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RESEARCH ARTICLE

CYTO-SENSITIVITY OF SOME LOCALLY GROWN PIGEON PEA [*Cajanus cajan* (L.) Millsp] TO AMIPROPHOS METHYL TREATMENT AS A GUIDE FOR IMPROVEMENT

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ABSTRACT

Effects of mutagens on the chromosomes of crop plants are a good indicator to deciphering the extent to which they can be utilized for crop improvement. The aim of this research work was to evaluate the cyto-sensitivity of two landraces of pigeon pea [*Cajanus cajan* (L.) Millsp] to amiprophos methyl (APM). The seeds from the two varieties were soaked in different concentrations of APM (4ppm, 6ppm and 8ppm) for 24, 48 and 72 hours, respectively. From the control experiment, it was observed that the diploid chromosome number was 2n=22. This diploid was also observed in other treated cells not affected by the APM treatment. The APM treatment caused varying types of chromosomal aberrations or abnormalities, which was observed to be concentration and time – dependent. The following chromosome abnormalities were observed viz,; disoriented chromosomes, clumped chromosome, laggards, sticky chromosomes, irregular chromosomes with bridges, inhibited chromosomes, elongated chromosomes, curved chromosomes; thick and sticky chromosomes. Chromosome doubling (polyploidy; 4n = 44) was also observed in seeds of brown pigeon pea treated with 6ppm of AMP for 24 hours; 6ppm of AMP for 48 hours and 8ppm of AMP for 48 hours for the white variety, respectively. Our result also revealed that the total percentage chromosomal aberrations increased with increase in the concentration of the mutagen and majorly on the duration of soaking, the variety notwithstanding. Succinctly, the chromosomal aberrations caused by the treatment of the seeds with amiprophos methyl (APM) notwithstanding, the induction of polyploidy to these pigeon pea landraces is significant in crop breeding and improvement if meticulously exploited.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Millsp] is a multipurpose legume crop and is well adapted even in marginal lands (Joshi *et al*., 2009). According to the Center for New Crops and Products (2002), pigeon peas serve as important food and excellent protein source in developing tropical countries. Additionally, the seeds and sometimes the pods are eaten as vetegtable and also used as flour additive in soups and rice. A species of bacteria, *Rhizobia* is capable of fixing 41 to 280kg/ha of nitrogen due to their intrinsic capacity to symbiotically associate with the root nodules of pigeon peas. Parrotta (2001) reported the use of their leaf preparations for the treatment of jaundice, inflammation and sores of the mouth. The high adaptability, heritability, genetic variability and nutritive values reported of locally grown pulses (landraces) (Udensi *et al*., 2011a; Udensi *et al*., 2011b) calls for concerted efforts towards their genome manipulation and improvement.

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It has been observed that genetic variability is very cardinal to successful breeding programme in vegetatively and sexually propagated plants. This variability can be naturally or artificially induced [chemically, (irradiations – gamma rays, x-rays, etc.)], producing mutants. These mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with yield and quality traits (Ahloowalia and Maluszynski, 2001). Cytogenetic studies are pivotal for obtaining information pertaining the role and effect of various mutagens and elucidating the responses of various genotypes to a particular mutagen (Khang and Tyagi, 2009). According to Mahandjiev *et al.* (2001), induced mutations have great potentials and serve as a complementary approach in genetic improvement of crops. Khan and Al-Qurainy (2009) reported the use of induced mutations in the improvement of major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. The ability of these mutagens to penetrate the cell of living organisms to interact with the DNA molecules produces the general toxic effects associated with their mutagenic properties. The induction of chromosomal

damages leading to bridge formation during mitotic division might however, increase phenotypic aberration. These chromosomal effects caused by these mutagens might result to polyploidy induction, which according to Ranney (2006) is an intriguing phenomenon in plant that has provided an important pathway for evolution and speciation. Ranney (2006) observed that all polyploids posses a certain amount of genetic redundancy. The implication is that extra copies of genes can mutate and diverge resulting in new traits without necessarily compromising essential functions. Owing to the urgent need to complement biotechnological approaches in plant breeding, especially in Nigeria using affordable and available techniques such as mutation breeding, it becomes therefore imperative to investigate the cytological effect of amiprophos methyl (APM) treatment on pigeon pea landraces, which will serve as a guide for their improvement.

