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Assessment of the Ploidy Level Diversity by Chloroplast Counts in Stomatal Guard Cells of Potato (*Solanum tuberosum* L.) Mutants

Emmy Chepkoech[1*], Miriam G. Kinyua[1], Oliver Kiplagat[1], Julius Ochuodho[2], Souleymane Bado[3], Zachary Kinyua[4], Stephen Kimno[1] and Michael Chelulei[1]

[1]Department of Biotechnology, University of Eldoret, Kenya.
[2]Department of Seed, Crop and Horticultural Sciences, P.O.Box 1125-30100, Eldoret, Kenya.
[3]Department of Plant Science, Phytoprise GmbH, Raimundgasse 25, A-7400, Oberwart, Austria.
[4]Kenya Agricultural, Livestock and Research Organization (KALRO) Headquarters, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EC, MGK, OK, JO, SB and ZK designed the study, authors EC, SK and MC performed the statistical analysis, authors EC and MGK wrote the protocol and authors EC, OK, JK, SB and ZK wrote the first draft of the manuscript. Authors EC and SK managed the analyses of the study. Authors EC, SK and MC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Potato (*Solanum tuberosum* L.) is the most important staple food in the world and plays an important role in food and nutritional security. Induced mutation generates variation within potato germplasm to widen the genetic base for breeding purposes. Polyploidy modifies both the genotype and phenotype of an organism, generating diverse changes that consequently transform the potato production. Potato has chromosomes with different ploidy levels which can be determined by counting chloroplasts in stomatal guard cells.
Study Design: The study was carried out in completely randomized block design.
Place and Duration of Study: Department of Biotechnology, University of Eldoret, between February 2015 and July 2016.

**Corresponding author: E-mail: emychepkoech@gmail.com;*

Methodology: The study involved 163 potato mutants developed from three commercially grown Kenyan potato varieties; Asante, Mpya, and Sherekea irradiated using gamma rays from 60Co source under different dose rates. Three middle leaves of greenhouse-grown plants were randomly selected for chloroplast counts in ten pairs of stomata guard cells on the lower surface of the leaf. Data on the number of chloroplast counts per mutant was calculated as a percentage of the parents or control and descriptive analysis.
Results: The results indicate that the number of ploidy level distribution was decreasing in diploids and triploids and were increasing in tetraploids from M1V1, M1V2 to M1V3 in all the potato mutant populations.
Conclusion: This shows that mutation induction generates genetic variations from which desired mutants may be selected based on the appropriate breeding strategies.

Keywords: Potato; ploidy; mutants; chloroplast counts; diversity.

1. INTRODUCTION

The cultivated potato (*Solanum tuberosum* L.) is the second most important staple food in Kenya after maize and the world's fourth major food crop after wheat, rice and maize [1,2,3]. In Kenya, potato is mostly grown by small scale farmers as source of food, employment and cash crop, therefore plays an important role in food security and provides high energy, protein as well as substantial amount of vitamins and minerals [3].

The cultivated potato, *Solanum tuberosum* species range from the diploid chromosome number 2n=2x=24 to pentaploid level 2n=5x=60 while the wild species include all the ploidy levels in addition to hexaploid 2n=6x=72 [4,5,6,7]. The cultivated potato, *Solanum tuberosum* L. is an autotetraploid (4 EBN (Endosperm Balance Numbers)) with probably four interchangeable alleles at a given locus with the possibility of intralocus interactions (heterozygozity) and interlocus interactions (epistasis) occurring [8] which are important in breeding to improve certain traits [9,10]. Potato crossability is affected by ploidy species type and EBN which ranges from 1 to 4 with diploid and tetraploid having 2 and 4 EBN respectively [11]. The level of heterozygozity is influenced by how different the four alleles are within a locus; the more diverse they are, the higher the heterozygozity and the greater the number of interlocus interactions, the greater the heterosis [8,9,10]. This makes conventional potato breeding cumbersome and time consuming effort and hence less successful when compared to other important crops, making it an excellent crop plant to improve by mutation or genetic transformation.

