Radiation induced hormesis and its cytological evaluation in sunnhemp *Crotalaria juncea* L.

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ABSTRACT: The present study documents ionizing radiation induced hormesis and its cytological evaluation via study of microsporogenesis in *Crotalaria juncea* L. (sunnhemp). Morphometric traits viz. plant height, seedling height, seed yield, lateral branches, number of pods and biomass were analyzed. A concomitant study of radiation response on the cytology of the plant has been done via studying pollen mother cells at different stages of microsporogenesis. Doses used were 100, 200, 300, 400, 500 and 600 Gy with respect to control. Doses in the range of 100 to 300 Gy were shown to induce hormesis. Cytological aberrations had a stabilizing trend upto the hormetic dose but showed a sharp increase at chronic doses.

KEYWORDS: Cytology, Hormesis, microsporogenesis, morphometric traits, cytology

The evolution of terrene species has transpired in an inescapably radioactive macrocosm. It is a pervasive and perpetual specter of the universe. As inhabitants of the larger cosmos we are daily subjected to low radiation rates, which in many cases accounts for the continuance and existence of life itself. Having said so, it would be unwise to rule out all radiation as detrimental to the living biota. As the most energetic component of the electromagnetic spectrum (10 keV to several hundred keV) with the highest penetration power gamma rays comprise a paramount source of radiation. Plant radiostimulation or hormesis is enhanced plant growth after seed exposure to low dose irradiation (Grodzinskii 1989). The term 'hormesis' has its genesis from the Greek word 'hormaein' meaning 'to excite'. The concept of radiation hormesis was first elucidated by T. D. Luckey (1982) and envisages the following (a) Biopositive effects of low dose radiation (b) Radioadaptive response whereby radiation also acts on the organism as a stress. Invigorating impacts of low dose exposure are altogether disparate from noxious repercussions of chronic exposure. However a pre-exposure to a low dose boosts the cell's defense mechanism and acclimatizes the cell against future radiation exposure. This gives a semblance of a diminished radiation response. Expansive dose range exploitation makes plants a feasible option over animals for scrutinizing the hormetic dose. A radiostimulant low dose is defined as any doses from environmental radiation level and the threshold that marks the boundary between positive and negative biological effects (Luckey 2003). The sensitivity of ionizing radiation is directly proportional to the size of the cell nucleus or chromosome volume. The larger the chromosome volume the more sensitive the material is to radiation (Sparrow *et al.* 1963). Lacking plant diversity and eventual erosion of genetic resources has given added impetus to the field of induced mutagenesis. Slow-paced recurrence of

spontaneous mutations in nature has necessitated a shift in focus of research strategy towards expeditious attainment of genetic diversity. The main advantage of the potential of mutation breeding is the genetic modification of one or more characters without changing resources. (Wani and Anis 2008). Radiation sources are the most sought after implements for augmentation of genetic diversity. Pre-sowing seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Selenina and Stepanenko 1979). Mitotic and meiotic cytological studies are irrefutable indicators to authenticate mutagenic efficacy. Induction of meiotic chromosomal aberrations is propitious in unravelling mechanistic of their heritability through successive generations and phenotypic manifestation. There is ample experimental data validating the low dose stimulatory response (Sumira *et al.* 2011; Kumar and Gupta 2007; Kumar and Yadav 2010; Kumar and Srivastava 2011). *Crotalaria juncea* L. (2n=16) of family Fabaceae popularly known as sunnhemp, is a multipurpose fibre and forage crop It is an excellent source of green manure recently being worked upon as a biofuel (Kamireddy *et al.* 2013) due to its high heating value. It has also been shown to be a natural metal hyperaccumulator in the roots and leaves with a promising potential in phytoremediation (Pereira *et al.* 2002). The present study aims to assess the mutagenic potency of gamma rays and screen out doses eliciting a hormetic response in *Crotalaria juncea* L