MATERIALS AND METHODS

This experiment was carried out between January and September, 2011in the Cytology Laboratory of the Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria. Seeds of brown and white "fiofio" [Pigeon pea- *Cajanus cajan* (L.) Millsp] were purchased from local farmers in Nsukka, Enugu State, Nigeria while Amiprophos methyl (APM) was obtained from VICAM Chemicals Ltd., Nigeria. Cytological studies were carried out according to the protocol of Kumar and Srivastava, (2010) with major modifications. The seeds were soaked in different concentrations of APM (4ppm, 6ppm and 8ppm) for 24, 48 and 72 hours, respectively in each of the treatment regimen. Thereafter, the soaked seeds were laved under running tap water. The treated seeds were grown in petri dishes lined with moist cotton wool along with their controls. On germination and subsequent root development, the root tips were harvested at intervals of 6.30am- 7.00; 7.30-8.00 and 8.30- 9.00am each day (This was to establish the best time for the harvest of root tips for local pigeon pea).

Pretreatment of the healthy harvested root tips were done in 8-hydroxylquinoline for 3hrs and then fixed in Carnoy's fixative (1:3glacial acetic acid: alcohol) for 24 hrs. The root tips were hydrolyzed in 1NHCl solution at 60ºC for 10 minutes and then preserved in 70ºC alcohol. They were stained in 2% aceto-orecin. Slides were viewed under light microscope and photomicrographs were taken for analysis.

RESULTS

Seeds of pigeon pea landraces [*Cajanus cajan* (L) Millsp] were exposed to amiprophos methyl (APM) treatment at 4ppm, 6ppm and 8ppm for 24, 48 and 72 hours, respectively. From the control experiment, it was observed that the diploid chromosome number was 2n=22 (Plate 1) for the two varieties. This diploid was also observed in other treated cells not affected by the APM treatment. The APM treatment caused varying types of chromosomal aberrations or abnormalities, which was observed to be concentration and time – dependent. The following chromosome abnormalities were observed viz,; disoriented chromosomes, clumped chromosome, laggards, sticky chromosomes, irregular chromosomes with bridges, inhibited chromosomes, elongated chromosomes, curved chromosomes, clumped and curved; thick and sticky chromosomes (Plates 2- 10) the variety notwithstanding. Chromosome doubling (polyploidy; 4n = 44) was also observed in seeds of brown pigeon pea treated with 6ppm of AMP for 24 hours; 6ppm of AMP for 48 hours and 8ppm of AMP for 48 hours for the white variety, respectively (Plate 11). The frequencies of chromosomal abnormalities in the APM treated seeds is presented in Table 1. The result revealed that the total percentage chromosomal aberrations increased with increase in the concentration of the mutagen and majorly on the duration of soaking, the variety

Table 1: Frequencies of chromosomal aberrations in the amiprophos methyl (APM) treated seeds of *Cajanus cajan* (L) Millsp landraces