Polyploids have played a significant role in evolving and exploring genetic diversity in crop plants particularly potato. Polyploids can be obtained by exploring spontaneous doubling events in nature such as triploids, tetraploids, pentaploids, hexaploids and octaploids available. Polyploids can also be created artificially by *interploid* and interspecific hybridization, somatic doubling by colchicine, endosperm culture in vitro and through mutation induction [12,13].

Mutation induction generates genetic variations from which desired mutants may be selected. The use of induced mutations is highly effective in enhancing genetic variability and has been used to develop improved cultivars of seed and vegetatively propagated crops, trees and other crops [14]. Sometimes, different ploidy levels can be observed within a single species. Thus, the determination of a ploidy level is not only important in the study of relative relationships of species, but also is very important in producing interspecies hybrids, and in genetics studies and breeding programs.

2. MATERIALS AND METHODS

2.1 Development of Mutant Population

2.1.1 Parental lines

Three commercial potato varieties namely; Asante, Kenya Mpya and Kenya Sherekea were obtained from Kisima farm. The three potato varieties used had various characteristics as described. Asante has intermediate dormancy, fairly tolerant to late blight, long oval white smooth skin colour, shallow eye depth, poor tuber storage, matures between 90 – 120 days and tuber yield is between 35 – 45 t/ha. Kenya Mpya has short dormancy, highly tolerant to late blight, shallow eye depth, cream white skin colour with pink eyes, early tuberization and matures early at 90 - 105 days, oval or round

tubers with large tuber size yielding between 35 – 45 t/ha. Kenya Sherekea has intermediate dormancy, highly tolerant to late blight and viruses, good storability, medium eye depth, red skin colour and oblong or round high tuber number per plant that matures between 105 – 120 days yielding between 40 – 50 t/ha [15]. A total of 30 tubers each of the three potatoes were sent to the Plant Genetics and Breeding Laboratory (PGBL) at IAEA/FAO Laboratories, Seibersdorf, Austria, where they were grown at the greenhouse to initiate *in vitro* shoot cultures as described by Bado et al. [16].

2.1.2 Irradiation methods

A radio-active cobalt-60 (^{60}Co) (gamma source) with a low dose rate of 2 gray per minute (Gy/min) were used to induce mutations. The optimal dosage for mutation induction, GR_{30} and GR_{50} (30% and 50% growth reduction respectively) as well as LD_{30} and LD_{50} (30% and 50% lethality dose) were determined for each approach as described by Owoseni et al. [17], Mba et al. [18], Kodym et al. [19], Bado et al. [16] to assess the susceptibility of each potato cultivar with regard to gamma irradiation. Two *in-vitro* radio-sensitivity tests were developed involving different tissues for irradiation of potato mutation induction as described by Bado et al., [20]. The irradiation dose rates performed on the two *in-vitro* radio-sensitivity tests for the three potato varieties were 0, 3, 6, 9, 12 and 15 Gy for Asante, 0, 5, 6, 10 and 15 Gy for Kenya Mpya and 0, 3, 5, 10, 12, 15, 20, and 30 Gy for Kenya Sherekea according to Bado et al. [20]. After mutation induction, a total of 570 mutant microtubers (Asante 230, Kenya Mpya 160, Kenya Sherekea 180) were developed from the three potato varieties and were transported to Kenya, University of Eldoret for establishment.

2.1.3 Establishment of M1V1 and surviving putative mutants in the greenhouse

A total of 570 mutant microtubers (M_1V_1) were received from PGBL and were established at the University of Eldoret (UoE), Biotechnology Green House Research facility on autoclaved loam sandy soil. Each mutant at M1V1 generation was planted on 10 × 9 mm polythene bag. There was no replication of the mutant microtubers because each does not maintain the same genetical constitution after irradiation. Induced potato plants derived from microtubers were therefore assumed to be different. The coding of the M_1V_1 plants were developed based on the number of irradiated stakes that survived and was then advanced to the subsequent generations. The tubers obtained at M_1V_1 were advanced to M_1V_2 and M_1V_3 mutant generations at the greenhouse.

