Nucleotypic Effects in Different Genotypes of *Vicia sativa* L.

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Abstract: Seven cultivar varieties of *Vicia sativa* L. were used. Nucleotypic effects of telophase (2C) nuclear DNA amounts on seed weight, percent mitotic index, stomatal frequency/mm², chlorophyll a and b content, root growth and shoot growth were determined.

No correlation was observed between the DNA contents and the percent mitotic index, chlorophyll a and b content, or root growth of the cultivar varieties. However a significant correlation was observed between DNA content and the seed weight, stomatal frequency and shoot growth of the cultivar varieties.

Key Words: *Vicia sativa*, Nuclear DNA Content, Nucleotypic Effect

**Introduction**

Study of the nuclear DNA content of different plant species generally reveals that the DNA content of each genome is constant and species-specific (1, 2).

Microspectrophotometric determinations have shown that if the nuclear DNA content per genome is different in families and species of the same family, then it is also different between different varieties of the same species (3-11). Such great variation is known to exist within angiosperm families like *Ranunculaceae, Droseraceae* and *between genera* and species in *Graminae, Leguminosae* and *Compositae* (12).

Significant intraspecific variations in DNA content per cell have been reported in different plant genotypes, varieties and ecotypes, e.g., *Picea glauca, Pinus banksiana, Picea sitchensis,*
Pisum sativum, Pisum fulwum and Vicia faba, including some of its mutants (13). Significant interspecific differences in DNA content per cell were demonstrated between some species of Vicia and Lathyrus (14).

This variation in DNA content is causally correlated with different phenotypic characters. This relationship between DNA content and phenotypic characters has been identified as the “nucleotypic effect”. The term nucleotype was coined to define those conditions of the nuclear DNA which affect the phenotype independent of its encoded informational content. Clearly, nuclear DNA influences the phenotype in 2 distinct ways: first, by expressing its genic content, and second, by physically affecting its mass and volume (15, 16, 17). Thus, the correlations between DNA C-value and chromosome, nucleus and cell size and mass are all largely nucleotypic effects.

The DNA C-value in angiosperms has been shown to correlate with many widely different nuclear, cellular, tissue and even organismal phenotypic characters including the number of chloroplasts per stomatal guard cell (18), seed weight (17), the rate and duration of the DNA synthesis phase (19, 20), the duration of the mitotic (20) and meiotic cycle (16), minimum generation time (1, 16), radiosensitivity (21), radiation-induced mutation rates (22), ecological and phenological factors (23), and the optimum environment and geographical ranges of species (12, 24).

In one previous study (25), different lines of Vicia sativa L. were examined and differences in these lines were determined in terms of 2C DNA content. Based on this work, the aim of the present study was to investigate nucleotypic effects in different cultivars and lines of Vicia sativa L.

Materials and Methods

In this study, several cultivars and lines of Vicia sativa L. were used as research material. The lines and cultivars used for the study are as follows:

<table>
<thead>
<tr>
<th>Uludağ 31-4</th>
<th>Karaelçi</th>
<th>Nilüfer 17-1</th>
<th>28-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emir 20-1</td>
<td>Tazza</td>
<td></td>
<td>142</td>
</tr>
</tbody>
</table>

The seed weights of the cultivars and lines were determined by weighing a total of 50 seeds. The seeds were germinated (26), and root tips 1-2 cm in length were cut off and immediately fixed in a freshly prepared solution of 3:1, absolute ethyl alcohol: glacial acetic acid for 24 h at 4°C (then thoroughly washed in distilled water before being stored in 70% ethanol at 4°C. The roots were hydrolysed in 1 N HCl at 60°C for 10 minutes and stained in Feulgen for 1.5 hours at room temperature (4, 7). The roots were rinsed in distilled water and washed for 10 minutes in 3 changes of SO2 water. Preparations were made from darkly stained root tips. Squashes of the root tips of Allium cepa were concurrently stained for each group of slides and were used as the standards. The coverslips were removed by the liquid nitrogen method and they were embedded in entellan.
Measurements were obtained from well-flattened telophases having clearly separated in the squashes at a wavelength of 550 nm using a Reichert-Zetopan microspectrophotometer. For each cultivar and line, 25 nuclei were measured on each preparation. The total extinction of the nucleus was calculated using the one-wavelength/two area technique, by the equation (27):

\[ E_{1A_1} = (\log 1/T) \cdot [(1-T)A_1 / (1 - T_1)] \]

The nuclear DNA content was also calculated for the preparation of Allium cepa. Assuming that the 2C value of Allium cepa was 33.5 picograms (28), the absorption values for each of the lines and cultivars were converted into absolute amounts.

