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CYTOLOGICAL EFFECTS OF TWO VARIETIES OF *LATHYRUS SATIVUS* L. EXTRACTS ON ONION ROOT TIP CELLS

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Abstract

Lathyrus sativus L. causes lathyrism both in animals and human due to the presence of the neurotoxin β-ODAP (β-N-Oxalyl-L-α, β-diaminopropionic acid). This study was performed to find out the toxic effect of grass pea on onion root tip cells. The root lengths of onion were reduced as the concentrations of different plant parts extracts of grass pea increased. The percentages of chromosomal abnormalities, NV and ICV of onion root tip cells showed an increasing trend with the increasing of different extracts concentrations. The mitotic index and chromosomal abnormalities in treated onion root tip cells were significantly different than that of control. The mitotic index and chromosomal abnormalities had a reverse relationship. Almost all the treated root tip cells of onion showed this mito-depressive effect. From this findings, it may concluded that β-N-Oxayl-L- α, β-diaminopropionic acid (Lathyrogens) in *Lathyrus sativus* may be responsible for mitotic depression and chromosomal abnormalities on the root tip cells of onion.

Key words: Cytological effect, grass pea, onion, β-ODAP, somatic cell.

Introduction

Lathyrus sativus L. (Grass pea) is pulse crops which have extensive economic significance, especially in developing countries including Bangladesh, India, Pakistan, Nepal and Ethiopia (Kumar et al. 2011). Grass pea is commonly grown for its grain and also used as fodder or green manure. The over consumption of this crop causes constant paralyze of lower limbs which is known as neurolathyrism because of the existence of neurotoxin called β-ODAP (β-N-Oxayl-L-α, β- diaminopropionoic acid) (Lambein 2009). When the seeds are grinded into flour, which is then used in baking or cooking, β-ODAP may not be removed. Unfortunately, successful therapies for detoxification frequently lead to decreased in nutritional consistency. Though it has much importance but it has little toxic effect. So, we should do extensive research to find out the toxic level of grass pea which will be very helpful for the common people. Bhalla et al. (1973) treated onion root tips with different concentrations of tobacco smoke condensate and observed a number of abnormalities in somatic chromosomes. Pandya (1975) worked on the effect of *Celosea argentea* extracts on root and shoot growth of bajra seedlings and observed that the aqueous extracts of fresh leaves, stem and root caused inhibition being more at higher concentrations. He also reported that the leaf and root extracts were more toxic than the stem extracts. Grass pea is chosen as it is easily available in south-west Bengal and whether they can be used as an alternative model for cytogenotoxic study. This plant has been chosen as a model for studying cytological effects of anti-cancerous drugs (Samanta et al. 2015). The present study deals the determination of cytological effects of two varieties of *Lathyrus sativus* L. extracts on onion root tip cells.

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Materials and Methods

Plant materials

The experiment was carried with moisture free seeds of the two varieties of *Lathyrus sativus* L. (BARI Khesari-2 and BARI Khesari-3).

Procedure for preparation of different types of extracts

Leaf extracts: The seeds of *Lathyrus sativus* L. were sown in pots, when the plants were about to bloom, 100 gm of matured leaves were plucked and made into pulp using pestle and mortar. Then the concentration (10%, 20% and 50%) of the extract was prepared by dissolving calculated quantity of extract in distilled water.

Seed extract: Dried seeds were made into powder using blender. 100 ml of distilled water was added to 10 gm of powder and stirred for one and half hour with the help of a magnetic stirrer. Then the solution was filtered. The filtrate was used as a stock solution and different strength of solution (5.0%, 12.5%, 25.0%, 50.0% and 100%) was made adding a known amount of distilled water.

Stem extract: First, collected stem were soaking in the 24 hours then make pest with mortar and pestle. 100 ml of distilled water was added to 10 gm of pest and stirred for one and half hour with the help of a magnetic stirrer. Then the solution was filtered. The filtrate was used as a stock solution and different strength (10%, 20% and 50%) of solution was made adding a known amount of distilled water.

