Biomonitoring of Genotoxic Effect of Glyphosate and Pendimethalin in *Vigna mungo* Populations

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Summary Genotoxicological biomonitoring of *Vigna mungo* populations in the Banda district in Uttar Pradesh, sprayed with the herbicides glyphosate and pendimethalin, was done by studying their meiotic behavior and morphological characters and comparing them with untreated populations.

The herbicides caused a change in the frequency of different meiotic phases at a given time, and also induced various anomalies. Disturbed polarity, spindle disturbance, bridges, and laggards were common in both the populations. Micronuclei were found in a large number of cells. Pollen fertility was less in the glyphosate-treated group as compared to the pendimethalin-treated and control groups. A comparative account of the correlation between percentage anomaly and pollen fertility as indicators of genotoxicity of the two herbicides has been described in this paper.

Key words Biomonitoring, Herbicide, Pendimethalin, Glyphosate, Meiosis, Pollen fertility.

A heterogeneous mixture of agrochemicals (pesticides, herbicides and fertilizers) is used by farmers all over the world for enhancing the yield of crop. The control of pest, weeds and disease is necessary for increasing agricultural productivity, and the incessant and persistent use of chemicals with this aim has resulted in their accumulation in their original or broken-down forms in the environment, as well as in the exposure of flora and fauna to the chemicals' subtoxic effects due to the absence of absolute selectivity in their action. Herbicides are used indiscriminately for management of weeds. They can be either selective or non-selective, and have been reported to affect non-target crop plants. They are also considered as potential chemical mutagens (Dragoeva *et al.* 2012).

Herbicide application is most effective for protection of plants from weeds and has contributed significantly to enhance agricultural productivity and crop yield, but there are reports of its adverse effect on meiotic behavior and pollen fertility in plants. Higher plants provide a valuable genetic assay for monitoring the toxicity of herbicides (Enan 2009). Biological monitoring provides a useful tool to estimate genetic risk developing from herbicide treatment (Fiskesjo 1985), as plant assays are highly sensitive to many environmental pollutants, including heavy metals (Fiskesjo 1988). Genotoxicological biomonitoring assessment was performed on *Vigna mungo* populations in the Banda district of Uttar Pradesh, sprayed with two herbicides—glyphosate and pendimethalin. *Vigna mungo* is an important legume crop of the family Fabaceae. It is a short duration summer pulse crop and requires 90–120 d from planting to maturity. Adequate rainfall is required from planting to maturity to pod formation. The crop grows best in fertile, sandy loamy soil, and is sown in July and harvested in October. The *Vigna mungo* populations were treated with two different herbicides, glyphosate and pendimethalin, and similar plots were left untreated as control. A comparative study of percentage anomaly during meiosis in pollen mother cells and pollen fertility

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as indicators of genotoxicity of the two herbicides was done.

Glyphosate is a broad spectrum non-selective herbicide. It is usually formulated as an isopropyl salt. It has been established that it inhibits the shikimic acid pathway, which prevents plants from synthesizing three aromatic amino acids that are useful for the growth and survival of plants. Pendimethalin is a selective herbicide used to control annual grasses and broadleaf weeds. It is a meiotic inhibitor which inhibits cell division, and the induction of micronuclei may occur. Chromosomal aberrations can be accepted as indicators of genetic damage induced by herbicides. Micronuclei and reduction in pollen fertility have been reported in *Moringa oleifera* by Silva *et al.* (2011).

Genetic damage at the chromosomal level may cause a change in chromosome number or structure, and can be monitored as chromosomal aberrations or micronuclei frequency.

Materials and methods

Study area

The experiment was conducted in a free land area in the Banda district of U.P., which lies between latitude 24°53' and 25°55'N and longitude 80°07' and 81°34'E. The site belongs to Mar and Kabar soils under the agro-ecological zone of River Ken and Yamuna River. Environmental disturbance has been reported in the last few decades, and the climate is extremely hot in the summer and bitterly cold in the winter. Rainfall has no fixed arrival and this has badly affected the agriculture of this district.

Land preparation and design of experiment

The land for the experiment was ploughed several times to obtain a good tilt condition. The land was leveled several times and all weeds were manually removed. The total area of the field was 900 square ft and the total number of plots was nine. The size of each plot was 100 square ft. All the treatments of the experiment were assigned at random to each plot.

Sowing and germination of seeds

The seeds were soaked in water for 24h, and then blotted and mixed with soil. They were sown in a random block design in three replicates. The germination of the seeds started 3 d after sowing, and after 5 d, the maximum number of seeds were germinated.

