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EFFECT OF 2,4-D AND ISOPROTURON ON CHROMOSOMAL DISTURBANCES DURING MITOTIC DIVISION IN ROOT TIP CELLS OF *TRITICUM AESTIVUM* L.

The widespread use of the herbicides for weed control and crop productivity in modern agriculture exert a threat on economically important crops by way of cytological damage to the cells of the crop plant or side effects, if any, induced by the herbicides. In the present communication, author describes the effects of 2,4-D and Isoproturon on chromosomal morphology in mitotic cells of Triticum aestivum L. The wheat seedlings were treated with range of concentrations (50–1200 ppm) of 2,4-D and Isoproturon for 72 h at room temperature. In the mitotic cells, twelve distinct chromosome structure abnormalities were observed over control. The observed irregularities were stickiness, c-mitosis, multipolar chromosomes with or without spindles, fragments and bridges, lagging chromosomes, unequal distribution of chromosomes, over contracted chromosomes, unoriented chromosomes, star shaped arrangement of the chromosomes, increased cell size and failure of cell plate formation. The abnormalities like stickiness, fragments, bridges, lagging or dysjunction, unequal distribution and over contracted chromosomes meet frequently.

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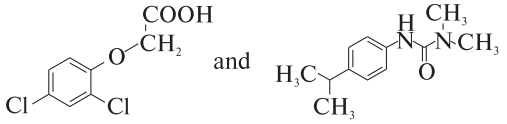
Introduction. The crop field or cereal plants were usually treated with some kind of herbicides [1]. The herbicides have different mode of action at the same site or other parts of the crop plant [2]. The herbicides might interrupt the photosynthesis by competing with Quinone B (QB) binding region of D1 protein in photosystem II (PSII) complex [3–5], production of proteins, isoprenoids, flavonoids, lipids or fatty acids [6]. The interruption in fatty acid assembly results in inhibition of cell division [7]. The herbicides might inhibit cell division in several ways [8–11]. The uses of herbicides in cereal crops were become very popular and common for the reason that tremendous crop protection, satisfactory residual action, wide-range weed control, flexibility in application timing and the most important cost-effective. The extensive use of the herbicides in modern agriculture exerts a threat on crops by a way of cytological damage to the cells of the crop plant or any side effects induced by the herbicides. In the past different workers had used different herbicides to study their mutagenic, genotoxic and carcinogenic potential in experiments both carried out *in vivo* and *in vitro* conditions [12–20]. After the first *Allium* test [21], many herbicides had been tested for mitotic activities on different plants taking the *Allium* test as a reference [22, 23]. The present communication was aimed to study the effects of 2,4-D and Isoproturon on mitotic root tip cells of *Triticum aestivum* L. which is an important cereal crop plant and used as a staple food in different parts of the world. Moreover, the importance of the crop plant could be understood by the use of wheat spike as a symbol for Food and Agriculture Organisation (FAO). Both 2,4-Dichlorophenoxy acetic acid (2,4-D) and 3-(4-isopropyl phenyl)-1,1-dimethyl urea (Isoproturon) are systemic herbicides and belong to the phenoxy and urea group respectively. The structural formulae are



respectively. They translocate throughout the plant via roots and leaves. The herbicides could be applied pre or post emergence for control of annual or perennial broad leaf competitive weeds and grasses.

Materials and Method. 2,4-Dichlorophenoxy acetic acid and 3-(4-isopropyl phenyl)-1,1-dimethyl urea were used in present analysis to make a systematic inquest in to the selected crop

