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In vitro screening of gamma rays induced mutant population in rice for peg-induced drought stress

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Abstract
The effects of gamma irradiation on rice genotype Rajendra Mahsuri 1 for drought tolerance capability were investigated. In this study a system for *in vitro* Screening of gamma induced mutant rice lines were optimized using polyethylene glycol (PEG-6000). Exposure to PEG-6000 solutions has been effectively used to mimic drought stress. The effect of different concentration of PEG-6000 (0%, 5%, 10%, 15% and 20%) on seed germination of wild type of Rajendra Mahsuri 1 were studied. The decrease in onset and rate of germination followed by their growth declines with increasing concentration of PEG-6000. The optimum concentration of PEG-6000 for screening irradiated Rajendra Mahsuri-1 lines was found to be 15%. The germination followed by survival of mutant lines was evaluated on 15% PEG-600. Five M2 lines each of different doses of γ-irradiation *viz.* 350 *Gy*, 400 *Gy*, 450 *Gy* and 500 *Gy* were used for *in vitro* screening. The maximum survival % of seedlings were observed in 450 *Gy* followed by 400 *Gy*. The survival % of seedlings on 350 *Gy* and 500 *Gy* were found to be less than 10%. Mutant rice line differed greatly in terms of germination followed by survival on 15% PEG-6000 which can be correlated their tolerance to drought stress.

Keywords: *Oryza sativa* L. gamma irradiation, rajendra mahsuri 1, peg-6000, drought tolerance

Introduction
Rice (*Oryza sativa* L.) is the staple food in approximate thirty three developing countries. It provides 27% of dietary energy and 20% of dietary protein in the developing world. It is estimated that the world needs to produce 40% more rice to feed the population by 2025. Rice productivity and quality are severely compromised by various abiotic and biotic stresses. Among different abiotic stresses, drought stress frequently limits rice production worldwide.
Rice is a moisture-hungry crop. It consumes twice the water needed to grow corn or wheat. Producing 1 kg of rice requires from 3,000 to 5,000 L of water (Cantrell and Hettel, 2004) [5]. By 2025, however, a “physical water scarcity” is expected in Asia’s more than 2 million hectares (Mha) of irrigated dry-season rice and 13 Mha of irrigated wet-season rice, and most of Asia’s 22 Mha of irrigated dry-season rice will be hampered by “economic water scarcity” (Tuong and Bouman, 2002) [24]. The ongoing climatic change process is likely to further worsen the scenario in these rice growing areas (Swamy *et al*, 2012) [21]. As drought is one of the main constraints to high yields also in rainfed-production systems in both the lowlands and the uplands, there is a need to develop new varieties of rice which can withstand drought with minimum or no yield penalties. The development of new rice genotypes tolerant to drought stress will increase and stabilize yield, and could save water (Zhou *et al*. 2006) [29].
The availability of landraces represents a powerful source of adapted drought tolerance genes donors for breeding (Kumar *et al.* 2009; Thomson *et al.* 2010) [12, 22]. Unfortunately, these genotypes, normally, have many undesirable agronomic traits and low yield potential (Thomson *et al.* 2010) [22]. The responses to environmental stress in plants are complex and multigenic, and the functions of many induced genes are still a matter of conjuncture (Bray, 2002) [4]. Because of this complexity, selection and breeding of drought-tolerant varieties are extremely difficult (Anami *et al*, 2009; Tirado and Cotter, 2010) [2, 23]. Induction of mutations by various mutagens is one way to generating large number of variability and has been successfully employed in rice (Kadhimi *et al*., 2016) [1]. But mutant screening involves the evaluation of a large number of mutant plants to identify the rare mutant individuals that meet the desired trait. The screening for drought tolerance under field condition is time consuming and labour intensive. Therefore, there is an urgent need to develop a simple and effective early screening method (Kim *et al*. 2001) [28]. For drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, such as polyethylene glycol (PEG) (Money 1989; Rao and Jabeen 2013; Turkan *et al*. 2005). [14, 19 25] These agents have no detrimental or toxic effects on the plant.

