conducted according to $p < 0.05$.

Results and Discussion

Spike measurements of F_1 plants and their parents were shown in Table 1. F_1 plants, which had values between the parents in spike length, spikelet number per spike and spike weight, had low values in terms of grain number and weight per spike. This is because the unbalanced chromosome number in F_1 plants prevents pollen development and fertilization (Kihara, 1982).

The germination percentage of parents was 100%, while that of F_2 seeds was 76.7%. Despite the 76.7% germination rate of the F_2 seeds, the resulting plants exhibited poor development, with the majority failing to produce stalks, spikes, or grains. The low germination percentage and poor plant characteristics observed in the F_2 generation may be attributed to several factors, including the compression of the embryo by the endosperm, the inability of the endosperm to develop sufficiently to support the embryo, and the presence of different chromosomes in each seed. Of the 184 F_2 seeds that germinated, 60 plant were observed to develop. Observations and measurements were conducted and correlated in 23 F_2 plants for which nuclear DNA measurements could be obtained.

The chromosome numbers of 23 F_2 plants were calculated based on of the quantity of nuclear DNA. Hayashi et al. (2009) underscored the practical utility of nuclear DNA quantification in determining the chromosome number of plants. The mean nuclear DNA content of hexaploid parent with 42 chromosomes was found to be 11,749.86 pg, while that of tetraploid parent with 28 chromosomes was 7,847.86 pg (Figure 1). The mean nuclear DNA content of F_2 plants with 28 chromosomes was 7870.39 pg, while those with 35 chromosomes demonstrated a mean of 9784.23 pg, and those with 42 chromosomes exhibited a mean of 11632 pg. 28-chromosome F_2 plants had a higher amount of nuclear DNA compared to the parent values, while 42-chromosome F_2 plants demonstrated a lower amount of nuclear DNA compared to the 42-chromosome parent. This observed increase in the number of chromosomes can be attributed to the higher amount of nuclear DNA. The disparate chromosome numbers observed in the F_2 generation can be attributed to the formation of three D-genome groups resulting from crosses between hexaploid and tetraploid wheat. In certain instances, chromosome elimination has also been documented. The first group's progenies (2n = 4x = 28) lost all seven D-genome chromosomes. Progenies with a medium number of D-genome chromosomes (total chromosomal numbers ranging from 2n = 29 to 41) make up the second category. Two copies of each of the seven D-genome chromosomes (2n = 6x = 42) were kept by the third group. The three sets of wheat hybrids created from pentaploids can either self or backcross with one of the parents, based on the breeding program's goal, which could be the creation of durum or bread wheat lines. To create elite durum lines, lines from the first group of hybrids, for instance, can be selfed or backcrossed with a durum parent (Padmanaban et al., 2017).

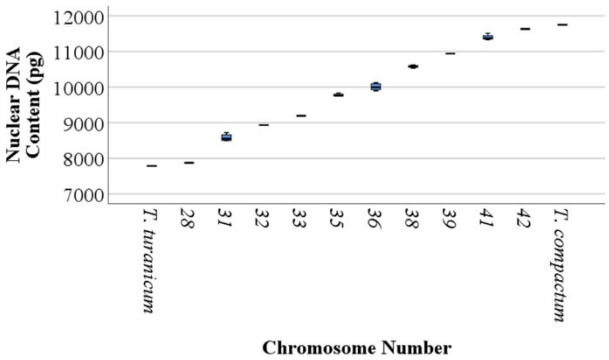

Figure 1. Nuclear DNA content of F_2 plants

Morphological, physiological and agronomic characteristics were also determined in 23 F_2 plants whose nuclear DNA contents were determined. Of these, one plant was found a chromosome number of 28, 32, 33, 39, and 42, four plants displayed a chromosome number of 31, three plants exhibited a chromosome number of 35, 38, and 41, and five plants demonstrated a chromosome number of 36. The varying number of chromosomes in F_2 plants indicated that a certain number of chromosomes were lost during meiosis of the F_1 pentaploid plants. It is expected that plants with a chromosome number of 35 or more will show evidence of a set of D genome. Prior research on this topic has indicated that each F_2 population derived from an F_1 pentaploid has a unique pattern in accordance with the frequency distribution of plants with a specific number of chromosomes. However, the conservation of D chromosomes in F_2 plants is dependent on the parental genotypes of the original cross (Martin et al., 2011; Padmanaban et al., 2018; Mohamed et al., 2020).