Percent mitotic indexes were determined from root tips (25). The chlorophyll a and b contents were established by extracting into chloroform: acetone (29, 30), and the stomatal frequency (31, 32) of the cultivars and lines was established. Moreover, the cultivars and lines of Vicia sativa were germinated in a growth system at 25°C with a photoperiod of 16/8 h (light/dark). After germination, the root lengths (from the cotyledonary node to the tip) were measured up to 7 days and their stem lengths (from the cotyledonary node to the plumula) were measured up to 14 days (33).

Results

The 2 C nuclear DNA contents of the 7 cultivars and lines of Vicia sativa L. determined by feulgen microspectrophotometry are shown in Table 1. The 2C nuclear DNA contents were found to be different between the cultivars and lines. While the line exhibiting the highest DNA content was 142 (4.902 ± 0.143 pg), the line with the lowest DNA content was Uludağ 31-4 (3.206 ± 0.865 pg). According to a tukey test carried out on the 2 C DNA content of the cultivars and lines, cultivars of Karaelci, Tazza and 142 are in one group and the lines Emir 20-1, Nilufer 17-1 and Uludağ 31-4 are in the other. Line 28-1 is also in those groups (Table 1). Variance analysis was used to compare the DNA contents and the differences were found to be significant for the 2 groups formed as a result of this analysis (F = 25.94; p < 0.001 Table 1).

<table>
<thead>
<tr>
<th>Names of cultivars and lines</th>
<th>2C DNA content (pg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>4.902 ± 0.143 a</td>
</tr>
<tr>
<td>Kara elçi</td>
<td>4.840 ± 0.488 a</td>
</tr>
<tr>
<td>Tazza</td>
<td>4.626 ± 0.398 a</td>
</tr>
<tr>
<td>28-1</td>
<td>4.102 ± 0.383 ab</td>
</tr>
<tr>
<td>20-1 (Emir)</td>
<td>3.801 ± 0.288 b</td>
</tr>
<tr>
<td>17-1 (Nilufer)</td>
<td>3.326 ± 0.435 b</td>
</tr>
<tr>
<td>31-4 (Uludağ)</td>
<td>3.206 ± 0.865 b</td>
</tr>
</tbody>
</table>

*: Species are arranged according to DNA content.

*: According to the tukey test, the same letters represent the same groups.
The seed weights of the cultivars and lines were determined (Table 2). The line with the highest seed weight was 28-1 (67.112 ± 3.560 mg) and the line with the lowest seed weight was Nilufer 17-1 (48.276 ± 2.467 mg). A regression analysis was performed on the DNA contents and seed weights, and a positive relationship between them was found ($F_{\text{Reg}} = 11.822; p < 0.05$; Figure 1).

The percent mitotic indexes of the cultivars and lines were determined (Table 2). The % mitotic index ranged from 7.021 ± 1.258 to 9.473 ± 0.807. According to regression analysis, there was no significant relationship between the DNA contents and the % mitotic index ($F_{\text{Reg}} = 2.130; p > 0.05$; Figure 2).
The mean stomatal frequency of the cultivars and lines per mm² was determined (Table 2). The mean stomatal frequency of the cultivars and lines ranged from 249.623 – 19.842 to 154.050 – 28.736. According to regression analysis, the relationship between the DNA contents and mean stomatal frequency of the cultivars and lines was statistically significant ($F_{\text{Reg}} = 5.025; \ p < 0.05$; Figure 3).

The chlorophyll a and b content of the cultivars and lines of *Vicia sativa* L. were determined (Table 2). Regression analysis was carried out on the values. According to the regression analysis, no significant relationship was found between the DNA contents and chlorophyll a ($F_{\text{Reg}} = 0.0216; \ p > 0.05$; Figure 4) or chlorophyll b contents ($F_{\text{Reg}} = 0.061; \ p > 0.05$; Figure 5).

