standard (Gymnemagenin, Sigma-Aldrich) and 10% aqueous leaf extract of different accessions (Gs_1-Gs_{20}) of *G. sylvestre*. Quantification of gymnemic acid in leaves of mentioned accessions was carried out by high-performance liquid chromatography method.[4] The aqueous leaf extract was prepared by heating (70°C) 10 g decoction in 100 ml distilled water for 6 h followed by filtration process. Volume of filtrate was made up to 100 ml by adding distilled water. For treatment, presoaked seeds were divided into 26 lots of 50 seeds each. Five lots were treated with different dilutions of gymnemic acid standard, twenty lots with aqueous leaf extracts of different accessions, and remaining lot was maintained in distilled water as a control. The duration of treatment is about 6 h. Treated seeds were washed with distilled water thoroughly and kept in Petri-dishes on moist blotting paper for germination. Radicle from germinated seed was fixed in Carnoy's fluid (3 alcohol: 1 acetic acid) between 1:20 and 1:25 pm and preserved in 90% alcohol. Cytological preparations were made by the following hematoxylin squash procedure. Random scoring was made from the ten different microscopic fields in ten root tips for determination of the frequency of mitotic index and chromosomal aberrations. Pooled data were statically analyzed using on STATISTICA (version 6.0, Statsoft Inc., Oklahoma, USA) software.

RESULTS AND DISCUSSION

Exposure of *B. campestris* seeds to different dilutions of standard gymnemic acid and crude leaf extracts of *G. sylvestre* accessions promotes decrease in mitotic index (MI) and various types of chromosomal anomalies, namely, condensed prophase (CP), clumped metaphase (CM), metaphase cleft (MC), fragmentation (FR), anaphase with persistent nucleolus (AP), disturbed anaphasic polarity (DA), laggard (LA), bridge (BR), binucleated cell (BI), etc. For prior case, decrease in mitotic indices was found dose depended [Table 1]. Range of chromosomal aberrations noted higher for the later case [Table 2]. Surprisingly, chromosomal FR showed a positive correlation with gymnemic acid percentage present in treatment solution [Figures 1-3]. Reductions in mitotic index have been attributed to inhibition of DNA synthesis at S-phase and formation of irregular and disorganized phragmoplast.[5] Stickiness/clumping have been resultant of entanglement of interchromosomal chromatin fibers or defective functioning of specific type of nonhistone proteins involved in chromosomal separation and segregation.[6] The persistence of nucleolus can be assumed, the extension of heterochromatin activity up to the stage of division or due to disturbance of equilibrium between nuclei and the nucleolus.[7] Lagging chromosome indicates a complete failure of spindle apparatus or formation of acentric chromosome, during exchange or delayed terminalization.[8] Treatments also induced clastogenic chromosomal aberrations in *B. campestris* represented by chromosomal FR and bridge at ana-telophase. Formation of ring chromosome and chromosomal bridges may be the result of reunion

Table 1: Mitotic index and different chromosomal anomalies in root tip of *Brassica campestris* treated with different dilutions of standard gymnemic acid (gymnemagenin) (average±standard error)

Treatment	MI	Percentage abnormality	Percentage of different type of anomalies			
			CP	CM	FR	Others
Control	8.44±0.22	0.00	0.00	0.00	0.00	0.00
0.01%	7.22±0.40	9.64±1.16	14.31	16.17	6.14	63.84
0.02%	6.43±0.45	11.87±1.73	13.88	14.01	7.71	64.40
0.04%	3.57±0.39	14.22±1.63	15.74	15.04	13.84	55.38
0.06%	2.95±0.22	16.11±1.97	14.61	14.17	17.48	53.74
0.08%	1.95±0.34	21.70±2.73	16.51	15.30	23.49	44.70

MI: Mitotic index; CP: Condensed prophase; CM: Clumped metaphase; FR: Fragmentation

Table 2: Percentage of different chromosomal anomalies in *Brassica campestris* is root tip treated with leaf extracts of different accessions of *Gymnema sylvestre* (average±standard error)

