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In vitro methods for mutation induction in potato (*Solanum tuberosum* L.)

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Potato (*Solanum tuberosum* L.) is an important vegetable and staple crop worldwide and mainly propagated vegetatively. Breeding of potato is problematic and therefore induced mutation is an attractive means of improving the crop. *In vitro* culture systems, and especially the production of micro-tubers, are ideal for such purposes in potato improvement. Radio-sensitivity testing (growth reduction, GR and lethal dose, LD) allows the determination of irradiation treatments (Gy) for mutation induction. Three schemes incorporating *in vitro* techniques were tested for mutation induction in potato namely: 1) irradiation of cuttings without leaves and subsequent dissociation of chimeras to produce plantlets or micro-tubers on M_1V_2 (or further generation) plantlets, 2) irradiation of cuttings with leaves and direct induction of mutant micro-tubers, and 3) induction and irradiation of micro-tubers. Variability among the potato genotypes to gamma irradiation was recorded. Optimized irradiation treatments for mutation induction were established for the various tissues/propagules: cutting growth (GR_{50}, 9-6 to 20.6 Gy), cutting tuberization ability (LD_{50}, 7.3 to 13 Gy) and micro-tuber sprouting ability (LD_{50}, 20.6 to 54.8 Gy). Micro-tubers were found to be more resistant for *in vitro* mutation induction than *in vitro* cuttings. This study shows the susceptibility of different plant tissue/propagule and potato genotypes to gamma irradiation. Radio-sensitivity analyses showed that lower gamma doses are required when mutation induction is applied in combination with micro-tuberization.

Key words: Potato, gamma irradiation, stem cuttings, micro-tubers, *in vitro* tuberization.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important vegetable and staple food crop worldwide consumed by over one

billion people. Its annual production of over 368 million tonnes ranks fourth after maize, rice and wheat (Albiski et al., 2012; Food and Agricultural Organization (FAO), 2014). In addition to high starch levels, potato tubers contain significant amounts of antioxidants, protein, vitamins (C and E), macro- and micro- nutrients (calcium, magnesium, iron and zinc), polyphenols, carotenoids and tocopherols (Brown, 2005), which are important for the human diet.

Potato belongs to the family Solanaceae and depending on the purpose, can be propagated through seed, axillary buds, apical meristems, synthetic seeds, tubers, mini-tubers and micro-tubers (Sharma et al., 2007; Badoni et al., 2010). With over 160 potato species (wild and cultivated), the Solanaceae family has a large gene pool (Grüneberg et al., 2009). However, success in breeding new cultivars, utilising these resources, has been slow, mainly because the crop is clonally propagated and highly heterozygous. For example, potato breeding in China, which has the biggest potato production in the world, is based on a narrow genetic base due to common pedigrees of breeding materials (Cheng et al., 2010). This low genetic diversity among cultivars represents a serious limitation to crop improvement, especially in the emergence of new diseases, pests and climatic changes.

Potato is considered to be among the most important clonally propagated crops, including cassava, sweet potato, yam, taro, sugar cane, banana and plantain (Grüneberg et al., 2009).

Major problems affecting potato production are: low multiplication rates in the field under conventional (biological) seed production, and yield loss due to susceptibility to diseases and pests such as late blight disease, potato cyst nematode and Colorado beetle (Evans et al., 1992; Mahfouze et al., 2012). In developing countries, many traditional cultivars suffer from poor yield with reduced tuber size and have undesirable traits such as sunken eyes, which reduce their market value. Potato, *S. tuberosum* is a tetraploid, outbreeding species that maintains a high degree of heterozygosity; therefore, it is mainly propagated vegetatively. Consequently, biological seed, which is heterogeneous, does not present a suitable material for mutation induction in potato (Sharma et al., 2007). *In vitro* culture of vegetatively propagated crops in combination with radiation induced mutation has proven to be a valuable method to broaden genetic variability (van Harten and Broertjies, 1989; Elias et al., 2009; Cheng et al., 2010; Mahfouze et al., 2012; Yaycili and Alikamanoglu, 2012; Jankowicz-Cieslak et al., 2012). Ionizing radiation was indicated as a potent method to generate new genetic variability for crop improvement (Stadler, 1928; Ahloowalia and Maluszynki, 2001). Furthermore, the main aim and advantage of mutation induction in vegetatively propagated crops is the ability to change one or a few characteristics without changing the elite cultivar genetic background (Ahloowalia, 1995; Broertjes and van Harten, 1988), that is having a low mutational load.

Since the pioneering work of Asseyeva (1931) in potato, mutation induction in potato has produced mutants for diverse traits such as modified starch biosynthesis (Cieśla et al., 2002; Muth et al., 2008), increased yield (Al-Safadi et al., 2000; Li et al., 2005) modified histological and texture properties (Nayak et al., 2007), long shelf-life (Baskaran et al., 2007) and increased tolerance to abiotic and biotic stresses (Al-Safadi and Arabi, 2003,2007; Albiski et al., 2012). From 1931 to 2015 only 6 potato improved cultivars have been registered in the Food and Agricultural Organization/ International Atomic Energy Agency (FAO/IAEA) mutant's database (http://mvd.iaea.org). Most mutation induction, using physical and chemical mutagens, for potato improvement reported by previous studies was conducted using *in vitro* cuttings, *in vivo* tubers and mini-tubers. Today, the *in vitro* micro-tuber represents another major target for mutagenesis. This study therefore, aimed to developed mutation induction methods that target *in vitro* micro-tubers.

Prior to mutation induction, radio-sensitivity tests need to be performed to determine the optimal dose treatment for mutation induction. This consideration is even more important for vegetatively propagated crops, because of the impossibility to restore the genetic background by backcrossing. It is important to note that the mutagenesis reported by different studies on micro-tuber induction and gamma irradiation were for the purpose of enhancing the micro-tuber production with minimal genetic change (Al-Safadi et al., 2000; Li et al., 2005; Mahfouze et al., 2012). In this study, the susceptibility of 8 different potato genotypes (landraces and commercial cultivars) to gamma irradiation was determined. The data provide useful information in optimizing irradiation treatments for mutation induction, which may be applied to other genotypes.

MATERIALS AND METHODS

Plant

Eight potato (*S. tuberosum*) cultivars known to be grown in Kenya (Mpya, Sherekea and Asante), Lesotho (Basotho Pink, BP1, Up-To-

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Date and Mondial) and Morocco (Kondor) were used in this study. Important characteristics of these cultivars for improvement are given in Table 1.

Conventional tubers were used as starting material and these were supplied from FAO/IAEA Member States, National Institute of Nuclear Energy, and grown up in the greenhouse at the FAO/IAEA Plant Breeding and Genetics Laboratory (PBGL), Seibersdorf, Austria to provide shoots as donor material to initiate *in vitro* shoot cultures as described as follows.