They inhibit the plant's growth by reducing the water potential in a way similar to soil drying (Bressan *et al*., 1981) [19]. In present investigation, an attempt was made to optimize the concentration PEG-6000 for *in vitro* screening of Rajendra Mahsuri 1, a mega indica rice variety of Bihar, India. Further, the developed methods were used to screen gamma irradiated rice lines

Materials and Methods

Plant materials

Seeds of Rajendra Mahsuri 1, a mega rice variety of Bihar, were irradiated with gamma rays (350 Gy, 400 Gy, 450 Gy and 500 Gy). Irradiation was undertaken at the Bhabha Atomic Research Centre (BARC), Trombe, and Mumbai. Gamma irradiated seeds (M_1) were shown in rice filed to obtained M_2 lines. None irradiated seeds of Rajendra Mahsuri 1were used as control.

Optimization of PEG-600 for *in vitro* screening for drought tolerance

Dehusked seeds of non irradiated Rajendra Mahsuri 1 were surface sterilized as described previously (Prasad *et al*., 2016). Surface sterilized seeds were placed in a petriplates containing two layers of Whatman filter paper moistened with four different concentration of PEG-6000 (5%, 10%, 15% and 20%). Seeds growing only on water were considered as control. Survival of germinated of seeds were recorded at 7 days after sowing. The length of shoots was observed at 10 days after sowing. All the experiments were repeated three times.

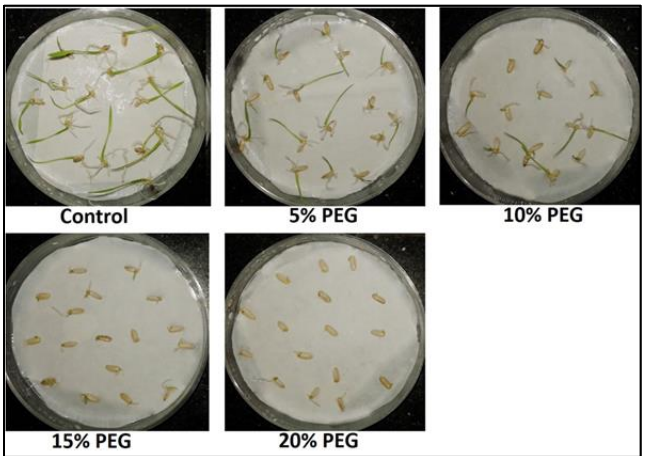

Fig 1: Survival of germinated seed on different concentration of PEG-6000 after 7 days of treatment

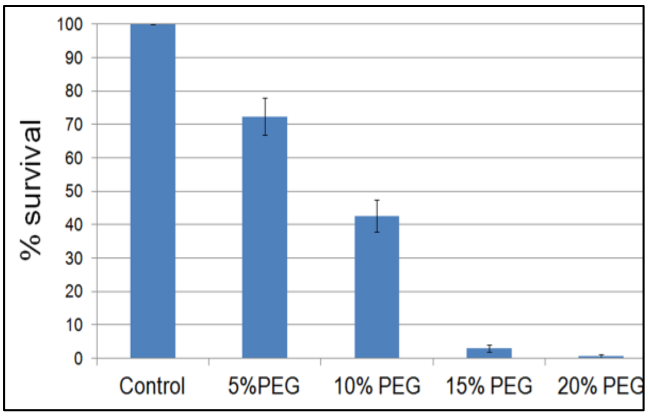

Fig 2: Optimization of PEG-6000 concentration for *in vitro* screening of drought tolerance in Rajendra Mahsuri 1

