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RESEARCH ARTICLE

Study of genotype dependent response against treatment of As+Se in barley

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SUMMARY

The present paper deals with the genotoxicity assessment of five partially tolerant and five non-tolerant accessions of *Hordeum vulgare* (barley) for the heavy metals arsenic and selenium in combination. The genotype dependent response was studied using root meristem cytology. These two heavy metals in combination influenced mitotic division inducing various kinds of anomalies.

Key Words : *Hordeum vulgare*, As+Se, Genotoxicity

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Heavy metals are natural components of the earth's crust. They cannot be degraded or destroyed. These metals have been used by humans for thousands of years. Although, several adverse health effects of heavy metals have been known for a long time exposure to heavy metals continues and is even increasing in some parts of the world particular in less developed countries though emissions (Hossn *et al.*, 2001). They enter our bodies via food, drinking water and air. The main threats to human health from heavy metals are associated with exposure to these heavy metals. Soil contamination with heavy metals is a worldwide problem now a days leading to agricultural losses and hazardous health problems, as these metals enter the food chain. Several adverse health effects of heavy metals have been known as long time exposure to heavy metals continues and is even increasing in most parts of the world, particularly in lesser developed countries. Almost all of them are dangerous to human health and to the plant growth. The present paper discusses the toxic impacts of As and Se in combination on cytology of some accessions of *Hordeum vulgare*, an economically important crop.

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MATERIAL AND METHODS

Sodium arsenate (Na_3AsO_4) and selenium dioxide (SeO_2) were used as sources of arsenic and selenium, respectively. Treatment with 10^{-3} M of As+Se solution was given to partially tolerant and non - tolerant accessions of *Hordeum vulgare* (Table A). For this root tips samples were collected from seeds germinated in control (Hoagland's solution) and treatment solution (10^{-3}M As+Se prepared in Hoagland's solution).

These root tips were fixed in acetic alcohol (3 parts absolute ethanol + 1 part glacial acetic acid) for at least 48 hours. Fixed root tip samples were stored in 70 per cent ethanol in refrigerator. After fixation, the root tips were boiled in 1N HCl and thereafter, smeared and squashed in 1 per cent aceto-carmine. For estimating the toxic effects of As+Se on the cytology of root meristem cells, the following parameters were

Table A : List of accessions of *Hordeum vulgare*.

Partially-tolerant			Non-tolerant		
Lab code	Accession	Source	Lab code	Accession	Source
B-48	K-195	DWR	B-110	IC 118653	NBPGR
B-68	K-387	DWR	B-114	IC 118663	NBPGR
B-69	K-470	DWR	B-124	IC 118696	NBPGR
B-169	K-169	DAC	B-126	IC 118698	NBPGR
B-177	A2-ADO	DAC	B-141	IC 138945	NBPGR

DWR=Directorate of Wheat Research, Karnal (Haryana), India; NBPGR= National Bureau of Plant Genetic Resources, New Delhi, India; DAC= Department of Agriculture, Canada.

analyzed:

- Mitotic index (MI),
- Active mitotic index (AMI),
- Type and frequency of mitotic anomalies, and
- Total mitotic anomaly (TMA).

These parameters were calculated using the below listed formulae :

$$\text{MI} = \frac{\text{Number of cells destined to divide}}{\text{Total number of cells}} \times 100$$

$$\text{AMI} = \frac{\text{Number of actively dividing cells (cells at metaphase and anaphase)}}{\text{Total number of cells}} \times 100$$

$$\text{Mitotic anomalies} = \frac{\text{Number of cells showing anomalies}}{\text{Total number of cells in active division}} \times 100$$

$$\text{TMA} = \frac{\text{Total number of cells showing anomalies}}{\text{Total number of cells in active division}} \times 100$$

A parameter called response co-efficient (RC) was calculated, using the following formula for estimating the toxicity imposed by (As+Se) treatments.

$$\text{Response co-efficient (RC)} = \frac{VT - VC}{VC}$$

(VT = value of the treated set; VC = value of the control set).

RESULTS AND DISCUSSION

Data for RCs related to the effect of As+Se on the cytological parameters are presented in Tables 1 and 2. Graphical representations of RCs for these parameters are depicted in Fig. 9-12 and photomicrographs showing mitotic anomalies induced by the treatment are presented in Fig.1-8.

