Short-term mutagenicity test by using IRAP molecular marker in rice grown under herbicide treatment

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ABSTRACT
Rice is an economically important plant as well as a model organism. The rice genome consists of 35% retrotransposons. Although most of the retrotransposons are inactivated through evolutionary processes, they can be activated under various biotic and abiotic stress conditions. The main objective of this study was to explore the effects of herbicides on retrotransposon activities and the usage of retrotransposons in short-term mutagenicity tests. In this study, bentazone and an MCPA (2-methyl-4-chlorophenoxyacetic acid)-containing herbicide was used. Plant samples were grouped into three classes: control (untreated), 1% and 2% herbicide treatment. Retrotransposon activities were investigated by using the inter-retrotransposon amplified polymorphism (IRAP) marker technique. IRAP analyses were performed for Houba (Tos5/Osr13) retrotransposon. Polymorphism ratios were calculated with the Jaccard similarity index, and the significance of polymorphism was evaluated by one-way analysis of variance (ANOVA). We observed that the polymorphism ratios ranged from 8%–90% for Houba among plant samples. ANOVA showed that these variable ratios were statistically significant. Bentazone and the MCPA-containing herbicide increased the retrotransposon activities, and they might be responsible for DNA mutations. This study indicated valuable data for establishing retrotransposon-based short-term mutagenicity test in rice with suitable retrotransposons such as Houba.

Introduction
Rice is an important model plant. Its diploid chromosome number is 24 and its genome size is about 430 Mb. The Rice Genome Annotation Project reported 55,986 rice loci, 16,941 of them containing transposons [1]. Herbicides are commonly used to eliminate or kill undesired plants to improve crop production and yield. However, they may also be harmful to the organisms living in the water, soil and even air [2,3]. Bentazone (3–isopropyl–1H–2,1,3–benzothiadiazin–4 (3H–one) and MCPA (2–methyl–4–chlorophenoxyacetic acid) are two active ingredients commonly used as a mixture in commercially available herbicides. Bentazone belongs to the chemical group of benzothiadiazole, acting as a photosynthetic electron transfer inhibitor [4]. The bentazone effects observed in plants range from a few days to one week depending on the species and the dosage [5,6]. MCPA is an acidic herbicide belonging to a larger class of phenoxy acetic acid herbicides that are widely used for cereal, tobacco and cotton production [7–9]. MCPA has also been examined in lifetime feeding studies in both rats (at target doses of 20, 80 and 320 ppm for 2 years) and mice (20, 100 and 500 ppm for 2 years), appearing to be non-carcinogenic [10]. However, it is reported to have some genotoxic activities [11]. As a result of these different studies, MCPA has been listed in the priority pollutants of the European Community and the US Environmental Protection Agency [12].

The genotoxic and mutagenic effects of herbicides have been commonly investigated by using several methods. One of them is the Ames test, which is widely used to analyse the mutagenic effects of chemicals. It is one of the most reliable short–term bacterial test systems. It is also not expensive and the results can be obtained very rapidly [13,14]. Another test is the Allium cepa root chromosomal aberration assay, using the genotoxic potential of chemicals in the environment [15]. The micronucleus (MN) test is also a useful short–term test to analyse environmental mutagenicity and genotoxicity. The formation of MN in root tip cells has been studied to identify the damage induced by various chemical agents [16].

Retrotransposons are classified into two groups depending on whether they have Long Terminal Repeats (LTRs) or not. Gypsy and Copia are two LTR-retrotransposon superfamilies and the most abundant retrotransposons in cereals. The ratio of Gypsy to Copia...
elements in rice is 4.9:1 [17,18]. Because of the fact that retrotransposons are important dynamic elements in genomes, they constitute a significant part of eukaryotic genomes and even cause genetic instability [19]. Moreover, these mobile elements could be easily affected by environmental conditions [20,21] and play important roles in defence mechanisms because LTRs contain cis-acting elements similar to motifs found in activated defence genes [22]. Such environmental stimuli could be in vitro tissue culturing and heat [23–25]. Moreover, rice DNA transposon mPing (rice DNA transposon) could be activated after cold and salt stress [26,27]. In addition, herbicides also affect plant growth and development [28]. Houba belongs to the Copia group and has 563 copies in the rice genome. Its total size is 6437 bp, including internal domains flanked by two LTR sequences (968 bp in length) [29]. Because of the high copy number, this retrotransposon is suitable for retrotransposon studies, especially in rice [30,31].

