Genetics and Plant Physiology – 2015, Volume 5(1), pp. 3–9
Special Issue – Project BG051PO001-3.3.06-0025 "Support for training and development of young competitive experts in the field of physiology, phytochemistry, genomics, proteomics and biodiversity of eukaryotic organisms"

Available online at http://www.ifrg-bg.com

CYTOGENETIC EFFECTS IN BARLEY ROOT APICAL MERISTEM AFTER EXPOSURE OF DRY SEEDS TO LITHIUM ION BEAMS

Nikolova I.[1*], M. Georgieva[1], K. Kruppa[2], M. Molnár-Láng[2], L. Liu[3], V. Manova[1], L. Stoilov[1]

[1]Department of Molecular Genetics, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bldg. 21, 1113 Sofia, Bulgaria

[2]Department of Plant Genetic Resources and Organic Breeding, Agricultural Research Institute, Hungarian Academy of Sciences, Martonvásár, Hungary

[3]Department of Plant Mutation Breeding & Genetic, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China

Received: *31 October 2014* Accepted: *23 January 2015*

Summary: In order to analyze the cytogenetic effects of high linear energy transfer radiation, dry barley seeds were irradiated with ^{7}Li-ions. Three reconstructed barley (*Hordeum vulgare* L.) karyotypes D-2946, T-46, T-29 and their parental genotype Freya were used in the study. Various types of chromosome aberrations were observed in metaphase preparations stained with Feulgen. Chromosomal aberrations of both chromatid and chromosome type were found which indicates that ^{7}Li-ions display S-independent mode of action. The frequency of chromosomal abnormalities was relatively low due either to the reduced clastogenic potential of the radiation doses applied or to the influence of cell cycle progression and elimination of the damaged cells. Fluorescence *in situ* hybridization with pTa71 and GAA repetitive DNA probes was performed. Hybridization with probe pTa71 revealed chromosome translocation between the two satellite chromosomes 5H and 6H resulting in a combination of both NORs containing ribosomal repeats in one and the same chromosome in line T-46 exposed to 45 Gy ^{7}Li-ions. As the observed abnormality equally affects the entire cell population of a single primary root this might be a plausible evidence of its spontaneous nature.

Citation: Nikolova I, M. Georgieva, K. Kruppa, M. Molnár-Láng, L. Liu, V. Manova, L. Stoilov, 2015. Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams. *Genetics and Plant Physiology*, 5(1): 3–9.

Keywords: chromosome aberrations, FISH, lithium ion beams.

Abbreviations: FISH – fluorescence *in situ* hybridization; GISH – genomic *in situ* hybridization; LET – linear energy transfer; NORs – nucleolus organizing regions; PCR – polymerase chain reaction.

*Corresponding author: i.i.nikolova@abv.bg

INTRODUCTION

At present there is no sufficient knowledge about the damaging capacity of high linear energy transfer (LET) radiation in plant genome. In comparison with low LET gamma rays, the higher potential of high LET radiation to induce chromosomal aberrations and micronuclei in root tip cells was demonstrated (Mei et al., 1994). Recently, the genetic effects and the influence of heavy ions (neon, argon, iron, carbon) on plant development in maize, rice, wheat and *Arabidopsis thaliana* were analyzed (Shi et al., 2010; Kazama et al., 2011). Complex effects of sparsely and densely ionizing radiation in plants were evaluated. The data showed that densely ionizing radiation was a more efficient damage inducer (De Micco et al., 2011). Various types of chromosome aberrations both in anaphase and metaphase were observed in root apical meristem after exposure of wheat dry seeds to carbon ion beams (Liu et al., 2013).

It was recently shown that the reconstructed barley karyotype D-2946 is a radiation-sensitive mutant line with reduced potential to maintain genome integrity in respect to DNA and chromosomal damage, including that produced by ^{7}Li-ions on DNA level (Stoilov et al., 2013). On the other hand, the information about the clastogenic potential of lithium ions in plant genome is rather scarce. The aim of the present study was to analyze the cytogenetic effects of high-energy ^{7}Li-ion beams in the radiosensitive barley line D-2946.