Analysis of the data related to RCs of the studied cytological parameters revealed that AMIs decreased in majority of the accessions (Fig. 9 and 10). Different types of mitotic anomalies observed during the present study included C-metaphase, late movement of chromosomes for metaphase alignment, clumped metaphase, fragmentation of chromosomes during metaphase, lagging of chromosomes, formation of chromatin bridge during anaphase, chromosome erosion during anaphase, and grouping of chromosomes during anaphase.

Heavy metals, arsenic and selenium are among the significant environmental pollutants and therefore are capable of antagonistically influencing the course of mitosis, as a result, seedling growth in plants. The cytological analyses related to somatic division in root meristem cells, gave information about the effects of As+Se on the rate of numerical increase of cells and it also provided details about the cytogenetic property of these heavy metals. During the present study, As+Se induced inhibition of AMI. This could result due to impaired functioning of genes or gene products regulating the cell cycle and its control. However, since only AMI was inhibited, therefore, it appears that genes/proteins inducing mitosis are more susceptible to this heavy metal combination than those inducing start of the cell cycle. The cytological and genetic effects of some of the heavy metals in animal and plant cells have been studied by various workers (Utsunomiya *et al*., 2002; EI-Ghamery *et al*., 2003; Kumar and Tripathi, 2003; Mansour and Kamel, 2005; Zou *et al*., 2006; Kumar and Tripathi, 2008; Chidambaram *et al*., 2009; Mumthas *et al.*, 2010; Pandey and Upadhaya, 2010; Tripathi and Kumar, 2010; Kumari *et al*., 2011; Srivastava and Jain, 2011; Choudhary *et al*., 2012; Eleftherios *et al*., 2012 etc.).

Treatment of As+Se in barley induced various mitotic anomalies related to chromatin agglutination, chromosome condensation, chromosome erosion and spindle anomalies. Chromatin agglutination may result in the formation of restitution nucleus, lagging of chromosomes and formation of chromatin bridges. Out of these the first could be probably due to the 'interchromosomal' stickiness, while the later two could be because of 'intrachromosomal' stickiness. As+Se induced significantly higher amount of chromatin agglutination. Similar observations were reported by Choudhary *et al*. (2012). C-metaphase was another common mitotic anomaly induced by As+Se either due to inhibition of spindle organization in some cells or due to disorganization of spindle after its organization (Sybenga, 1992). Various other workers also reported about the induction of C-metaphase during mitosis by heavy metals and other water pollutants (EI-Ghamery *et al*., 2003; Mansour and Kamel, 2005; Pandey and Upadhaya, 2010; Srivastava and Jain, 2011 etc). Chromosome fragmentation was presently observed in some of the treated sets. Several earlier workers also reported induction of chromosome fragmentation by heavy metals