Tissue culture conditions

Young shoots from greenhouse grown plants were harvested and used to initiate *in vitro* cultures after sterilization of axillary meristems with 70% ethanol for 10 to 20 s, 20% commercial bleach for 15mins, and three rinses with sterile distilled water, operations carried out in a laminar flow bench. The propagation medium was based on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 2% sucrose and 0.18% gelrite as gelling agent. The pH of the medium was adjusted to 5.8. One node cuttings were placed in test tubes for initiation (1 to 3 per tube) and subsequently also for micro-propagation. Developing shoots were sub-cultured every 2 to 3 weeks and maintained in controlled environment rooms with 16 h fluorescent light (65 µmol/m2/s; using cool white fluorescent tubes, Philips TLP 36/86, Philips, Amsterdam, the Netherlands) at 22° ± 2°C. Rounds of sub-culturing continued until sufficient plantlets were obtained for mutation induction.

Tuberization conditions

In order to compare genotypic differences in micro-tuber production and the susceptibility of micro-tubers to gamma irradiation, one node cuttings with one leaf were transplanted to a modified medium from Hoque (2010) consisting of MS basal medium supplemented with 4 mg/L Kinetin, 8% sucrose and 0.18% gelrite. Differential responses to culture conditions have been reported for different genotypes (Piao et al., 2003; Hoque, 2010; Nistor et al., 2010; Kodym et al., 2012). In order to minimize these differences an attempt was made to identify a common medium that would work sufficiently well for all the eight genotypes. This was selected from media with various cytokinin (kinetin, benzyl adenine purine, and chlorocholine chloride) combinations with one sucrose concentration (8%). The pH of the micro-tuber induction medium was adjusted to 5.7. The cultures were incubated in the dark at 22° ± 2°C and developing micro-tubers were harvested. Radio-sensitivity tests were carried out 5 to 6 weeks after initial culture.

Irradiation methods

Standard gamma cells (220, Atomic Energy of Canada Limited, Ottawa, Canada) with ^{60}Co source with a low emission dose rate of 2 or 7.07 Gy/min were used for irradiation. 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 potato genotype, using methods described by Kodym et al. (2012), to define the susceptibility. Three *in vitro* radio-sensitivity tests applicable to potato mutation induction were developed (Figure 1) involving different target tissues for irradiation, but also different patterns of regeneration:

Scheme 1A: *In vitro* single node stem cuttings (without leaves) were irradiated with 6 different doses and subjected to several rounds of *in vitro* shoot propagation to dissolve chimeras. Plantlets at the stage of M_1V_{3-4} were used for phenotypic and genotypic screening of mutants. Alternatively, in **Scheme 1B** after the dissolution of chimeras, micro-tubers were induced on the M_1V_{2-3} cuttings. Micro-tubers were used for field evaluation. Three replications with at least 20 uniform cuttings with one axial meristem per dose was selected and used to determine the optimal dose for mutation induction, the radio-sensitivity test with dose treatments ranging from 0, 5, 10, 15, 20 and 30Gy using 2 Gy/min gamma dose rate. After subsequent growth for a cycle of 2 to 3 weeks, plant height, fresh weight and number of nodes were recorded to assess the effects and the optimal dose of gamma irradiation. The plantlet height was used to determine optimal dosage for mutation induction as growth reduction GR_{30} and GR_{50}.

Scheme 2: *In vitro* single node stem cuttings (with leaves) were irradiated and induced to produce micro-tubers *in vitro* directly. The micro-tuber induction rate was used to determine the optimal dose for mutation induction using two replications of at least 36 cuttings with one axial meristem per dose ranging from 0, 3, 6, 9, 12 and 15 Gy using 2 Gy/min gamma dose rate. Tuberization was recorded as the number, weight and size of micro-tubers developed. The tuberization rate (%) was calculated as the number of nodal induced micro-tuber/number of planted × 100. Micro-tubers induced on *in vitro* plantlets at the stage M_1V_2 were used for mutation screening. The lethality dose, given as the reduction of the tuberization response at 30 and 50% (LD_{30} and LD_{50}) of the cuttings per genotype, were determined.

Scheme 3: *In vitro* micro-tubers were irradiated with 7 different doses. As a first step micro-tubers were produced in sufficient amounts and sorted for uniformity of size (medium and large) and weight. Radio-sensitivity tests were performed with 30 micro-tubers per dose using a wide dose range of: 0, 10, 20, 30, 40, 60 and 80 Gy using 7.07 Gy/min gamma dose rate. To facilitate sprouting, micro-tubers were placed on filter paper in Petri dishes moistened with 5 mg/L GA_3 and incubated in the dark for 24 h at 22° ± 2°C. Sprouting ability was assessed as a parameter to determine the vitality of the treated tissues. Micro-tubers were considered sprouted, when after 4 weeks the sprouting shoot length was equal or longer than the size of micro-tuber. The sprouting ability rate (%) was calculated as the number of micro-tuber sprouted/number of control micro-tubers sprouted × 100. Mutant plantlets at the stage of M_1V_1 cannot be used for phenotypic and genotypic screening of mutants as they are likely to be chimeric. Typical radio-sensitivity curves show genotype difference, but an enhancement of plant growth and tuberization at low doses, lethal effects at high doses were observed.

Statistical analyses

Analysis of variance (ANOVA) and least significant differences (LSD) of means (5% level) were performed using GenStat Release 9.2 for *in vitro* cuttings and tuberization and JMP statistics packages 12 for the micro-tubers sprouting ability.

RESULTS

Mutation induction schemes

Three mutation induction schemes were developed for *in vitro* tissues and organs of potato (Figure 1). In a first

Table 1. Characteristics of potato cultivars and target traits for their improvement by mutation induction.

Country	Cultivars	Characteristics	Traits to be improved
Kenya	Mpya	- Tuber yield 35-45 t/ ha - Oval/round, large size tubers - Early tuberization - Cream white skin colour with pink eyes - Shallow eye depth - Highly tolerance to late blight - Short dormancy - Requires 90 days to maturity	Increase yield
	Sherekea	- Tuber yield 40-50 t/ha - Oblong/round tubers - High number of tubers per plant - Red skin colour - Medium eyes depth - Highly tolerant to late blight and viruses - Good storability - Intermediate dormancy - Requires 105 -120 days to maturity	- Shorten dormancy period - Shorten the maturity period
	Asante	- Tuber yield 35-45 t /ha - Fairly tolerant to late blight - Intermediate dormancy Requires 90 -120 days to maturity	- Increase late blight tolerance - Shorten the maturity period - Shorten dormancy period
Lesotho	Basotho Pink	- High yielding - Sunken eye - Resistance to diseases and pests - Palatable - Drought tolerant - Intermediate dormancy - Requires 140 days to maturity	- Decrease eyes depth - Shorten maturity period - Increase frost tolerance
	BP1	- Acceptable yields - Moderately resistant to diseases and pests - Drought susceptible - Intermediate dormancy - Requires 90-120 days to maturity	- Reduce metallic taste - Increase drought and frost tolerance - Increase yield
	Up-to-date	- Acceptable yields - Moderately resistant to diseases and pests - Drought susceptible - Requires 110-120 days to maturity	- Reduce metallic taste - Drought and frost tolerance - Increase yield
	Mondial	- High yields - Susceptible to bacterial wilt - Taste palatable - Drought susceptible - Intermediate dormancy - Requires 90-120 days to maturity	- Increase wilt resistance - Increase drought tolerance

Table 1. Cont'd.