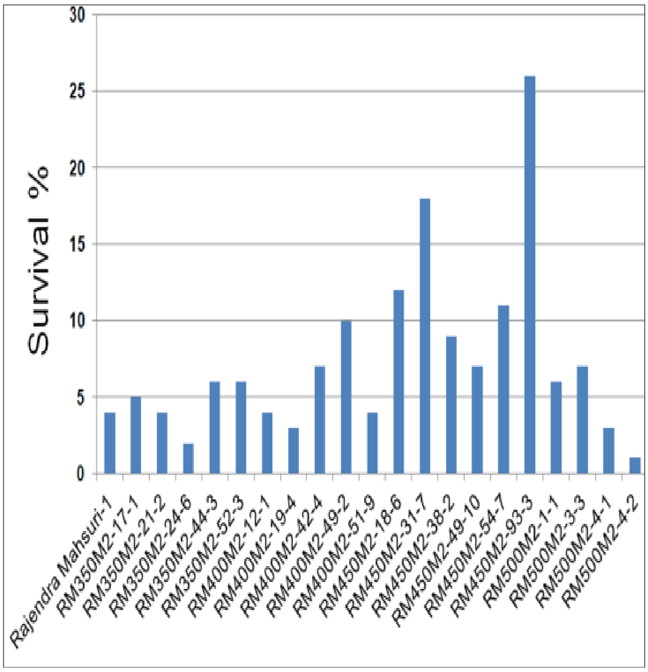

Fig 3: *In vitro* screening of gamma irradiated mutant rice lines

In vitro screening for drought tolerance

Dehusked seeds of M2 rice lines along with control Rajecdra Mahsuri 1 were germinated on 15% PEG-6000. Survival of germinated of seeds were recorded at 7 days after sowing.

Result

Optimization of PEG-6000 concentration for *in vitro* screening for drought tolerance

Optimization of correct doses of PEG-6000 is prerequisite for *in vitro* screening for drought tolerance in rice. Non irradiated seeds of Rajendra Mahsuri 1 were germinated on different concentration of PEG-6000. Seeds germinated on water were considered as control. The data of survival were taken on 7days after sowing. The survival % of germinated seeds decreased as concentration of PEG-6000 was increased. All the germinated seeds were survived are growing happily on water control (Figure 1, 2). The survival % varied differently on varying concentration of PRG-6000. Survival % was found to be 72.33%, 42.67 % and 6.33 at 5 %, 10 % and 15 %, respectively (Figure 1, 2). However, all the seeds were died in 20% of PEG-6000 (Figure 1, 2). Therefore, 15% PEG-6000 concentration were used in *in vitro* screening of rice mutant lines.

In vitro screening for drought tolerance

The *in vitro* screening of M2 populations was carried out by germinating seeds on PEG-6000 and analyzing the survival % as described in methods. Five M2 lines each of different doses of γ-irradiation *viz.* 350 Gy, 400 Gy, 450 Gy and 500 Gy were used for *in vitro* screening. The maximum survival % of seedlings were observed in 450 Gy followed by 400 Gy (Figure 3). The survival % of seedlings on 350 Gy and 500 Gy were found to be less than 10% (Figure 3).

Discussion

Drought is one of the most common environmental stresses that affect growth and development of plants which continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. Mutation breeding is an effective technique to increase resilience to drought in crops grown in drought-prone Countries. However, screening for drought tolerance under field condition is time consuming, labour intensive and the environmental influences that affect phenotypic expression of genotype. The *in vitro* screening method proves to be an ideal method to screen large set of germplasm with less effort, accurately and the growth pattern differences are due to genotypes with least environmental influences. Further, field screening requires full season field data and it's not always convenient or efficient, hence need to have simple and effective early screening method (Kim *et al*. 2001) [28].

