Cytogenetic and biochemical effects of imazethapyr in wheat 
(Triticum durum)

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Abstract: Mitotic abnormalities, chromosomal behavior, and protein content in wheat (Triticum durum) due to imazethapyr (IM) treatment were determined. Wheat seeds (variety HI-8663) were exposed to 5 IM concentrations (0.5, 0.89, 1.58, 2.81, and 5 ppm) according to the between paper method, in laboratory conditions. The protein content in the root-shoot axis and endosperm decreased significantly with the dosage increase. Cytological observations showed that the mitotic frequency in the root meristematic cells decreased parallel to the increase in concentrations. The most common types of the observed anomalies were sticky chromosomes, lagging chromosomes, scattered chromosomes, chromatin bridges, and micronuclei. The results revealed that the herbicide IM could affect biochemical parameters, mitotic frequency, and chromosomal behavior in wheat seeds.

Key words: Imazethapyr, Triticum durum, chromosomal abnormalities, mitosis, protein

Introduction

Food shortage is a serious global problem in the current century; thus, the agricultural sector has to increase crop grain production (1). Triticum durum, also called durum wheat or macaroni wheat, is the only tetraploid species of wheat having more commercial importance that is widely cultivated nowadays (2). Therefore, it is the most important and strategic cereal in most developing countries. Among all of the different pests, weeds are the most considerable constraint in crop production, responsible for heavy yield losses (3). Hand weeding and herbicide usage are the common weed control measures, especially in developing countries (4). Imazethapyr is an imidazolinone compound that inhibits the activity of the acetolactate synthase (ALS) enzyme (5). It is also used as a selective herbicide for controlling annual and perennial grasses and broadleaf weeds in chickpea and other legumes (6).

Residues of herbicides can lead to environmental pollution and have adverse effects on people and animals. Furthermore, the application of such chemicals in agricultural plants has harmful effects on their cytological mechanisms (7). HI-8663 (2n = 28) is an excellent new wheat variety with a high β-carotene content of 6.5 ppm and has remarkable stability over growing seasons and collations. The current study was designed and accomplished due to the agricultural importance of wheat, the need for laboratory studies, and to explore the effects of imazethapyr on the early growth parameters of wheat plants, especially the chromosomal abnormalities and biochemical changes of seedlings.
Materials and methods

The herbicide, imazethapyr (Pursuit™ 10% SL), has a molecular formula of $C_{15}H_{19}N_3O_3$ and was procured from BASF, India. The certified seed of wheat (*Triticum durum*), variety HI-8663, was obtained from the University of Agricultural Sciences, Dharwad, Karnataka, India. The germination studies were carried out according to the between paper method recommended by the International Seed Testing Association (8). The healthy wheat grains were surface-sterilized using 2% sodium hypochlorite solution for 5 min, followed by repeated washing with distilled water, 10-12 times, to remove the excess chloride. To overcome physiologic dormancy in wheat, the replicates for germination were placed in contact with the moist substratum and kept at a low temperature (5-10 °C) for an initial period of up to 7 days. Wheat grains, 10 comparably sized (seed sample of 100 seeds for each replicate), were placed in 10 petri dishes (9 cm), sterilized with sulfuric acid. The wheat grains were allowed to germinate in different 5 mL concentrations (0.5, 0.89, 1.58, 2.81, and 5 ppm) of imazethapyr prepared in Hoagland's nutrient solution, under clean bench conditions at 26 ± 2 °C for 4 days, in the dark. Dose range was selected based on field prescribed concentrations that could affect 10% to 95% of the seedlings with logarithmic intervals. From the fifth day onward, the germinated seedlings were exposed to 12 h of light intensity and allowed to grow for 15 more days. Out of the 400 seeds, 4 replicates were kept for each dose. The plants were watered with distilled water, when needed. The control group was treated only with Hoagland's nutrient solution.