Use of herbicide and treatment of plants

Two herbicides, glyphosate and pendimethalin, were used in the experiment. The plants were treated with different concentrations of the two herbicides after 21 d from the day of sowing. The experiment consisted of treatments maintained in three replicates. The plants were washed with a foliar spray of water 24 h after the time at which treatment was given.

Experimental studies

Stomatal frequency

For the study of the stomata, peels from the adaxial surface of the leaves were taken and mounted in a 70% saffranin glycerin solution.

$$\text{Stomatal frequency} = \frac{\text{Total number of stomata in 10 fields}}{10}$$

Pollen studies

For pollen studies, flower buds of the same age were taken randomly from the control and treated plants of each treatment, and fixed in 70% alcohol. Pollen that stained dark purple in malachite green was considered viable, while those that stained pale green were considered non-viable. The ratio of viable pollen to the total number of pollen grains counted for each treatment was expressed as pollen fertility %.

$$\text{Percentage pollen fertility} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Meiotic studies

For meiotic studies, flower buds of approximate size were fixed in 1:3 iron saturated acetic acid and absolute alcohol for 24 h and transferred to 70% alcohol. Slides were prepared by squashing the anther in an acetocarmine stain. Later, the slide was made permanent using the butanol schedule.

$$\text{Relative abnormality rate (RAR)} = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells observed}} \times 100$$

$$\text{Abnormality percentage} = \frac{\text{Number of any kind of abnormality observed}}{\text{Total number of abnormal cells}} \times 100$$

Harvesting and data collection

To evaluate yield, harvesting was done in October when the crop matured. Different yield contributing data were recorded from the mean of five harvested plants which were selected at random from each plot. Before harvesting, plant height, internodal length and pod length were measured by using a meter scale. The total number of nodes, branches, and number of pods were averaged over five plants. To evaluate yield, the number of seeds per pod was averaged over five plants.

Results

In this study, we have biomonitoring the genotoxic effect of two herbicides, glyphosate and

Table 1. Effect of foliar spray of herbicides on morphological and yield parameters in *Vigna mungo*.

Morphological characters	Control	Pendimethalin	Glyphosate	F Value
Plant height (cm.)	92.83±0.60	91.66±0.75	84±2.08	11.01*
Number of branches	15.33±2.02	12.66±1.45	9.33±1.20	9.5*
Number of nodes	17.5±0.28	15.83±0.44	11.5±0.76	114.19**
Internodal length (cm.)	5.5±0.25	3.7±0.25	2.9±0.1	126.8**
Number of pods	46±0.44	50±1.15	35.7±1.34	58.15**
Pod length (cm.)	5±0.05	4.2±0.08	3.7±0.14	3.48 ns
Number of seeds per pod	8.66±0.33	7±0.57	5.33±0.88	12.43*
Stomatal frequency	50.66±2.33	62.66±1.20	38.33±1.66	39.97**
Stomata length (μm)	58.7±1.78	55.96±1.49	54.93±0.90	2.00 ns
Stomata width (μm)	28.15±1.49	19.57±0.59	19.16±0.54	51.55**
Breath of guard cell (μm)	29.18±0.9	27.33±0.4	26.86±1.06	1.70 ns
Pollen diameter (μm)	155.86±1.03	145.93±1.10	143.23±0.37	8.26*
Pollen fertility (%)	96.63±1.09	94.56±1.37	92±1.28	2.98 ns

Values represent mean±SE; ns-non significant; *significantly different at $p \leq 0.05$ and **significantly different at $p \leq 0.05$ and $p \leq 0.01$

Table 2. Different types of chromosomal aberrations seen in meiosis in pollen mother cells in plants of *Vigna mungo* populations treated with herbicides, pendimethalin and glyphosate.

Meiotic anomaly (%)	Pendimethalin	Glyphosate
RAR	16.96	21
Disorientation	26.31	23.8
Bridge	15.78	9.52
Laggard	10.52	9.52
Spindle disturbance	21.05	19.04
Clumping	10.52	4.76
1 micronuclei	5.26	4.76
2 micronuclei	10.52	14.28
3 micronuclei	—	4.76
Binucleate cells	—	9.52

pendimethalin, in *Vigna mungo* populations, and significant differences in growth parameters were observed. All the parameters, viz., plant height, number of branches, number of nodes, internodal length, pod length, and number of seeds per pod, were higher in the control, followed by the pendimethalin-treated and then the glyphosate-treated plants, except for the number of pods, which were higher in the pendimethalin-treated plants (Table 1).