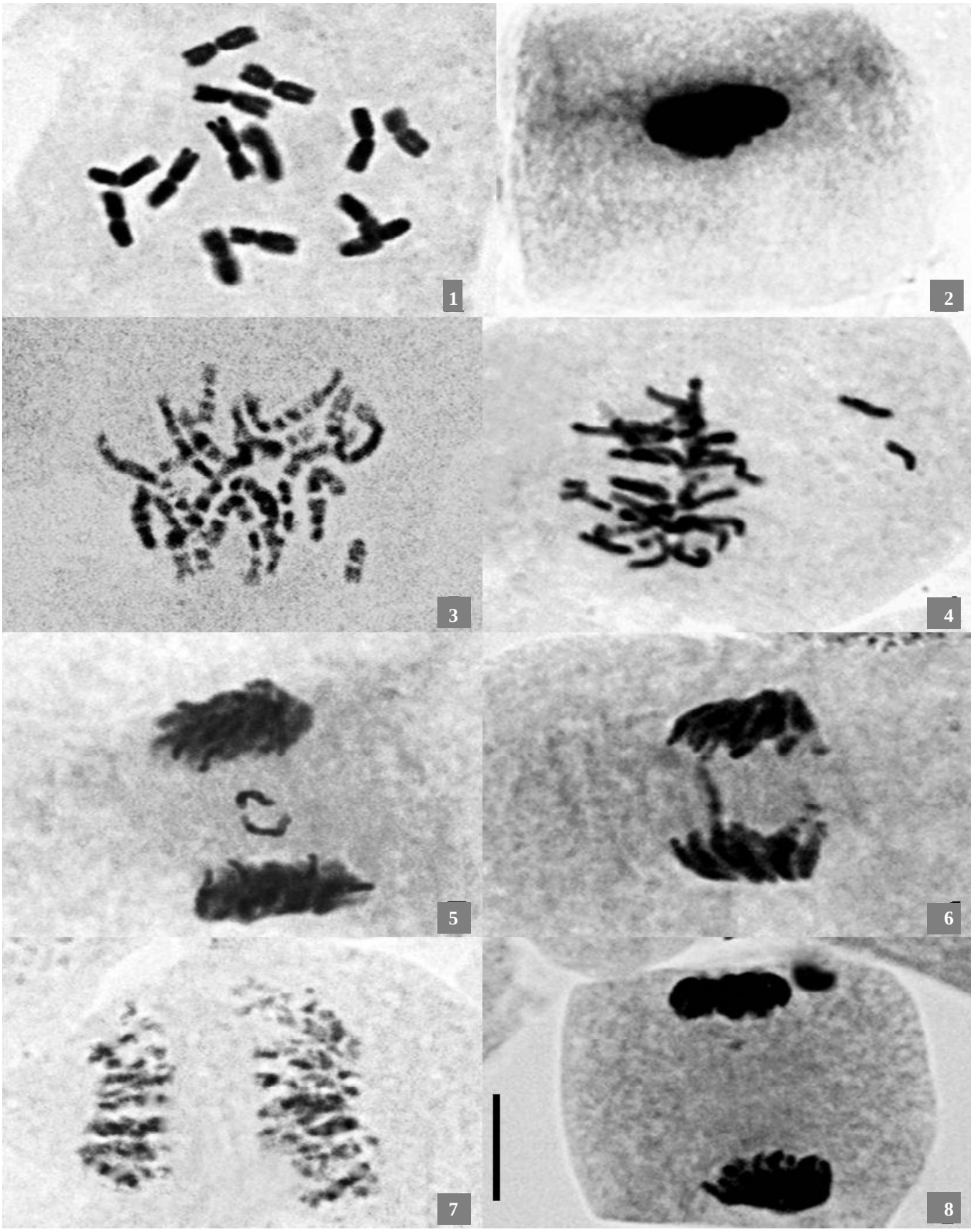
(Kumar and Tripathi, 2003; Mumthas *et al.*, 2010; Pandey and Upadhaya, 2010; Choudhary *et al*., 2012 etc.). The occurrence of fragments at metaphase may be attributed to the failure of broken chromosome to recombine.

Chromosome erosion was first reported by (Levan, 1938) and Levan and Tjio, (1948). Several other workers like Banerjee and Sharma (1983), Grover and Dhingra (1987), MacFarlane (1951), Roy *et al*. (1989), Somashekhar and Arekal (1983), etc. also reported its induction by various water pollutants. It was established by Pool *et al*. (1989) that several physical factors, carcinogens and mutagens in the environment may induce amplification of DNA sequences which can be located on the chromosome on non-staining regions. The presence of retarded movement of chromosome for metaphase alignment and lagging of chromosome suggested that As+Se could induce functional anomalies in spindle apparatus. EI-Ghamery *et al*. (2003), Zou *et al*. (2006), Kumar and Tripathi (2008), Chidambaram *et al*. (2009), Mumthas *et al.* (2010), Srivastava and Jain (2011), Choudhary *et al*. (2012) etc. also reported presence of laggards in mitosis treated with various heavy