The total protein content of the seedlings was determined according to the procedure by Lowry et al. (9) as follows: the blue color developed by the reduction of the phosphomolybdic-phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan, present in the protein, plus the color developed by the reaction of the protein with the alkaline cupric tartrate, were measured. The absorbance was read at 660 nm. The standard graph was plotted using bovine serum albumin and the amount of protein was calculated.

To study the effects of imazethapyr on somatic cells, the root tips of treated and untreated seedlings of wheat were harvested and pretreated with 8-HQ for 4 h. The root tips were fixed in Carnoy's solution II (alcohol:chloroform:acetic acid at a ratio of 6:3:1) for 24 h. The fixed root tips were preserved in 70% ethanol in a refrigerator. They were treated with 4% iron alum for 5 min and washed in 45% propionic acid, stained with a few drops of haematoxylin, and kept in a refrigerator for 4 to 5 h. The root tips were placed on a clean slide and squashed using 45% propionic acid based on the method of Levan (10). The mitotic index and frequency of abnormalities were calculated according to the method of Fiskesjö (11), by examining 500 cells per slide and calculating approximately 2000 cells. Triplicates were made for each concentration. The data were subjected to analysis of variance, using SPSS package version 16.0 with Tukey's honestly significant difference test at a level of 5%.

Results and discussion

The protein content in the root-shoot axis and endosperm of the wheat after a 15-day exposure showed a significant difference at a dosage of 0.5 to 5 ppm of IM (Table 1). Almost all of the seeds contain protein reserves for the nitrogenous supplies required by the young seedlings before they become able to absorb nitrogen through roots. The protein degradation to amino acids in the initial stages of seed germination helps in diverting amino acids towards the synthesis of new proteins/enzymes, cellular constituents, or translocation to the growing axis. During germination, the stored food materials in the cotyledons get hydrolyzed due to imbibitions of water and translocation into shoot and root axis, whereas the stored proteins are converted into amino acids due to the activity of proteolytic enzymes. These amino acids are utilized by developing seedlings to synthesize various enzymes and structural proteins. The proteolytic enzymes are synthesized in cotyledons during germination (12). When a plant is subjected to any biotic or abiotic stress factor, the first observed response is a decrease in its normal metabolic activities, along with a consequent reduction of growth. In this ‘alarm phase’ protein synthesis is an adversely affected anabolic process along with photosynthesis, transport of metabolites, uptake, and translocation of ions (13). The herbicide applied on seeds has a tendency to penetrate into plant tissues. There, it is transformed into metabolites, which are physiologically more

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active than the pattern compounds, and finally affect the seed health and quality. It has been opined that, after the ALS inhibition by herbicides, protein synthesis can only continue through an increase in the protein turnover; then the free amino acid pool shows a remarkable accumulation (14,15). However, they stated that IM had not decreased protein, and “de novo” synthesis of proteins had also inhibited in treated plants, showing that there would have been protein synthesis, but from the amino acids scavenged, mainly from the protein turnover. Furthermore, IM has been found to be a potent inhibitor of acetohydroxyacid synthase enzyme. This enzyme has an excellent herbicidal function and its phytotoxic effects can be reversed by exogenous application of valine, leucine, and isoleucine. This indicates that such imidazolinone herbicides are bound to the enzyme-pyruvate complex. The inhibition of acetohydroxyacid synthase by the imidazolinones could demonstrate the herbicidal effects of such compounds. If the imidazolinones inhibit the synthesis of valine, leucine, and isoleucine in vivo, there may be a rapid decrease in the pool size of such amino acids, which in turn could cause a decrease in protein synthesis (16). Consequently, imidazolinone herbicides cause a slowdown in the rate of cell division.

