INDUCTION OF MICRONUCLEI IN BARLEY ROOT MERISTEM BY THE ROOT EXTRACT OF *CONVULVULUS ARVENSIS*

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ABSTRACT

The presence of a common weed *Convulvulus arvensis* in the fields of barley leads to stunted growth and lower seed set in the crop. This is perhaps thought as an effect of competition for the available mineral resource of the soil as well as light and space constraints. However it has been observed that the presence of this weed causes a reduction in root branches and root area. It was therefore decided to assess the cytological aspects allelopathic effect of root exudates of *C. arvensis* on barley roots under controlled laboratory conditions simulating the field conditions. The results showed a dose dependent reduction in mitotic activity in root meristem of barley after treatment. Besides other cytological anomalies in dividing cells, micronuclei formation was observed in high percentage. Micronucleus formation leads to removal of genetic material from cells which might lead to cell death. This may be a method adopted by the weed to reduce root growth in the barley plants. The paper provides an insight into probable mechanisms of cytological allelopathy by the weed.
KEYWORDS: Allelopathy; Barley; *Convulvulus arvensis*; Cytotoxicity; Nuclear buds; Micronucleus; Weed

INTRODUCTION

Barley (*Hordeum vulgare* L. fam. Poaceae) is one of the ancient food grains also known as *Jau* in Hindi. It is one the most valuable cereal which comes just after rice, wheat and maize in terms of world production. In India it is grown as a Rabi crop planted during winters in low temperature. The crop is hardy and can tolerate temperature fluctuations, water stress and mild pH variations. The common weeds in this crop are *Convulvulus arvensis, Chenopodium album, Coronopus didymus* and *Anagalis arvensis.* Out of these, *Convulvulus* is very obnoxious and leads to reduction in crop yield if not removed in the beginning. *C. arvensis*, also known as *Hirankhuri* in Hindi, is a rhizomatous herb and belongs to Family Convulvulaceae. It is native to Europe and Asia. It has climbing or creeping herbaceous perennial stems growing to 0.5–2 metres in length, usually found at ground level, with small, white and pink flowers.

It is common knowledge that any weed reduces crop yield by competing for the available minerals, space, water and light. It is also well known that the weeds are much better competitors for resources than any crop plant. However, some weeds are known to reduce the competition by releasing chemicals that interfere with normal metabolic processes of the crop plants thus setting a disadvantage for them. These chemicals may also disturb the normal growth of plant roots so that their absorption is reduced. This phenomenon is observed in crop fields of barley where the plants growing around a weed plant have poor root development. Retardation of root growth means that there must be factors that interfere in cell division in root meristematic cells.

MATERIALS AND METHODS

The roots of *C. arvensis* were collected from the Botanical Garden of Shri Jai narain PG College, Lucknow. The collected material was washed in running water and then shade dried and powdered using a blender and stored in a dessicator. Fresh aqueous extract was prepared by mixing 0.1g of the powdered material in 100 ml distilled water and keeping it overnight to prepare the stock solution. Different concentrations of the extract, viz, 0.005, 0.01, 0.05 and 0.1% (w/v) were prepared in distilled water in similar manner.
Seeds of Barley (*Hordeum vulagre* var K10) were washed thoroughly in running water and then germinated on sterilized cotton wool. When the roots were 0.5 -1.0 cm long, they were treated with the various

concentrations of root extracts of *C. arvensis* for 12h. After treatment, the roots were plucked at 8:45 – 9:00 am and fixed in modified Carnoy's fluid (1 glacial acetic acid : 3 absolute alcohol) for 5 hours. The fixed root tips were then transferred to 70% ethanol and stored at $4^{O}C$ for cytological investigations. For slide preparation acetocarmine squash technique was used. Slides were observed for aberrations under microscope (Olympus CX21 FSI, Japan) fitted with digital camera interface.