Accessions	Percentage of gymnemic acid in leaf extract	MI	Percentage abnormality	Percentage of different type of anomalies									
				CP	CM	MC	FR	AP	DA	LA	BR	BI	Others
Gs-1	0.148	6.70±0.66	26.60±2.36	14.40	13.01	1.63	11.38	4.07	0.81	0.81	0.58	16.00	37.31
Gs-2	0.126	7.16±0.64	30.50±3.04	14.50	20.00	1.45	13.30	5.60	2.00	15.30	0.89	13.00	13.96
Gs-3	0.121	6.93±0.63	27.70±2.37	16.20	15.20	2.40	13.80	3.10	4.10	11.50	1.86	14.60	17.24
Gs-4	0.142	6.71±0.51	36.80±3.54	8.83	4.87	2.61	15.60	1.77	2.65	11.06	7.60	4.80	40.21
Gs-5	0.053	5.45±0.28	36.10±2.28	9.40	11.40	3.20	6.40	3.20	4.60	14.00	5.30	18.20	24.30
Gs-6	0.008	5.65±0.44	28.20±2.52	15.00	13.20	14.30	4.40	4.60	3.00	10.00	2.20	14.60	18.70
Gs-7	0.029	5.95±0.30	30.60±3.88	20.22	10.11	8.99	7.80	2.23	7.00	3.37	3.37	12.36	24.55
Gs-8	0.030	6.13±0.60	36.60±3.37	14.50	11.60	11.40	4.80	4.60	5.70	5.40	2.60	16.80	22.60
Gs-9	0.360	7.30±0.48	36.80±3.54	11.20	8.20	6.80	18.80	11.30	5.60	8.00	3.00	18.20	8.90
Gs-10	0.118	6.63±0.64	40.20±1.24	9.40	4.60	4.40	10.40	1.80	8.60	3.60	7.60	4.20	45.40
Gs-11	0.072	5.98±0.59	36.90±2.11	12.40	11.20	8.40	4.40	3.20	11.00	4.80	4.20	11.60	28.80
Gs-12	0.154	5.36±0.34	34.30±2.77	13.40	7.80	10.40	8.20	4.40	12.60	7.20	6.80	9.80	19.40
Gs-13	0.071	5.70±0.36	32.70±3.58	7.80	10.40	11.20	11.80	5.40	9.80	8.20	7.40	13.40	15.60
Gs-14	0.049	6.23±0.32	39.10±3.58	15.20	10.20	9.40	7.80	3.20	5.20	11.20	2.80	14.60	20.40
Gs-15	0.047	5.71±0.57	37.40±3.58	13.20	10.20	8.00	6.80	5.20	7.00	3.40	4.00	12.36	29.84
Gs-16	0.051	6.79±0.53	32.20±5.44	8.10	10.40	12.60	14.20	6.20	3.00	11.00	6.80	9.60	18.10
Gs-17	0.431	5.72±0.34	37.30±2.34	9.20	8.60	11.20	22.40	6.60	4.60	3.50	3.70	10.00	20.20
Gs-18	0.318	5.93±0.63	31.60±3.12	10.20	8.20	10.20	7.80	8.60	5.20	4.70	4.80	11.60	28.70
Gs-19	0.039	7.26±0.44	37.30±2.40	18.20	7.40	8.60	7.80	2.23	7.00	3.37	3.37	12.36	29.87
Gs-20	0.067	6.37±0.59	38.20±3.03	11.40	7.40	12.40	8.40	8.60	7.20	8.20	2.60	11.20	22.60

MI: Mitotic index; CP: Condensed prophase; CM: Clumped metaphase; MC: Metaphase cleft; FR: Fragmentation; AP: Anaphase with persistent nucleolous; LA: Laggard, BR: Bridge; BI: Bi-nucleated cell; DA: Disturbed anaphasic polarity

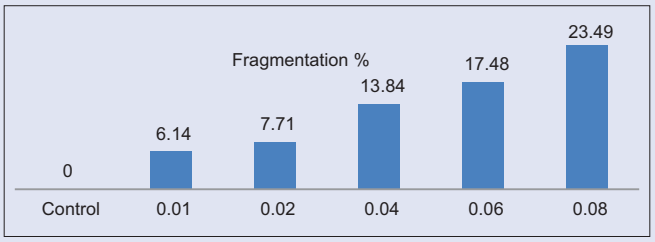

Figure 1: Relative association of chromosomal fragmentation and different dilutions of standard gymnemic acid (gymnemagenin)

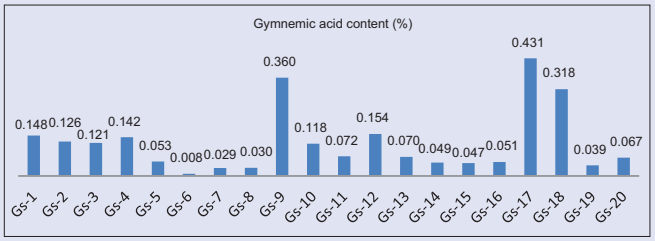

Figure 2: Percentage of gymnemic acid in different accessions of *Gymnema sylvestre*

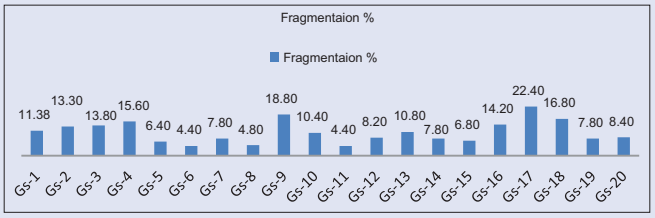

Figure 3: Percentage of chromosomal fragmentation after treatment with leaf extracts of different accessions of *Gymnema sylvestre*

of broken chromosome ends.[8] The formation of binucleated and multinucleated cells in treated material may be due to inhibition of cytokinesis process and other metabolic disorders.[9] Binucleate cells are otherwise interpreted as a consequence of inhibited cell cycle, in which chromosome DNA is replicated but not distributed in usual way.[9] Higher range of mitotic anomalies in second treatment probably due to the presence of various types of cytotoxic metabolites in the leaf extract.

CONCLUSION

Above results suggested that gymnemic acid and its allied biomolecules possibly responsible for chromosomal FR. Direct correlation between the percentage of gymnemic acid in treatment solution and percentage of chromosomal FR also validate above result. This astonishing result of aforesaid experiment may be utilized for the development of novel methodology or marker system for identification of high active principle yielding accessions of *G. sylvestre*.

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Conflicts of interest

There are no conflicts of interest.

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