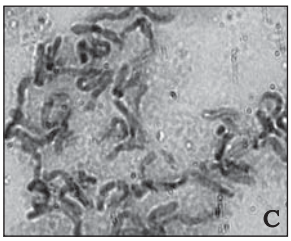


Fig. 1. Normal mitosis

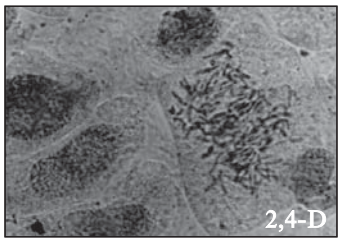


Fig. 2. Chromosome stickiness, 50 ppm

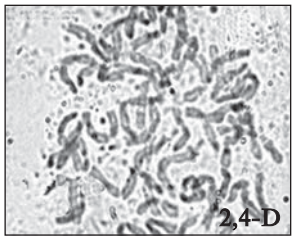


Fig. 3. C-metaphase, 100 ppm

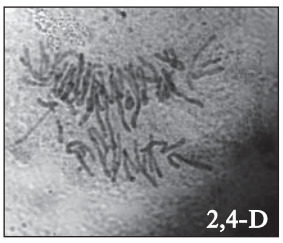
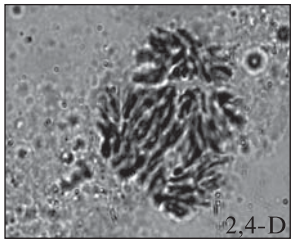


Fig. 4. Unequal and unorinted chromosomes, 200 ppm

plant. Both the herbicides were used against annual grasses and broad leaved weeds in crop fields. *Triticum aestivum* L. characterized by having large sized chromosomes selected for the present study. Seeds of the *T. aestivum* L var. HUW 234, HUW 468 and HUW 533 were collected from the Department of Genetics and Plant Breeding, Institute of Agricultural Science, Banaras Hindu University, Varanasi, India. Seeds were germinated in petridishes with different concentrations (50, 100, 200, 400, 800, 1200 ppm) of both the herbicides and control for 72 h at 24 ± 2 °C. The root tips (size 1.5–2.0 cm) of the germinated seedlings were collected and rinsed with distilled water. The root tips were fixed in ethanol: acetic acid (3:1) solution at 4 °C in refrigerator for 24 h followed by washing in distilled water before transferring in 70 % alcohol for further use. The slides were prepared in 2 % acetocarmine for the study of mitotic abnormalities.

Result. The deviations observed than normal mitosis (Fig. 1, magnification for all figure, ×1000) were prearranged in figure 2–28. The twelve different types of chromosomal irregularities were recorded The irregularities found at the ranged concentrations (50–1200 ppm) of 2,4-D and Isoproturon herbicides treated were stickiness (50 ppm, Fig. 2, 14), c-mitosis (100 ppm, Fig. 3, 15), increased cell size (100 ppm, Fig. 15), unequal distribution or unoriented chromosomes (200 ppm, Fig. 4, 16), longitudinal gaps or splits among chromosomes (Fig. 5, 17) and over contraction of the chromosomes at 400 ppm (Fig. 5, 17), fragments and bridges (800 ppm, Fig. 7–10 and 18–20), lagging or dysjunction chromosomes (800 ppm, Fig. 18–20), multipolarity (1200 ppm, Fig. 13, 21), star shaped structure of chromosomes (1200 ppm, Fig. 11, 22) and the failure of the cell plate formation (1200 ppm, Fig. 24–26).



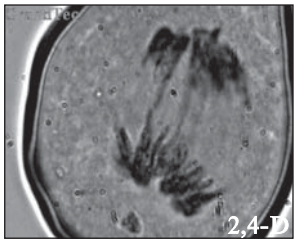


Fig. 5, 6. Longitudinal gaps and overcontraction in chromosomes, 400 ppm

Discussion. Wheat is an important cereal crop. Many herbicides are being used in the field to check the weeds. These herbicides come in contact with soil then main crop and causes abnormalities at morphological, physiological, biochemical and cytological levels [24–27]. 2,4-D and Isoproturon were common herbicides used in the crop fields. The effects of these herbicides were illustrated at mitotic level in root tip cells of wheat seedlings (Fig. 2–28). Root tip cells are considered to be good experimental material to study the herbicidal action on the mitotic stages for any mitotic aberrations, as the chromosomes are susceptible to external stimuli of environmental changes [28]. Chromosome susceptibility to environmental changes might suggest that wheat quality may get deteriorated by chromosome disturban ces. Cytological illustrations were also suggested that the tested herbicides can create some risk to human health and environment, if exposed for prolonged [29, 30].