The results of the present study are in line with the effects of isoproturon on wheat (17), as well as of metribuzin, butachlor, and chlorimuron-ethyl on wheat and maize (18) and of malathion on lentil (19). The toxic effects of Cd on plants is expressed in the form of the activity inhibition of various enzymes involved in metabolism, which consequently lowers the level of the amino acid pool (20). On the other hand, pendimethalin and metobromuron caused an increase in the leaf and seed protein of cowpea, respectively, while metolachlor and prometryne were found to be most inhibitory to seed protein development in cowpea (21). However, in another study, it was indicated that imazethapyr, imazamox, and bentazone did not affect the crude protein rate of *Trifolium resupinatum* during the first year, while in the second year an important effect on the crude protein rate was demonstrated (22). The inhibition of germination and subsequent growth of the seedling by the herbicide IM may attribute to the impairment of the biochemical processes of the seedling due to herbicide treatment. The observed reduction in the protein content of the shoot-root axis of wheat indicates that such herbicides interfere in the synthesis of proteins during germination. However, the increase in protein content of the wheat endosperm shows interference in the synthesis of hydrolic enzymes during the germination (23,24).

In the present study, the mitotic index decreased in response to an increase in concentrations of the IM herbicide in wheat crop compared to the control (Table 2). The maximum mean value of the mitotic

### Table 1. Effect of different concentrations of imazethapyr on the protein content of wheat seedlings.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Days</th>
<th>Root-shoot axis (mg/g F.Wt.)</th>
<th>Endosperm (mg/g F.Wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4th</td>
<td>8th</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.48 ± 2.12a</td>
<td></td>
<td>30.42 ± 2.01a</td>
<td>35.61 ± 2.31a</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>17.44 ± 1.32b</td>
<td>27.89 ± 1.18b</td>
</tr>
<tr>
<td>0.89</td>
<td></td>
<td>13.39 ± 0.54c</td>
<td>20.62 ± 1.03c</td>
</tr>
<tr>
<td>1.58</td>
<td></td>
<td>11.89 ± 0.69d</td>
<td>17.39 ± 0.98d</td>
</tr>
<tr>
<td>2.81</td>
<td></td>
<td>8.81 ± 0.63e</td>
<td>10.71 ± 0.87e</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>6.92 ± 0.37f</td>
<td>7.68 ± 0.61f</td>
</tr>
</tbody>
</table>

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, when subjected to SPSS ver. 16.0, according to Tukey’s mean range test at the 5% level.
Table 2. Imazethapyr induced mitosis stages and chromosomal abnormalities in root tip cells of HI-8663 variety of *Triticum durum*.