The descriptive statistics of the examined traits are presented in Table 2. The studied population indicates considerable variability. For the traits where the coefficient of variation is less than 15% (plant canopy temperature, leaf relative water content, plant height, spikelet number per spike), the observed variability is low. The values obtained for these traits exhibited a high degree of correlation. As the skewness values are less than 2, it can be stated that a normal distribution is present for all traits. Furthermore, the values below the mean for leaf chlorophyll content, relative water content, spikelet number per spike, grain number per spike, and spike harvest index are the majority. Additionally, the kurtosis values are negative, indicating that the normal curve is flattened.

Table 1. Spike characteristics of F_1 plants and their parents

Genotypes	SL	SNS	GNS	SW	GWS
T. compactum	6.2	19.4	45.2	2.08	1.58
T. turanicum	11	22	34	2.44	1.58
T. compactum x T. turanicum	7.3	21.2	20.8	2.03	0.93

SL: spike length, SNS: spikelet number per spike, GNS: grain number per spike, SW: spike weight, GWS: grain weight per spike

Table 2. Descriptive statistics of the characteristics analyzed in the study

Variable	Min	Max	Mean	SE	SD	Variance	CV%	Skewness	Kurtosis
CHL	238.0	485.0	385.0	13.0	65.2	4257.3	16.95	-0.53	-0.11
CT	16.700	25.000	18.986	0.433	2.167	4.69	11.41	1.67	2.32
SN	37.00	60.00	43.52	1.32	6.62	43.76	15.20	0.96	0.03
RWC	59.64	85.79	76.29	1.42	7.12	50.71	9.33	-0.63	-0.27
FLA	16.88	46.80	30.72	1.80	8.98	80.56	29.21	0.48	-0.72
PH	119.00	147.00	127.72	1.46	7.30	53.29	5.72	0.98	0.84
SL	4.750	9.500	6.172	0.188	0.938	0.879	15.19	1.93	6.08
SNS	15.500	24.500	20.040	0.396	1.979	3.915	9.87	-0.26	0.81
GNS	22.00	48.00	37.66	1.48	7.39	54.66	19.63	-0.86	0.05
SDI	25.00	45.99	31.05	1.01	5.04	25.42	16.24	1.63	2.55
SW	0.920	3.530	1.657	0.105	0.526	0.277	31.76	1.84	5.95
GWS	0.3500	2.3300	1.0308	0.0843	0.4214	0.1776	40.88	1.11	2.95
SHI	21.81	79.25	61.34	2.46	12.31	151.59	20.07	-1.75	3.90
TGW	9.46	60.60	27.08	1.84	9.22	84.94	34.03	1.82	6.96

*CHL: flag leaf chlorophyll content, CT: canopy temperature, SN: stomata number, RWC: leaf relative water content, FLA: flag leaf area, PH: plant height, SL: spike length, SNS: spikelet number per spike, GNS: grain number per spike, SDI: spike density index, SW: spike weight, GWS: grain weight per spike, SHI: spike harvest index, TGW: thousand-grain weight