2.2 Plant Materials and Sample Preparation

Representative plants from the M_1V_1, M_1V_2 and M_1V_3 mutant generations for each mutant were randomly selected at 2 months after planting and subjected to ploidy level determination as described by Ordoñez, [21]. The experiment was carried out at least 5 times on each sample per treatment (clone). One to five leaves of the apical part of the plant were collected from selected plants. The samples were placed in a petri-dish containing a filter paper moistened with distilled water covering the bottom of the lid. One or two drops of iodine solution (iodine-potassium iodide) were placed in the center of a slide. The surface cell layer from the under surface of the leaf was peeled off. Then, epidermal peels taken from the abaxial side near the vein structure using a pair of fine tweezers was immediately placed on the slide. After 2 min, a cover slip was mounted and gently pressed down and observations were made under a light microscope at 100X magnification.

2.3 Sample Observation and Data Collection

Chloroplast counts in each of the two guard cells of stomata was scored in ten stomata per sample indicating average ploidy level as described by Ordoñez, [21] (Table 1).

Table 1. Scale to determine the ploidy level of potato genotypes

Chloroplast number in stomatal guard cells	Ploidy
6 to 8	Diploid (2n=2x=24)
9 to 11	Triploid (2n=3x=36)
12 to 14	Tetraploid (2n=4x=48)

Source: Ordoñez, [21]

2.4 Data Analysis

Data on the number of chloroplast counts on the stomatal guard cells per mutant was calculated as a percentage of the parents or control. The data on chloroplast number in stomatal guard cells were analyzed separately for M_1V_1, M_1V_2 and M_1V_3 and subjected to ANOVA using SAS version 9.1. The means were compared based

on Duncan Multiple Range Test (DMRT) whenever differences were significant at 95% confidence level. The ploidy level distribution by chloroplast counts were also computed using descriptive analysis (Box and Whisker plots).

3. RESULTS AND DISCUSSION

The percentage distribution of chloroplast counts in stomatal guard cells at M_1V_1 generation of Asante mutants ranged from 42% to 90% and was the most widely distributed compared to Mpya (range from 60% to 96%) and Sherekea (range from 66% to 98%) mutants (Fig. 1).

The variation in distribution of chloroplast counts in stomatal guard cells in the genotypes within a species may be due to the differential genotypic responses on the physical and biochemical tissue contents such as size, water content, DNA content, nuclear volume as a result of mutagen effects which are random [17,22,16,23]. The chloroplast counts distribution in M_1V_1 generation in the three mutant populations was found to differ for diploids, triploids and tetraploids compared with M_1V_2 and M_1V_3 generations. This can be attributed to damage or energy absorbed by the cells after irradiation or confounding effects which include reliance on dominant or hemizygous alleles in the first generation. Phenotypic observations at first generation can be influenced by environmental conditions, epigenetic variation and the presence of genotypic heterogeneity or chimerism after mutagenesis [17,24].

The results from the box and whisker plots (Fig. 2) showed that the chloroplast number in the stomatal guard cells ranged from diploids (2n = 2x = 24) through triploids (2n = 3x = 36) to tetraploids (2n = 4x = 48) in the different potato mutants in all the generations and could be attributed to irradiation effects. At M1V3 generation, the average chloroplast number counts showed that the mutant populations were tetraploids (2n = 4x = 48). These observations were in agreement with Ugent, [25] who reported that cultivated potatoes have frequent genetic exchange among ploidy levels, a high incidence of interspecific hybridization and a high level of diversity among cultivars. Other studies have shown consistency with the present findings that cultivated potato is an autotetraploid (2n = 4x = 48) species [9,10]. Jaskani and Khan, [26] in potato and Usman et al. [27] in grapefruit reported that the number of stomatal guard cells was directly proportional to ploidy level of the plants.