Table 2: Cytological response of (As+Se) treatment on barley

Partially tolerant						Non-tolerant				
Acc.	Tre./ Para.		Mean ± SE	Range	RC	Acc.	Tre./ Para		Mean ±SE	Range	RC
B-84	Co	MI	100.00 ± 0.00	100.00-100.00		B-110	Co	MI	100.00±0.00	100.00-100.00	
		AMI	10.42±0.60	9.09-12.78				AMI	9.92±0.43	8.52-10.99	
		TMA	0.00±0.00	0.00-0.00				TMA	0.00±0.00	0.00-0.00	
	Tr	MI	99.42±0.15	99.06-100.00	-0.01		Tr	MI	99.38±0.08	99.05-99.51	-0.01
		AMI	4.79±0.36	3.89-.91	-0.54			AMI	5.43±0.47	4.29-7.19	-045
		TMA	7.78±2.98	0.00-16.67	-			TMA	10.98±3.18	0.00-22.22	-
B-68	Co	MI	100.00±0.00	100.00-100.00		B-114	Co	MI	99.92±0.08	99.61-100.00	
		AMI	8.43±1.17	4.66-12.50				AMI	6.33±0.96	2.63-8.63	
		TMA	0.00±0.00	0.00-0.00				TMA	0.00±0.00	0.00-0.00	
	Tr	MI	99.49±0.02	99.41-99.55	-0.01		Tr	MI	99.44±0.07	99.12-99.58	0.00
		AMI	5.25±0.46	4.02-7.06	-0.38			AMI	4.56±0.45	3.51-6.25	-0.28
		TMA	8.82±2.06	0.00-12.50	-			TMA	14.05±1.40	10.00-18.18	-
B-69	Co	MI	100.00±0.00	100.00-100.00		B-124	Co	MI	100.00±0.00	100.00-100.00	
		AMI	8.11±0.58	6.33-10.34				AMI	7.01±0.32	5.63-7.58	
		TMA	3.83±2.26	0.00-1250				TMA	0.00±0.00	0.00-0.00	
	Tr	MI	100.00±0.00	100.00-100.00	0.00		Tr	MI	99.40±0.02	99.33-99.46	-0.01
		AMI	6.40±1.03	4.69-10.96	-0.21			AMI	5.84±0.45	4.27-7.10	-0.17
		TMA	12.58±2.23	9.09-22.22	2.28			TMA	17.27±3.01	10.00-28.57	-
B-169	Co	MI	99.85±0.09	99.62-100.00		B-126	Co	MI	100.00±0.00	100.00-100.00	
		AMI	4.79±0.30	4.27-6.10				AMI	6.12±0.40	5.31-7.78	
		TMA	0.00±0.00	0.00-0.00				TMA	0.00±0.00	0.00-0.00	
	Tr	MI	100.00±0.00	100.00-100.00	0.00		Tr	MI	100.00±0.00	100.00-100.00	0.00
		AMI	5.03±0.48	3.94-6.84	0.05			AMI	5034±0.57	3.08-6.86	-0.13
		TMA	8.49±3.31	0.00-18.18	-			TMA	11.19±2.87	0.00-18.18	-
B-177	Co	MI	99.88±0.12	99.38-100.00		B-141	Co	MI	99.84±0.09	99.61-100.00	
		AMI	5.73±0.60	3.18-7.06				AMI	6.03±0.27	5.20-6.69	
		TMA	0.00±0.00	0.00-0.00				TMA	0.00±0.00	0.00-0.00	
	Tr	MI	99.48±0.14	99.12-100.00	0.00		Tr	MI	99.73±0.11	99.52-100.00	0.00
		AMI	4.18±0.24	3.50-5.02	-0.27			AMI	4.04±0.43	2.79-5.46	-0.33
		TMA	18.77±5.87	0.00-37.50	-			TMA	17.94±3.53	11.11-33.33	-