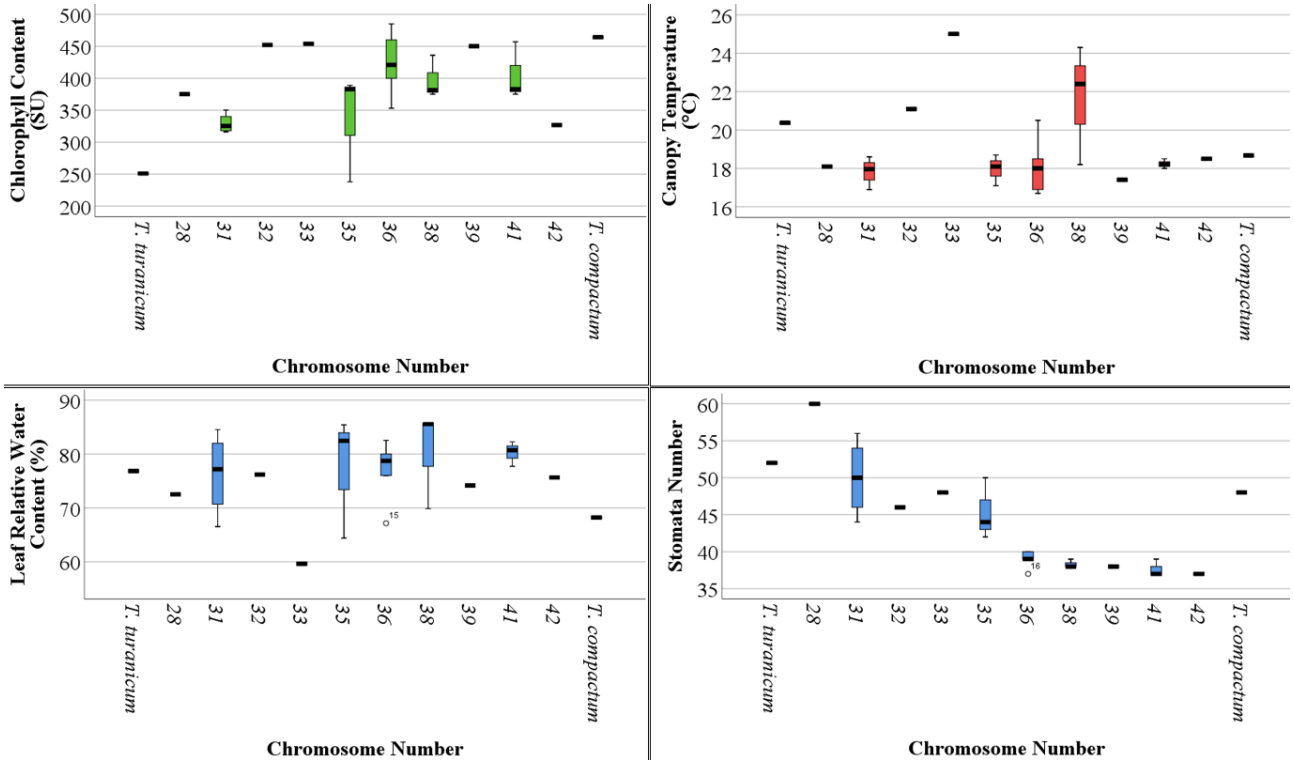

Figure 2. Changes in physiological characteristics according to the difference in chromosome numbers

It was observed that the examined traits exhibited variation among plants with identical chromosome numbers. The increase in recombination of AB chromosomes in the F_2 generation of pentaploids derived from hexaploid and tetraploid crosses results in a significant expansion of genetic variation. Chromosome rearrangements lead to complex responses, including changes in the epigenome and activation of transposons and increased chromosome recombination, resulting in a wide variation (Padmanaban et al., 2017; Muenchrath et al., 2023). The highest leaf chlorophyll content was observed in plants with 36 chromosomes, while the lowest canopy temperature was observed in plants with the same chromosome number. The highest leaf relative water content was found in plants with 38 chromosomes, and the highest stomata number was observed in plants with 28 chromosomes (Figure 2). The range of chlorophyll content observed among F_2 plants is 238 to 485 spectrum units (SU). Flag leaf chlorophyll content is higher in *T. compactum* (464 SU). One F_2 plant displayed higher levels of chlorophyll content than *T. compactum*. No F_2 plant exhibited lower chlorophyll content than *T. turanicum* (251 SU), with values between those of the parents (Figure 2). The temperature of the canopy was determined to be 20.37°C in *T. turanicum* and 18.67°C in *T. compactum*. It is hypothesized that the lowest canopy temperature observed in plants with 36 and 39 chromosomes may be associated with the D genome. The relative water content values of the F_2 plants ranged from 59.64% to 85.79%, while the parental values were recorded at 68.2% in *T. compactum* and 76.85% in *T. turanicum* (Figure 2). When the data were analyzed according to chromosome number,

the lowest relative water content was observed in plants with 33 chromosomes, while the highest was observed in plants with 38 and 41 chromosomes. Additionally, differences were noted in the relative water content values of F_2 plants with the same chromosome number.