MATERIALS AND METHODS

In this study, 3 reconstructed barley (*Hordeum vulgare* L.) lines: D-2946, their parental translocation lines T-46 and T-29, as well as the initial genotype Freya, were used.

Metaphase analysis

Conventional Feulgen staining was performed for visualization of metaphase chromosomes. Cytological procedures for scoring of chromosome aberrations were performed as previously described (Gecheff, 1989).

Dry barley seeds were irradiated with ^{7}Li-ions (doses of 20 and 45 Gy) and germinated in Petri dishes at 24°C for specific time intervals – 38, 39, 41 and 42 h after seeds imbibition. The primary roots were incubated in 0.025% colchicine saturated with 1-bromonaphthalene for 2 h prior to fixation with ethanol-acetic acid (3:1, v/v). To cover the first cell cycle after irradiation the material was fixed for different periods during germination as specified above. After hydrolysis in 1N HCl for 9 min, the roots were stained with Shiff's reagent for 1 h (Feulgen method) and macerated in 4% pectinase. Chromosome aberrations were scored in metaphase using temporary squash preparations by Olympus microscope BX-41. For each fixation period at least 100 random cells were scored. Non-irradiated seeds from the respective lines served as a control.

Fluorescence in situ hybridization

Fluorescence *in situ* hybridization (FISH) was performed essentially as described by Molnár-Láng et al. (2000).

Before hybridization, the DNA probes were labeled by Nick translation or polymerase chain reaction (PCR). The following repeated sequences were used as probes for FISH: (1) GAA satellite sequences amplified from barley genomic DNA, labeled with biotin-11-dUTP by PCR (green signal); (2) clone pTa71, containing the 18S–5.8S–26S rDNA repeat unit isolated from *Triticum aestivum*, labeled simultaneously with 50% digoxigenin-16-dUTP and 50% biotin-11-dUTP by nick translation (yellow signal). Digoxigenin and biotin signals were detected by anti-digoxigenin-rhodamine and streptavidin-fluorescein isothiocyanate (FITC), respectively. The slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 1μg/ml) and mounted in Vectashield antifade. Images were captured and analyzed by Image Pro plus 5.1 software and a Spot CCD camera attached to a Zeiss Axioscope 2 epifluorescence microscope.

RESULTS

Metaphase analysis

Various types of chromosome aberrations, both of chromatid and chromosome type, were observed in metaphase such as isolocus breaks (Fig. 1a), chromatid breaks (Fig. 1b), dicentrics with fragments (Fig. 1c), chromatid translocation and ring chromosomes (Fig. 1d). Multiple damages of the chromosomal complement were also detected (Fig. 1e). The frequency of chromosomal damage was relatively low – between 10% and 30% for different