The mutagenic effects caused by herbicides or various chemicals are generally tested cyogenetically. On the other hand, some effects at the DNA level could not be detected with cyrogenetic tests [32]. Molecular markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) have been commonly used to detect genotoxic and mutagenic effects at the DNA level [33–36]. However, the inter-retrotransposon amplified polymorphism (IRAP) marker technique has some advantages as compared to these markers. IRAP is better suited to revealing large changes in the genome than other markers such as RFLP AFLP, and ISSR. Moreover, unlike RAPD, IRAP is reproducible, cheaper and more easily applicable than AFLP [30,37]. In this report, we aimed to show retrotransposon activation under herbicide application. Moreover, we tested that a plant-specific retrotransposon (Houba for rice) might be used for short-term mutagenicity test.

Materials and methods

Plant material and herbicide treatments

Rice seeds (Oryza sativa L. cv. Beser) were germinated in Petri dishes containing moist filter paper. At the 7th day after germination, commercially available herbicide that contains bentazone and MCPA (in a mixture of 400 and 60 g/L, respectively) was applied to the filter paper as 1% (4 μg/mL bentazone and 0.6 μg/mL MCPA) and 2% (8 μg/mL bentazone and 1.2 μg/mL MCPA) concentrations for 7 days. Experiments were carried out in three groups: control (untreated), 1% and 2% herbicide treatments (Figure 1).

DNA isolation and IRAP analyses

Roots and leaves of three seedlings from each group were harvested and gDNAs were isolated according to Pervaiz et al. [38]. The quality and quantity of the gDNA samples were measured by Nanodrop 2000c and the concentrations were equalised to 10 ng/μL. IRAP primers were originally designed by our research group based on the accession number AFS37365.1 obtained from the National Center for Biotechnology Information (NCBI). The primer sequences are Houba F: 5’-CTTCGAGGGGCTAAGGGCCC-3’ and Houba R: 5’-GTTCGACCAAGCGCAGGGTC-3’. Polymerase chain reaction (PCR) was performed with Sapphir-eAmp Fast PCR Master Mix (Takara, RR350A) in a total volume of 20 μL, containing 20 ng of template DNA, 10 pmol/μL forward and reverse primers and PCR-grade distilled water. The PCR conditions were as follows: one cycle of initial denaturation at 94 °C for 2.5 min followed by 30 cycles of 94 °C for 30 s, 51 °C for 30 s and 72 °C for 3 min. The reactions were completed with one cycle of final extension at 72 °C for 7 min. The PCR products were run in 8% polyacrylamide (29:1 Acrylamide: Bis) gel in 1 x TBE buffer at 200 V for 5 h. Gels were stained in 1 x TBE buffer containing 0.5 μg/mL ethidium bromide and photographed on a UV transilluminator.

Estimation of genomic polymorphism

IRAP bands were scored visually and polymorphism ratios were calculated by Jaccard’s coefficient [39]. The significance of polymorphism ratios was evaluated by using one-way analysis of variance (ANOVA) against the null hypothesis (Null hypothesis: Bentazone and MCPA...
treatments do not cause retrotransposon polymorphism). Differences were considered statistically significant at a probability level of $p < 0.05$. Samples were classified into three groups as control, 1% and 2% treatments. Further, ANOVA statistical test was performed to compare statistical significance among groups depending on the homogeneity of variance.

Results and discussion

Our Houba IRAP analyses resulted in 24 bands ranging from $\sim 250$ to $\sim 1500$ bp (Figure 2). The polymorphism ratios calculated with Jaccard’s coefficient by using these band profiles are presented in Table 1. In all samples, the polymorphism ratios ranged from 8% to 90%. Because of this high polymorphism range, we evaluated all individuals within each group (control, 1% or 2% herbicide–treated groups).