Bilgrami et al. (2015) referred that increased expression of the 'D' genome of tetraploid wheat caused a decrease in net photosynthetic rate and chlorophyll content (Del Blanco et al., 2000), while the 'A' genome chromosomes of wheat carried genes controlling high net photosynthetic rate, water use efficiency and reduced transpiration rate (Zhang et al., 2000), and that photosynthetic (Austin et al., 1987) and transpiration rate (Huang et al., 2007) occasionally decreased with increasing ploidy levels of wheat during the development process. Prior research has indicated that the D genome plays a role in net photosynthetic rate, gas exchange, flag leaf area, and chlorophyll content (Del Blanco et al., 2000; Mao et al., 2018). A general tendency has been observed whereby the density of stomata decreases and the size of the stomata increases as the ploidy level of the plant increases (Shao et al., 2009). Aryavand et al. (2003) noticed significant differences in stomatal density between tetraploid and hexaploid subspecies of flag leaves. In the present study, the number of stomata per mm^2 ranged from 37 to 60. In general, a decrease was observed in parallel with the increase in chromosome number. While no prior reports exist regarding a correlation between chromosome number and stomatal number, this may be attributed to the shift in ploidy level from tetraploid to hexaploid concomitant with the change in chromosome number from 28 to 42 (Figure 2).

The largest flag leaf area was observed in *T. compactum*, with an average of 46.8 cm². The flag leaf area of F_2 plants ranged from 16.88 to 46.8 cm², as illustrated in Figure 3. The plants with 28 and 41 chromosomes had highest flag leaf area, while the lowest in 32 and 33 chromosomes. The mean plant height of the studied population was 127.72 cm, with a maximum height of 147 cm and a minimum height of 119 cm (Figure 3). The parents had a plant height of 128.83 cm (*T. compactum*) and 131 cm (*T. turanicum*), respectively. The presence of plants with plant heights that deviate from the aforementioned values in the F_2 population can be attributed to transgressive segregation and the influence of the environment.