Methods

Morphological observation: Morphological data were obtained mainly from living materials. The length of root considered for their differentiation in particularly the characters. Data were recorded from 10 plants of each type randomly from their habitat. The length of a single root from as selected plant was measured in centimeter (cm).

Mitotic index, nuclear phenotype and chromosomal abnormalities

Root tip cells of *Lathyrus sativus* L. were examined and the temporary slides were prepared by haematoxylin method following the Haque et al. (1976).

Mitotic Index values were expressed in percentage as follows; $\mathrm{MI} = \frac{\text{No. of dividing cells}}{\text{No. of total cells}} \times 100$,

Nuclear volume (NV) = $\frac{4}{3}\left[\pi r^3\right]$,

Interphase chromosome volume (ICV) = $\frac{\text{NV}}{\text{2n no. of chromosome in somatic cell}}$ were calculated as described by Fiskesjo (1993) and Nayar et al. (1971).

The abnormalities were bridge, fragment, laggard etc. Then the data was recorded in the notebook and transferred into percentage by following formula.

$$\% \text{ of Mitotic abnormalities} = \frac{\text{Total Number of abnormal cell}}{\text{Total number of cells studied}} \times 100.$$

Results

Root lengths of onion

In control situation, the root lengths of onion bulbs increased vary rapidly. The mean values of root length of onion in control medium after 24, 36, 48, 60 and 72 hours were 1.281, 1.97, 2.441, 3.041 and 3.81 cm, respectively. But under different concentrations of various types of extracts (seed, leaf & stem) of *Lathyrus,* sprouting and root elongation of onion were slow. The mean values of root length of onion in control medium after 24, 36, 48, 60 and 72 hours were 1.28, 1.97, 2.44, 3.04 and 3.81 cm, respectively. In different concentration of various types of seed extract, root length of onion after 24, 36, 48, 60 and 72 hours were 1.51, 1.43, 1.97, 2.57 and 3.19 cm, respectively at 5% of seed extract; mean value of root length were 0.87, 0.93, 1.03, 2.54 and 2.24 cm, respectively at 12% of seed extract; mean value of root length were 0.64, 0.70, 0.81, 1.11 and 1.13 cm, respectively at 25% of seed extract; mean value of root length were 0.26, 0.37, 0.37, 0.40 and 0.52 cm, respectively at 50% of seed extract and 0.14, 0.19, 0.19, 0.25, 0.24 cm, respectively at 100% of seed extract (Table 1).

Table 1. Root lengths (in cm) of onion treated with different concentrations of seed extract at different treatment hours along with control.

Concentration (%)	Root lengths (in cm) at different treatment hours (h)				
	24	36	48	60	72
Control	1.26±0.07	1.87±0.07	2.34±0.04	3.05±0.10	3.85±0.15
5	1.15± 0.02	1.43±0.12	1.97±0.06	2.57±0.20	3.191±0.19
12	0.87±0.02	0.93±0.16	1.03±0.06	2.54±0.07	2.24±0. 19
25	0.64±0.06	0.70±0.12	0.81±0.06	1.11±0.04	1.13±0.19
50	0.26±0.07	0.37±0.04	0.37±0.03	0.40±0.06	0.52±0.04
100	0.14±0.02	0.19±0.10	0.19±0.01	0.25±0.02	0.24±0.02

In different concentration of various types of leaf extract root length on onion in control medium after 24, 36, 48, 60 and 72 hours were 0.97, 1.10, 1.53, 1.83 and 2.60 cm, respectively at 10% of leaf extract; mean value of root length were 0.741, 0.79, 0.87, 1.15 and 1.57 cm, respectively at 20% of leaf extract; mean value of root length were 0.351, 0.431, 0.41, 0.57 and 0.631 cm, respectively at 50% of leaf extract (Table 2).