In the control group plants, the polymorphism ratios were variable (from 8% to 39%). When we compared the leaves derived from different individuals, the polymorphism rates were 14% (between L1 and L2), 20% (between L2 and L3) and 31% (between L1 and L3). Moreover, in the root samples, we also observed polymorphism rates of 8% (between R1 and R2), 23% (between R1 and R3) and 39% (R2 and R3). Thus, we concluded that Beser variety of rice has a natural polymorphism among individuals for Houba retrotransposon. In addition to these polymorphism ratios, the roots and leaves of the same plant also showed different band profiles. The polymorphism rates were 33%–37% in these tissues, indicating that each tissue could have its own retrotransposon profile. Therefore, it is proposed that retrotransposons might have a role for organ differentiation. Slotkin et al. [40] studied the transposable elements (TE) in Arabidopsis thaliana pollen and reported a reprogramming role of TE during plant development. Another experiment was performed by Fernandez et al. [41] and they concluded that cis–activation processes caused by class–II transposable elements in grapevine plants could be a mechanism to produce somatic cell variation. Our results were supported by these experiments. Moreover, these findings are also consistent with our previous studies. In those studies, we analysed barley roots and leaves originating from the same plant and observed different IRAP profiles in the samples [42,43]. Although variable polymorphism ratios were observed in the control plants, these polymorphism ratios were found as insignificant in the ANOVA test.

The polymorphism rates in the 1% herbicide-treated samples (from 24% to 57%) were clearly higher than that in the control samples. In the 1% herbicide-treated plants, similar to the control plants, the individuals and the tissues of the same plant had polymorphic bands. When the control samples were compared with the 1% herbicide-treated samples, the highest polymorphism

![Figure 2. IRAP-PCR results of Houba; L, leaf; R, root; C (--), PCR negative control. Numbers indicate individual plants.](image)

| Table 1. Houba retrotransposon polymorphism ratios among groups calculated with Jaccard’s coefficient. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Control | 1% Treatment | 2% Treatment |  |
|  | L1 | R1 | L2 | R2 | L3 | R3 | L1 | R1 | L2 | R2 | L3 | R3 | L1 | R1 | L2 | R2 | L3 | R3 | L1 | R1 | L2 | R2 | L3 | R3 |
| L1 | – | 34 | 14 | 34 | 31 | 37 | 47 | 55 | 50 | 55 | 35 | 47 | 58 | 60 | 80 | 65 | 81 | 64 |
| R1 | – | 34 | 8 | 29 | 23 | 56 | 56 | 65 | 63 | 63 | 63 | 56 | 59 | 61 | 83 | 67 | 90 | 58 |
| L2 | – | 37 | 20 | 27 | 47 | 55 | 50 | 55 | 40 | 47 | 58 | 60 | 86 | 71 | 81 | 57 |
| R2 | – | 33 | 39 | 58 | 56 | 67 | 58 | 65 | 50 | 61 | 56 | 84 | 61 | 90 | 53 |
| L3 | – | 33 | 35 | 60 | 47 | 67 | 45 | 53 | 56 | 65 | 65 | 85 | 70 | 80 | 55 |
| R3 | – | 50 | 47 | 56 | 56 | 67 | 58 | 53 | 50 | 68 | 56 | 90 | 75 | 85 | 60 |
| L1 | – | 57 | 28 | 50 | 34 | 24 | 60 | 62 | 52 | 75 | 67 | 63 | 60 |
| R1 | – | 37 | 24 | 50 | 53 | 39 | 53 | 45 | 56 | 28 |
| L2 | – | 28 | 28 | 37 | 47 | 50 | 56 | 47 | 50 | 40 |
| R2 | – | 45 | 34 | 60 | 39 | 61 | 45 | 63 | 37 |
| L3 | – | 42 | 45 | 62 | 68 | 60 | 63 | 59 |
| R3 | – | 67 | 47 | 75 | 60 | 70 | 52 |
| L1 | – | 50 | 47 | 37 | 50 | 47 |
| R1 | – | 59 | 31 | 53 | 33 |
| L2 | – | 36 | 25 | 47 |
| R2 | – | 50 | 30 |
| L3 | – | 50 |