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**Table 2. Mean Values ± Standard Deviations of phenotypic characters in *Vicia sativa* L. cultivars and lines.**

<table>
<thead>
<tr>
<th>Names of cultivars and lines</th>
<th>% Mitotic Index</th>
<th>Seed Weight (mg)</th>
<th>Stomatal frequency /mm²</th>
<th>Chlorophyll a (mg l⁻¹)</th>
<th>Chlorophyll b (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>7.528 ± 1.024</td>
<td>65.592 ± 2.815</td>
<td>187.644 ± 25.974</td>
<td>7.497 ± 0.610</td>
<td>8.690 ± 5.762</td>
</tr>
<tr>
<td>Kara Elçi</td>
<td>9.473 ± 0.807</td>
<td>54.360 ± 2.682</td>
<td>216.617 ± 30.079</td>
<td>6.803 ± 0.636</td>
<td>8.253 ± 4.637</td>
</tr>
<tr>
<td>Tazza</td>
<td>8.360 ± 0.828</td>
<td>66.648 ± 2.503</td>
<td>239.028 ± 12.426</td>
<td>7.183 ± 3.509</td>
<td>7.820 ± 1.931</td>
</tr>
<tr>
<td>28-1</td>
<td>7.021 ± 1.258</td>
<td>67.112 ± 3.560</td>
<td>249.623 ± 19.842</td>
<td>6.803 ± 0.626</td>
<td>8.490 ± 5.646</td>
</tr>
<tr>
<td>Uludağ 31-4</td>
<td>7.281 ± 1.541</td>
<td>51.476 ± 1.572</td>
<td>203.383 ± 11.893</td>
<td>7.403 ± 2.286</td>
<td>8.383 ± 4.089</td>
</tr>
</tbody>
</table>

²: Species are ordered according to DNA content.
The root lengths (from cotyledonary node to the tip) of the cultivars and lines were measured up to the 2, 4 and 7th days (Table 3). No significant relationship was found between root length on the second day and DNA contents ($F_{\text{reg}} = 1.120; \ p > 0.05$; Figure 6). No significant relationship was found between DNA content and root length on the fourth day ($F_{\text{reg}} = 2.436; \ p > 0.05$; Figure 7) or on the seventh day ($F_{\text{reg}} = 1.684; \ p > 0.05$; Figure 8).

While there was a negative though not significant correlation between the 2C DNA contents and root lengths of the cultivars and lines in the early period of development (2nd day). There was a positive a but not significant relationship among these characters in more advanced stages of development (Figures 6, 7 and 8).
Stem lengths from the cotyledon node to the plumula of the cultivars and lines were measured up to 3, 6, 9 and 14 days (Table 4). According to regression analysis, there was a negative and significant relationship between 2C DNA contents and stem lengths on the 3rd day ($F_{\text{Reg}} = 13.199; \ p < 0.001$; Figure 9). A positive relationship was established between telophase DNA contents and stem lengths on the 6th day ($F_{\text{Reg}} = 3.787; \ p > 0.05$; Figure 10). There was a significant and positive relationship between 2C nuclear DNA contents and stem lengths on the 9th day ($F_{\text{Reg}} = 21.035; \ p < 0.001$; Figure 11) and 14th day ($F_{\text{Reg}} = 27.59; \ p < 0.001$; Figure 12).

Table 3. Mean root growth and standard deviation of *Vicia sativa* L., according to day.

<table>
<thead>
<tr>
<th>Cultivars and Lines</th>
<th>2nd day</th>
<th>4th day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kara Elçi</td>
<td>11.571 ± 3.207</td>
<td>40.285 ± 9.725</td>
<td>73.00 ± 14.376</td>
</tr>
<tr>
<td>28-1</td>
<td>49.500 ± 22.150</td>
<td>79.666 ± 23.395</td>
<td>119.500 ± 22.204</td>
</tr>
<tr>
<td>20-1 (Emir)</td>
<td>19.230 ± 8.105</td>
<td>50.384 ± 14.466</td>
<td>98.846 ± 30.768</td>
</tr>
<tr>
<td>17-1 (Nilüfer)</td>
<td>28.580 ± 6.487</td>
<td>64.416 ± 19.266</td>
<td>131.166 ± 20.467</td>
</tr>
<tr>
<td>31-4 (Uludağ)</td>
<td>21.384 ± 7.113</td>
<td>60.846 ± 10.270</td>
<td>93.307 ± 12.612</td>
</tr>
</tbody>
</table>

$^z$: Species have been arranged, according to DNA content.
As it is shown in the figures, there was a negatively significant relationship between stem lengths and the DNA contents of the cultivars and lines in the early period of development, and the gradual increase in the positive correlation was significant with more advanced development.