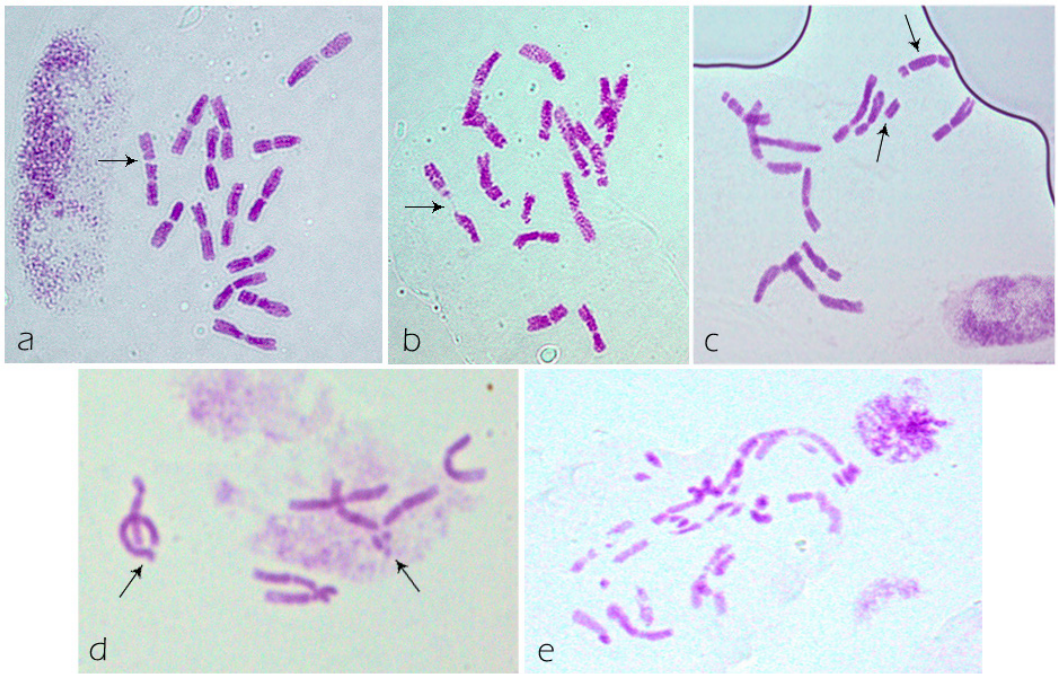

Figure 1. Chromosomal aberrations induced in barley by ^{7}Li-ions (marked with arrows): a – isolocus breaks without sister chromatid reunion, b – chromatid breaks, c – dicentrics with fragments, d – chromatid translocation and ring chromosome, e – multiple damage of the chromosome complement.

periods during germination. This could be a consequence of either reduced clastogenic potential of the radiation doses applied in dry barley seeds or the possible elimination of the damaged cells during cell cycle progression.

Fluorescence *in situ* hybridization

Hybridization with labeled probe pTa71 complementary to 18S-5.8S-26S rDNA revealed a chromosome translocation between the two satellite chromosomes 5H and 6H, resulting in a combination of both NORs containing ribosomal repeats in one and the same chromosome in the line T-46 which was exposed to 45 Gy ^{7}Li-ions (Fig. 2). The observed abnormality concerned identically the entire cell population of one single primary root of the line, which might be a plausible evidence of its spontaneous nature.

DISCUSSION

The main objective of this study was to analyze the clastogenic potential of lithium ions in barley genome. Our data showed that the chromosomal aberrations induced in root meristematic tissue were both of chromatid and chromosome type (Fig. 1). The data on the spectrum of the chromosomal abnormalities showed that ^{7}Li-ions displayed S-independent, non-delayed mode of action. The frequency of damaged cells with aberrations was lower than that observed after treatment with similar doses of gamma-rays (unpublished results). This could be a consequence of either reduced clastogenic potential of the radiation doses applied or the possible influence of repair activities during germination.