MATERIALS AND METHODS

Seeds of *Crotalaria juncea* variety Swastika were obtained from Sunnhemp Research Station, Pratapgarh, Uttar Pradesh. The seeds were exposed to different doses of gamma rays viz. 100, 200, 300, 400, 500 and 600 Gy from Co⁶⁰ source at National Botanical Research Institute,

Fig. 1. Radiation response on height

Lucknow. Post-irradiated seeds were pre-soaked for 12 h with suitable control seeds maintained in distilled water and sown in experimental pots under greenhouse conditions in replicates of three with 8 seeds each. For meiotic studies, young floral buds in the pre-anthesis stage were fixed in Carnoy's fixative II (3 parts absolute ethanol: 1 part glacial acetic acid) for 24 h and subsequently preserved in 70 % ethanol at 4°C. Slides were prepared by anther squash method using 2% acetocarmine. The frequency of meiotic chromosomal abnormalities was scored in 200–400 pollen mother cells from each anther. Morphological data from each replicate per dose along with control were evaluated. SPSS16.0 for Windows was used for Statistical Analysis. The data of all parameters was analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range test. Results with $p < 0.05$

Fig. 3. Radiation response on number of pods and biomass

Fig. 2. Radiation response on seed yield and lateral branches

were considered to be statistically significant. Sigma Plot 13 was used for graphical illustration.

OBSERVATION

Morphological Observation Parameters viz. seedling height, plant height at maturity, number of lateral branches per replicate, seed yield per replicate, number of pods per replicate, biomass per replicate were evaluated in the present study. Increment in seedling height (in cm) at doses viz. 100 Gy (19.8), 200 Gy (19.63) and 300 Gy (21.46) was significant as compared to control (16.5) with a maximum at 300 Gy. The plant height (in cm) showed an increasing response upto 500 Gy. Highest increase was observed at 100 Gy (151.0) and 300 Gy (131.0) with respect to control (128.0). The trend of plant height and seedling height has been shown in Fig. 1. The number of lateral branches per replicate showed a remarkable increase at doses 200 Gy (14.00) and 300 Gy (14.00) as compared to control (5.00). A statistically significant increase in seed yield (in g) was observed at 200 Gy (17.46) and 300 Gy (18.93) with respect to control (13.77). The dose response for lateral branches and corresponding seed yield displayed a synchronous trend represented in Fig. 2. The above ground biomass (in g) and number of pods also displayed synchrony upto 300 Gy beyond which the trends were different. The above ground biomass (in g) was highest at 200 Gy (142.33) and 300 Gy (153.33) with respect to control (128.0), seed yield was highest at 200 Gy (17.46) and 300 Gy (18.93) with respect to control (13.77) represented in Fig. 3

Cytological Observation Meiosis in *Crotalaria juncea* L. ($2n=16$) was perfectly normal in control plants with eight bivalents at the equatorial plate at metaphase I (Fig. 4-1) and 8:8 separation at anaphase I (Fig. 4-2). Plants in gamma irradiated sets displayed varying degrees of chromosomal abnormalities distributed in all planes of

Fig. 4: Cytological Plate

1. Normal Metaphase I, 8 bivalents ($2n=16$), 2. Normal Anaphase I (8:8 separation), 3. Normal Anaphase II, 4. Precocious movement at metaphase I, 5. Unipolarity at metaphase I, 6. Forward and Laggard Movement of chromosome at Anaphase I, 7. Incomplete Single Bridge at Anaphase I, 8. Asynchronous Division at metaphase II, 9. Unoriented Metaphase II, 10. Stickiness at metaphase II with precocious movement, 11. Tripolarity at Anaphase II, 12. Pentapolarity at Anaphase II. Scale bar shows $6.818\mu\text{m}$.