Morocco	Kondor	- High yields - Early bulking - High resistance to drought - Good resistance to *Potato Virus Y* - Shorten dormancy - Requires 110-120 days to maturity	- Increase resistance to common scab: *Streptomyces scabies*

step radio-sensitivity tests were conducted on different tissues (one node cutting with and without leaf and micro-tuber). In a second step different patterns of regeneration were compared. *In vitro* mutant plantlets or micro-tubers may be used for phenotypic or genotypic mutant screening.

Effects of gamma irradiation on in vitro cuttings

Analysis of variance of plant height on seedlings grown from *in vitro* cuttings of six potato genotypes exposed to different gamma irradiation dose (Scheme 1 and Figure 1) was significant ($P<0.05$) among irradiation treatments, genotypes and the interactions of dose*genotypes (Table 2). The results indicated that increasing doses of gamma irradiation progressively inhibited the growth of stem cuttings. The potato genotypes showed different responses (Table 3 and Figure 2). The effects of gamma rays were more pronounced on rooting, plant height and fresh weight than number of nodes and leaves for each genotype. Since further sub-culturing and chimera dissolution could be performed only with differentiated plantlets, plant height was considered for optimum dosage determination. A significant effect of irradiation on the plantlet height was recorded in the six potato genotypes (Table 3). Plant height showed growth retardation at relatively high doses such as 15 and 20Gy whereas a relatively low dose of 5Gy enhanced the growth of cultivars Mpya, Kondor and Mondial, which exhibited better growth than their respective untreated controls (Table 3 and Figure 2). Cuttings irradiated at doses equal to or above 15 Gy exhibited a very low root induction rate, and an undifferentiated shoot growth was recorded which had further negative impact on micro-tuber production. The plantlet height was used in determination of optimal dosage for mutation induction according to Kodym et al. (2012) as growth reduction GR_{30} and GR_{50} (Table 3). The results showed an expected variation in response among the cultivars: Mpya (GR_{50} = 20.6 Gy) and Sherekea (GR_{50} = 18.0 Gy) were relatively more radio-resistant than the susceptible genotypes BP1 (GR_{50} = 9.7 Gy) and Up-To-Date (GR_{50} = 9.9 Gy); whereas the cultivars Mondial (GR_{50} = 14.5 Gy) and Kondor (GR_{50} = 13.9 Gy) exhibited moderate resistance to gamma irradiation (Figure 2 and Table 3).

Effects of gamma irradiation on the tuberization

The effects of irradiation on micro-tuberization (Scheme 2 and Figure 1) showed that the untreated stem cuttings from cultivars Kondor and Basotho Pink had about 100% tuberization whereas other cultivars reached about 80% (Figure 3). A significant effect of gamma irradiation was recorded on tuberization of five potato genotypes and gamma irradiation doses ($P<0.05$) (Table 4). Increasing the applied dose of gamma irradiation diminished the tuberization response of all genotypes (Table 5 and Figure 3). However, a relatively low dose of 3 and/or 6 Gy increased tuberization rate of all potato genotypes except of Basotho Pink. The estimated LD_{50} showed that genotype BP1 was relatively more resistant to gamma irradiation than other genotypes. While genotypes Basotho Pink, Kondor and Mondial were moderately resistant, the genotype Mpya was susceptible (Table 5 and Figure 3).

Effects of gamma irradiation on sprouting ability of in vitro micro-tuber

Micro-tuber production showed variations in shape (oval and spherical), size (small, medium and big) and skin color (cream, purple, white with red spots, yellow) (Figure 4). Variability was also observed in initiation time and micro-tuber position on the stem (basal, axial or apical). Uniform micro-tubers were selected for radio-sensitivity tests (Scheme 3, Figure 1). The sprouting ability of micro-tubers was affected significantly by gamma irradiation dose and genotype (Table 6). The size of the micro-tuber had no effect on sprouting ability and the number of eyes sprouted was not significant, while emerged eyes ranged from 1 to 4 independently of the irradiation dose applied. Analysis of variance of sprouting ability was significant with the gamma irradiation dose and genotype by least significant differences of means testing ($P<0.05$) (Table 6). Doses of 10 and 20 Gy stimulated the sprouting ability of micro-tubers of genotypes Kondor and Basotho Pink, respectively. All doses above 40 Gy were completely