Chromosome stickiness was very frequent in both herbicides treated at the 50 ppm concentration. The distinct views have been given by the different workers support the abnormality [31]. The abnormality contemplates mentally the physiological influence of the treated herbicides. It had been suggested that stickiness was the physiological

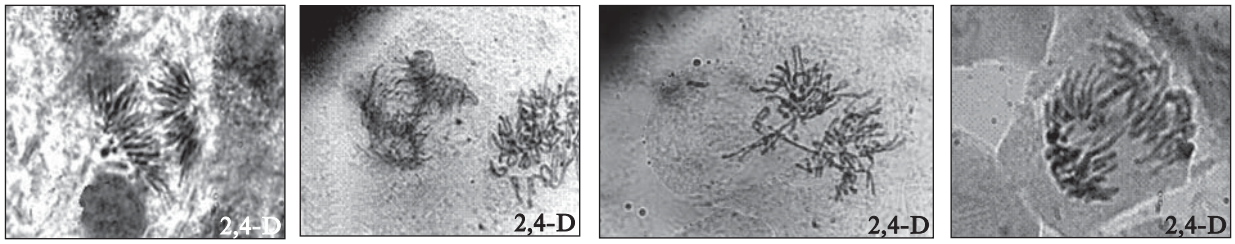


Fig. 7–10. Fragments, bridges, lagging or dysjunction chromosomes, 800 ppm

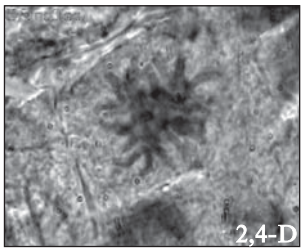


Fig. 11. Star-shaped structure of chromosomes, 1200 ppm

effect of the herbicides [32, 33] and also might be the consequence of complicate tangling of inter-chromosomal chromatin thread. This brings to the sub-chromatid association with the chromosomes [34]. Apart from the above, stickiness might be also a result of the action of these herbicides on the proteins of the chromosomes [35] and may lead to other kinds of chromosomal abnormalities like unequal distribution of chromosomes or un-oriented chromosomes at 200 ppm concentration of both herbicides treatment (Fig. 4, 16), over-contraction of the chromosomes at 400 ppm (Fig. 5, 17) in case of both the herbicides treatment, formation of longitudinal gaps or splits among the chromosomes at 400 ppm in case of both the herbicides (Fig. 5, 17), formation of fragments and bridges at 800 ppm in both herbicides treatment (Fig. 7–10 and 18–20), and the star-shaped structure of the chromosomes at 1200 ppm in both herbicide treatment (Fig. 11, 22). Therefore, for all these irregularities in the chromosome, sticky chromosomes might be used as a measurement of the cytotoxicity of the herbicides which ultimately might lead to cell death [36, 37]. A number of herbicides are known for artificial induction of sticky chromosomes [38, 39].

In c-mitosis or stathmokinese (Fig. 3, 15), as the case in both the herbicides treatment occurred at 100 ppm concentration, the chromosomes unable to move onward stages from beginning to end like prophase, metaphase, anaphase and telophase. The herbicides might be affecting the function of spindle but not its structure [40–42]. c-mitosis makes known that the herbicides hinder the spindle formation having a resemblance to the consequence of an action of colchicine [43]. The act of bringing on c-mitosis by artificial means occurring often and meet frequently at this concentration with spindle spoil was a sign of turbogenic effect [44]. In a species with high chromosome number and economically important plant such like wheat ($2n = 42$), needs much effort to establish precisely whether sister chromatids existing apart or intact in c-mitosis division figure of the chromosomes. The distinct herbicides examined in plant root tip cells able to persuade c-mitosis of the chromosomes [45, 46].