In Fig. 3, the percentage distribution of chloroplast counts based on the dosage rates showed that 12 Gy in Asante, 5 Gy in Mpya and 3 Gy in Sherekea potato mutants displayed the largest percentage chloroplast counts distribution. The variation in response of potato genotypes to increasing gamma irradiation dose could be due to the sensitivity (Mpya and Sherekea) or persistent of the genotypes or irradiation methods used with effects on plant growth and development.

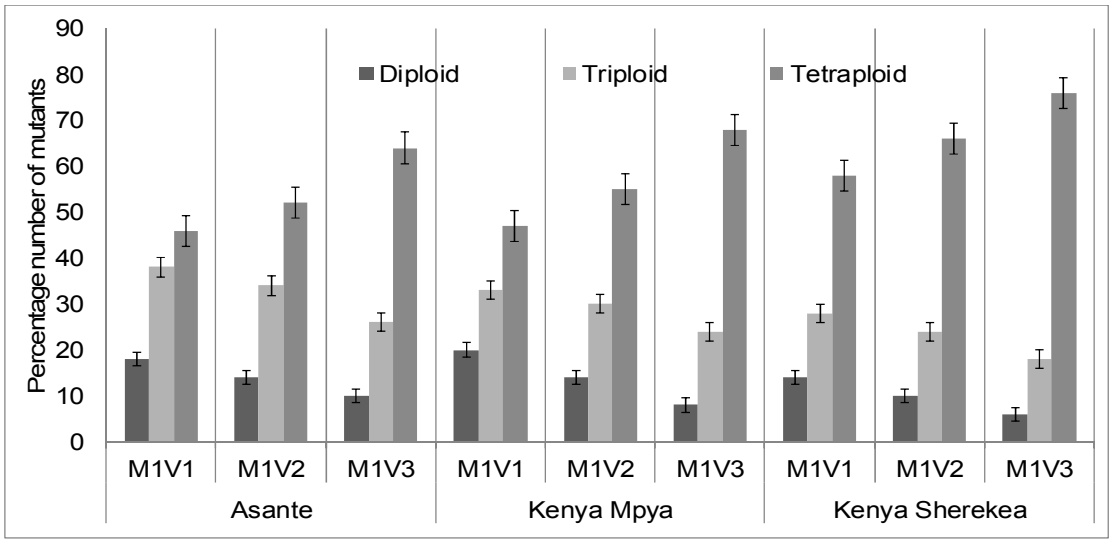

Fig. 1. Percentage number of Asante, Mpya and Sherekea potato mutants at various generations (M_1V_1, M_1V_2 and M_1V_3) based on chloroplast counts in stomatal guard cells