Table 2. Root lengths (in cm) of onion treated with different concentrations of leaf extract at different treatment hours along with control.

Concentration (%)	Root lengths (in cm) at different treatment hours (h)				
	24	36	48	60	72
Control	1.26±0.07	1 .87±0.07	2.34±0.04	3.05±0.10	3.85±0.15
10	0.97±0.06	1.11±0.10	1.53±0.06	1.83±0.07	2.6±0.18
20	0.74±0.039	0.71±0.08	0.87±0.06	1.15±0.06	1.57±0.06
50	0.35±0.028	0.43±0.03	0.41±0.03	0.57±0.03	0.63±0.04

In different concentration of various types of stem extract root length on onion in control medium after 24, 36, 48, 60 and 72 hours were 1.16, 1.41, 1.93, 2.13 and 2.83 cm, respectively at 10% of stem extract; mean value of root length were 0.99, 1.12, 1.52, 1.98 and 2.61 cm, respectively at 20% of stem extract; mean value were 0.72, 0.79, 0.94, 1.22 and 1.821 cm, respectively at 50% of stem extract. These results showed that the extract of was more effective for inhibition of the root elongation of onion (Table 3).

Table 3. Root lengths (in cm) of onion treated with different concentrations of stem extract at different treatment hours along with control.

Concentration (%)	Root lengths (in cm) at different treatment hours (h)				
	24	36	48	60	72
Control	1.26±0.07	1 .87±0.07	2.34±0.04	3.05±0.10	3.85±0.15
10	1.16±0.07	1.41±0.12	1.93±0.05	2.13±0.04	2.83±0.16
20	0.99±0.10	1.12±0.09	1.52±0.05	1.98±0.07	2.61±0.11
50	0.72±0.04	0.79±0.02	0.94±0.03	1.22±0.02	1.82±0.02

Nuclear phenotype (Nuclear volume and interphase chromosome volume)

Nuclear volume and interphase chromosome volume from the root tip cells of onion treated by the different extract of *Lathyrus sativus* L. were determined below:

Onion root tip treated with seed extract

Nuclear volume was determined from the root tip cells of onion treated with seed extract of *L. sativus* L. which are shown in Table 4. The nuclear volume of onion root tip cells treated with seed extracts of *Lathyrus sativus* L. were found to be 103.39±1.81 μ^3 at 12%, 135.54±0.61 μ^3 at 25%, 130.45±0.97 μ^3 at 50% and 181.05±0.32 μ^3 at 100%. The nuclear volume of the treated root tip cells of onion ranged from 103.0±0.99 μ^3 to

181.05±0.32 μ^3. In contrast, nuclear volume was 103.53±0.767 μ^3 in the control. Data on nuclear volume showed an increase of the nuclear volume with an increase the concentrations of seed extracts (Table 4).

Table 4. Mean values of nuclear volume (μ^3) and interphase chromosome volumes (μ^3) of onion root tip cells treated with different concentration of seed, leaf and stem extracts of *Lathyrus sativus* L. along with control.

Type of extract	Concentration (%)	Nuclear volume (μ^3) ($\bar{x}$±SE)	Interphase chromosome volume (μ^3) ($\bar{x}$±SE)
	Control	103.53±0.767	7.39±0.556
Seed	5	103.39±0.49	7.38±0.36
	12.5	118.83±1.001	8.45±0.080
	25	135.54±0.61	9.68±0.044
	50	130.45±0.97	8.32±0.07
	100	181.05±0.32	12.92±0.036
Leaf	10	97.71±0.65	6.98±0.188
	20	106.8±0.53	7.62±0.172
	50	113.80±0.345	8.18±0.101
Stem	10	78.97±0.35	6.19±0.38
	20	86.74±0.51	7.52±0.108
	50	105.30±0.573	7.91±0.927

Interphase chromosome volume (ICV) was determined from the root tip cells of onion treated with water extract of different parts of *L. sativus* L. The ICV of onion root tip cells treated with seed extracts of *Lathyrus sativus* L. were found to be 7.38±0.36 μ^3 at 5%, 8.45±0.80 μ^3 at 12%, 9.68±0.044 μ^3 at 25%, 9.32±0.07 μ^3 at 50% and 12.92±0.036 μ^3 at 100%. In the control, it was 7.39±0.556 μ^3. ICV data show an increase in the interphase chromosome volume with a rise in seed extract concentrations.