Chromosomes of anaphase stage in mitotic division existing apart with the help of spindle filament. The important component of the same is aster and two asters form a spindle fibre [47]. The herbicide propham treatment of *Haemanthus* endosperm cells indicated the chromosomes move

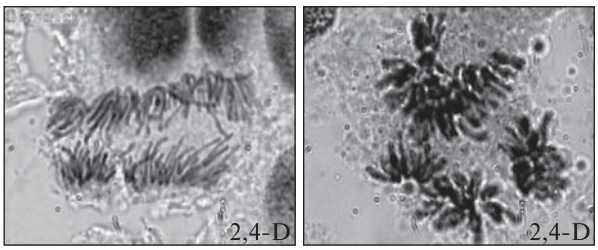


Fig. 12, 13. Multipolar chromosomes, 1200 ppm

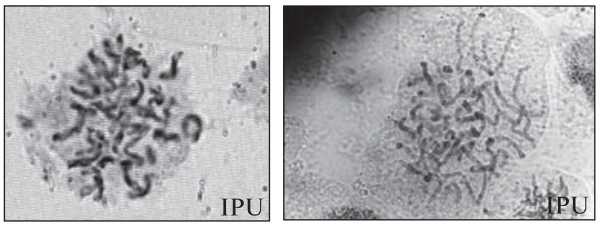


Fig. 14. Chromosome stickiness, 50 ppm

Fig. 15. C-metaphase and increased cell size, 100 ppm

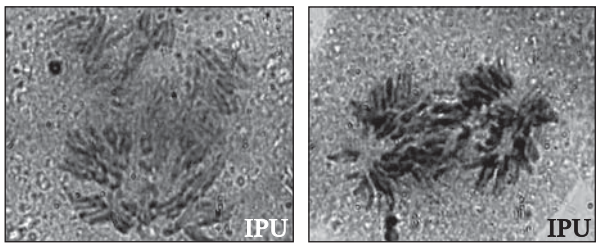


Fig. 16. Unequal and unoriented chromosomes, 200 ppm
Fig. 17. Longitudinal gaps and overcontraction in chromosomes, 400 ppm

to several parts within the cell instead of two poles [48–50]. These multiple poles might be composed of microtubules which acting along with the lines diverging from the centre and seems to function as «minispindles» within the cell [50]. It is believed that herbicides 2, 4-D and Isoproturon may influence the scattering of microtubule organizing centre (MTOC) from their normal bipolar existence [50]. This disturbance in MTOCs might be the source for lagging chromosomes or dysjunction in both the herbicides at 800 ppm concentration (Fig. 7–10 and 18–20). Multipolar division images (Fig. 13, 21) at 1200 ppm in both the herbicides might be the consequence of not complete suppression of spindle function. The process of origin by which chromosomes arranged in a particular form in multipolar division images was not known. But it has been reported that the other herbicides having a resemblance to the function of 2,4-D and Isoproturon used to study the effect on spindle function [51, 52].

The failure to reunion of the broken segments of chromosome with same chromosome from which it originated brings to the formation of fragments (Fig. 7–10 and 18–20). The arrangement of these fragments into the bridges at anaphase stage ascribes to unequal separation (Fig. 4, 16) of chromosome at their respective poles or they form dicentric chromosomes. Sticky chromosomes (Fig. 2, 14) might be the source for fragmentation and bring an artificial change in the framework of chromosome synthesis [53]. The breakage and reunion of chromosomes show the presence of clastogenic action of 2,4-D and Isoproturon [54].