The *in vitro* screening method using PEG has been proved to be very effective method for studying the effect of water stress on seed germination and seedling growth characters (Hadas, 1976; Aquila *et al*., 1984; Kim et al., 2001; Van den Berg and Zeng, 2006; Radhouane, 2007) [7, 3, 28, 27, 18] and simple cost effective method to screen large set of germplasm within very less time period and accurately (Kim *et al*., 2001; Kulkarni and Deshpande, 2007) [28, 11]. Several methods have been developed to screen drought tolerant germplasm in plant species. Based on the literature available, PEG is considered as a superior chemical to induce water stress (Kaur *et al*, 1998). Polyethylene glycol (PEG) molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress in crop plants (Carpita *et al*, 1979 [6]; Turkan *et al*, 2005 [25]; Landjeva *et al*, 2008 [13]; Rauf *et al*, 2006) [20]. One of the important speculations is that a positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments was noted (Kosturkova *et al*, 2014) [10].

In present investigation, standardization of optimal concentration of PEG-6000 for induction of artificial drought was carried out. The optimization of PEG concentration is crucial as it varies with the crop and genotypes (Kulkarni *et al*., 2007). In our study 15% PEG-6000 concentration was found to be optimum for Rajendra Mahsuri 1 rice genotype.

In this study, the reduction in germination followed by survival % with increase PEG was observed. Similar results like reduction in germination rate with the increase PEG were noted in different crops (Kadhimi *et al*., 2016; Basha *et al*., 2015; Kaur *et al*, 1998) [1, 15, 9]. The artificial induction of drought using PEG however is dependent on the concentration and varies with the crop and genotype.

The γ-irradiated genotypes (450 *Gy*) showed the maximum values of survival % at 15% PEG-6000. However, the minimum survival % was observed in non-irradiated seeds on 15% PEG-6000. Therefore, γ-irradiation has been widely used widening genetic variability (Jan *et al*., 2013) leading to either improving or developing stress tolerance.

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References

1. Kadhimi AA, ALhasnawi AN, Isahak A, Ashraf MF, Mohamad A, Mohtar WWY. *et al*. Gamma radiosensitivity study on MRQ74 and MR269, two elite varieties of rice (Oryza Sativa L.) Life Sci J. 2016; 13(2):85-91.
2. Anami S, Block M, Machuka J, Lijsebettens M. Molecular improvement of tropical maize for drought stress tolerance in Sub-Saharan Africa. Crit Rev Plant Sci. 2009; 28:16-35.
3. Aquila AD, Pignone D, Carella G. Polyethylene Glycol 6000 Priming Effect on Germination of Aged Wheat Seed Lots. Biologia Plantarum. 1984; 26:166-173.
4. Bray EA. Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. Plant Cell Environ. 2002; 25(2):153-161.
5. Cantrell RP, Hettel GP. New Challenges and Technological Opportunities for Rice-Based Production Systems for Food Security and Poverty Alleviation in Asia and the Pacific. Presented at the FAO Rice Conference, FAO, Rome, Italy. 2004, 12-13.
6. Carpita N, Sabularse D, Monfezinos D, Delmer DP. Determination of the pore size of cell walls of living plant cells. *Sci*. 1979; 205:1144-1147.
7. Hadas A. Water uptake and germination of leguminous seeds under changing external water potential in osmoticum solution. J Exp Bot. 1976; 27:480-489.
8. Jan ST, Parween R, Hameed TO, Siddiqi, Mahmooduzzafar. Effects of presowing gamma irradiation on the photosynthetic pigments, sugar content and carbon gain of *Cullen corylifolium* (L.) Medik. Chilean J Agric Res. 2013; 73:345-350.
9. Kaur S, Gupta AK, Kaur N. Gibberellic acid and kinetin partially reverse the effect of water stress on germination and seedling growth in chickpea. Plant Growth Regul. 1998; 25:29-33.
10. Kosturkova G, Todorova R, Dimitrovai M, Tasheva K. Establishment of Test for Facilitating Screening of Drought Tolerance in Soybean. Series F. Biotechnologies. 2014, 18.
11. Kulkarni M, Deshpande U. *In Vitro* Screening of Tomato Genotypes for Drought Resistance Using Polyethylene Glycol. Afric J Biotechnol. 2007; 6(6):691-696.
12. Kumar A, Verulkar S, Dixit S, Chauhan B, Bernier J, Venuprasad R. *et al*. Yield and Yield-attributing Traits of Rice (Oryza sativa L.) under Lowland Drought and Suitability of Early Vigor as a Selection Criterion. Field Crops Res. 2009; 114(1):99-107.
13. Landjeva S, Neumann K, Lohwasser U, Borner A. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol. Plantarum. 2008; 52:259-266.
14. Money NP. Osmotic pressure of aqueous polyethylene glycols: relationship between molecular weight and vapor pressure deficit. Plant Physiol. 1989; 91:766-769.
15. Basha PO, Sudarsanam G, Madhu M, Reddy S, Sankar NS. Effect of peg induced water stress on germination and seedling development of tomato germplasm. Int J Recent Sci Res. 2015; 6(5):4044-4049.
16. Prasad BD, Kumar P, Sahni S, Kumar V, Kumari S, Kumar P. *et al*. An improved protocol for agrobacterium-mediated genetic transformation and regeneration of indica rice (*Oryza sativa* L. var. rajendra kasturi). *J Cell Tissue Res*. 2016; 16(2):5597-5606.
17. Bressan RA, Hasegawa PM, Handa AK. Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Sci Lett. 1981; 21:23-30.
18. Radhouane L. Response of Tunisian autochthonous pearl millet (Pennisetum glaucum (L.) R. Br.) to drought stress induced by polyethylene glycol (PEG) 6000. Afric J Biotechnol. 2007; 6:1102-1105.
19. Rao S, Jabeen FTZ. *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (*Saccharum*