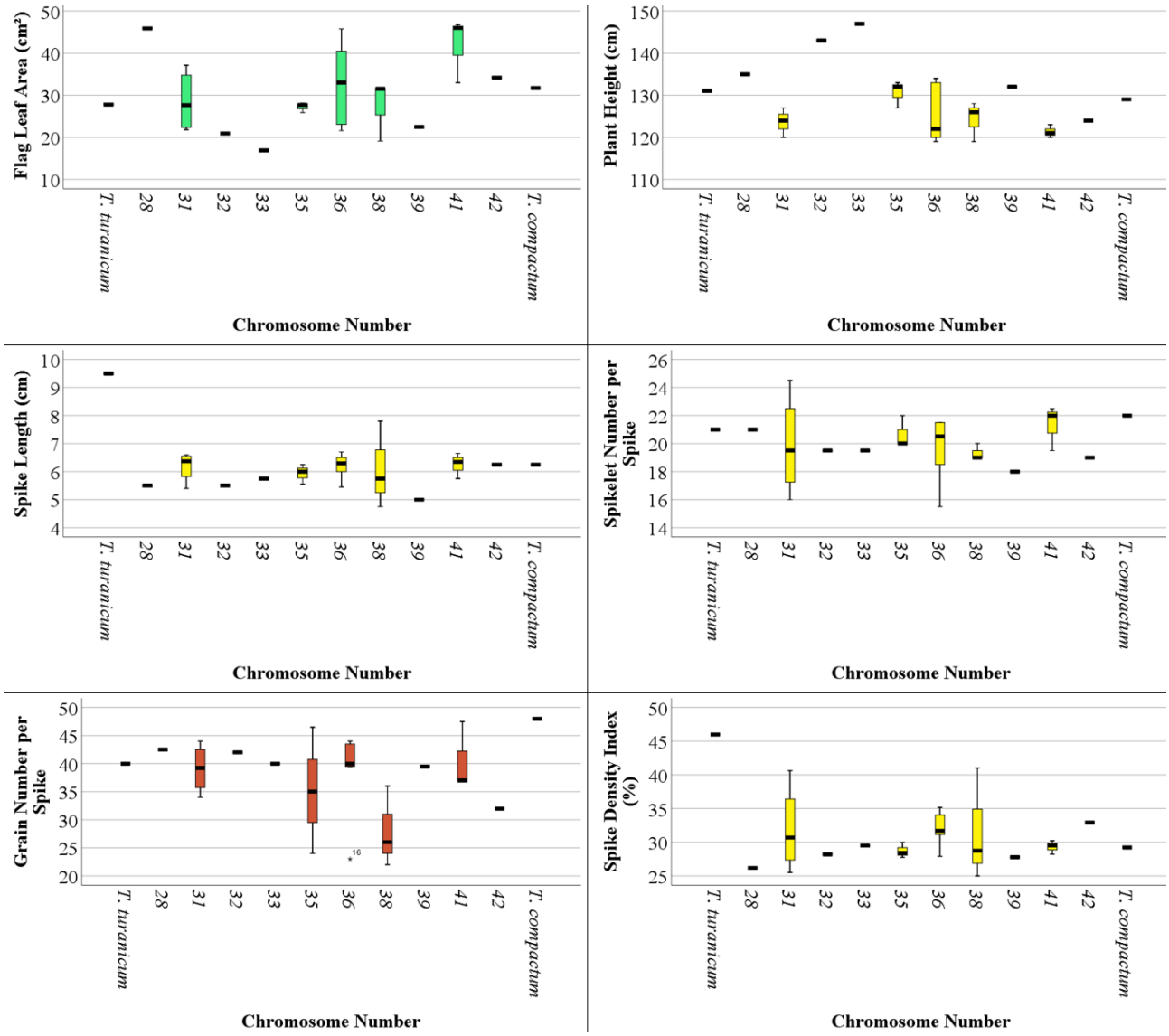

Figure 3. Changes in morphological characteristics according to the difference in chromosome numbers

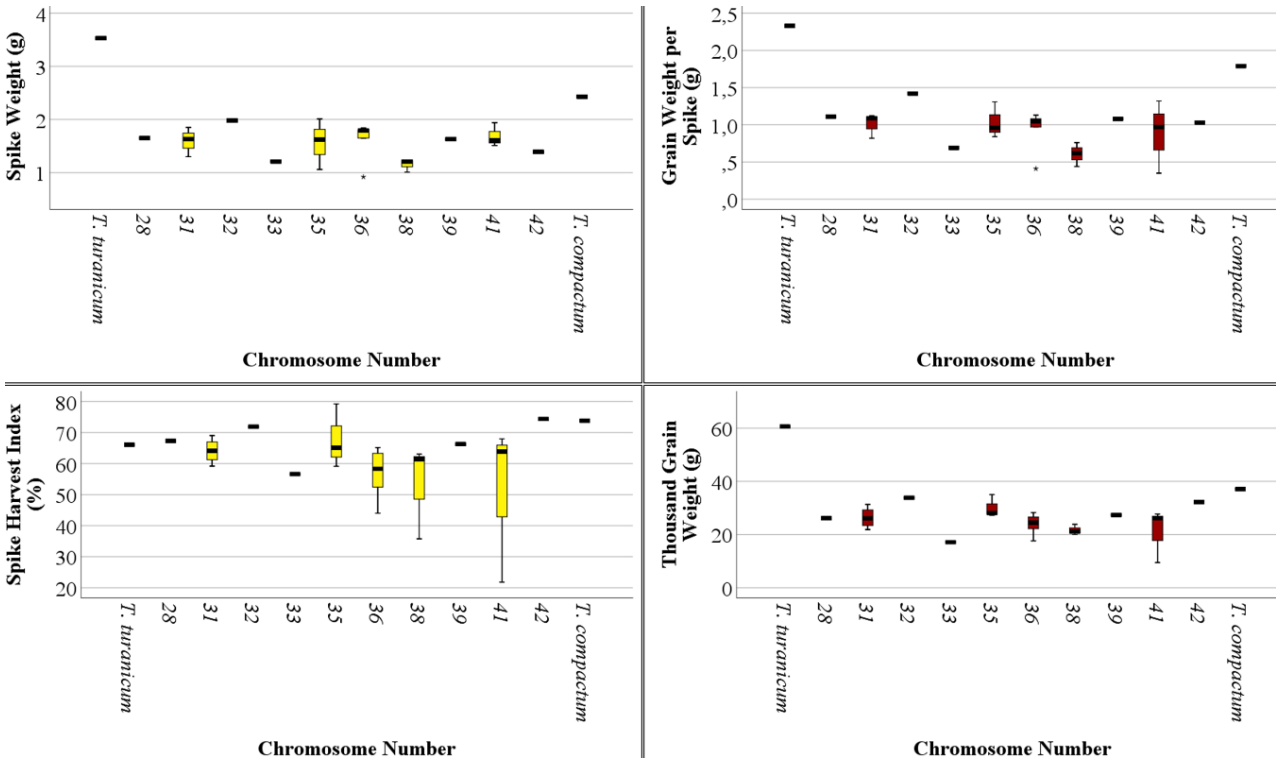

Figure 4. Changes in primary yield traits according to the difference in chromosome numbers