Table 1. Radiation induced meiotic chromosomal aberrations in pollen mother cells of *Crotalaria juncea* L. sunnhemp

Dose (Gy)	Total PMC count	Abnormal PMC count	Metaphasic Abnormalities Anaphasic Abnormalities												Tab %
			Sc	Un	Asy	Uni	Pr	St	St	Lg	Trp	Pp	Dp	FM	
Ct	271	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	203	16	0.44±0.28 ^a	1.14±0.57 ^b	0.96±0.55 ^c	-	1.62±0.65 ^{ab}	-	0.70±0.42 ^a	0.45±0.23 ^a	0.33±0.18 ^c	-	0.22±0.11 ^b	-	5.90±0.74 ^c
200	235	23	-	2.73±0.66 ^{ab}	2.23±1.12 ^{bc}	-	0.54±0.09 ^{ab}	-	-	-	1.51±0.32 ^{abc}	0.50±0.31 ^{ab}	2.40±0.76 ^a	0.77±0.42 ^a	9.78±0.53 ^d
300	240	20	0.24±0.14 ^a	1.96±1.15 ^{ab}	1.12±0.06 ^c	0.74±0.65 ^b	0.26±0.13 ^{ab}	-	-	-	0.90±0.32 ^{bc}	-	2.68±0.11 ^a	-	8.67±1.18 ^d
400	244	40	1.01±0.68 ^a	1.62±0.69 ^{ab}	1.07±0.59 ^c	3.80±0.71 ^a	1.92±1.17 ^a	0.87±0.44 ^b	-	0.23±0.11 ^{ab}	1.92±1.07 ^{abc}	3.48±1.93 ^{ab}	-	0.23±0.11 ^{ab}	16.20±0.60 ^c
500	259	38	0.44±0.26 ^a	2.86±0.69 ^{ab}	4.47±0.49 ^a	1.45±1.92 ^b	-	3.43±0.43 ^a	-	-	2.80±0.36 ^{ab}	3.58±1.79 ^{ab}	-	-	19.07±0.55 ^b
600	263	58	1.22±0.37 ^a	3.70±0.47 ^a	3.60±0.11 ^{ab}	-	-	3.20±0.26 ^a	-	-	3.67±1.13 ^a	4.10±0.38 ^a	3.49±0.32 ^a	-	22.53±0.45 ^a

PMC=Pollen Mother Cell, Sc=Scattering, Un=Unorientation, Asy=Asynchronous division, Uni=Unipolarity, Pr=Precocious movement, St=Stickiness, Lg=Laggard, Trp=Tripolarity, Pp=Pentapolarity, Dp=Disturbed polarity, FM=Forward Movement, Tab=Total Abnormality

Data represent the mean value ± standard error. Values followed by different superscripts within same column differ at P<0.05 between treatments by the DMRT

division. Although the abnormality percent increased upto 200 Gy showing a stabilizing effect at 300 Gy. At the initial doses the total abnormality percent was more or less constant viz. 100 Gy (5.90%), 200 Gy (9.78%), 300 Gy (8.67%) but it showed a drastic increase from 400 Gy onwards viz. 400 Gy (16.20%), 500 Gy (19.07%), 600 Gy (22.53%). The chromosomal abnormalities observed were scattering, unorientation (Fig. 4-9), asynchronous division (Fig. 4-8), precocious movement (Fig. 4-4, 10), unipolarity (Fig. 4-5), stickiness (Fig. 4-10), laggard movement (Fig. 4-6, 7), tripolarity (Fig. 4-1), pentapolarity (Fig. 4-12), disturbed polarity, forward movement (Fig. 4-6, 7). Stickiness and anaphasic polarity disturbance were amongst the most pre-dominant abnormality scored. The percentage of metaphasic stickiness was found to be greater than the anaphasic stickiness with a higher incidence at chronic doses. Amongst the polarity disturbances the percentage of pentapolarity was greater than the percentage of tripolarity. At chronic doses of 500 Gy and 600 Gy the chromosomal abnormalities were considerably increased as compared to the previous doses. The abnormalities have been summarized in Table 1.

DISCUSSION

Genomic instability is a consequence of heightened agglomeration of genome modifications. The inception and continuity of genomic instability engages numerous signaling pathways.

The relative contribution of the different pathways depends upon the genetic background of the irradiated cell or organism (Watson *et al.* 1997).