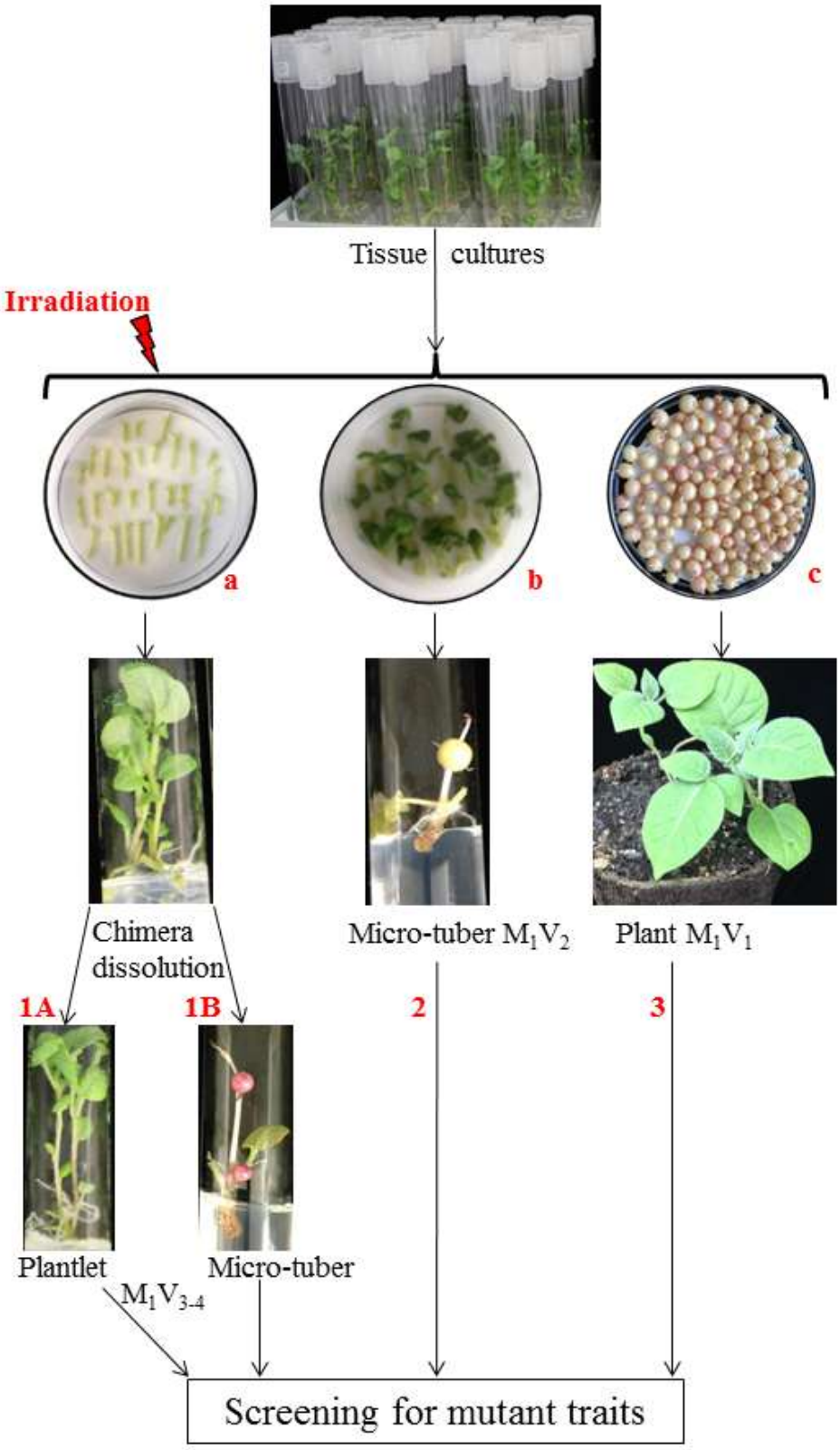

Figure 1. Mutation induction schemes for *in vitro* tissue cultures of potato: irradiation of a) cuttings with leaves and b) cuttings without leaves, c) micro-tubers; and screening of either *in vitro* plantlets or micro-tubers using strategies 1A or 1B, 2 and 3.

Table 2. Analysis of variance for effects of gamma irradiation doses on *in vitro* plantlet height of six potato genotypes.

Source of variation	DF	Mean Square	F-Value	P-value
Dose (Gy)	5	64.8253	216.78	<0.001
Genotype	5	2.3271	7.78	<0.001
Dose (Gy)*Genotype	25	1.8048	6.04	<0.001
Residual	70	0.2990		
Total	107			

Table 3. Mean and standard deviation of *in vitro* plantlet height (cm), and the respective mutation induction dose (GR_{30} – GR_{50}) for six potato genotypes.

Dose (Gy)	Genotype					
	BP1	Kondor	Mondial	Mpya	Sherekea	Up-to-Date
0	6.07±0.81^{a}	3.33±0.15^{b}	4.40±1.47^{b}	3.70±0.46^{b}	3.73±0.12^{a}	6.60±1.13^{a}
5	5.47±0.32^{b}	4.30±0.26^{a}	5.07±1.63^{a}	5.23±1.12^{a}	3.53±0.06^{a}	5.60±0.75^{b}
10	2.37±1.20^{c}	2.43±0.32^{c}	2.80±0.50^{c}	3.03±0.85^{c}	2.57±0.06^{b}	2.93±1.44^{c}
15	1.30±0.30^{d}	1.17±0.06^{d}	2.10±0.56^{d}	3.17±0.60^{c}	2.47±0.50^{b}	2.00±0.72^{d}
20	0.50±0.10^{e}	0.73±0.06^{e}	0.60±0.20^{e}	2.37±0.32^{d}	1.70±0.26^{c}	0.43±0.49^{e}
30	0.00±0.00^{f}	0.10±0.00^{f}	0.00±0.00^{f}	0.23±0.06^{e}	0.40±0.10^{d}	0.17±0.06^{e}
CV%	21.3					
LSD	0.3635					
GR_{30} (Gy)	6.8	10.5	11.5	16.5	13.4	6.7
GR_{50} (Gy)*	9.6^{c}	13.8^{b}	14.5^{b}	20.6^{a}	18.0^{b}	9.9^{c}

*Values in the same column followed by the same letter are not significantly different.

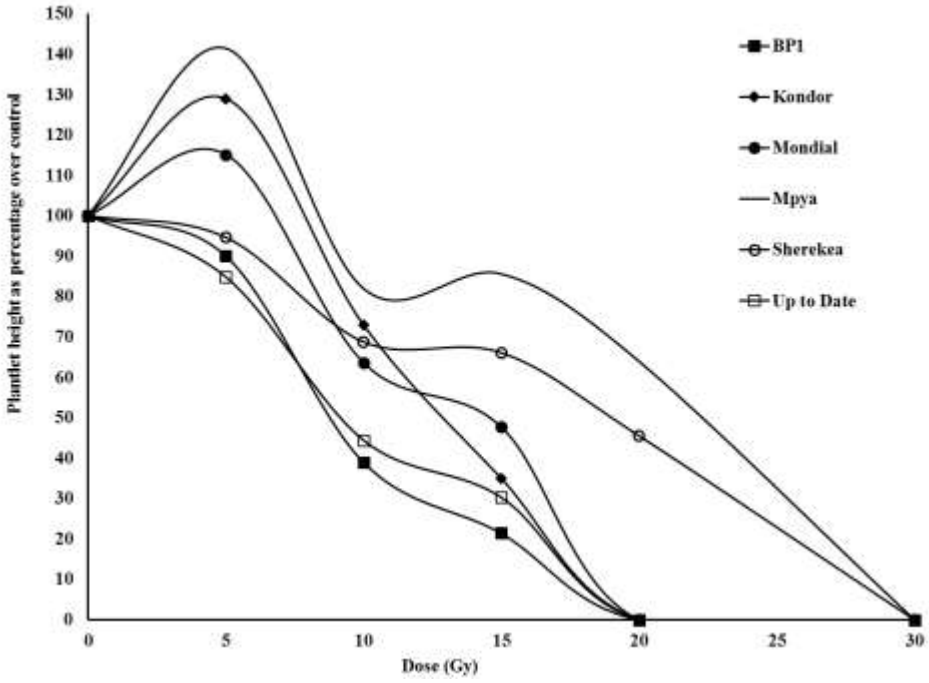

Figure 2. Effects of gamma irradiation on plant height of six potato genotypes after three weeks growth.

