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*Full Length Research Paper*

# Effects of Herbicide Attribut (propoxycarbazone-sodium) and Surfactant BioPower (sodium alkylether-sulphate) on DNA amounts of *Triticum aestivum* L. cv. Pehlivan by Flow Cytometry

**Gulden Yılmaz[1], Ozlem Aksoy[2], Cıler Merıc[1], Feruzan Dane[1]**

1Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne/Turkey
2 Kocaeli University, Faculty of Science and Arts, Department of Biology, İzmit/Kocaelı

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**In the present study, the effects of herbicide Attribut (propoxycarbazone-sodium) and an anionic surfactant BioPower (sodium alkylether-sulphate) on DNA amounts of *Triticum aestivum* L. cv. Pehlivan were investigated and compared by flow cytometric method. For the experiments, *Triticum aestivum* were treated with Attribut at the concentrations of 0.21 mM, 0.42 mM, 0.82 mM and 1.68 mM, respectively. In order to evaluate the combined effect of BioPower and Attribut, 0.25% BioPower was added in each of applied Attribut doses. The nuclear DNA content of these specimens was analyzed by flow cytometry. Propidium iodide-stained nuclei were analyzed on an EPICS XL (Beckmann Coulter) model flow cytometer. The nuclear DNA content (2C-value) of the treated specimens were given. DNA amount was shown to decrease in the treatment of (FD×4) 1.68 mM Attribut while it increased in the treatment of (FDS×4) 1.68 mM Attribut + 0.25% BioPower.**

**Keywords:** flow cytometry, genotoxic effect, propoxycarbazone-sodium, sodium alkylether-sulphate

## INTRODUCTION

Attribut (propoxycarbazone-sodium) is a herbicide being developed for post emergent use on wheat. Control of weed grasses in cereals is a major problem in agriculture. Propoxycarbazone-sodium as the active substance now offers the farmer an opportunity to exert outstandingly effective control aimed particularly at brome grasses (*Bromus* species), blackgrass (*Alopecurus myosuroides*) and bentgrass (*Apera spica-venti*), as well as an opportunity for selective control of the perennial couchgrass (*Elymus repens*) (Müler, 2002). Its mode of action is inhibition of acetolactate synthase (ALS).

Surfactants are capable of exerting inhibitory as well as stimulatory effects upon plant growth and behavior (Parr and Norrman, 1965). In this study, BioPower, an anionic surfactant, is used to improve the effectiveness of foliar applied herbicide (propoxycarbazone-sodium) by reducing the surface tension of aqueous systems.

Flow cytometry is an analytical tool widely used to obtain detailed informations on bioprocesses, light-scattering, fluorescence and absorbance measurements. In recent years, flow cytometry has become the preferred technique for estimating the nuclear DNA content because of its

*Corresponding Author's: Email: guldenyilmaz2009@yahoo.com

ease, quickness, and accuracy in determining nuclear DNA contents of more than 100 major crop plant species (Rayburn et al., 1989; Heslop-Harrison, 1995; Arumuganathan and Earle, 1991a). Vogel et al. (1999) used flow cytometry to determine the base DNA content of the genomes in the perennial Triticeae. The amount of DNA in plant cells is expressed in picograms (pg) as a "C" value (Bennett and Smith, 1976). The letter C stands for a "constant" or the amount of DNA in a haploid nucleus or genome; 2C values represent the DNA content of a diploid somatic nucleus. In the present study, possible effects of Attribut and BioPower on the amount of genetic material of *Triticum aestivum* L. cv. Pehlivan were evaluated for the first time by using flow cytometry method for treated seeds with different doses of Attribut alone or in combination with BioPower to reveal any synergistic effect, if present.

## MATERIALS AND METHODS

### Plant materials

Preferable fresh leaves of *Triticum aestivum* L. cv. Pehlivan were collected from young plants (3-4 week-old, 40 mg leaves of *Triticum aestivum)*, while + 20 mg *Hordeum vulgare* was used as the standard. The obtained plant tissues were placed into a Petri dish existing on ice. 1 ml of solution (A) [24 mL $MgSO_4$ buffer (ice-cold), 25 mg dithiothreitol, 500 µL propidium iodide stock (5.0 mg propidium iodide in 1.0 mL double distilled $H_2O$) and 625 µL Triton X-100 stock (1.0 g Triton X-100 in 10 mL $ddH_2O$)] were added into the Petri dish and the plant tissues were cut into tiny pieces in the liquid with the help of a sharp knife or a razor. The obtained solution was transferred into a micro-centrifuge tube with a 30-33 µm filter. Micro-centrifuge tubes were centrifuged at high rate (14 000 rpm) for a short while (25-30 sec) and the corresponding supernatant was discharged. Then, the sediment formed at the bottom of the micro-centrifuge tube was dissolved in 400 µm of solution (B) [7.5 mL solution A; 17.5 µL RNase (DNase free)]. These samples were incubated at 37 ºC for 20 minutes and then analyzed by the flow cytometry.