Onion root tip treated with leaf extract

Nuclear volume was determined from the root tip cells of onion treated with leaf extract of *L. sativus* L. The NV of onion root tip cells treated with leaf extracts of *Lathyrus sativus* L. were found to be 97.71±0.65 μ^3 at 10%, 106.80±0.53 μ^3 at 20%, 103.80±0.345 μ^3 at 50%. In control, it was 103.53±0.767 μ^3. Data on the unclear volume of onion root tip cells showed an increase in nuclear volume with an increase in leaf extract concentrations.

Interphase chromosome volume (ICV) of onion root tip cells treated with leaf extracts of *Lathyrus sativus* L. were found to be 6.98±1.88 μ^3 at 10%, 7.62±0.172 μ^3 at 20% and 8.18±0.101 μ^3 at 50% leaf extract but 7.39±0.556 μ^3 was in control. Data on ICV of onion root tip cells showed an increase in the interphase chromosome volume with an increase in leaf extract concentration.

Onion root tip treated with stem extract

The nuclear volume of onion root tip cells treated with stem extracts of *Lathyrus sativus* L. were found to be 78.97±0.35 μ^3 at 10%, 86.74±0.51 μ^3 at 20%, 105.30±0.573 μ^3 at 50% stem extract. The nuclear volume of the treated root tip cells of onion ranged from 86.74±0.51 μ^3 to 105.30±0.573 μ^3. In control, nuclear volume was 103.53±0.767 μ^3. Data on the nuclear volume of root tip onion cells showed an increase in nuclear volume, with an increase in stem extract concentrations.

The interphase chromosome volume of onion root tip cells treated with stem extracts of *Lathyrus sativus* L. were found to be at 6.19±0.38 μ^3 10%, 7.52±0.108 μ^3 at 20% and 7.91±0.927 μ^3 at 50% stem extract. In control, it was 103.53±0.767 μ^3. Data on interphase chromosome volume of root tip cells of onion showed an increase of the nuclear volume with an increase the concentrations of seed extracts like the above mention cytological parameters.

Mitotic index and chromosomal abnormalities

Mitotic index and abnormalities from the root tip cells of onion treated by the different extract of *Lathyrus sativus* L. were determined below:

Onion root tip treated with seed extract

The mitotic index of onion root tips cells treated with seed extract of *Lathyrus sativus* L. were found to be 15.36±0.35% at 5%, 15.23±0.59% at 12%, 10.16±0.86% at 25%, 9.8±0.38% at 50% and 5.4±0.22% at 100%. Percentage of chromosomal abnormalities of root tip cells treated with seed extract of *Lathyrus sativus* L. were 1.00±0.333% at 5%, 1.33±0.272% at 12%, 1.33±0.272% at 25%, 1.96±0.89% at 50%, and 2.33±0.272% at 100%, which are shown in Table 5.

Onion root tip treated with leaf extract

The mitotic index of onion root tips cells treated with leaf extract of *Lathyrus sativus* L. were found to be 12.36±0.463% at 10%, 11.97±0.488% at 20% and 9.157±0.438% at 50%. Percentage of abnormalities of root tip cells treated with leaf extract of *Lathyrus sativus* L. were1.96±0.0894% at 20%, 2.12±0.113% at 50%, which are shown in Table 5.