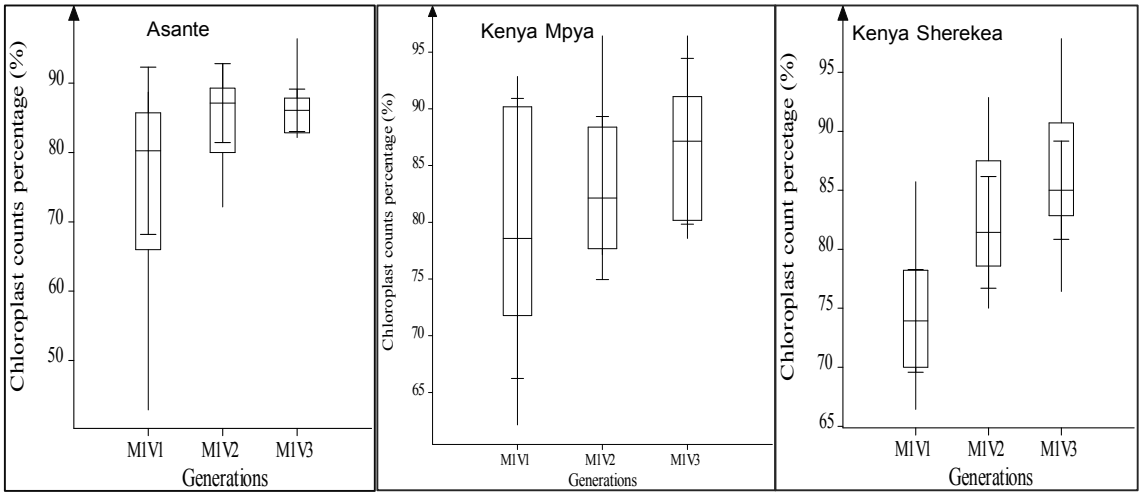

Fig. 2. The ploidy level distribution by chloroplast counts in stomatal guard cells of Asante, Mpya and Sherekea potato mutant populations across all the generations (M_1V_1, M_1V_2 and M_1V_3) using box and whisker plot

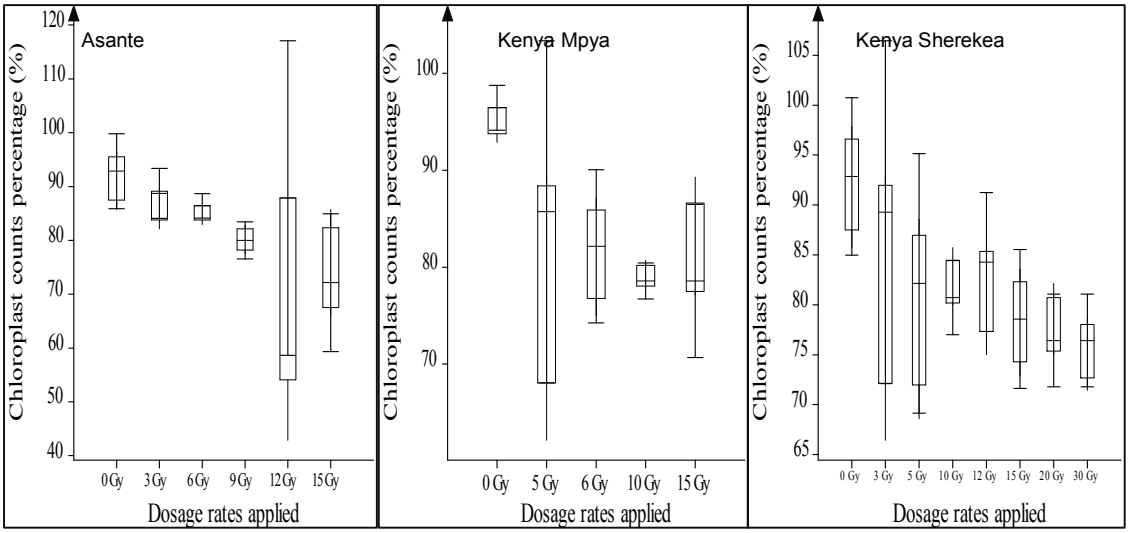

Fig. 3. The ploidy level distribution by chloroplast counts in stomatal guard cells of Asante, Mpya and Sherekea potato mutant populations across the various dosage rates applied using box and whisker plots

The percentage distribution of chloroplast counts showed decreasing rate as the gamma irradiation dose increases across all the potato mutants. Increasing gamma irradiation treatments have inhibitory effects and can cause chromosomal damage or change in plant meristematic cells, cell cycle deceleration and mitotic delay, which considerably influence general plant regeneration and development. Though at higher radiation doses causes DNA damage more frequently and enhances mutation frequency but mostly lethal to the plant [28,29,30, 20].

4. CONCLUSION

In this study, the use of chloroplast counts in the stomatal guard cells in assessing diversity of potato mutants appeared to be an important strategy to identify polyploids to develop germplasm bank for breeding programmes. Chloroplast count method is very easy, rapid and reasonable for ploidy level analysis. Gamma irradiation treatment has been proved to affect chromosome variability as shown by chloroplast counts in stomatal guard cells.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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