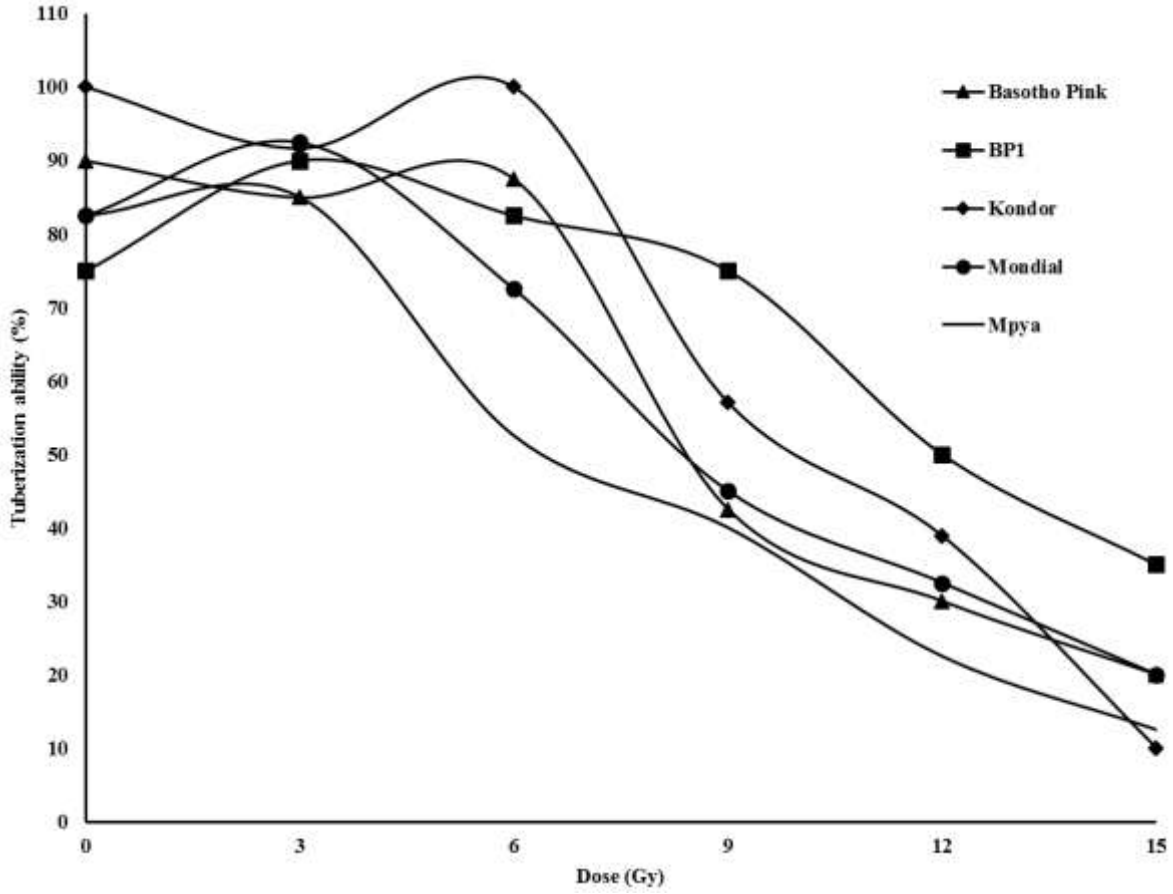

Figure 3. Effects of gamma irradiation on micro-tuber production of five potato genotypes after six weeks.

Table 4. Analysis of variance for effects of gamma irradiation doses on *in vitro* tuberization ability of five potato genotypes.

Source of variation	DF	Mean Square	F-Value	P-value
Dose (Gy)	5	0.842059	163.84	<0.001
Genotype	4	0.067864	13.20	<0.001
Dose (Gy)*Genotype	20	0.019673	3.83	<0.001
Residual	29	0.005140		
Total	59			

lethal for genotypes Mpya and Sherekea, whereas in other genotypes growth was severely retarded. Genotype differences for sprouting ability were observed among the genotypes investigated. Thus the genotypes Mpya and Up-To-Date were more radio-susceptible with observed LD_{50} of 20.6 and 26 Gy. Genotypes Asante, BP1 and Sherekea were moderately radio-resistant with LD_{50} between 32.4 and 35.5 Gy in comparison to relatively radio-resistant genotypes Basotho Pink and Kondor with 41.1 and 54.8 Gy, respectively to gamma irradiation (Table 7 and Figure 5).

DISCUSSION

Effects of increasing doses of gamma irradiation on *in vitro* cuttings of potato genotypes showed a significant growth decrease. High dose treatments of cuttings may also affect subsequent micro-tuber production when adopting Scheme 1B (Figure 1) due to difficulties in sub-culturing undifferentiated nodes. Plantlet height was negatively correlated with increasing applied dosage of gamma irradiation. These results agree with reports of Kodym et al. (2012) that plant height determined by cell

Table 5. Mean and standard deviation of *in vitro* tuberization ability rate (%), and the respective mutation induction dose (LD_{30} – LD_{50}) for five potato genotypes.

Dose (Gy)	Genotype				
	Basotho Pink	**BP1**	**Kondor**	**Mondial**	**Mpya**
0	90.00±0.00^{a}	75.00±7.00^{c}	100.00±0.00^{a}	82.50±4.00^{b}	82.50±4.00^{a}
3	85.00±7.00^{a}	90.00±0.00^{a}	91.67±4.00^{b}	92.50±4.00^{a}	85.00±7.00^{a}
6	87.50±4.00^{a}	82.50±11.00^{b}	100.00±0.00^{a}	72.50±4.00^{c}	52.50±11.00^{b}
9	42.50±11.00^{b}	75.00±7.00^{c}	57.03±0.06^{c}	45.00±7.00^{d}	40.00±14.0^{c}
12	30.00±14.00^{c}	50.00±0.00^{d}	38.89±0.07^{d}	32.50±1.00^{e}	22.50±4.00^{d}
15	20.00±7.00^{d}	35.00±0.00^{e}	10.00±0.00^{e}	20.00±7.00^{f}	12.50±10.00^{e}
CV%	11.9				
LSD	05.986				
LD_{30}(Gy)	6.4	7.6	8.7	5.7	4.0
LD_{50}(Gy)*	9.4^{ab}	13.0^{a}	10.5^{ab}	9.2^{bc}	7.3^{c}

*Values in the same column followed by the same letter are not significantly different.