**Discussion**

The telophase DNA contents of several cultivars and lines of *Vicia sativa* were determined, and the differences in their DNA contents were established (Table 1). Interspecific and
Intraspecific variation in DNA content has been reported in several plant genera (1, 7, 10, 14, 34, 35).

Intra- and interspecific variation have been established in the 2C and 4C DNA contents of numerous plant species by different researchers (1, 2). The majority of organisms have more DNA than is needed for coding their structural and metabolic proteins. In studies carried out on nuclear DNA content in different plant species, different researchers have contributed different ideas about the origin of interspecific and intraspecific variation of DNA contents in different plant species (2, 7, 10, 14, 23, 27, 30).

### Table 4. Mean stem growth (standard deviation of Vicia sativa L. cultivars and lines.

<table>
<thead>
<tr>
<th>Name of Cultivars and Lines</th>
<th>Stem Growth (mm)</th>
<th>3rd days</th>
<th>6th days</th>
<th>9th days</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>45.400 ± 7.691</td>
<td>116.600 ± 21.334</td>
<td>176.100 ± 49.807</td>
<td>207.900 ± 57.744</td>
<td></td>
</tr>
<tr>
<td>Kara Elçi</td>
<td>50.000 ± 16.466</td>
<td>131.875 ± 19.533</td>
<td>185.875 ± 58.222</td>
<td>214.125 ± 31.957</td>
<td></td>
</tr>
<tr>
<td>28-1</td>
<td>73.923 ± 13.961</td>
<td>131.307 ± 24.404</td>
<td>188.307 ± 35.434</td>
<td>210.076 ± 37.053</td>
<td></td>
</tr>
<tr>
<td>Emir 20-1</td>
<td>49.555 ± 13.135</td>
<td>86.222 ± 15.434</td>
<td>142.000 ± 35.089</td>
<td>277.222 ± 33.618</td>
<td></td>
</tr>
<tr>
<td>Nilüfer 17-1</td>
<td>58.257 ± 12.965</td>
<td>114.076 ± 13.775</td>
<td>147.000 ± 21.629</td>
<td>163.923 ± 16.199</td>
<td></td>
</tr>
<tr>
<td>Uludag 31-4</td>
<td>62.193 ± 15.777</td>
<td>120.538 ± 41.009</td>
<td>160.923 ± 46.313</td>
<td>187.076 ± 38.690</td>
<td></td>
</tr>
</tbody>
</table>

2: Species are arranged, according to DNA content.

![Figure 9. The relationship between 2C DNA content and stem growth on the 3rd day.](image-url)
This variation may occur as a result of conditions like underreplication of heterochromatin (some sequences of DNA do not replicate), decrease of highly repetitive sequence amount (36), saltatory replication, unequal cross-over, transposition, mutation, deletion (37), or an adaptation mechanism in reaction to changes in environmental factors (differences in microhabitat, climate, manure, etc.) (15, 33).

In the works of Raina and Rees, wide variations are recorded in DNA measurements for some diploid strains of the genus *Vicia* belonging to the *Fabaceae* family, and it has been shown that in *Vicia sativa*, there is approximately 4.50 pg of 2C DNA. It was found that the diploid...
The chromosome number of *Vicia* species is 10, 12 and 14 and there are differences in several species in terms of 2C nuclear DNA content at variety level in the *Vicia* genus. For example, *Vicia grandiflora* var. *kitaibeliana* has 7.20 pg DNA and *Vicia grandiflora* var. *grandiflora* has 9.05 pg DNA. In the present study, no direct correlations were observed between chromosome number and 2C DNA content.

Akpınar and Bilaloğlu discovered a significant variation in the 2C DNA contents of 9 *Vicia* species from 26 populations, and established that *Vicia cracca* ssp. *cracca* has 13.08 pg and *Vicia cracca* ssp. *tenuifolia* has 11.83 pg of 2C nuclear DNA content. They determined differences in 2C nuclear DNA content among subspecies and lines of *Vicia sativa* (25).