Variety of Pigeon pea	Conc. of AMP (ppm)	Soaking duration (hr)	Types of chromosomal aberrations									Total % aberration
			Number of cells observed									
			35	40	41	38	36	42	37	43	42	
			DSC	CUC	LA	STC	IRC	INC	ELC	CRC	TSC	
		24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	O	48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		72	0.00	0.00	2.44	0.00	0.00	0.00	0.00	0.00	0.00	2.44
		24	8.57	4.88	0.00	2.63	11.11	0.00	0.00	0.00	0.00	27.19
	4	48	Chromosome doubling									
		72	11.43	0.00	12.20	0.00	2.78	2.38	0.00	0.00	2.38	31.17
		24	Chromosome doubling									
Brown	6	48	11.43	7.50	7.32	15.79	0.00	0.00	0.00	2.33	4.65	49.02
		72	14.29	10.00	9.76	18.42	5.56	0.00	0.00	6.97	7.14	72.14
		24	2.85	12.50	12.20	0.00	0.00	0.00	0.00	0.00	0.00	27.55
	8	48	5.71	15.00	14.63	2.63	0.00	0.00	0.00	0.00	9.53	47.50
		72	8.57	17.50	17.07	10.53	0.00	14.29	0.00	4.65	14.29	87.53
		24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	O	48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		24	2.85	5.00	7.31	7.89	0.00	0.00	0.00	2.32	0.00	25.37
	4	48	5.71	7.50	9.75	13.16	2.78	2.38	0.00	0.00	2.38	43.66
		72	11.42	12.50	12.20	15.79	8.33	4.76	2.70	0.00	7.14	74.84
		24	2.85	7.50	0.00	5.26	0.00	2.38	0.00	2.32	0.00	20.31
White	6	48	Chromosome doubling									
		72	8.57	5.00	9.76	13.16	5.56	0.00	2.70	6.97	7.14	58.86
		24	2.85	12.50	14.63	0.00	0.00	0.00	0.00	2.33	0.00	32.31
	8	48	Chromosome doubling									
		72	5.71	10.00	17.07	10.53	0.00	14.29	0.00	4.65	11.90	74.15

KEYS: DSC = Disoriented chromosome; CUC = Clumped chromosome; LA = Laggard; STC = Sticky chromosome; IRC = Irregular chromosome; INC = Inhibited chromosome ELC = Elongated chromosome; CRC = Curved chromosome; TSC = Thick and sticky chromosome.

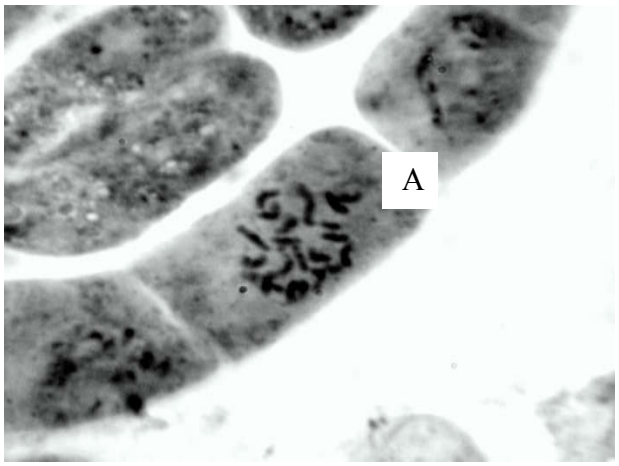

Plate 1: Metaphase chromosome from controls (2n = 22)