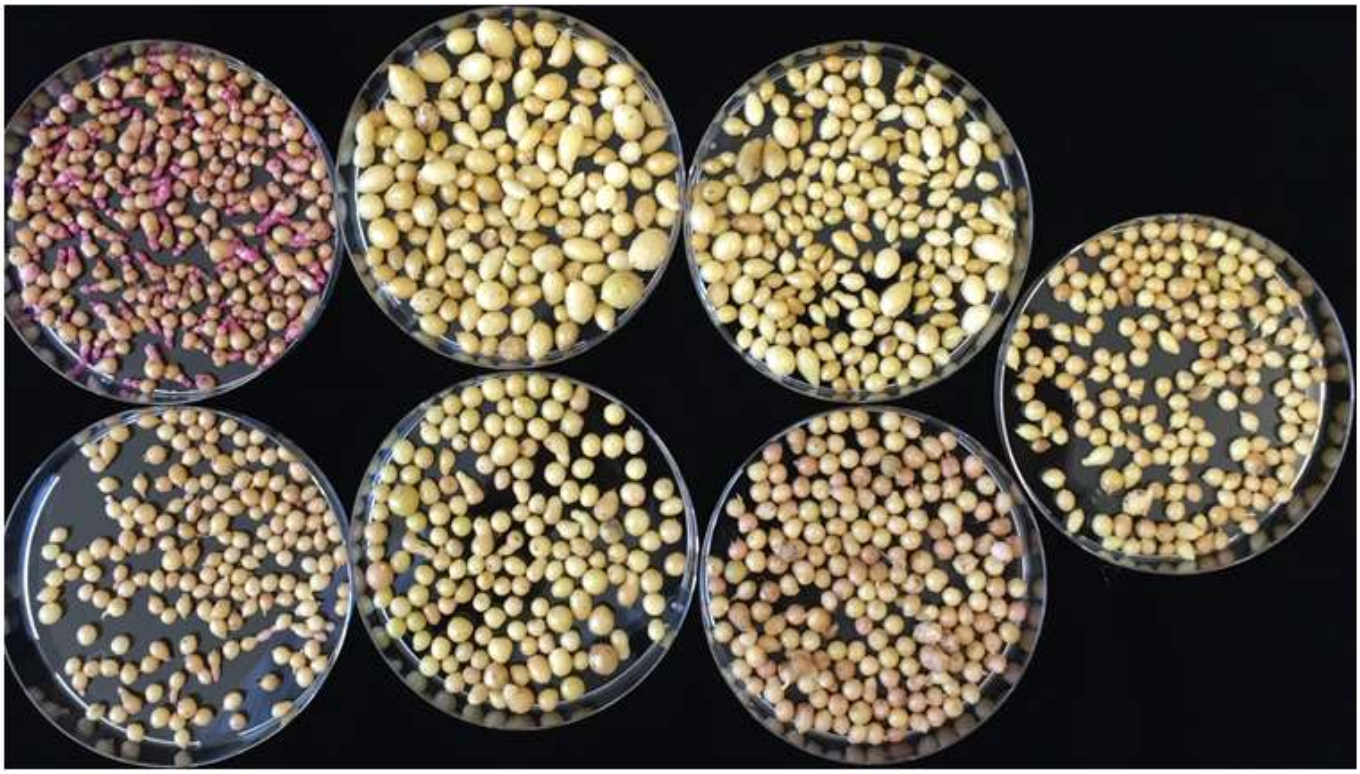

Figure 4. Micro-tubers of potato cultivars showing variation in shape, size and color): upper row, left to right: Basotho Pink, BP1, Up-To-Date, bottom row: Mpya, Asante, Sherekea and last to the right side: Kondor.

division as well as cell extension and provides a simple early measure of mutagenic treatment effects. Although reduced plant growth was recorded with increasing gamma irradiation dose for most genotypes, however the dose 5 Gy exerted a growth stimulation effect on the genotypes Kondor, Mondial and Mpya. The phenomenon of growth stimulation due to low irradiation treatments was recorded in the present study (Figure 2) and has been reported in M_1/M_1V_1 generation in many radio-sensitivity tests for seed and vegetatively propagated

Table 6. Analysis of variance for effects of gamma irradiation doses on sprouting ability of seven potato genotypes.

Source of variation	DF	ChiSquare	Prob>ChiSq	P-value
Dose (Gy)	1	83.158799	<0.001*	<0.00000
Genotype	6	18.460181	0.0052*	0.00518
Dose (Gy) *Genotype	6	1.6026054	0.9524	0.95239
Difference	13	127.0782	<0.0001	

Table 7. Sprouting ability rate (%) and the respective mutation induction dose (LD_{30} – LD_{50}) for seven potato genotypes.

Dose (Gy)	Genotype						
	Asante	Basotho Pink	BP1	Kondor	Mpya	Sherekea	Up-To-Date
0	76.92	88.00	75.00	96.97	86.96	90.48	92.31
10	75.00	88.00	65.00	100.00	78.26	80.95	83.33
20	53.85	88.46	50.00	93.55	65.22	68.42	65.39
30	57.69	65.39	40.00	75.76	21.74	42.86	46.15
40	57.69	50.00	20.00	68.75	4.17	27.27	11.54
60	28.00	21.43	10.00	34.38	0.00	23.81	7.69
80	11.54	8.33	0.00	25.00	0.00	20.00	4.00
LD_{30} (Gy)	12.9	27.9	15.4	41.3	13.9	15.7	17.05
LD_{50} (Gy)*	35.5^{bc}	41.1^{ab}	32.4^{bc}	54.8^{a}	20.6^{d}	33.8^{bc}	26.8^{cd}

*Values in the same row followed by the same letter are not significantly different.

crops (Al-Safadi and Simon, 1990; Wiendl et al., 1995; Paull, 1996; Jain et al., 2011; Cheng et al., 2010) confirming the present results. Generally, irradiation induced growth stimulation is observed with low dosage treatments, and is genotype dependant. Performing radio-sensitivity test on *in vitro* cuttings of three potato genotypes Al-Safadi and Arabi (2003) reported that 1, 5 and 10 Gy treatments of gamma rays stimulated post-irradiation plant growth. At higher dosages, DNA damage occurs more frequently and provides more mutation events but mostly lethal to plant survival (Preuss and Britt, 2003). The *in vitro* cutting radio-sensitivity test revealed resistant, moderately resistant and susceptible genotypes among the different potato genotypes. Genetic variation may explain the differential responses in different genotypes within a species, which are due to physical and biochemical characteristics of the tissue, such as propagule size, water content, DNA content, nuclear volume, etc.

In mutation induction, radio-sensitivity is performed with the purpose of selecting the optimal treatment for a specific genotype, that is, the dose that will provide the desired genotype (mutant trait in low mutational load genetic background) at a frequency that can be detected in a mutant population. This is especially important in vegetatively propagated crops - such as potato - as it is difficult to restore an elite genetic background by backcrossing. The optimal dose for mutation induction was found to be around 30 Gy for potato *in vitro* stem cuttings, which gave 50% reduction of the shoot length (Safadi and Arabi, 2003, 2007). The contrast of this high dose to observations in this study could be explained by the different genotype and also the radiation method and the application of the dose rate (chronic dose rate of 0.71 Gy/min) used. In order to produce the same relative biology effects, higher doses under chronic irradiation are needed than under acute irradiation because of plant tissue adaptation to irradiation (Esnault et al., 2010). The present data are in agreement with the results of Yaycili and Alikamanoglu (2012) on potato.