It is known that variation in DNA content influences certain phenotypic characters. In the present study, mitotic index, chlorophyll a and b content, stomatal frequency, root and stem length were determined and the relationships between these characters and DNA content were examined. In our study, there was a significant positive correlation between DNA content and seed weight (Figure 1). Bennett and Smith stated that there is a positive correlation between DNA content and seed weight in plant species. In one study of different cultivars, no significant correlation was reported between DNA amount and seed weight (1). It has been considered that an increase in cell size will affect the seed size in relation to the amount of DNA.

In a study carried out on different species, a correlation at the rate of 80% was established between DNA content and % mitotic index. Cavallini et al. (33) suggested that there are similar mitotic indexes in different cultivars of *Pisum sativum*. The duration of the mitotic cycle affects the DNA amount in *Festuca arundinacea*. The duration of the cycle is extended with a high DNA amount. However, it was also suggested that the number of cells which undergo mitotic division has no effect. In spite of the fact that the DNA contents were different in the population
which was studied, no significant differences in mitotic index were determined (37). Their findings support ours. As can be seen in Figure 2, no significant correlation was found between 2C DNA content and percent mitotic index.

The stomatal frequency of 7 cultivars and lines of *Vicia sativa* was determined (Table 2). A significant correlation was observed between the DNA content and stomatal frequency of the cultivars and lines (Figure 3). According to the results of the regression analysis of the between chlorophyll a and b contents and DNA amount of the cultivars and lines, no significant correlation existed between DNA amount and chlorophyll a and b content (Figures 4 and 5).

Butterfass (18) investigated chloroplast numbers per guard cell in plant species whose specific nuclear DNA contents had been determined by other researchers. He reported that a positive correlation existed between DNA amounts, and the chloroplast number also increased with an increase in DNA amount.

A significant positive correlation was established between 4C DNA content and chloroplast number per guard cell of stomata in 15 populations of *Zea mays ssp. mays* (38).

In populations of *Lathyrus odaratus* growing at different location around the world, a significant correlation was observed between 2C DNA content, pollen volume and chloroplast number per guard cell (2). Even though chlorophyll content no increased in connection with the increase in stomata number and chloroplast number, no significant differences were established between the cultivars and lines, and no relationships were reported in terms of DNA contents.

In the present study, root lengths on the 2nd, 4th and 7th days were determined (Table 3). While no significant negative correlation existed between DNA amount and root lengths in the early growth period (2nd day), this relationship showed no significant positive correlation with the progress of the growth periods (Figures 6, 7 and 8).

Quite a significant positive correlation was observed between 4C nuclear DNA content and root growth in early growth periods in *Pisum sativum* by Cavallini at al., but this correlation was non-significant by the 5th day of growth (33).

The stem lengths on the 3rd and 6th and 9th and 14th days of growth in this plant species were determined (Table 4). A significant negative correlation was established between DNA amount and stem lengths of the cultivars and lines in the early growth period (3rd day), and a significant positive correlation was established between them with the progress of the growth periods (6th, 9th and 14th days) (Figures 9-12).

It was determined that quite a positive significant correlation existed between 4C nuclear DNA amount and stem growth in *Pisum sativum*. It has been reported that this was nonsignificant after the 20th day (33).

The mitotic cycle is long in species which have a high DNA content and large cell size. The mitotic cycle is short in species containing little DNA, but having a small cell size. In the early
growth period, the growth of species with low DNA content is more vigorous by means of quick division. If we assume that cell elongation observed during the growth phases promotes development, it is not erroneous to emphasise that cell growth in DNA-enriched varieties contributes more to growth rate in general. Cavallini et al. reported that there were differences in the cell growth of different tissues according to the DNA amount in the pea (33). It was determined that the cell size was greater with larger amounts of DNA.

In research on Festuca arundinacea, a negative correlation was shown between root growth and leaf surface and DNA amount (37). Moreover, a positive correlation was shown between culm height and flowering time and DNA amount (37). Such observed differences related to growth indicate that this effect alone will not explain the subject of DNA amount.

Our experiments indicate that quantitative nuclear DNA intraspecific variability may affect the phenotype, even when plants are grown under controlled and optimal conditions. This influence may be more effective when growth conditions are limited. It is possible for the influence of nucleotypic effects upon phenotypic characters to be evaluated. Moreover, more information is necessary as to the source of interspecific variation, by C-banding, and on the determination of heterochromatin.

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References