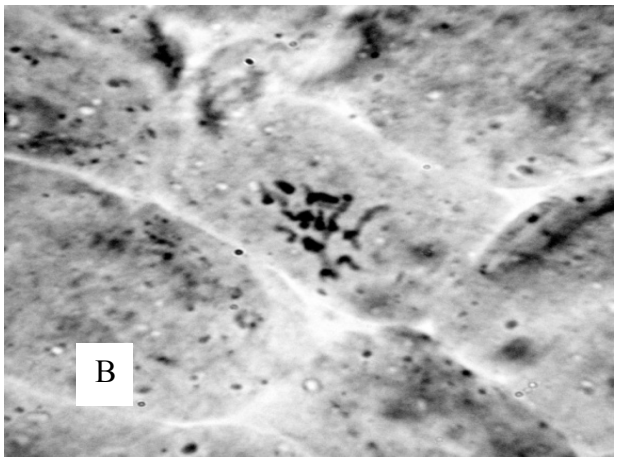

Plate 2: Disoriented metaphase chromosome

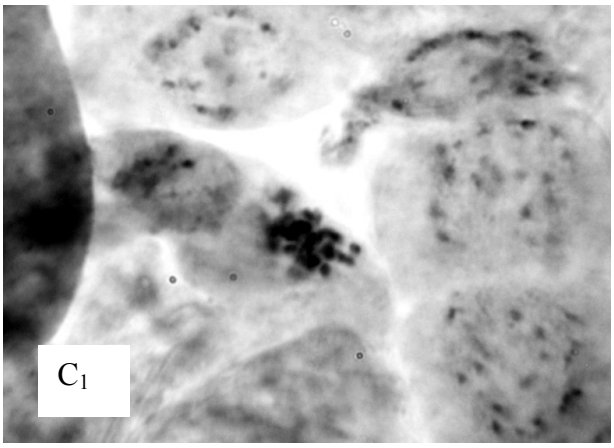

Plate 3a: Clumped chromosomes

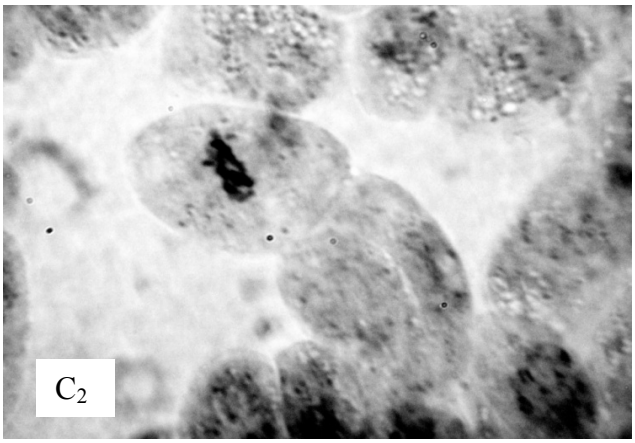

Plate 3b: Clumped chromosome

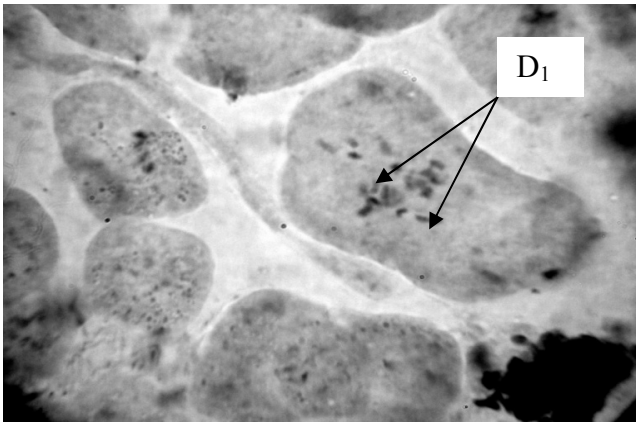

Plate 4a: Laggards

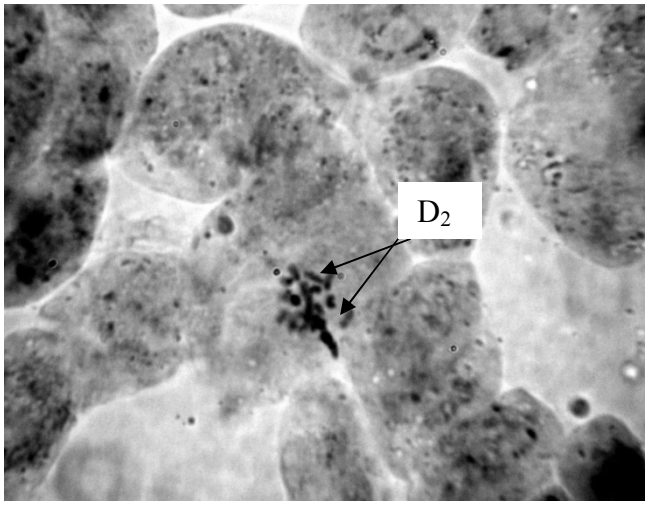

Plate 4b: Laggard

notwithstanding. During the preparation of the slides, it was observed that the best time for the harvest of the root tips of these local pigeon pea varieties was between 8.30 – 9.00am.