Two different schemes have been developed to induce and isolate potato mutants after mutagenesis on *in vitro* stem cuttings (Scheme 1A and B, Figure 1). A plantlet is the final product of chimera dissolution in Scheme 1A and may be subject to screening for desirable mutant traits (Safadi and Arabi, 2003, 2007; Esnault et al., 2010). Scheme 1B involves micro-tubers as the final products. The advantages of micro-tubers in comparison to plantlets are manifold (Nistor et al., 2010). In fact, the advantage of using micro-tubers in strategy 1B, 2 and 3

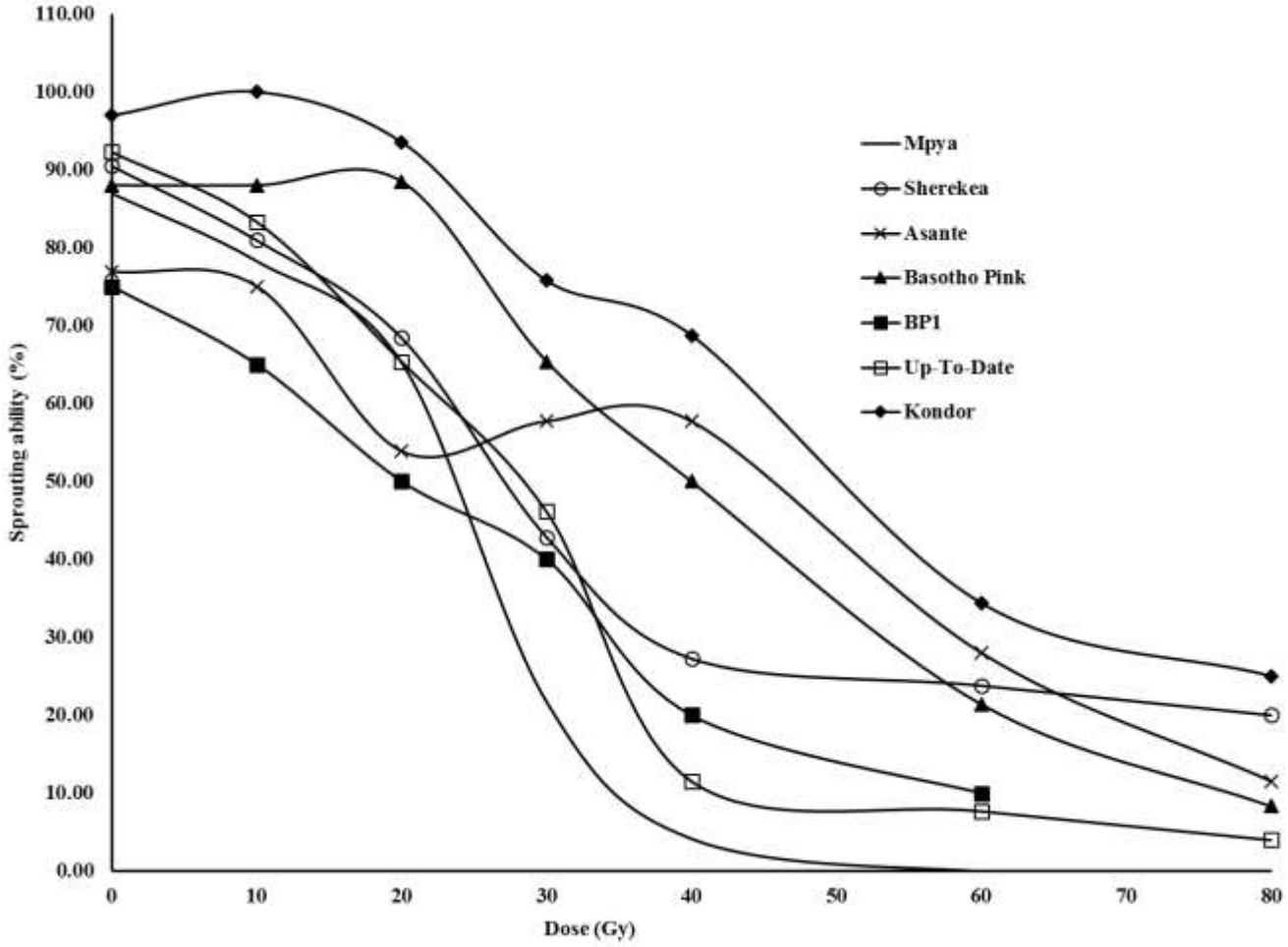

Figure 5. Effects of gamma irradiation on micro-tubers sprouting of seven potato cultivars after treatment with GA_3 and 4 weeks incubation.

over *in vitro* plantlets in strategy 1A is the higher vigour and vitality of micro-tubers. The production can be carried out all year-round; there is no need for immediate plant production as micro-tubers can be stored, and micro-tubers are easy to transport (Nistor et al., 2010) (Table 8).

The loss of *in vitro* plantlets before reaching field trials recorded during the acclimatization step is much higher, whereas micro-tubers sprouting ability can be enhanced to 100% using GA_3 and was even shown to enhance yield (Pruiski et al., 2003). Although *in vitro* plantlets can be maintained without sub-culturing for up to 2 months, they are sensitive to stress such as drought during acclimatization. However, micro-tubers withstand handling better and do not dry out as rapidly as plantlets. Micro-tubers are generally dormant and can be transported or shipped over long distances and stored for over 6 months. Additionally, micro-tubers may be used in early screening, which reduces field labour to evaluate the mutant lines. Over all, large scale handling like mutant population of plantlets requires more laboratory space and manpower for maintenance in comparison to micro-tubers (Table 8).

Advantages of micro-tubers were taken into consideration at the early stage during assessment effects of gamma irradiation on the tuberization. This study revealed genotype variation in tuberization capacity (Figure 3), as previously reported by Ahloowalia (1994, 1999) who investigated the ability of 15 potato cultivars to form mini-tubers. However, micro-tuber weight and size were also significantly determined by the genotypes (data not shown). Various weight, size and eyes of micro-tubers were recorded in each treatment and compared to untreated samples. These parameters are not recommended for Scheme 3 because the scattering of data for weight and size found for each dose makes the assessment difficult. In fact, similar findings on micro-

Table 8. Comparison of advantages and disadvantages of the three *in vitro* mutation induction schemes for potato.