In the present study, the application of herbicides 2,4-D and Isoproturon pointed out the root tip protuberances at 200 ppm (Fig. 27) and total hindrance for further growth of root tip at

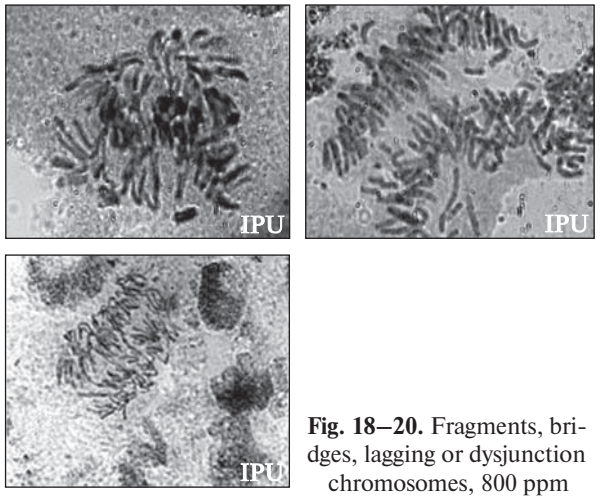


Fig. 18–20. Fragments, bridges, lagging or dysjunction chromosomes, 800 ppm

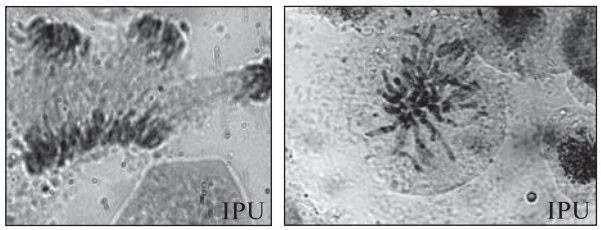


Fig. 21. Multipolar chromosomes, 1200 ppm
Fig. 22. Star-shaped structure of chromosomes, 1200 ppm

1200 ppm (Fig. 28). This abnormal mass protuberance might be a sign of increased root tip width and probably persuade for increase in cell size [55, 56].

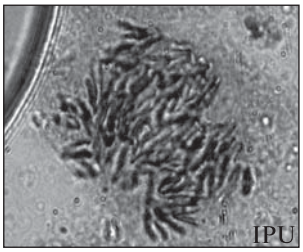


Fig. 23. Unequal division in chromosomes and pole formation, 1200 ppm

Haemanthus endosperm cells treated with propham herbicide failed to obtain the standard cell plate. Similarly, colchicine produced an effect to a particular arrangement not having all parts of the cell plate i.e. cross walls in cells of *Triticum aestivum* L. [57]. If cells were in involuntary course i. e. in cytokinesis at the time of colchicine application, the spoiled phragmoplast microtubule would not assign this limited amount of cell plate establishment precisely [57] and owing to that binucleate cells might be formed (Fig. 24– 26). Cell plate might be absent in longer exposure of herbicides. Consequently, the

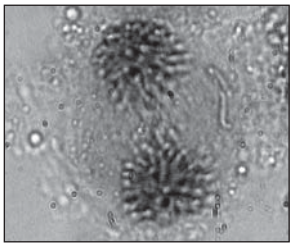
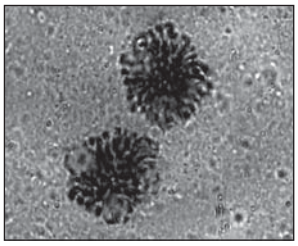




Fig. 24–26. Binucleate cell formation probability, 1200 ppm

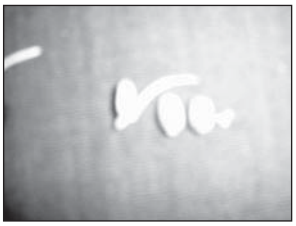



Fig. 27. Bulbous structure at 200 ppm might be the source for increased cell size

Fig. 28. Growth inhibition at 1200 ppm

cell plate abnormality is the satisfactory outcome of the disruption and disorientation of cell plate formation at the higher concentration of the herbicides treatment.