DISCUSSION

Crop development and improvement is very pivotal in the sustainability and food security of the Sub-Saharan African countries especially, Nigeria. Though there have been so many advances in the use of molecular and biotechnological approaches to this regards, there are still big chasm created between the developed and under-developed world as pertains to the availability of these improved crops, which undoubtedly can be bridged through mutation breeding (Mahandjiev *et al.,* 2001) because of affordability and availability of instrumentations in these regions. Though our work in this report only x-rays the cytological effects of amiprophos methyl, its ultimate goal is to improve these landraces through this technique. It is obvious that during interphase of a mitotic cycle, DNA precursor molecules are incorporated into the chromosomes and histone proteins are formed. Consequently, the harvest time for mitotic metaphase chromosome between 8.30 - 9.00am observed implies that DNA synthesis must have

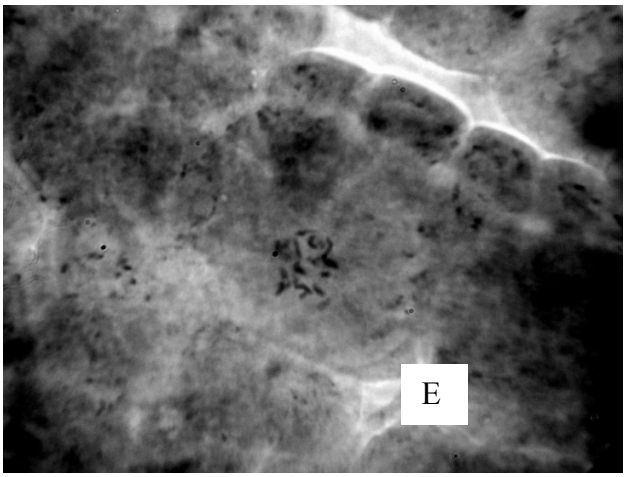

Plate 5: Sticky chromosome