<table>
<thead>
<tr>
<th>IM concentration (ppm)</th>
<th>Total number of cells</th>
<th>Mitotic frequency (%)</th>
<th>Prophase (%)</th>
<th>Metaphase (%)</th>
<th>Anaphase (%)</th>
<th>Telophase (%)</th>
<th>Total abnormalities (%)</th>
<th>Laggard chromosome (%)</th>
<th>Scattered chromosome (%)</th>
<th>Chromosomal bridge (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5890</td>
<td>6.30 ± 0.34a</td>
<td>53.40 ± 4.35c</td>
<td>21.66 ± 1.10e</td>
<td>14.44 ± 1.35e</td>
<td>10.50 ± 0.98a</td>
<td>0.50 ± 0.02a</td>
<td>0.19 ± 0.08d</td>
<td>0.31 ± 0.02bc</td>
<td>0.00 ± 0.00e</td>
</tr>
<tr>
<td>0.5</td>
<td>5735</td>
<td>5.80 ± 0.25b</td>
<td>50.96 ± 3.65f</td>
<td>29.37 ± 1.85a</td>
<td>12.37 ± 1.23a</td>
<td>9.00 ± 0.89f</td>
<td>0.71 ± 0.03c</td>
<td>0.10 ± 0.01c</td>
<td>0.29 ± 0.01c</td>
<td>0.11 ± 0.01d</td>
</tr>
<tr>
<td>0.89</td>
<td>5213</td>
<td>5.20 ± 0.31c</td>
<td>58.16 ± 4.12d</td>
<td>22.31 ± 1.12d</td>
<td>11.33 ± 1.65c</td>
<td>8.20 ± 0.75c</td>
<td>0.93 ± 0.04d</td>
<td>0.21 ± 0.01cd</td>
<td>0.31 ± 0.02bc</td>
<td>0.00 ± 0.00c</td>
</tr>
<tr>
<td>1.58</td>
<td>4965</td>
<td>4.30 ± 0.13d</td>
<td>60.10 ± 5.37c</td>
<td>24.18 ± 1.65b</td>
<td>8.40 ± 1.75c</td>
<td>7.32 ± 0.63d</td>
<td>1.12 ± 0.10c</td>
<td>0.23 ± 0.02c</td>
<td>0.20 ± 0.01d</td>
<td>0.21 ± 0.01c</td>
</tr>
<tr>
<td>2.81</td>
<td>4450</td>
<td>3.10 ± 0.18e</td>
<td>60.92 ± 4.68e</td>
<td>23.67 ± 1.87c</td>
<td>8.60 ± 1.32d</td>
<td>6.81 ± 0.52c</td>
<td>1.75 ± 0.012c</td>
<td>0.29 ± 0.02bc</td>
<td>0.31 ± 0.02bc</td>
<td>0.32 ± 0.02b</td>
</tr>
<tr>
<td>5</td>
<td>3865</td>
<td>2.21 ± 0.17f</td>
<td>73.22 ± 5.36c</td>
<td>16.42 ± 1.23f</td>
<td>5.04 ± 0.97c</td>
<td>5.32 ± 0.45f</td>
<td>2.24 ± 0.19c</td>
<td>0.52 ± 0.03b</td>
<td>0.41 ± 0.02e</td>
<td>0.39 ± 0.02b</td>
</tr>
</tbody>
</table>

Mean ± SD followed by the same superscript are not statistically significant between the concentrations, when subjected to SPSS ver. 16.0, according to Tukey’s mean range test at the 5% level.
index was observed in the control (6.3%), while it decreased from 5.8% to 2.81% at a dosage of 0.5 to 5 ppm. Although mitotic cells were observed in the treated root tips, it was lower than that of the control root tips. Our study revealed that IM affects the normal sequence of cell division in treated plants. The reduction of mitotic activity seems to be a common effect of most herbicides tested for their action on mitosis. Many herbicides have been reported to cause such effects, such as isoproturon, dithiopyr, and 2,4-D on wheat (25,26); koriskol dust on barley (27); imazethapyr on *Vicia faba* (28); pendimethalin, cypermethrin, and fenvalerate on *Allium cepa* (29,30); and fusilade on lentil (31). Herbicide inhibition causes a disruption in protein synthesis, which in turn leads to interference in DNA synthesis and cell growth. The inhibition of cellular division by ALS-inhibiting herbicides may cause a decrease in the root growth. An alteration of the intermediate metabolite pools or the ATP levels after the ALS inhibition could cause the inhibition of cellular division (32). The mitodepressive effect of the pesticide may be due to interference of the pesticide in the normal process of mitosis by reducing the number of dividing cells. The inhibition in the mitotic index (MI) may be due to interference of the herbicide in the normal sequence of cell division, which prevents or reduces the number of cells entering the prophase stage (33).

In the somatic cells of wheat, the IM inhibited mitosis and blocked it at the prometaphase, as well (Table 2). In the control plants, the prophase was 53.40%, while it was 73.22% in the 5 ppm treatment. Among the treatments, frequency of metaphase, anaphase, and telophase decreased. The maximum mean value of metaphase was 29.37% at the 0.5 ppm concentration, while the maximum values of anaphase and telophase were 14.44% and 10.50%, respectively, at the control. Herbicides are known to act as mitotic poisons by blocking mitosis in meristematic regions. The IM inhibited mitosis and also arrested the division process at prometaphase in wheat. Our results are in line with the effects of fusilade in lentil (31) and of *Allium cepa* affected by tribunil (34), which showed an increase in prophase and root meristematic cells. The plants treated with herbicides causing disruption of cell division have mitotic stages present, but sometimes one or more stages, that are normally present, will be absent or aberrant. The inhibition of cell division is a secondary effect caused by the disturbance of a plant’s metabolic process (31).