Onion root tip treated with stem extract

The mitotic index of onion root tips cells treated with stem extract of *Lathyrus sativus* L. were found to be 9.071±0.927% at 10%, 7.91±0.272% at 20% and 6.25±0.358% at 50%. Percentage of abnormalities of root tip cells treated with leaf extract of *Lathyrus sativus* L. *were* 1.33±0.272% at 20%, 1.66±0.293% at 50%, which are shown in Table 5.

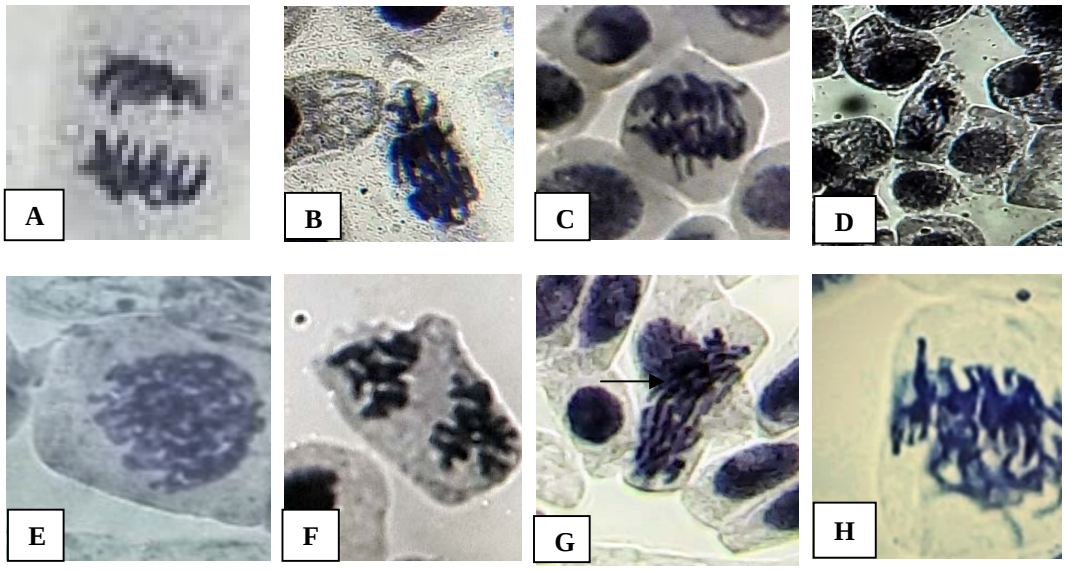

Fig. 1(A-H): Different chromosomal abnormalities by different concentrations along with control of, seed, leaf and stem extracts in onion root tip cells. A = Normal anaphase in control, B = Vagrant and bridge chromosome in late anaphase, C = laggard and bridge chromosome in early anaphase, D = Sticky single bridge chromosome at anaphase, E = Nuclear budding at interphase, F = Disoriented anaphase G = Multiple bridge in anaphase and H = Ring chromosome at metaphase.

Table 5. Mean values of mitotic index and abnormalities volumes of onion root tip cells treated with different concentration of seed, leaf and stem extracts of *Lathyrus sativus* L. along with control.

Type of extract	Concentration (%)	Mitotic index (%) ($\bar{x}$±SE)	Abnormalities (%) ($\bar{x}$±SE)
	Control	17.76±.332	0
Seed	5	15.36±.35	1.00±.333
	12.5	15.23±.59	1.33±.272
	25	10.16±.86	1.33±.272
	50	9.8±.38	1.96±.089
	100	5.4±.22	2.33±.272
Leaf	10	12.36±.463	0
	20	11.97±.488	1.96±.0894
	50	9.16±.438	2.12±.113
Stem	10	9.07±.489	1.66±.293
	20	7.91±.9217	1.33±.272
	50	6.25±.358	2.12±.113

Discussion

Root lengths of onion treated with different concentration of various types of extract of *L. sativus* L. along with the control were recorded. Root elongation rates were found decrease with the increases of the concentrations of various extracts. This study showed that the extract of seed was more effective for inhibition of the root elongation of onion. Debnath and Mukharji (1982) reported decreased root growth of rice with the increase of the concentrations. Different concentrations of various extracts of grass pea were used on onion root tip cell due to the study of nuclear volume and interphase chromosome volume. This study showed that the interphase chromosome volume was raising with the attack rates of the concentration. The raising rates were not established significant for different extract but for various concentrations the rates were significant.