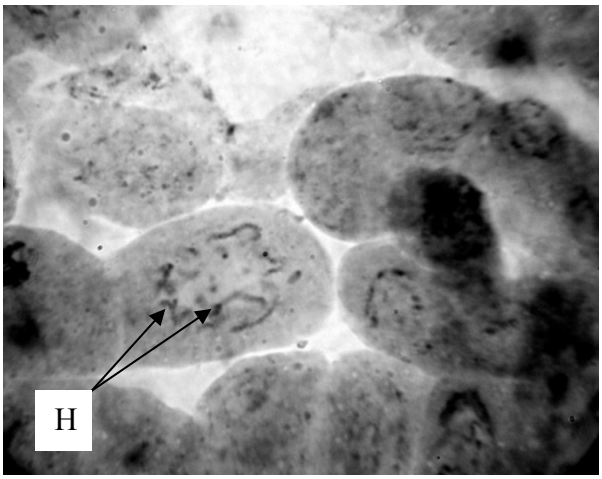

Plate 8: Elongated chromosomes

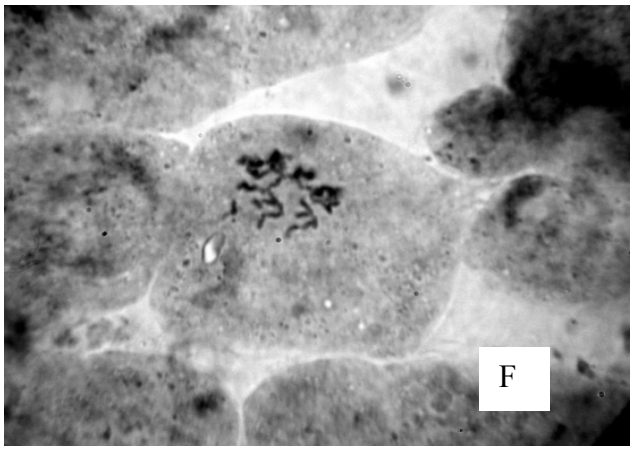

Plate 6: Irregular and lagging chromosome

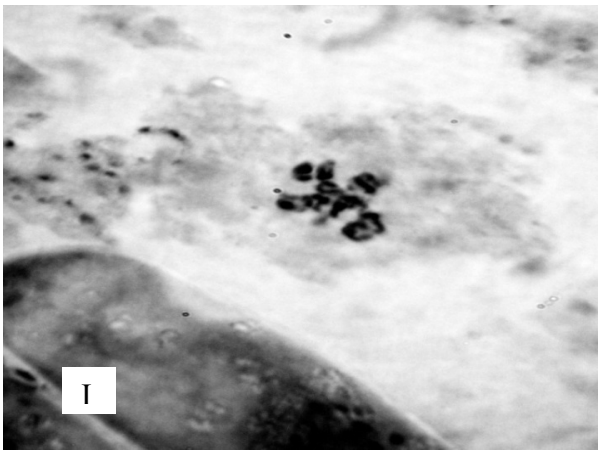

Plate 9: Clumped and curved chromosomes

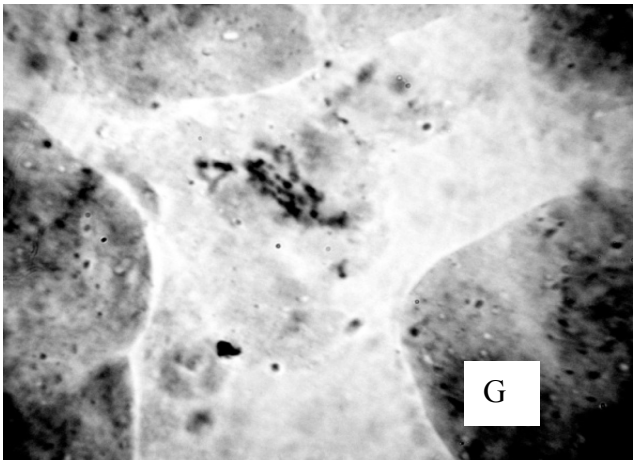

Plate 7: Inhibited metaphase chromosome

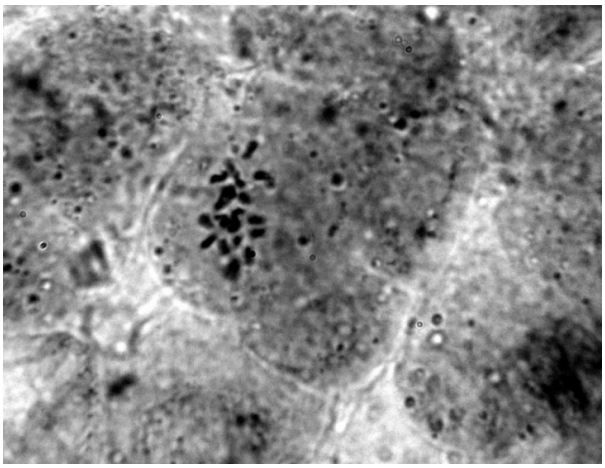

Plate 10: Thick and sticky chromosome

been completed, thus paving way to the other phases of mitosis. It is important to point out at this juncture that the time of harvest of root tips of different plant species is time-specific. The chromosome number of any plant species is a significant biological blueprint of a species. The diploid chromosome (2n = 22) obtained in our report confirms earlier reports by Panigrahi *et al.* (2007), Mallikarjuna *et al.* (2006) on *Cajanus cajan* (L) Millsp. The reported chromosomal aberrations in this study also corroborate the reports of Khan and Al-Qurainy (2009), Khan and Goyal (2009), Siddiqui *et al.* (2007) though using different mutagens and plant species.

