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Electron beam irradiation revealed genetic differences in radio-sensitivity and generated mutants in groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Electron beam accelerators are being used for many industrial applications including food and agriculture. A 10 MeV linear accelerator facility was standardized for low dose application 0.1–1 kGy) in pulse mode using unscanned scattered beam for irradiation of groundnut seeds for mutation breeding. Using this facility, 50% growth reduction (GR_{50}) dose was standardized in five groundnut genotypes. There were significant differences for radio-sensitivity among these genotypes. Seed mutagenesis of two groundnut genotypes, TG 26 and TG 68 with electron beam has generated one large seeded and four high yielding mutants in preliminary field trials.

1. Introduction

Ionizing radiation-induced mutation breeding has been an efficient tool for crop improvement. Globally, the use of induced mutants (obtained from both physical and chemical mutagens) in plant breeding has resulted the official release of 3220 improved mutant varieties in almost 210 different crop species (Mutant Variety Database (MVD) available at https://www.mvd.iaea.org; Bado et al., 2015). Radiation mutation breeding includes the use of X rays, beta rays, gamma rays, ion beam, laser, neutrons and electron beam. Among these, gamma rays were extensively used for induced mutation breeding in crop plants and have resulted in the release of around 50% of the above mentioned crop varieties. Recently, high power linear electron accelerators in the range of 500 keV – 10 MeV energy have gained importance for various applications that include surface irradiation, food preservation, medical sterilization, cross linking of polymers, graft polymerization etc. (IAEA-TECDOC, 2004; IAEA, 2008; Mittal, 2012). These accelerators work in switch-on-off mechanism like in X-ray facilities. It produces electron beam, which can be used for irradiating materials in a high-throughput manner. Gamma irradiators are permanent source of radiation. Whereas, electron accelerator have prompt radiation which can be stopped by switching off the source of electrons and radio frequency power (Mittal, 2012). A similar linear electron accelerator based facility is presently operational at Raja Ramanna Centre for Advance Technology (RRCAT), Indore, India. It is being used for various industrial high dose applications including food irradiation (http://www.rrcat.gov.in/technology/accel/mal/arpf.html). The same facility is used in the present study at low dose for studying mutagenesis and radio-sensitivity in groundnut genotypes.

Groundnut is an important oilseed crop extensively grown for food, feed and oil purpose in India. It is an allotetraploid (AABB, 2n = 4x = 40) crop plant and originated from a natural cross event between *Arachis duranensis* (A genome donor) and *Arachis ipaensis* (B genome donor) followed by chromosome doubling (Moretzsohn et al., 2004). Because of this, along with its highly self pollinated nature, the genetic base of groundnut crop is very narrow. Thus the radiation induced mutation breeding has been used since decades to broaden the genetic variability (Badigannavar et al., 2002; Badigannavar and Mondal, 2007). Several ionizing radiation sources like X rays (Gregory, 1955; Bora et al., 1961), gamma rays (Mouli and Patil, 1976), neutrons (Shivraj et al., 1962; Wang et al., 2015) and laser (Bozhan et al., 1997) were used in groundnut to induce genetic variability and to develop novel mutants and varieties. Use of electron beam in mutation breeding was limited due to the availability of the source and particularly the problem of getting low dose delivery unit for mutation breeding applications. Previously, electron beam was used to induce genetic variability in rice (Guo et al., 1982), barley (Xu et al., 1983), soybean (Li et al., 1988), sorghum (Lu et al., 1995) and adzuki bean (Luo et al., 2012). Till now, no reports are available towards the use of electron beam for induced mutagenesis in groundnut.

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Optimization of absorbed dose is the first step in radiation induced mutation breeding programme for any crop plants. Mba et al. (2010) suggested that optimal dose should be the one that achieves the optimum mutation frequency. Tshilenge-Lukanda et al. (2012) described that the optimum dose can be determined by recording percentage of seed germination, epicotyl and hypocotyl length and /or seedling height. In epigeal germination of dicot plants like groundnut, the total seedling height can be taken as an indicator of genotypic response to a mutagen (Konzak et al., 1972; Kodym et al., 2011). Present study explores the possibility of the use of linear electron accelerator for induced mutagenesis in groundnut (*Arachis hypogaea* L.). The study was undertaken in five groundnut genotypes to determine optimal dose of electron beam and to ascertain their radiosensitivity to electron beam irradiation in comparison to gamma rays. Further, effectivity of electron beam in generating desirable mutants was discussed.

2. Materials and methods

2.1. Groundnut genotypes

Well dried seeds (7 – 10% moisture content) of five genotypes, TAG 24, TG 26, TG 51, TG 68 and TG 69 were taken for the present study. Of them, TAG 24, TG 26 and TG 51 are mutant varieties and registered in MVD (IDs 1575, 1580 and 2938, respectively). The seeds were multiplied at experimental field facility, BARC, Trombay, Mumbai. TAG 24, TG 26 and TG 51 are high yielding varieties with desirable traits and are commercialized across the country. TG 68 and TG 69 are disease resistant breeding lines with better yield potential. These genotypes were bred at Bhabha Atomic Research Centre (BARC), Mumbai. The seeds were kept in a dessicator containing 60% glycerol/water mixture for 10 days at room temperature to ascertain the uniform level of moisture in seeds. Uniform seeds from each genotype were chosen separately for electron beam and gamma ray irradiation.