The stomatal frequency of the adaxial surface of the leaves in the pendimethalin-treated plants was higher than the control and glyphosate-treated plants. It was 62.66 ± 1.20 in the pendimethalin-treated and 38.33 ± 1.66 in the glyphosate-treated plants. Pollen fertility was 94.56% in the pendimethalin-treated and 92% in the glyphosate-treated plants as compared to the control plants (96.63%), corresponding to a percent decrease of 4.63% in the glyphosate-treated and 2.07% in the pendimethalin-treated plants.

A significant difference in meiotic parameters was revealed by RAR (Table 2). RAR was higher in the glyphosate-treated (21%) and lower (16.96%) in the pendimethalin-treated plants. The abnormality % in the control plants was negligible, while several aberrations (Fig. 1A–O) were observed in the herbicide-treated populations. Disorientation, bridge, laggard, spindle disturbance, and clumping percentages were higher in the pendimethalin-treated populations as compared to the glyphosate-treated ones. Micronuclei were found in both treatments, but the number of cells with micronuclei was higher in the glyphosate-treated populations.

Binucleate cells were found in the glyphosate-treated population with a percentage of 9.52. Stickiness was observed at metaphase I and II. Bridge and laggard were dominant at anaphases I and II. Disorientation was highest among all anomalies, found in 26.31% of pendimethalin-treated and 23.8% of glyphosate-treated plants. Percentage of clumping was higher in the pendimethalin-treated plants (10.52%) compared to the glyphosate-treated ones (4.76%). The nature of aberrations generated was different in the two populations, which along with the RAR, can be used as a biomonitoring system.

Discussion

Higher plants provide a valuable genetic assay system for screening and monitoring environmental pollutants. They are recognized as excellent indicators of the mutagenic effects of environmental chemicals, and are applicable for the detection of environmental mutagens. A number of plant bioassays have been identified for *in situ* environmental monitoring (Uhl *et al.* 2003). The most common assays used for studying mutagenicity of various pollutants in plants are based on the detection of chromosomal aberrations in *Allium cepa* (Fiskesjo 1985), *Tradescantia* (Ichikawa 1992), *Vicia faba* (Kanaya *et al.* 1994) or *Zea mays* (Grant and Owens 2006). Higher

Fig. 1. Different types of chromosomal abnormalities in pollen mother cells of plants of *Vigna mungo* sprayed with pendimethalin and glyphosate. (A) Normal prophase, (B) normal metaphase, (C) normal anaphase I, (D) normal metaphase II, (E) normal anaphase II, (F) clumped metaphase I, (G) disorientation in metaphase I, (H) spindle disorientation in metaphase II, (I) bridge formation in anaphase I, (J) bridge formation in anaphase II, (K) disorientation in anaphase II, (L) anaphase II with laggard, (M) telophase I with one micronuclei, (N) telophase I with two micronuclei, (O) binucleate cell.

plants are suitable for cytological analysis, and responses seen in plant tests are highly correlated with those seen in other biological systems, making the plant test good for evaluating the genotoxicity of environmental samples (Grant 1999, Sadowska *et al.* 2001).

Genotoxicity of herbicides has been reported by several researchers in several plants, such as by Boyle and Evans (1974) in *Secale-cereale* L., Tartar *et al.* (2006) in *Allium cepa* L and *Allium sativum* L, and by Mansour (1984) in *Vicia faba*. The cytogenetic effect of 2,4-D was assessed as

chromosome abnormalities in the meiosis of *Vicia faba* (Khilman 1975) and barley plants (Khalatkar and Bhargava 1985). The mutagenic activity was assessed using cytogenetic and pollen viability endpoints in *Allium cepa* and *Oryza sativa* (Pavlica *et al.* 1991).

The two herbicides studied here induced a decrease in morphological parameters at similar doses of application. The effect of herbicides on morphological characters has been studied by many workers in various plants, viz., by Santos *et al.* (2007) in different eucalypt clones and Fayinminnu *et al.* (2010) in *Vigna unguiculata*. There are few reports on the effect of herbicides in the *Vigna* species, including *Vigna angularis* (Soltani *et al.* 2005) and greengram (Zaidi *et al.* 2005, Boe *et al.* 1991, Khan *et al.* 2006).

It was observed that all the parameters are higher in the control as compared to the pendimethalin-treated and glyphosate-treated plants, except for the number of pods and stomatal frequency, which were higher in the pendimethalin-treated plants. The frequency of stomata on the leaf surface is an important biomonitoring trait, as the herbicide is taken up by the plant through the stomata, and any increase or decrease in stomata frequency can be considered to be a direct effect of herbicide treatment. Ferreira *et al.* (2007) have reported an increase in stomata frequency in sugarcane cultivars, while Cali (2009) observed a decrease in stomata frequency.