The cytological observation from onion root tip cells revealed that the various extract of *Lathyrus sativus* L. have a significant depressive effect. It was also observed that percentage of dividing cells decreased with the increasing of the different concentration. Jain and Sarbhoy (1987) observed the impact of chlorinated hydrocarbon in the *Lens* and *Pisum* and they found a significant fall in mitotic index. Sudharsan and Reddy (1971) also made such kind of observation. They recorded that several chemicals are found to have cytological defects in different plant extracts. They named this category of chemicals lathyrogens in their research, which are possibly present in the form of β-N-Oxayl-L-α, β-diaminopropionic acid and homoarginine. Quder et al. (1986) reported that *Lathyrus sativus* L. produces a toxic compound called β-N-Oxayl-L-α, β-diaminopropionic acid, which contributes to neurolathyrism through excessive human consumption. Shehab (1980) and Elena (2012) have investigated the mitotic effects of *Teucricum pilosum* extract and herbicide, gradually in onion root tip cells that had antimitotic influence. Different phases have been observed with mitotic effects for the vaccinated, untreated *Allium cepa* root tip cells. In the highest mitotic index, untreated content was found. But the mitotic index was shown to decrease when the root tips were treated with extract from different parts of *Lathyrus sativus*. The lowest mitotic index was 5.40%, measured at 100% seed extract concentrations of *Lathyrus sativus*. The mitotic index and chromosome abnormalities had a reverse relationship. In this study, the cytological effect of *Lathyrus sativus* on *Allium cepa* roots was determined based on chromosomal aberrations and mitotic index. It was so cleared that each steeping concentration of various extracts was significantly effective to decrease the percentage of dividing cells. Cytological results revealed that the various extracts of *Lathyrus sativus* L. had a cytological effect on the chromosomes of onion root tip cells. Most of the depression cells found in the present study were precocious separation of chromatid-bridge and vagrant chromosomes. The percentages of aberrations were found to increase with the increase of the steeping concentration of different extracts. Thus, it is sure that the extracts of *Lathyrus sativus* L. are capable of causing stickiness and may form a potential source to bring a new translocation and consequent change in nuclear set up (Sudharsan and Reddy 1971).

In this present investigation lagging chromosomes, ring chromosome, sticky chromosome and chromatid bridges were also found as chromosomal abnormalities in mitosis. When the increase of the concentration of

different extracts the percentages of chromosomal abnormalities were found to increase. Data on abnormalities of root tip cells of onion showed an increase of the abnormalities with an increase the concentrations of stem extracts. Results found in this investigation showed that the various extract was more effective to increase the abnormalities and decrease the mitotic index. The above mentioned data showed a graduals mito-depressive effect on the root tips of onion. Therefore, the present investigation stated that mitotic inhibition and chromosomal abnormalities were occurred in the root tip cells of onion may be due to the toxicity of β-N-Oxayl-L-α, β-diaminopropionic acid (Lathyrogens).

Conclusion

The purpose of this investigation was to determine the toxic impact of grass pea on onion root tip cells. It was observed that when the concentrations of various extracts increased the root length of onion decreased and the nuclear and interphase chromosome volume was increased. The percentages of chromosomal abnormalities were increased with the increase of the steeping concentration of the examined species. It was observed that the mitotic index and chromosomal abnormalities had a reverse relationship. The findings stated that mitotic depression and chromosomal abnormalities were occurred in the root tip cells of onion due to the presence of an endogenous neurotoxic non-protein amino acid (β-ODAP) in *Lathyrus sativus*.

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Conflict of interest: The authors declare that there is no competing interest.

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