Note: L indicates leaf and R indicates root. Same numbers indicate leaves and roots belonging to one and the same plant. The experiment was repeated three times for each group.
ratio was 67%. The IRAP result of the 2% herbicide-treated plants was similar to that in the 1% group (from 25% to 59%) but the polymorphism ratios were higher than in the 1%-treated samples. These results suggest that that herbicide treatment could enhance the retrotransposon activities in rice with increasing concentration. Moreover, the highest polymorphism ratio was 90% between control and 2% herbicide-treated plants. The statistical analyses showed that the polymorphism ratios between the control and 1% plants were not significantly different. On the other hand, we found significant differences between the control and 2% herbicide-treated plants. These results showed that bentazone and MCPA-containing herbicides promoted Houba retrotransposon activities with increasing concentrations. Moreover, we compared 1% treatment with 2% by using ANOVA, indicating insignificant results.

Many tests have been developed to identify the harmful effects of chemicals in plants, animals, water etc. These tests are bioindicators to measure the pollution and also evaluate the effects of toxic and mutagenic substances in the environment [44] but most of them are time-consuming and costly. Short-term assays have been proposed as rapid response to evaluate carcinogens and/or mutagens [45]. Moreover, plant-based assays are regarded as short-term and low cost. Several plants such as Zea mays, Arabidopsis thaliana, Tradescantia and especially Allium cepa have been used and found to be as sensitive and reliable as other tests [46].

Short-term tests can be used not only for chemical agents (herbicides, pesticides etc.) but also for assessment of the effects of plants (especially medically important plants) on other organisms. For example, Tirloni et al. [47] studied the effects of Casearia sylvestris in Wistar rats by the MN test, comet assay and tumour marker analyses. They concluded that this plant did not cause any mutagenic and genotoxic effects or any alterations in important tumour markers.

On the other hand, Sharif et al. [48] analysed the genotoxic and mutagenic effect of Kalanchoe laciniata using Ames and MTT assays, revealing mutagenic and cytotoxic potential of the aqua-methanolic and n-hexane extracts of this plant. In addition to medicinally important plants, the effects of waste waters have been widely investigated. Radić et al. [49] investigated the surface and wastewater genotoxicity using the A. cepa test, revealing modifications of the A. cepa roots in addition to mitotic and chromosomal aberrations. Iqbal et al. [50] analysed petroleum refinery wastewater in terms of the cytotoxicity and mutagenicity in A. cepa and Ames tests, respectively. They proposed that A. cepa and Ames tests were very sensitive for the evaluation of toxicity before and after treatment. Similarly, A. cepa and comet assay were used for evaluation of genotoxic compounds into mineral water stored in polyethylene terephthalate bottles [51].

In addition to Allium test, the effects of pesticides and herbicides have been commonly analysed by using the MN test and comet assay on plants, fish and also bacteria [47,52,53]. However, these tests are generally used for the identification of herbicide effects. In this study, we investigated the potential effects of Benztazone herbicide on rice in terms of retrotransposon movements. Generally, the comet assay is considered to be more sensitive than piscine MN test [54–56]. As a result of our study, we conclude that retrotransposon movements could be more sensitive than other methods because of identification at the DNA level. Our results are in agreement with Dimitrov et al. [57], who reported that carcinogens increased the Ty1 retrotransposon mobility in Saccharomyces cerevisiae. Moreover, they concluded that the Ty1 short-term test showed positive responses. Similarly, Pesheva et al. [58,59] also demonstrated that chemical carcinogens also activate Ty1 retrotransposition. In plants, Finatto et al. [60] reported short-term responses of Nipponbare seedlings to iron excess, including activation of genes and also LTR retrotransposons. Based on all these reports, we suggested that the IRAP marker technique could be used for short-term mutagenicity tests with suitable retrotransposons to detect large changes at the DNA level in the plant genome.

Conclusions

We report that IRAP can be used to evaluate herbicide genotoxicity at the DNA level and is suitable for short-term mutagenicity tests in plants. For this purpose, specific retrotransposons found in a target genome should be chosen and retrotransposon-based molecular markers should be used. This study showed valuable data related to retrotransposon-based short-term mutagenicity test in rice with suitable retrotransposons such as Houba. In addition to rice, this technique could easily be applied to other plants. Our results are expected to contribute valuable knowledge about short-term mutagenicity test in terms of retrotransposons.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Kawahara Y, de la Bastide M, Hamilton JP, et al. Improvement of the *Oryza sativa Nipponbare* reference genome using next generation sequence and optical map data. *Rice*. 2013 [cited 2018 Apr 26];6:4. DOI:10.1186/1939-8433-6-4.