The effect of treatment with carbon ion beams in *Arabidopsis* genome

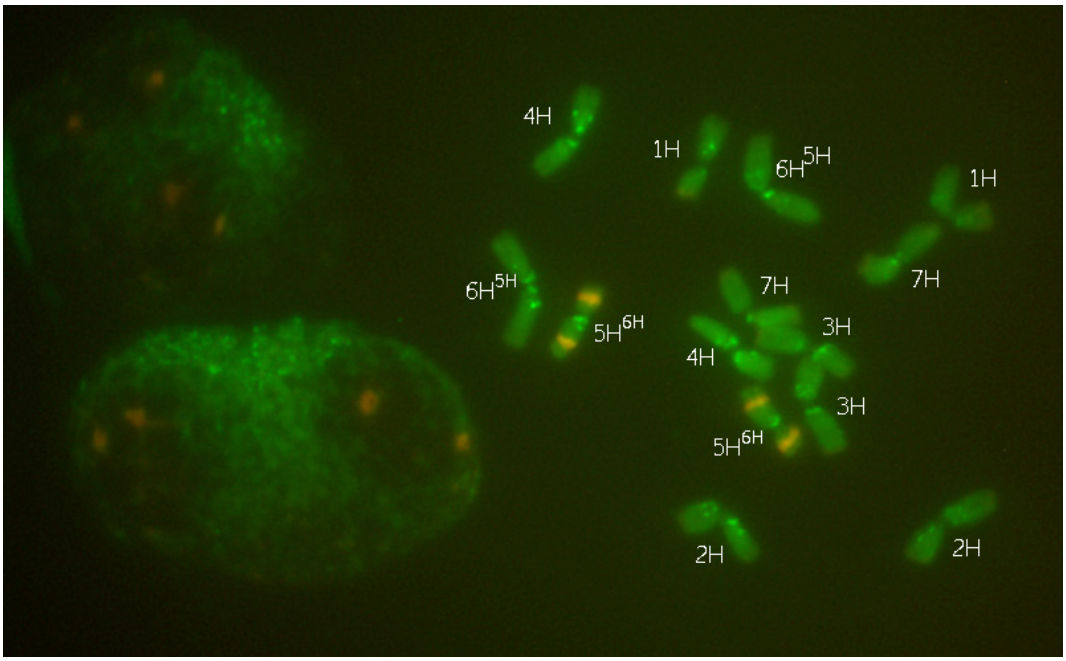

Figure 2. Spontaneous translocation combining both NORs in one and the same chromosome, observed in line T46. FISH analysis was performed with DNA probes GAA (green signal) and pTa71 (yellow signal).

revealed high mutation frequency and broad mutation spectrum. Based on PCR and sequencing analyses it was established that 50% of all mutants produced by ion beams possessed large DNA alterations, while the rest contained mainly point mutations (Tanaka et al., 2010). Moreover, it was shown that heavy ion beams induced a number of mutant *Arabidopsis* phenotypes even at low irradiation doses and short exposure. These studies revealed also that Fe-ion irradiation tended to produce complex mutations like chromosomal rearrangements or large deletions (Kazama et al., 2013). Another study revealed that the number of chromosomal breaks observed at 50 Gy $^{20}Ne^{10+}$ were about 8 times more than those induced by X-rays (Kikuchi et al., 2009).

It was found that the chromosome constitution affected the distribution pattern of induced aberrations along the individual chromosomes (Gecheff et al., 2000; Gecheff et al., 2001). One of the potential "hot spots" for induction of chromosomal damage in barley genome was identified to be in the vicinity of the nucleolus organizing regions (NORs) of the satellite chromosomes 5H and 6H (Gecheff et al., 2009). Karyotype reconstruction based on translocation between the two NOR-bearing chromosomes was previously reported in barley and pepper (Nikoloff et al., 1977; Scaldaferro et al., 2014).

We established that hybridization with labeled probe pTa71 visualized chromosome translocation between the two satellite chromosomes 5H and 6H, resulting in a combination of both NORs. The observed chromosomal reconstruction combined ribosomal repeats in one and the same chromosome in line T-46. This abnormality was detected within the cell population of only a single primary root out of the whole irradiated material which is a strong evidence for its spontaneous nature. We suppose that the observed alteration is most probably a consequence of initial gamma irradiation arising in the late progenies of line T-46. As it is known that double strand break levels and mutation frequency after treatment with high LET radiation are elevated (Shikazono et al., 2003; Stoilov et al., 2013), this might be an appropriate environment for the appearance of the detected spontaneous chromosomal rearrangement.

ACKNOWLEDGEMENTS

The Operational Programme Human Resources Development (2007–2013), the European Social Fund and the Ministry of Education and Science of Bulgaria (Grant № BG051PO001-3.3.06-0025) are thanked for financial support.

We are thankful to Prof. K. Gecheff for the critical reading of the manuscript.

REFERENCES

De Micco V, C Arena, D Pignalosa, M Durante, 2011. Effects of sparsely and densely ionizing radiation on plants. *Radiat Environ Biophys*, 50: 1–19.