3. Electron beam facility

The experimental irradiation facility at RRCAT, Indore, India is based on 10 MeV electron linac. This was operated in electron mode in energy range of 7–10 MeV and in X-ray mode at 7.5 MeV with varying power levels up to 3 kW. The linac was mounted in horizontal configuration with a fast current transformer at the exit to monitor the accelerated beam current coming out from the linac. A vacuum chamber (scanner) was installed at downstream. A thin titanium foil (thickness $\sim$ 50 μm) at exit end of the scanner served as the beam window for transporting the electrons from vacuum to atmosphere. The accelerated electron beam has a diameter of $\sim$ 20 mm at the exit of the beam window. To deliver dose to the process load, usually the electron beam was scanned inside a scanning horn and uniform dose was achieved at higher dose level (> 500 Gy). However, the seed samples required comparatively lower dose (50 − 600 Gy) with precise control and better resolution. The conventional beam scanning with product transportation through conveyor movement was not found suitable for this case. To meet the above requirements, the accelerator was operated at nominal energy of 8.5 MeV with 200 mA beam current, 5 Hz pulse per repetition (PPR) with reduced pulse width of 5 μs in un-scanned condition. Beam scattering foils were placed in beam path to get uniform radiation field in a reference plane, at 80 cm away from the window foil. Seeds of different groundnut genotypes packed in small plastic packets were placed in uniform radiation field and were irradiated to single seed thickness (approx. 7–10 mm). The seeds were irradiated with varying absorbed doses 50, 100, 200, 300, 400, 500 and 600 Gy. Control seeds were kept outside the accelerator facility.

4. Electron beam radiation field characterization and dosimetric measurements

Before conducting the electron beam irradiation, characterization of radiation field (measurement of uniform field width, beam energy etc.) was carried out by conducting dosimetric measurements in accordance with ISO/ASTM 51649 (2005). GEX, USA make radio-chromic film dosimetry system consisting of Genesys 20 spectrophotometer (ThermoFisher Scientific, Waltham, USA) with calibration standard set (calibration traceable to NIST), B3 radiochromic films, gafchromic films and film heat treatment system, were used for dose measurement as per the procedure recommended in ISO/ASTM 51275 (2002). Response of these films was linear in the dose range 1 − 100 kGy (B3 film) and 1 − 1000 Gy (Gafchromic film).

For irradiation of seed samples at low dose, scattering technique with un-scanned beam (as mentioned above) was used to get uniform radiation field for the seed sample packed in small packets. An online dose monitoring system was installed at the exit of the linac to deliver precise dose delivery to the seed samples in stationary state. Pulse by pulse current signal was then integrated and monitored by downstream electronics and software (Petwal et al., 2013). The integrated value of the charge over a period of time, termed as 'Monitor Units' (MUs) were calibrated against the absorbed dose measured at reference plane. The absorbed dose at reference plane was measured by using the alanine EPR dosimetry system. Dose delivered by each pulse at reference plane was found to be 1.1 Gy/pulse. The pulse width was 5 μs. The instantaneous peak dose rate was significantly high $\sim 2.2 \times 10^5$ Gy/s. Time averaged dose rate value was found to be 330 Gy/min.

5. Gamma irradiation of groundnut seeds

Samples of groundnut seeds of TAG 24 and TG 51 were exposed to gamma radiation at doses of 50, 100, 150, 200, 300, 350, 400, 450 and 500 Gy in a ^{60}Co Gamma Cell 5000 irradiator (BRIT, Mumbai, India) at the dose rate of 40.9 Gy/min. Irradiated and non-irradiated samples were immediately used for further experiments. Dosimetry was carried out by cerric-cerrous sulphate dosimeter calibrated with Fricke's dosimeter.

6. Germination test of irradiated seeds and growth of seedlings

The biological effects of the electron beam or gamma rays treatment on groundnut seeds were studied based on radicle length during germination and shoot and root length at seven days after germination. Briefly, the experiment was set up in a completely randomized design with two replications. Standard germination test was practiced in sterile petri-plate containing moistened 3 mm-blotting paper. Two replications of 20 seeds were placed in petri-plate and incubated at room temperature (25 °C) in dark for 3 days. After taking germination percentage, radicle length was measured. The germinated healthy seedlings were then transferred to Gibson tube containing 0.5X Steinbergs solution. The tubes were kept again at dark for two days for root establishment and then transferred to growth chamber maintained at 29 °C, 90% relative humidity, 12 h light (with a gradient light intensity of 40 $\mu mol.m^{-2}.s^{-1}$ from 7.00 to 11.00 AM, 115 $\mu mol\ m^{-2}\ s^{-1}$ from 11.00 to 12.00 PM, 165 $\mu mol\ m^{-2}\ s^{-1}$ from 12.00 to 15.00 PM, 115 $\mu mol\ m^{-2}\ s^{-1}$ from 15.00 to 17.00 PM and 40 $\mu mol\ m^{-2}\ s^{-1}$ from 17.00 to 18.00 PM) and 12 h dark. Seedling height (epicotyl length + hypocotyl length) and root length were measured after five days of growth.

7. Mutation breeding for TG 26 and TG 68

Using the experiences from the above radiation sensitivity experiments, dry seeds (250 seeds for each treatment) of TG 26 and TG 68 were treated with 150, 200 and 250 Gy of electron beam to create