[2] Ereofeeva EA. Hormesis and paradoxical effects of wheat seedling (*Triticum aestivum L*) parameters upon exposure to different pollutants in a wide range of doses. *Dose-Response*. 2014;12:121–135.

[3] EFSA (European Food Safety Authority). Conclusion on the peer review of the pesticide risk assessment of the active substance bentazone. *EFSA J*. 2015 [cited 2018 Apr 26];13:4077. DOI:10.2903/j.efsa.2015.4077.

[4] EPA Reregistration Eligibility Decision (RED): Bentazon, EPA 738-R-94-029. Office of prevention, pesticides and toxic substances. Washington (DC): U.S. Environmental Protection Agency; 1994.

[5] Fleming AA, Banks PA, Legg JG. Differential response of maize inbreds to bentazon and other herbicides. *Can J Plant Sci*. 1988;68:501–507.

[6] Bradshaw LD, Barrett M, Poneleit CG. Physiological basis for differential bentazon susceptibility among corn (*Zea mays*) inbreds. *Weed Sci.* 1992;40:522–527.

[7] Flox C, Garrido JA, Rodriguez RM, et al. Mineralization of herbicide mecoprop by photoelectro-fenton with UVA and solar light. *Catal Today*. 2007;129:29–36.

[8] Vergili I, Barlas H. Removal of 2,4-D, MCPA and metalaxyl from water using lewatit VP OC 1163 as sorbent. *Desalinization*. 2009;249:1107–1114.

[9] Poll C, Pagel H, Devers-Lammani M, et al. Regulation of bacterial and fungal MCPA degradation at the soil-litter interface. *Soil Biol Biochem*. 2010;42:1879–1887.

[10] Bellet EM, Van Ravenzwaay B, Pigott G, et al. Chronic dietary toxicity and oncogenicity evaluation of MCPA (4-Chloro-2-methylphenoxyacetic acid) in rodents. *Regul Toxicol Pharm*. 1999;30:223–232.

[11] Elliott B. Review of the genotoxicity of 4-chloro-2-methylphenoxyacetic acid. *Mutagenesis*. 2005;20:3–13.

[12] Nabholz JV. Environmental hazard and risk assessment under the United States Toxic Substances Control Act. *Sci Total Environ*. 1991;109:649–665.

[13] Maron DM, Ames BN. Revised methods for the salmonella mutagenicity test. *Mutat Res*. 1983;113:173–215.

[14] Liman R, Akyil D, Eren Y, et al. Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/ *Salmonella* and *Allium* test. *Chemosphere*. 2010;80:1056–1061.

[15] Bianchi J, Fernandes TC, Marin-Morales MA. Induction of mitotic and chromosomal abnormalities on *Allium cepa* cells by pesticides imidaclopid and sulfentrazone and the mixture of them. *Chemosphere*. 2016;144:475–483.

[16] Turkoglu S. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutat Res*. 2007;1:4–14.

[17] International Rice Genome Sequencing Project. The mapped-based sequence of the rice genome. *Nature*. 2005;436:793–800.

[18] Tian Z, Rizzon C, Du J, et al. Do genetic recombination and gene density shape the pattern of DNA elimination in rice long terminal repeat retrotransposons? *Genome Res*. 2009;19:2221–2230.

[19] Wicker T, Sabot F, Hua-Van A, et al. A unified classification system for eukaryotic transposable elements. *Nat Rev Genet*. 2007;8:973–982.

[20] Alzohairy AM, Yousef MA, Edris S, et al. Detection of LTRretrotransposons reactivation induced by in vitro environmental stresses in barley (*Hordeum vulgare*) via RT–qPCR. *Life Sci*. 2012;9:5019–5026.

[21] Alzohairy AM, Gyulai G, Jansen RK, et al. Transposable elements domesticated and neo functionalized by eukaryotic genomes. *Plasmid*. 2013;69:1–15.