In the plant height comparisons conducted according to the number of chromosomes, it was observed that plants with 31 and 41 chromosomes had values that were similar to one another. The longest plant height was observed in plants with 33 chromosomes, while the plant heights of plants with 31, 36, 41, and 42 chromosomes were close the each other (Figure 3). The spike length values of the plants examined in this study varied between 4.7 - 9.5 cm. The measured values were observed to fall between those of tetraploid and hexaploid parents, or below these values. While no plants exceeded the parental values in length, the spike structures of the F_2 plants were more similar to those of the maternal parent, *T. compactum*. The lowest spike length was observed in plants with 38 and 39 chromosomes, whereas the highest spike length value was noted in plants with 41 and 42 chromosomes (Figure 3). It has been reported that the D genome of wheat has the potential to have a positive effect on flag leaf area, spike length, and plant height. These effects may depend on the D chromosome sources (Mohamed et al., 2020). The presence of plants similar to hexaploid parents in terms of these traits, as well as the observation that the number of chromosomes in these plants is greater than 35, can be explained by this information.

The number of spikelets exhibited a similar range in the parents, with a slight variation. However, in the F_2 plants, there was a notable discrepancy, with a range of 15.5 to 24.5 spikelets observed (Figure 3). Additionally, there were differences in the number of spikelets observed between plants with the same chromosome number. The highest number of spikelets was observed in plants with 28 and 41 chromosomes. It has been reported that certain genes with a beneficial impact on spikelet formation are present in the A genome (Muqaddasi et al., 2019). The fertility of the spikelets is of particular importance in determining the number of grains per spike. The number of grains per spike in F_2 plants showed considerable variation, with a range of 22 to 48 grains per spike. This value was found to be closely aligned with the parent's average (Figure 3). Additionally, the grain numbers per spike values differed between F_2 plants with the same chromosome number. It was observed that plants with a chromosome number less than 35 exhibited a higher spike grain number. This phenomenon can be attributed to the presence of both sterile and fertile chromosome combinations. Özkan and Genç (1998) reported that plants with 35 chromosomes had a lower spike grain number. Spike density, a crucial spike morphological trait linked to wheat yield, is calculated by dividing the number of spikelets per spike by the spike length (Liu et al., 2020). Spike density values varied between 25 and 46, with a general tendency to fall between the parental values (Figure 3). As with other traits, F_2 plants with disparate values were observed, despite the chromosome number being identical. However, the values of plants with 41 chromosomes showed a high degree of similarity. The highest spike density was observed in plants with 42 chromosomes.

The spike weight values varied between 0.97- 3.53 g, with the F_2 plants exhibiting lower values than the mean of the parents. Additionally, it was observed that spike weights exhibited variation among the F_2 plants with the same chromosome number. The highest spike weight was observed in plants with 35 chromosomes (Figure 4). The grain weight per spike of F_2 plants was found to be markedly lower than that of their parents (Figure 4). The grain weight per spike was found to be 1.79 g in the hexaploid parent and 2.33 g in the tetraploid parent, with the highest weight of 1.42 g observed in plants with 32 chromosomes. Additionally, grain weight per spike

differed according to the number of chromosomes. Spike harvest index values varied between 21.81% and 79.25% and were highest in plants with chromosome numbers 32 and 42 (Figure 4). The thousand-grain weights of F_2 plants displayed a considerable range, with values between 9.46 and 35.0 g, and were notably lower than those observed in the parental plants. The highest thousand-grain weights were observed in plants with 32 and 42 chromosomes. The observed variation in spike characteristics among F_2 plants can likely be attributed to differences in chromosome content. The observed reduction in spike yield is likely a consequence of sterility. As reported by Wang et al. (2005), this sterility is due to the sterility of pollen grains.