High doses of radiation can promote epigenetically silencing of adaptive response genes. (Scott *et al.* 2009). Epigenetic changes are meiotically heritable and mitotically stable alterations in gene expression that include DNA methylation, histone modification and RNA associated silencing (Jaenisch and Bird 2003). DNA methylation is concurrent with a quiescent chromatin state and subdued gene expression.

Several studies have enumerated that pre-exposure to low irradiation dose remodels radiosensitivity downsizing the aberration frequency. This phenomenon called adaptation is allied to a defense mechanisms for extenuating genotoxic damage via more efficient detoxification of free radicals, DNA repair systems, induction of new proteins in irradiated cells with a conditioning dose and enhanced anti-oxidant production (Coleman *et al.* 2005).

It is known that re-population is an important mechanism of post-radiation recovery in plants (Grodzinskii 1989). Injured cells in a population are restored by healthy cells by engaging extra ordinarily non-dividing cells into multiplication. Replacement of damaged or dead cells by repopulation has been attributed to the function of a genetically determined system that appeared during evolution to ensure seed adaptation to changes in environmental conditions (Kondo 1988).

It has been proposed that DNA repair processes induced by the low dose radiation exposure are error free processes (Mitchel 1995). Exposure to low doses of ionizing radiation caused a temporary inhibition in DNA synthesis (Feinendengen *et al.* 1987). The transitory cessation of DNA synthesis lengthens the recovery period for the cell and generates free radical scavengers.

Plant height is primarily a quantitative trait controlled by a polygene. Each gene contributes small effects which is called the genetic additive effect (Abdullah *et al.* 2009). Increased phosphorus (P) uptake by plants may be a probable cause of increase in height observed in the present study. Increased tendency in number of branches was reported by Kumar and Srivastava (2011) in *Sesbania cannabina* and Yousef *et al.* (1998) in chamomile.

If the dose is too low, there will not be enough mutation because of low mutation frequently and results in small mutated sector (Nazir *et al.* 1998). Modulation in photosynthesis in irradiated plants might partly contribute to increased growth (Wi *et al.* 2007).

Chronic irradiation acutely affects pre-meiotic and post-meiotic stages due to a raised nuclear volume. Meiotic pairing and reduction tend to enhance the damage wrought by aberrations which may survive in diploid somatic cells. (Iglesias-Andreu *et al.* 2012). At chronic irradiation levels the irreversible damage occurs rendering the cell's defense mechanism defunct.

Gaulden (1987) postulated that sticky chromosomes may result from the defective functioning of 1 or 2 types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of the mutagen on the proteins (induced stickiness). In the latter case the proteins co-ordinating chromosomal condensation in meiotically active cells are the most prone target sites. Since stickiness was evident from metaphase I onwards it would suffice to say that the mutagen probably acted during the prophase to metaphase I transition. Ionizing property of gamma radiations disassembles bonds stabilizing the nucleosome complex thereby depolymerizing DNA which culminates in stickiness.

Indeed, chromosomes present as unpaired univalents at the first meiotic division have been reported to undergo premature sister chromatid separation (i.e., equational division), and/or to lag at anaphase, and/or to induce metaphase arrest (Darlington 1939).

The presence of bridges with or without fragment both at anaphase I/II could be interpreted as due to paracentric inversions (Sinha and Godward 1972). Bridges might also have arisen due to breaks in two chromosomes followed by union of the centric fragments (Shreekrishna 2006) or due to stickiness of chromosome at metaphase and their failure to separate at anaphase. Variation in seed yield may appertain to abnormal microsporogenesis (Pagliarini and Pereira 1992) producing non-viable gametes.

CONCLUSION

From the foregoing study it can be concluded that gamma radiations succeeded in inducing invigorating outcomes at lower doses. In the present study the optimum doses which elicited a significant hormetic response in sunnhemp were 200 Gy and 300 Gy. Mutagenic efficiency was most appropriate at dose of 300 Gy with a low level of abnormality induction but a considerably high increment in morphometric traits as compared to the non-radiated control.

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