Scheme	Advantages	Disadvantages
1A	1. *In vitro* screening for abiotic and abiotic stresses 2. Plant phenotyping 3. Advanced generation development	1. Chimera dissolution 2. Laborious maintenance 3. Incubation room space 4. Long period of mutant population development 5. Population size limited to original mutation induction size 6. Acclimatization losses 7. Specialized shipment
1B	1. – 3. as in 1A 4. Easy handling and transport micro-tubers	1. 5. as in 1A 6. Requires optimal tuberization conditions
2	1. Short period of population development 2. Easy handling and transport micro-tubers 3. Generation ready to be screened (nutritional, tuber shape, color) 4. Limited laboratory work	1. May require a pre-treatment for sprouting ability 2. Requires optimal tuberization conditions 3. Population size limited to original mutation induction size
3	1. 2. as in 2 3. May require less micro-tubers for large population size, since the population size is factor of number emerged eyes per tubers 4. Very limited laboratory work	1. 2. as in 2 3. Developed population is at first generation M_1V_1. 4. May be seasonal advanced in field

tuber weight and size after gamma irradiation of stem cuttings before tuberization were already reported (Al-Safadi et al., 2000; Li et al., 2005; Mahfouze et al., 2012). Rarely more than one micro-tuber was produced *per* cutting; which is convenient when adopting a 'single seed descendant' type approach in advancing potato lines from micro-tubers. Therefore, the tuberization capacity of cuttings was used to distinguish the radio-susceptibility of potato genotypes. Optimal dose established (LD_{50}) for mutation induction exhibited the relative resistance of genotype BP1, Basotho Pink, Kondor and Mondial to be moderate resistant, whereas Mpya was found to be radio-susceptible to gamma irradiation. A similar stimulation of micro-tuber induction by gamma irradiation was previously reported in different potato genotypes (Al-Safadi et al., 2000; Li et al., 2005; Al-Safadi and Arabi, 2003; Mahfouze et al., 2012). In this study the stimulation dose varied between potato genotypes, but remained below or equal to 10Gy under *in vitro* culture conditions. In addition to enhancing the tuberization rate, low gamma dose affects positively affected the content of ascorbic acid, reducing sugars and proteins of micro-tuber (Li et al., 2005). Low irradiation doses were reported to stimulate plant growth through enhanced physiological activity (Roy et al., 2009).

It is important to note that the mutagenesis reported by different studies on micro-tuber induction and gamma irradiation were for the purpose of enhancing the micro-tuber production with minimal genetic change (Al-Safadi et al., 2000; Li et al., 2005; Mahfouze et al., 2012). On the contrary, the attempt in this study was to determine the optimal dose treatments for mutation induction with the objective of generating mutant populations for screening in potato improvement, a unique approach to our knowledge. The most effective doses could be compared to stimulation doses. The optimal dose for mutation induction using scheme 2 (Figure 1) allowed the production of M_1V_2 generation micro-tubers. The advantage of this scheme is the direct production of micro-tubers at a stage, which can be screened for micro-tuber size, color, sprouting ability. In addition, micro-tubers may be evaluated for their biochemical content, but it is important to note that screening technique may be destructive (Table 8). An advantage of micro-tubers is they are easily multiplied and therefore reserve clones may be developed.

Effects of gamma irradiation on the sprouting ability of *in vitro* micro-tubers are comparable to seed mutagenesis with regard to dosage applied and shipment constraints. This represents an advantage for potato mutation

breeding, when the mutagenesis facility is not available in a given laboratory. Thus the study aimed to establish optimum dose for this type of potato propagule (Scheme 3 and Figure 1). The results of optimal dose (LD_{50}) established for the seven potato genotypes are comparable to gamma irradiation reported for mini-tubers of potato cultivar Shepody (Cheng et al., 2010). Doses of 10, 20 and 30 Gy promoted sprouting in mini-tubers whereas 60 Gy caused no sprouting. This study presents the first data on potato micro-tuber irradiation. Micro-tubers also have similar susceptibilities to gamma irradiation as mini-tubers. The radio-sensitivity test on white yam mini-tuber showed a 50% lethality dose around 40 Gy for gamma irradiation (Nwachukwu et al., 2009), which matches our findings on micro-tubers irradiation with Kondor and Basotho Pink genotypes. However, in micro-tuber gamma irradiation Asante, BP1 and Sherekea were moderately resistant and Mpya and Up-To-Date susceptible genotypes. That variable response recorded with micro-tubers exhibits the same genetic variation observed among potato genotypes with *in vitro* cuttings in the two other schemes. The M_1V_1 plants produced by scheme 3 have the advantage of increasing the population size because of the number of sprouted eyes per micro-tuber. However, the following generation is recommended for screening and selection. Thus Table 8 summarizes the advantages and disadvantages of the three strategies adopted in this study as guidelines regards to irradiation facility availability, population development, screening and selection of mutants.

The mechanisms of mutation induction caused by irradiation are complex and discussed in Lagoda (2012) and Shu et al. (2012). For mutation breeding optimal irradiation doses should be applied to induce adequate genetic changes to allow for efficient selection of desirable mutants (Nwachukwu et al., 2009; Sparrow, 1961). However, different genotypes and different tissues/propagules may show a different susceptibility to irradiation (Ahloowalia and Maluszynski, 2001). The present study corroborates previous studies by showing that cultivars respond differently depending on the tissues/organs that are subjected to irradiation. In fact, micro-tubers like mini-tubers show more resistance to gamma irradiation than most other propagules used in potato breeding.

Mutation induction optimum dose as 50% of growth reduction or lethality dose of gamma irradiation for potato mutation breeding after evaluation of the susceptibility of various genotypes are 10 to 21 Gy, 7 to 13 Gy and 20 to 55 Gy respectively for gamma irradiation of stem cuttings, tuberization and micro-tuber sprouting ability. Results in this study showed a higher susceptibility of the tuberization process to gamma irradiation than shoot growth. On the other side, micro-tuber sprouting ability was most resistant to gamma irradiation. Three schemes were evaluated here, the advantages and disadvantages for potato mutation breeding are given in Table 8. The importance of micro-tuber versus plantlets has been discussed at length with regard to development, handling of putative mutant populations and their yield enhancement reported by different researchers (Nistor et al., 2003; Pruski et al., 2003).

Conclusions

In vitro tissue culture combined with mutation induction proved to be effective in inducing useful mutants in vegetatively crops. Mutagen dose/concentration and the plant tissue/propagule used in the mutagenesis treatment are key factors for the successful improvement of potato through induced mutation. Potato currently lags behind other crops in improvement via plant mutation breeding. Three schemes have been developed to exploit *in vitro* cultures in potato mutation induction. Genotypic variation was recorded with respect to radio-sensitivity. The choice of scheme will depend on available facilities, the ability to develop, handle large mutant populations and screening for desired mutant types.

Conflict of Interests

The authors have not declared any conflict of interests.

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