The different kinds of chromosomal abnormalities induced by IM in wheat in the present study increased along with the increase in concentration of IM (Table 2). While the abnormality ratio of the control plants was 0.5%, it was 0.71% to 2.24% among the treatments. The most common types of observed anomalies were sticky chromosomes, lagging chromosomes, scattered chromosomes, chromatin bridges, and micronuclei (Figure 1). Formation of micronuclei was observed at the 5 ppm concentration. The highest frequency of sticky chromosome was obtained through the application of 2.81 ppm (0.83%), while it was not observed in the control. The IM was effective in bridge formation especially at higher doses, while the control and 0.89 ppm treatment did not show any chromatin bridge. The lagging chromosome showed a minimum frequency at a dosage of 0.5 ppm (0.10%) and a maximum frequency at a dosage of 5 ppm (0.52%). The occurrence of scattered chromosomes was maximum (0.41%) at a dosage of 5 ppm, while the minimum frequency (0.20%) was at dosage of 1.58 ppm. Micronuclei (0.1%) were seen only in the 5 ppm concentration.

Chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and chromosomes. As the result, there is an intermingling of fibers, while chromosomes become attached to each other by subchromatid bridges (35). The chromosome stickiness and clumping have been reported following treatment with a number of chemicals including imazethapyr in *Vicia faba* (28); 2,4-D and isoproturon on wheat (36); copper mine, afugan, and atrazine in *Allium cepa* (37-39); and maleic hydrazide in *Trigonella foenum-graecum* (33). Such stickiness could be due to the depolymerization of nucleic acid caused by mutagenic treatments or to a partial dissociation and altered pattern of organization of nucleoprotein. The stickiness of chromosomes makes their separation and free movement incomplete; thus, they remain connected by bridges (33).

Chromosome bridges have been reported following treatment with a number of chemicals including copper chloride on *Helianthus annuus* (34),
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Imazethapyr on Vicia faba (28), afugan on Allium cepa (38), maleic hydrazide on Helianthus annuus (40), maleic hydrazide on Trigonella foenum-graecum (33), and atrazine on Allium cepa (39). The presence of chromosome bridges may be due to stickiness or the formation of dicentric chromosomes caused by breakage and reunion (33).

Laggard chromosomes have been reported in Vicia faba treated with imazethapyr (41), in Allium cepa exposed to copper mine (37), in Allium cepa affected by afugan (38), and in Allium cepa treated with atrazine (39). Kaymak (40) suggested that precocious movement of chromosomes and laggards may be attributed to the failure of the spindle apparatus to organize in a normal way.

Inceer et al. (37) reported that the wastes of copper mine induced some abnormalities including scattered chromosomes in the root tip cells of Allium cepa. They also suggested that such chromosomal regulations can affect the vigour, fertility, yield, or competitive ability of the exposed plants.

Micronuclei have been reported following treatment with a number of chemicals including pesticides with phenolic compounds in Allium cepa (42), imazethapyr herbicide in Vicia faba (28), and afugan in root tip cells of Allium cepa (38). It is known that micronuclei are true mutagenic aspects that may lead to a loss of genetic material. This mutagenic effect is estimated as a percentage of micronuclei formed in the interphase (28).

Generally, it can be concluded that the IM has harmful effects on the root tip cells of wheat. Furthermore, the increase in herbicide concentration leads to certain irreversible cytogenetic effects in plants.

Figure 1. Chromosomal abnormalities in wheat root tip cells induced by imazethapyr.

(a) Scattered chromosome (10 ppm)  (b) Laggard chromosome (1 ppm)
(c) Chromosomal bridge (10 ppm)  (d) Sticky chromosome (2.5 ppm)
(e) Micronuclei (5 ppm)
Acknowledgments

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References


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