### METHODS

The procedures described by Arumuganathan and Earle (1991b) were used to determine DNA content per nucleus. Briefly, the procedure consists of preparing suspensions of intact nuclei by chopping plant tissues and lysing protoplasts in a $MgSO_4$ buffer mixed with DNA standards and staining the nuclei with propidium iodide (PI) in a solution containing DNase-free RNase. Fluorescence intensities of the stained nuclei were measured by flow cytometry. Values for nuclear DNA content were estimated by comparing fluorescence intensities of the nuclei of the test population with those of a diploid barley (*Hordeum vulgare* L. cv. Hitchcock) or hexaploid wheat (*Triticum aestivum* L. cv. Arapahoe) internal DNA standard that was included with the tissue being tested.

### Data collection

The prepared material was analyzed in the Faculty of Medicine of Trakya University EPICS XL (Beckmann Coulter) model flow cytometer. Mean position of $G_0/G_1$ (nuclei) peak of sample and internal standard were determined by analyzing the data by CellQuest software. The mean DNA content per plant was calculated based on the 1000 scanned nuclei. The formula used for converting florescence values to DNA content was as follows: Nuclear DNA content=(mean position the of unknown peak)/(mean position of known) × DNA content of known standard. Seven samples of leaves treated with seed treatment including control group were analyzed for DNA content per accession. One seedling per accession was analyzed triple (subsample a,b,c) to obtain an estimate of laboratory precision which was 0.01 pg for this study.

### Statistical analysis

Statistical evaluation was conducted with the help of STATISTICA AXA 7.1 statistics program with a serial number of AXA507C775506FAN3. Conformance of the measurable data to the normal distribution was checked by single sample Kolmogorov Smirnov test and Shapiro Wilk test and then, t test and variance analysis were used in independent groups for those showing normal distribution in comparison between the groups. Kruskal-Wallis variance analysis and Mann Whitney U test were used in comparison between the groups not showing normal distribution. Median (Min-Max) values and arithmetical mean ± standard deviation were provided as descriptive statistic. Significance limit was specified as ($p \leq 0.05$) for all statistics.

## RESULTS AND DISCUSSION

The fresh leaf tissues which had treated by the doses given in the table were taken for the analysis. DNA amounts were determined for each sample at least three times. The results were showed in Table 1.

Flow cytometric analysis could provide a rapid and accurate analytical tool in order to determine relationship between herbicides and toxicity to characterize and categorize the health effects caused by herbicides and surfactants. There were many investigations about toxic effects of pesticides on cell division (Treshow, 1970; Hocking and Thomas, 1979; Hossain et al., 1980). It was determined that Antracol fungucide increased cell division

**Table 1.** DNA amounts (2C) (picogram) Mean, ± SS, Median, (Min-Max) values of control and Attribut and BioPOWER treated groups were given.

| GROUP | DNA AMOUNTS (2C) (picogram) Mean. ± SS, Median, (Min-Max) |
|---|---|
| CONTROL | 33.98 ± 0.365, 33.99 (33.61 – 34.34) |
| (FD) 0.42 mM Attribut | 34.30 ± 0.717, 34.12 (33.69 – 35.09) |
| (FD×2) 0.84 mM Attribut | 33.19 ± 0.638, 33.53 (32.45 – 33.58) |
| (*) (FD×4) 1.68 mM Attribut | 32.75 ± 0.281, 32.78 (32.45 – 33.01) |
| (FDS) 0.42 mM Attribut +0.25% BioPower | 34.80 ± 0.191, 34.83 (34.60 – 34.98) |
| (FDS×2) 0.84 mM Attribut + 0.25% BioPower | 34.30 ± 1.568, 35.06 (32.50 – 35.35) |
| (*) (FDS×4) 1.68 mM Attribut + 0.25% BioPower | 34.22 ± 0.851, 34.38 (33.30 – 34.98) |

Notes: Each arithmetical mean value represents the mean of 3 independent experiments. DNA amounts of wheats a month after sowing in pots were given. (*) indicates significant differences at the 5% level between values obtained under control and seed treated plants (P≤ 0.05).

(Steward and Krikorian, 1971). In our study DNA amount in leaves of *Triticum aestivum* L. cv. Pehlivan in (FD×4) dose was 32.75 pg and in (FDS×4) dose was 34.30 while in control was 33.98 pg. It appeared thet DNA amount decreased in the treatment of (FD×4) 1.68 mM Attribut in statistically significant amounts while it increased in the treatment of (FDS×4) 1.68 mM Attribut + 0.25% BioPower.

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