officinarum L.). Physiol Mol Biol Plants 2013; 19:261-268.
20. Rauf M, Munir M, Hassan M, Ahmad M, Afzal M. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. 2006; 6:971-975.
21. Swamy BPM, Kumar A. Sustainable rice yield in water-short drought-prone environments: Conventional and molecular approaches. In: Lee T S. Irrigation Systems and Practices in Challenging Environments. German: In Tech. 2012.
22. Thomson MJ, Ismail AM, Mc Couch SR, Mackill DJ. Marker Assisted Breeding. In Abiotic Stress Adaptation in Plants Pareek, A., S. K. Sopory and H. J. Bohnert (eds.), Netherlands: Springer. 2010; 451-469.
23. Tirado R, Cotter J. Ecological farming: Drought-resistant agriculture. In: Erwood S, Truchi N, Stabinsky D. Greenpeace Research Laboratories Technical Note. Amsterdam, Netherland: the Netherlands Greenpeace International. 2010.
24. Tuong TP, Bouman BAM. Rice production in water-scarce environments. To be published in proceedings ofthe Water Productivity Workshop, 12-14 November 2001, International Water Management Institute, Sri Lanka. 2002.
25. Turkan I, Bor M, Ozdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci. 2005; 168:223-231.
26. Turkan I, Bor M, Zdemir F, Koca H. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant P. acutifolius and drought-sensitive P. vulgaris L subjected to polyethylene glycol mediated water stress. Plant Sci. 2005; 168:223-231.
27. Van den Berg L, Zeng YJ. Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. Afr J Bot. 2006; 72:284-286.
28. Young-Jin Kim, Shanmugasundaram S, Song-Joon Yun, Ho-Ki Park, Moon-Soo Park A. Simple Method of seedling screening for drought tolerance in soybean. Korean J crop Sci. 2001; 46(4):284-288.
29. Zhou L, Liu Z, Li Y, Shunwu Yu, Hanwei Mei, Xiaoyan Xu. *Et al*. novel gene OsAHL1 improves both drought avoidance and drought tolerance in rice. Sci Rep. 2016, 1-15.