[22] Alzohairy AM, Sabir JSM, Gyulai G, et al. Environmental stress activation of plant long-terminal repeat retrotransposons. *Func Plant Biol*. 2014;41:557–567.

[23] Hirochiha H, Sugimoto K, Otsuki Y, et al. Retrotransposons of rice involved in mutations induced by tissue culture. *PNAS*. 1996;93:7783–7788.

[24] Cavrak VV, Lettnner N, Jamge S, et al. How a retrotransposon exploits the plant’s heat stress response for its activation. *PloS Genet*. 2014 [2017 Oct 03];10:e1004115. DOI:10.1371/journal.pgen.1004115.

[25] Ito H, Kim J-M, Matsunaga W, et al. A stress–activated transposon in *Arabidopsis* induces transgenerational abscisic acid insensitivity. *Sci Rep*. 2016 [2017 Oct 03];6:23181. DOI:10.1038/srep23181.

[26] Naito K, Zhang F, Tsukiyama T, et al. Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. *Nature*. 2009;461:1130–1134.

[27] Yasuda K, Ito M, Sugita T, et al. Utilization of transposable element as a novel genetic tool for modification of the stress response in rice. *Mol Breed*. 2013;32:505–516.

[28] Castro AJ, Carapito C, Zorn N, et al. Proteomic analysis of grapevine (*Vitis vinifera*) tissues subjected to herbicide stress. *J Exp Bot*. 2005;56:2783–2795.

[29] Vitte C, Panaud O, Quesneville H, LTR retrotransposons in rice (*Oryza sativa L*): recent burst amplifications followed by rapid DNA loss. *BMC Genom*. 2007 [2017 Oct 03];8:218. DOI:10.1186/1471-2164-8-218.

[30] Yüzbaşioğlu G, Yilmaz S, Gözukirmizi N. *Houba* retrotransposon-based molecular markers, tool for variation analysis in rice. *Turk J Agric For*. 2016;40:456–464.

[31] Yüzbaşioğlu G, Yilmaz S, Maraklı S, et al. Analysis of Hopi/ *Osr27* and *Houba/Tos5/Osr13* retrotransposons in rice. *Biotechnol Biotechnol Equip*. 2016;30:213–218.

[32] Khanna N, Sharma S. *Allium cepa* root chromosomal aberration assay: a review. *IJPBR*. 2013;1:105–119.

[33] Piraino F, Aina R, Palin L, et al. Air quality biomonitoring: assessment of air pollution genotoxicity in the province of Novara (North Italy) by using *Triolium repens* L. and molecular markers. *Sci Total Environ*. 2006;372:350–359.

[34] Abdelmigid HM. Qualitative assessment of cadmium stress using genome template stability in *Hordeum vulgare*. Egypt J Genet Cytol. 2010;39:291–303.

[35] Mutlu F, Mutlu B. Genotoxic effects of cadmium on tolerant and sensitive wheat cultivars. *JEB*. 2015;36:689–694.

[36] Sobieh SS, Kheirallai ZMH, Rushdy AA, et al. *In vitro* and *in vivo* genotoxicity and molecular response of silver nanoparticles on different biological model systems. *Caryologia*. 2016;69:147–161.

[37] Schulman AH, Flavell AJ, Paux E, et al. The application of LTR retrotransposons as molecular markers in plants. *Methods Mol Biol*. 2012;859:115–153.
[38] Pervaiz ZH, Turi NA, Khaliq I, et al. A modified method for high-quality DNA extraction for molecular analysis in cereal plants. Genet Mol Res. 2011;10:1669–1673.

[39] Jaccard P. Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat. 1908;44:223–270.

[40] Slotkin RK, Vaugh M, Borgers F, et al. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell. 2009;136:461–472.

[41] Fernandez L, Torregrosa L, Segura V, et al. Transposon-induced gene activation as a mechanism generating cluster shape somatic variation in grapevine. Plant J. 2010;61:545–557.

[42] Maraklı S, Yılmaz S, Gozukırmızı N. BARE1 and BAGY2 retrotransposon movements and expression analyses in developing barley seedlings. Biotechnol Biotechnol Equip. 2012;26:3451–3456.