The raw data for the variables were converted by principal component analysis (PCA) into principal factors representing different proportions of data variability. Consequently, the data were reduced and transformed into principal components. In the initial stage of the analysis, the principal component factors were equal to the number of variables under study, which was 15 in this case. Subsequently, these factors transformed the raw data into five factors, with the first factor contributing the most variability. The initial five factors collectively explained 79.8% of the variance in the population and were thus deemed suitable for further data analysis, as they exhibited eigenvector values exceeding 1.0 (Table 3). The application of the variamax rotation method served to enhance the reliability of the results. The loadings of the first two components were plotted using the underlying factors that exhibited the greatest variability.

The contribution of different characters to the principal components varies. About Factor 1, all characters exhibited a positive contribution, with the exception of flag leaf area, chlorophyll content, canopy temperature, leaf relative water content, and nuclear DNA content. In contrast, only eight components contributed positively for Factor 2, while five components contributed negatively for Factors 3 and 4 (Table 3). The initial component, designated as primary yield components, exhibited the highest factor loadings for the variables spike weight, grain weight per spike, spike harvest index, and thousand-grain weight. The second component was constituted by spike length and spike density, which are important secondary yield components. The third component included chlorophyll content, stomatal number, and nuclear DNA content. The fourth component included flag leaf area, number of spikelets, and number of grains per spike. The fifth component included canopy temperature, leaf relative water content, and plant height. Figure 5 depicts the loading plot of the initial two principal components, with the examined traits represented on the x-axis. The weight plane is interpreted in accordance with the correlation between each variable and each principal component. Accordingly, variables that are in close proximity are positively correlated, whereas variables that are 180 degrees apart are negatively correlated. Furthermore, no correlation exists between variables that are 90 apart, indicating that the variables are independent of one another. Consequently, a positive correlation is observed between chromosome number (nDNA) and canopy temperature, flag leaf area, chlorophyll content, and leaf relative water content. Conversely, a negative correlation is evident between the number of grains per spike, grain weight per spike, thousand-grain weight, and spike weight. The flag leaf chlorophyll content, which is positively correlated with chromosome number, was found to be highest in plants with 32 and 33 chromosomes. Flag leaf-related traits in wheat, such as flag leaf area and chlorophyll content, are quantitative traits with a complex genetic basis and are significantly influenced by environmental factors. The genes that control these traits were found to be predominantly located in the A and B genomes (Yan et al., 2020). Although there is a correlation between them, the lack of a corresponding increase or decrease in parallel with the number of chromosomes can be explained by this situation.

Table 3. Contribution of eigenvector value and principal component axes to variation

Variable	Factor1	Factor2	Factor3	Factor4	Factor5	Communality
FLA (cm2)	-0.161	-0.031	0.003	0.742	-0.385	0.726
CHL (SU)	-0.066	0.235	-0.719	-0.116	0.269	0.662
CT (°C)	-0.143	0.034	0.016	0.010	0.607	0.390
SN	0.217	0.076	0.848	0.187	0.256	0.872
LRWC (%)	-0.165	-0.067	-0.039	0.044	-0.801	0.676
PH (cm)	0.132	0.055	0.160	-0.301	0.724	0.662
SL (cm)	0.202	-0.900	0.169	0.154	-0.130	0.920
SNS	0.123	0.053	0.139	0.850	-0.085	0.767
SW (g)	0.740	-0.528	0.092	0.298	0.124	0.939
GNS	0.546	0.082	-0.076	0.583	0.388	0.800
GWS (g)	0.940	-0.233	0.133	0.138	0.114	0.988
SHI (%)	0.780	0.481	0.173	-0.149	-0.020	0.891
SDI (%)	0.111	-0.882	0.092	-0.388	-0.093	0.959
TGW (g)	0.855	-0.305	0.254	-0.144	-0.100	0.920
nDNA	-0.136	0.108	-0.840	0.141	-0.221	0.804
Variance	3.2982	2.3367	2.1502	2.0989	2.0904	11.9744
% Var	0.220	0.156	0.143	0.140	0.139	0.798