In the present investigation, the two herbicides induced a decrease in pollen fertility as compared to the control, and glyphosate caused greater reduction. Similar observations have been reported by Jabee *et al.* (2008) and Kaymak and Gokalp (2005) in *Allium cepa*, Pline *et al.* (2003) in cotton, and by Thomas *et al.* (2004) in corn. Variation in size of pollen was also observed.

Meiotic behavior was studied as an important biomonitoring trait for the assessment of genotoxicity of the two herbicides. Various aberrations were found in the meiotic cells of sorghum in response to atrazine and 2,4-D (Liang *et al.* 1969), in *Vicia faba* in response to terbutryn (Badr *et al.* 1987), and in *Trigonella foenum-graecum* in response to sodium azide (Kapoor and Srivastava 2010). A significant difference in meiotic behavior was revealed by RAR between pendimethalin- and glyphosate-treated plants. The RAR increased as a result of the two herbicide treatments as compared to the control. The abnormalities observed were clumping, laggards, bridge, spindle disorientation, micronuclei formation and binucleate cells in varying frequencies.

In the present investigation, both treatments produced similar types of abnormalities, but the percentage of abnormality was different for each treatment. Stickiness may be due to the depolymerisation of nucleic acids, caused by herbicidal treatment or the partial dissociation of nucleoprotein alteration in their pattern of organization (Evans 1962), or by partial dissociation of nucleoproteins (Kaufman *et al.* 1955). Jayabalan and Rao (1987) suggested that stickiness might be a consequence of disturbance in cytochemically balanced reactions. Some types of gene mutations that cause incorrect coding of some non-histone protein involved in chromosomal organization can lead to chromosomal clumping. It may also be possible that the mutagen itself reacts with histone proteins and brings about a change in surface property of the chromosome due to improper folding of DNA, thereby causing them to clump or stick (Grant 1978).

The clumped nuclei are formed due to disturbances at the cytochemical level. Clumping of the chromosome can interfere with normal arrangement at metaphase, and separation may lead to the formation of bridges. Occurrence of chromosome bridges may be due to stickiness or formation of dicentric chromosomes caused by breakage and reunion. When the spindle fibers pull the chromosome towards the pole, the bridges can break into fragments, which then form micronuclei. The presence of micronuclei is a result of acentric fragments of the chromosome and failure in normal spindle formation (Degrossi and Rizzoni 1982). Bridges and laggards could have arisen due to delayed termination of stickiness of chromosomal ends (Kaur and Grover 1985). Laggards may result in the formation of micronuclei at telophase I. Acentric fragments or laggards may result in the formation of micronuclei at telophase II, and ultimately in variation in number and size of pollen grains resulting from mother cells.

Disorientation is reported to be due to disturbances in orientation of the spindle apparatus. The anomaly was probably due to the disturbance in orientation of the spindle apparatus resulting from the toxic effects of herbicides.

It has been shown that for different plant species, the decline in seed production is correlated with meiotic irregularities (La Fleur and Jalal 1992). Spindle-related aberrations probably cause greater damage to the viability of gametes, and the clumping of the chromosome seems to be an appropriate, possible adaptation to circumvent the damage to the gametes. The yield of seeds are greater in pendimethalin-treated populations than in glyphosate-treated ones. Clumping is more commonly found in pendimethalin-treated populations. As meiosis progressed, the number of interconnections or clumping decreased. The absence of clumping in the later stage of meiotic division, coupled with high pollen viability and yield, led to a conclusion that the clumping of the chromosome during early stages could be a strategy for survival and not an aberration. Binucleate cells are more commonly found in the glyphosate-treated plants, but did not result in a corresponding increase in crop yield. Hence, the observed clumping does not seem to be deleterious; instead, it correlates with the formation of fertile pollen grains. The occurrence of chromosome clumps was previously associated with sterility and degeneration. However, in *C. annum* and *C. frutescence*, a high percentage frequency of chromosome clumping had no effect on pollen viability or seed yield, which were high in the two species (Falusi 2006).

The purpose of this study was to biomonitor the genotoxic effect of the two herbicides, glyphosate and pendimethalin, in *Vigna mungo* populations, and to assess which herbicide has a greater detrimental effect on the plants. It was found that glyphosate causes more detrimental effects compared to pendimethalin on applying the same dose of treatment. The result of the experiment indicates that the highest yield and growth were obtained by using pendimethalin (greater than those in the control), and the lowest yield was obtained by using glyphosate. An assessment of the different anomalies in PMC cells treated with the two herbicides help us to assess their toxic effect. Chromosomal aberrations, stomatal frequency, and pollen fertility are efficient tools for biomonitoring the adverse effects of any agrochemical, and the study of any of these parameters can help in determining the suitability of an herbicide for a particular crop in a specific geographical area.

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