[43] Cakmak B, Maraklı S, Gozukırmızı N. SIRE1 retrotransposons in barley (Hordeum vulgare L.). Russ J Genet. 2015;51:661–672.

[44] Matsumoto ST, Mantovani MS, Malagutti MI, et al. Assessment of the genotoxic and mutagenic effect of chromium residues present in tannery effluents using the micronucleus and comet assay in Oorechomis niloticus and chromosomes aberrations in of Allium cepa. Genet Mol Biol. 2006;29:148–158.

[45] Bajpayee M, Pandey AK, Parmar D, et al. Current status of short-term tests for evaluation of genotoxicity, mutagenicity, and carcinogenicity of environmental chemicals and NCEs. Toxicol Mech Methods. 2005;15:155–180.

[46] Grant WF, Zinov’eva-Stahevitch AE, Zura KD. Plant genetic test systems for the detection of chemical mutagens. In: Stich H, San R, editors. Short-term tests for chemical carcinogens topics in environmental physiology and medicine. New York (NY): Springer-Verlag; 1981. p. 200–216.

[47] Tirloni CAS, Traesel GK, Livero FAR, et al. Short-term carcinogenesis evaluation of Casearia sylvestris. Rev Bras Farmacogn. 2017;27:603–610.

[48] Sharif A, Akhtar MF, Akhtar B, et al. Genotoxicity and cytotoxic potential of whole plant extracts of Kalanchoe laciniata by Ames and MTT Assay. EXCLI J. 2017;16:593–601.

[49] Radić S, Stipaničev D, Vujčić V, et al. The evaluation of surface and wastewater genotoxicity using the Allium cepa test. Sci Total Environ. 2010;408:1228–1233.

[50] Iqbal M, Nisar J, Adil M, et al. Mutagenicity and cytotoxicity evaluation of photo-catalytically treated petroleum refinery wastewater using an array of bioassays. Chemosphere. 2017;168:590–598.

[51] Ceretti E, Zani C, Zerbini I. Comparative assessment of genotoxicity of mineral water packed in polyethylene terephthalate (PET) and glass bottles. Water Res. 2010;44:1462–1470.

[52] de Castilhos Ghisi N, Cestari MM. Genotoxic effects of the herbicide Roundup® in the fish Corydoras paleatus (Jenyns 1842) after short-term, environmentally low concentration exposure. Environ Monit Assess. 2013;185:3201–3207.

[53] Franco-Bernardes MF, Rocha OP, Pereira LC, et al. The herbicides trifluralin and tebuthiuron have no genotoxic or mutagenic potential as evidenced by genetic tests. Environ Sci Pollut Res. 2017;24:24029–24037.

[54] Hartmann A, Elhajouji A, Kiskinis E, et al. Use of the alkaline comet assay for industrial genotoxicity screening: comparative investigation with the micronucleus test. Food Chem Toxicol. 2001;39:843–858.

[55] Dixon DR, Pruski AM, Dixon LR, et al. Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis. 2002;17:495–507.

[56] Klobucar GV, Pavlica M, Erben R, et al. Application of the micronucleus and comet assays to mussel Dreissena polymorpha haemocytes for genotoxicity monitoring of freshwater environments. Aquat Toxicol. 2003;64:15–23.

[57] Dimitrov M, Venkov P, Pesheva, M. The positive response of Ty1 retrotransposition test to carcinogens is due to increased levels of reactive oxygen species generated by the genotoxins. Arch Toxicol. 2011;85:67–74.

[58] Pesheva M, Krastanova O, Staleva L, et al. The Ty1 transposition assay: a new short-term test for detection of carcinogens. J Microbiol Methods. 2005;61:1–8.

[59] Pesheva M, Krastanova O, Stamenova R, et al. The response of Ty1 test to genotoxins. Arch Toxicol. 2008;82:779–785.

[60] Finatto T, de Oliveira AC, Chaparro C, et al. Abiotic stress and genome dynamics: specific genes and transposable elements response to iron excess in rice. Rice. 2015 [2017 Oct 03];8:13. DOI:10.1186/s12284-015-0045-6.