Gecheff K, 1989. Multiple reconstruction of barley karyotype resulting in complete cytological marking of the chromosome complement. *Theor Appl Genet*, 78: 683–688.

Gecheff K, T Hvarleva, N Papazova, 2000. Chromosome position effect

in the expression of ribosomal genes in barley. *Bian Report of IGE, NCAS*, p.23

Gecheff K, N Papazova, 2001. Reconstructed karyotypes as a tool for identification and manipulation of barley genome. *Biotechnol Biotec Eq*, 15 (2): 27–32.

Gecheff K, V Manova, G Bonchev, M Kitanova, M Vlahova, L Stoilov, 2009. Position-specific effects in the action of mutagenic agents on the chromosomes of barley (*Hordeum vulgare* L.). *Genetics & Breeding*, 37: 3–13.

Kazama Y, T Hirano, H Saito, Y Liu, S Ohbu, Y Hayashi, T Abe, 2011. Characterization of highly efficient heavy-ion mutagenesis in *Arabidopsis thaliana*. BMC *Plant Biology*, 11: 161–170.

Kazama Y, T Hirano, K Nishihara, S Ohbu, Y Shirakawa, T Abe, 2013. Effect of high-LET Fe-ion beam irradiation on mutation induction in Arabidopsis thaliana. *Genes Genet Syst*, 88: 189–197.

Kikuchi S, Y Saito, H Ryuto, N Fukunishi, T Abe, H Tanaka, H Tsujimoto, 2009. Effects of heavy-ion beams on chromosomes of common wheat, *Triticum aestivum*. *Mutat Res*, 669 (1-2): 63–66.

Liu Q, Z Wang, L Zhou, Y Qu, D Lu, L Yu, Y Du, W Jin, W Li, 2013. Relationship between plant growth and cytological effect in root apical meristem after exposure of wheat dry seeds to carbon ion beams. *Nucl Instrum Methods Phys Res*, 305: 9–15.

Mei M, H Deng, Y Lu, C Zhuang, Z Liu, Q Qiu, Y Qiu, TC Yang, 1994. Mutagenic effects of heavy ion radiation in plants. *Adv Space Res*, 14(10): 363–372.

Molnár-Láng M, G Linc, RB Friebe, J Sutka, 2000. Detection of wheat–barley translocations by genomic *in situ* hybridization in derivatives of hybrids multiplied *in vitro*. *Euphytica*, 112: 117–123.

Nicoloff H, M Anastassova-Kristeva, G Künzel, 1977. Changes in nucleolar organizer activity due to segmental interchanges between satellite chromosomes in barley. *Biol Zbl*, 96, 223–227.

Scaldaferro MA, M Grabiele, JG Seijo, H Debat, MV Romero, DA Ducasse, AR Prina, EA Moscone, 2014. Efficiency of cytogenetic methods in detecting a chromosome rearrangement induced by ionizing radiation in a cultivated chili pepper line (*Capsicum baccatum* var. pendulum – Solanaceae). *Int J Radiat Biol*, 90(1): 104–112.

Shi J-M, J-G Guo, W-J Li, M Zhang, L Huang, Y-Q Sun, 2010. Cytogenetic effects of low doses of energetic carbon ions on rice after exposures of dry seeds, wet seeds and seedlings. *J Radiat Res*, 51: 235–242.

Shikazono N, Y Yokota, S Kitamura, C Suzuki, H Watanabe, S Tano, A Tanaka, 2003. Mutation rate and novel *tt* mutants of *Arabidopsis thaliana* induced by carbon ions. *Genetics Society of America*, 163: 1449–1455.

Stoilov L, M Georgieva, V Manova, L Liu, K Gecheff, 2013. Karyotype reconstruction modulates the sensitivity of barley genome to radiationinduced DNA and chromosomal damage. *Mutagenesis*,

28: 153–160.

Tanaka A, N Shikazono, Y Hase, 2010. Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate and spectrum of mutation phenotype for mutation breeding in higher plants. *J Radiat Res*, 51: 223–233.