Calculation of Active Mitotic index (%) and Total Abnormality percentage was done using the following formulae.

$$\text{Active Mitotic Index \% (AMI)} = \frac{\text{No. of Actively dividing Cells}}{\text{Total no. of cells observed}} \text{ X } 100$$

(Actively dividing cells are cells that are in Metaphase, Anaphase or Telophase)

$$\text{Total Abnormality \% (TAb)} = \frac{\text{No. of Cells showing chromosomal Abnormalities}}{\text{Total no. of Actively Dividing Cells}} \text{ X } 100$$

RESULTS AND DISCUSSION

During the present study, it was observed that the root extracts of *C. arvensis* were able to elicit cytoxic response in barley root meristematic cells which was at par with chemical mutagens. There was a clear cut dose based decrease in AMI in all treated root cells. There was also an increase in the chromosomal abnormalities with increase in the concentrations of the extract. There were many abnormalities observed in the cells which include unorientation in metaphase, bridges and laggards in anaphase as well as stickiness at all stages of division (Table 1). However the most peculiar of all abnormalities was presence of a high number of micronuclei in dividing cells (Table 2).

Table 1: Active Mitotic Index and Total Abnormality percentage

Treatment (%)	Total cells observed	Total Actively Dividing Cells	Total No. of cells with abnormalities	AMI ± SE (%)	TAb ± SE (%)
CONTROL	481	205	2	42.62 ± 0.38	0.75 ± 0.18
0.005	462	171	45	37.01 ± 0.34	26.34 ± 0.56
0.01	482	163	58	33.82 ± 0.65	35.56 ± 0.27
0.05	479	106	42	22.13 ± 0.54	40.00 ± 0.25
0.1	470	94	50	19.36 ± 0.32	55.14 ± 0.33

SE=standard error at ($p<0.05$)

The micronuclei formation was also accompanied by formation of nuclear buds or appendages originating from the outer membrane of nuclear envelop. It was also peculiar that mostly these micronuclei were formed more in the cells during interphase stage rather than mitotic stages. These may be a result of the selective entrapment of extra chromosomal amplified DNA by the nucleus and which can probably end in micronucleation during S-phase (Shimizu et.al.,1998). Micronuclei that are formed in actively dividing cells might be derived from metaphase fragmented chromosomes or anaphase laggards and bridges that are left outside daughter nuclei in telophase. Thus they may be considered as "a fair index of fragmentation of chromosomes" by cytotoxic agents (Sparrow & Singleton, 1953). A study of previous literature on micronuclei provides evidences that they may also be formed from extra-chromosomal elements called double minutes (DMs) which are common in human cancer cells (Eckhardt et.al., 1994). Other studies suggest that micronuclei may be formed due to non-incorporation of chromosome fragments (Fenech, 2007, Leme et.al, 2008) or entire chromosome (Fenech & Crott, 2002) into normal dividing chromosomes.

Nuclear budding is therefore a process which removes these micronuclei amplified DNA and is therefore a marker of gene amplification (Tanaka & Shimizu, 2000). Most nuclear buds, are formed from "interstitial or terminal acentric fragments, possibly representing nuclear membrane entrapment of DNA that has been left in cytoplasm after nuclear division or excess DNA that is being extruded from the nucleus" (Lindberg et. al., 2007). Other studies suggest that these nuclear buds arise as a result of the excessive production of nucleic acids and proteins, induced by the cytotoxicants (Hellgren & Morré, 1992).

Table 2: Presence of Micronuclei and Nuclear buds in cells under different treatments

Treatment (%)	Percentage of cells with nuclear buds and micronuclei ± SE
CONTROL	0.00
0.005	18.13 ± 0.21
0.01	36.24 ± 2.12
0.05	42.33 ± 1.22
0.1	34.42 ± 1.08

SE=standard error at ($p<0.05$)

CONCLUSION

It may be concluded that *Convulvulus arvensis* which is a common weed of barley crop fields has a definite allelopathic effect on root growth of barley. The weed is seen to reduce root growth of barley plants in its vicinity. It is definitely a better competitor for minerals and space but it also produces certain chemicals that interfere in the normal cell division of root meristem of barley. This reduces root growth and brings the barley plants to a disadvantageous condition. The roots of *C. arvensis* has certain chemicals which are released in the rhizosphere and they can enter root meristem of barley. Once inside they either act on the spindle of produce certain reactions that cause chromosome damage. Damaged chromosomes are not included in normal mitotic division and hence they form micronuclei. These micronuclei get entrapped in nuclear membrane and form nuclear buds. These buds and micronuclei were clearly seen during the study of barley root cells treated with *Convulvulus* root extracts. The study brings out a probable mechanism of achieving competitive advantage by the weed over the barley plant.

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