Co=Control; Tr=Treatment; MI= Mitotic index; AMI= Active mitotic index and TMA= Total mitotic anomaly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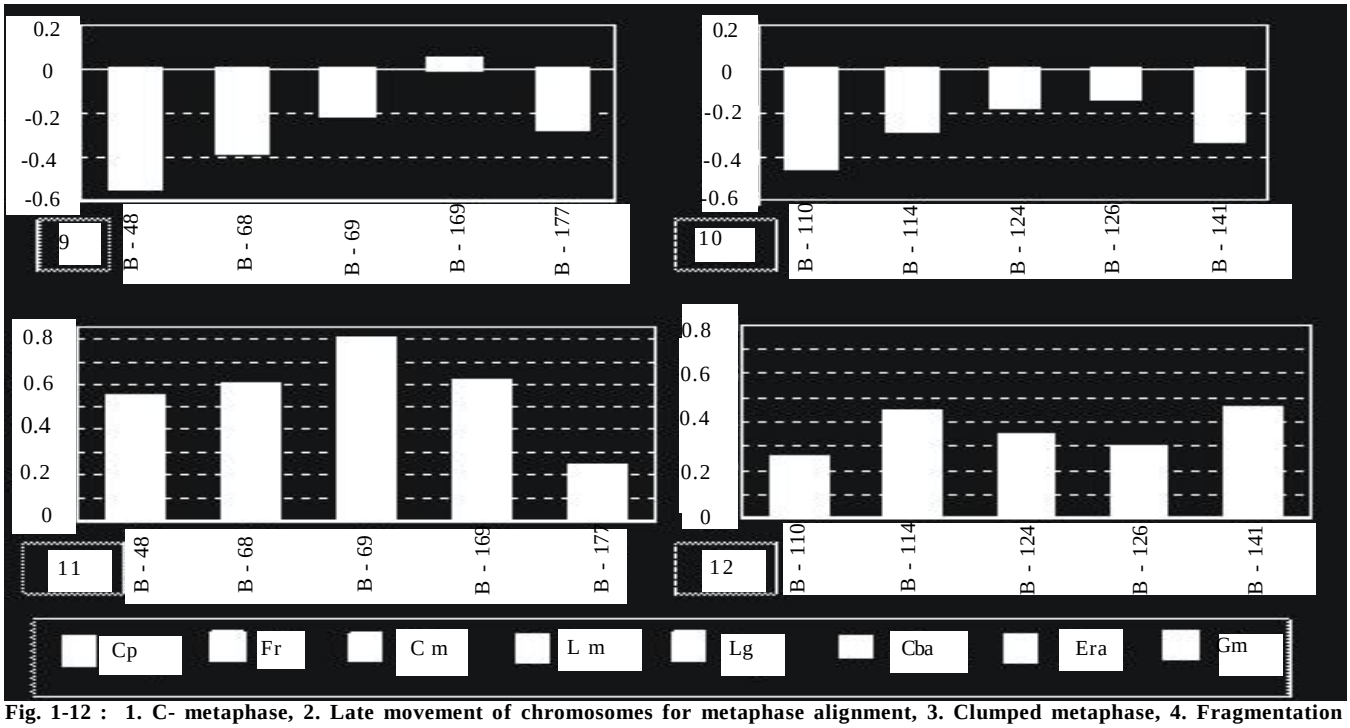

Fig. 1-12 : 1. C- metaphase, 2. Late movement of chromosomes for metaphase alignment, 3. Clumped metaphase, 4. Fragmentation of chromosomes during metaphase, 5. Lagging of chromosomes, 6. Formation of chromatin bridge during anaphase, 7. Chromosome erosion duringanaphase, 8. Grouping of chromosomes during anaphase, 9. Graphical presentation of RCs for AMIs of partially tolerant accessions, 10. Graphical presentation of RCs for AMIs of non-tolerant accessions, 11. Graphical presentation of frequency distribution (%) of the types of mitotic anomalies in partially tolerant accessions, and 12. Graphical presentation of frequency distribution (%) of the types of mitotic anomalies in non-tolerant accessions. (Bar=10µm for Figs. 1-8).

Table 2 : Frequency (%) distribution of As+Se induced mitotic anomalies in barley

Acc.	Tre.	Cp	Fr	Cm	Lm	Lg	Cba	Era	Gm
				Partially tolerant					
B-48	Co	-	-	-	-	-	-	-	-
	Tr.	-	0.21	0.16	0.09	-	-	-	0.09
B-68	Co	-	-	-	-	-	-	-	-
	Tr.	0.18	-	-	0.25	0.18	-	-	-
B-69	Co	-	-	-	-	-	-	-	-
	Tr.	-	-	0.27	-	-	0.36	-	0.18
B-169	Co	-	-	-	-	-	-	-	-
	Tr.	0.08	-	-	0.18	-	0.36	-	-
B-177	Co	-	-	-	-	-	-	-	-
	Tr.	-	-	0.16	-	-	0.09	-	-
				Non-tolerant					
B-124	Co	-	-	-	-	-	-	-	-
	Tr.	-	-	-	-	-	0.15	-	0.20
B-126	Co	-	-	-	-	-	-	-	-
	Tr.	0.18	-	-	0.12	-	-	-	-
B-141	Co	-	-	-	-	-	-	-	-
	Tr.	-	-	0.09	-	0.12	-	0.16	0.09
B-110	Co	-	-	-	-	-	-	-	-
	Tr.	0.16	-	-	0.10	-	-	-	-
B-114	Co	-	-	-	-	-	-	-	-
	Tr.	-	-	0.08	-	0.08	-	0.29	-

Cp= Clumping during metaphase; Fr= Fragmentation of chromosome during metaphase; Cm= C- metaphase; Lm= Late movement during metaphase; Lg= Lagging at anaphase; Cba= Chromatin bridge during anaphase; Era= Chromosome erosion during anaphase; Gm= Grouping at metaphase

metals. The formation of bridges could be attributed to chromosome stickiness and to chromosome breakage and reunion (Mumthas *et al.*, 2010)

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