It has been reported that almost all the mutagenic treatment induce a high degree of stickiness, which might be due to the delay in chromosome separation cased by disturbances at cyto-chemical level. Additionally, disoriented chromosomes at various stages of cell division could be due to the disturbance in the behavior of spindle or its dissolution. According to Khan and Al-Qurainy (2009), chromosome stickiness arises from improper folding of the chromosomes into single chromatin as a result of which chromatin fibers intermingle and become attached to each other by means of sub-chromatid bridges and incidence of lagging chromosomes increases. This

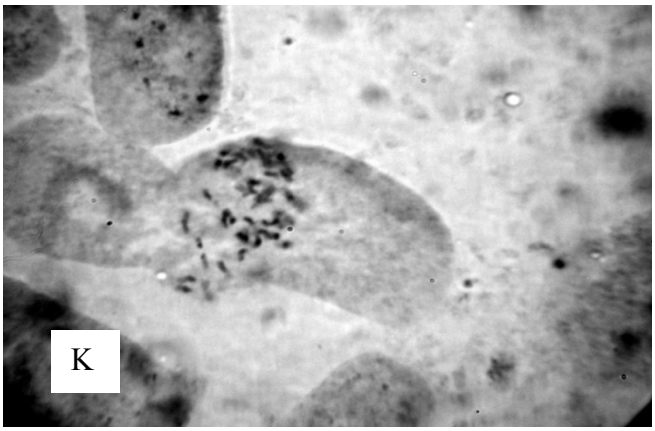

Plate 11: Chromosome doubling (2n = 44)

might be the reason underlying the frequency of the lagging chromosomes observed in this present study. Important to recall is the fact that spindle fiber organization and movement during cell division is an ATP- dependent process. Due to the reduced DNA synthesis and less availability of ATP, the spindle fiber organization in APM treated root tips cells may be affected, leading to the poor organization of chromosomes at the metaphase plate and migration of chromosomes towards the respective poles during anaphase (Khan and Al-Qurainy, 2009). It might be this deformity in spindle formation and chromosomes separation during mitosis may have resulted in high percentage frequency of chromosomal aberrations as observed in our work. Percentage frequencies of aberrations reported in Table 1 suggest that increasing the duration of soaking, concentration notwithstanding increases aberration frequency. This could be explained with the understanding that APM was given enough time to penetrate the cells of seeds to interact with the DNA molecules. Suffice it to say that the induction of chromosome aberrations by APM might be disadvantageous to pigeon pea improvement since the abnormal chromosome may not complete the mitotic cycle thereby reducing the productivity chances of the crop. According to Ranney (2006) chromosome doubling (polyploidy) is an intriguing pathway leading to plant evolution and speciation. Most polyploids display heterosis relative to their parental species and may display novel variation or morphologies that may contribute to the processes of speciation and ecoloniche exploitation (Comai, 2005). Wendel (2000) reported that polyploids populations often demonstrate extensive genomic rearrangement including the origin of novel regions of DNA. Interestingly, there are a number of factors that may provide polyploids with adaptive and evolutionary advantages. Most importantly, poltploids can be significantly more heterozygous than their diploid counterparts. The degree of heterogousity may be a key factor in the growth performance and adaptability of a polyploidy (Ranney, 2006). Fortunately, the polyploid obtained in this research is tetraploid, which obviously could lead to better paring and furthermore higher performance. Ranney (2006) opined that polyploids exert varying effects on plants, which basically is based on the plant species, the degree of heterozyousity, the ploidy level, and the mechanism that relates to gene silencing, gene interactions, gene dose effects and regulation of specific traits and processes.

Conclusion

Succinctly, the chromosomal aberrations caused by the treatment of the seeds with amiprophos methyl (APM) notwithstanding, the induction of polyploidy to these pigeon pea landraces is significant in crop breeding and improvement if meticulously exploited.

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