There were reports on like genotoxic consequences in different plant materials processed to different herbicides by several workers [58–60]. Even after making known to genotoxicity, turbogenicity and clastogenecity, it is unknown to us what would be the momentous effects of these herbicicides, if any, on the ribosomes, mitochondria, chlorophyll, cytoplasm and other extrachromosomal bodies with distinct existence in the cell. Chromosomal abnormalities suggested that the chromotoxicity of the herbicides might be the reason for diversion of the plant from its original parental line [61–64]. These chromosomal irregularities may affect the competitive ability and vigour of the exposed plant. Moreover, the increase or accumulation of these herbicides in the soil or water (biomagnification) can lead to certain rough impacts on plant or environment and human health.

Conclusion. Herbicides were, often, used in crop field to increase the yield and growth of the cereal plants. Herbicides were studied worldwide in past for their effects on chromosome abnormalities in plants and animals, tumors and reproductive potential of the organisms, human health and environmental quality. The majority of the herbicides available in the market could be grouped into the mitotic disrupter herbicides including phenoxy and urea group of herbicides [65]. A good number of herbicides from the group were used to control the grasses and small seeded weed species in the crop field. A minute observation of herbicide treated meristematic root tip cells under microscope revealed a dose-dependent loss of microtubules [66]. The effect of herbicides on microtubules could be the cause factor for the production of rough cell wall, stickiness (Fig. 2, 14), c-metaphase (Fig. 3, 15), unequal or unoriented chromosomes at the poles (Fig. 4, 16), dysjunction (Fig. 7–10, 18–20), multipolar chromosomes (Fig. 13, 21) and star-shaped structure of the chromosomes (Fig. 11, 22). Microtubule spoiling herbicides have an immense prospective in screening for mutant plants with changed herbicide sensitivity in treated plants [67, 68]. It could play a remarkable role for agricultural significance worldwide [69, 70]. All the herbicides, eventhough, are chemically different but common in application and symptomatology. The end result of the treated herbicides was dose-dependent manner and representing a familiar means of action [71]. There might be some possibilities for the use of the herbicides as a selective cause for plant alteration or transformation and in conventional breeding method as a tolerant species [72–74]. Also, the herbicides could be used for the induction of tetraploidy which might lead to quality improvement for both ornamental and agronomic plant species [75, 76].

There is need to sufficiently scientific, sensitive and molecular approach to evaluate the contaminants of the herbicides or their derivatives which might be the cause for chromotoxicity, mutagenicity, carcinogenicity or turbogenicity of the herbicides in the organisms. Also need some long term test *in vivo* and *in vitro* to develop technologies that may improve the crop yield and at the same time preserve the resource base alongwith some important plant species which affected by the herbicides unknowingly.

Sanjay Kumar

ВЛИЯНИЕ 2,4-Д И ИЗОПРОТУРОНА НА ХРОМОСОМНЫЕ НАРУШЕНИЯ В ХОДЕ МИТОТИЧЕСКИХ ДЕЛЕНИЙ В КЛЕТКАХ КОНЧИКОВ КОРНЕЙ *TRITICUM AESTIVUM* L.

Широкое использование гербицидов для контроля сорняков и обеспечения продуктивности сельскохозяйственных культур в современном агропроизводстве представляет угрозу для хозяйственно важных растений из-за возможности возникновения вызываемых гербицидами цитологических повреждений клеток или побочных эффектов. В настоящей работе описывается влияние 2,4-Д и изопротурона на морфологию хромосом в митотических клетках пшеницы *Triticum aestivum* L. Проростки пшеницы были обработаны 2,4-Д и изопротуроном в широком спектре концентраций (50–1200 ppm) в течение 72 ч при комнатной температуре. По сравнению с контролем в митотических клетках было обнаружено 12 различных структурных аберраций хромосом. Среди них наблюдали слипание хромосом, к-митозы, мультиполярные хромосомы с митотическим веретеном и без него, фрагменты и мосты, отстающие хромосомы, неравное распределение хромосом, конденсированные хромосомы, дезориентированные хромосомы, звездоподобное распределение хромосом, увеличение размера клеток и невозможность формирования клеточной пластинки. Такие аномалии, как слипание хромосом, фрагменты, мосты, отставание или разъединение, неравное распределение и конденсированные хромосомы, встречались часто.

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