*CHL: flag leaf chlorophyll content, CT: canopy temperature, SN: stomata number, RWC: leaf relative water content, FLA: flag leaf area, PH: plant height, SL: spike length, SNS: spikelet number per spike, GNS: grain number per spike, SDI: spike density index, SW: spike weight, GWS: grain weight per spike, SHI: spike harvest index, TGW: thousand-grain weight

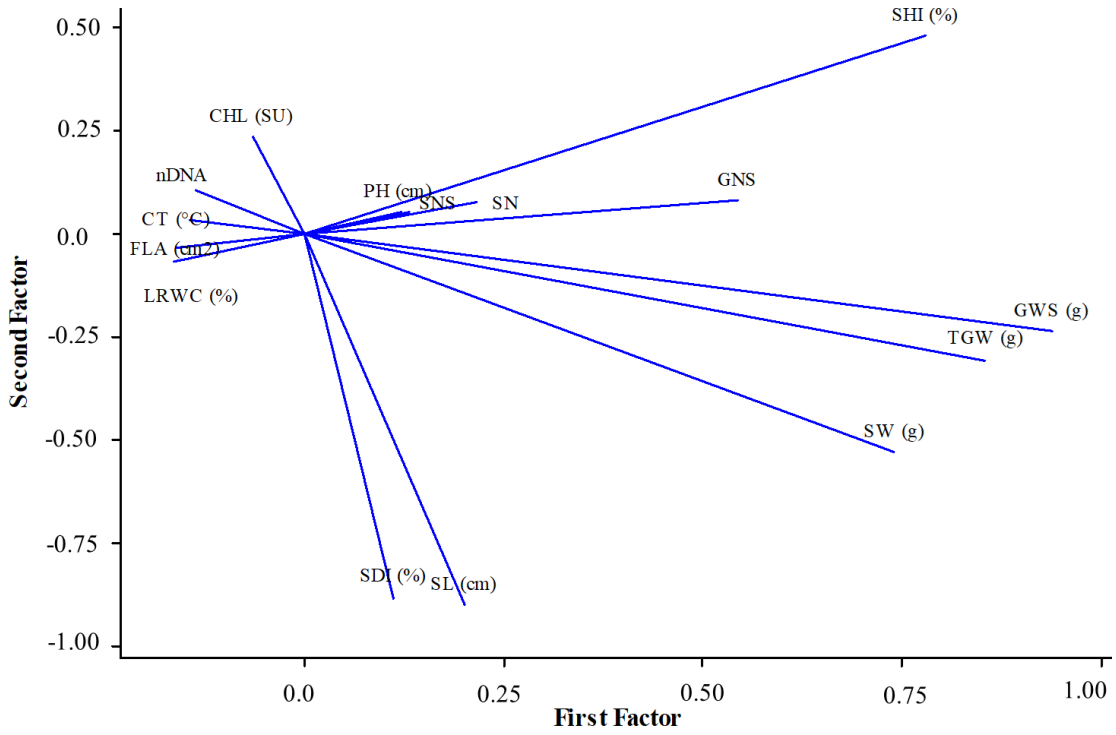

Figure 5. Plant properties in the loading plot described by the first two principal components

Conclusion

In the F_2 generation, plants with chromosome numbers ranging from 2n=28 to 2n=42 were produced. Despite the similarity in chromosome number among these plants, it is suggested that plants with identical chromosomes may exhibit variation in morphological and physiological traits, potentially due to the divergence of the D genome chromosome group. Additionally, these traits are influenced by environmental conditions and are quantitative traits with complex genetic mechanisms. In the pursuit of trait improvement through crosses between hexaploid and tetraploid wheats, the potential of pentaploid wheats should be given particular consideration. Pentaploid wheats have the potential to increase allelic diversity in the A and B genomes. In subsequent generations, a more comprehensive evaluation of plants with 35 chromosomes will provide valuable insights for breeding studies.

Declarations

This paper was presented in III. International (XV. National) Field Crops Congress.

Author Contribution Statement

Gülcan Eser: Project administration, data collection, investigation, statistical analysis, and writing the original draft; Oğuzhan Önal and Feyza Yıldırım: Data collection and investigation; İmren Kutlu: Project administration, supervision, conceptualization, methodology, writing the original draft, review and editing

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Conflict of Interest

The authors declare no conflict of interest.

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