



Assessment of Genetic Variability Estimates of Selected Traits in Irish Potato Mutants

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Authors' contributions

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

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ABSTRACT

Aims: For an effective potato breeding strategy, knowledge of the genetic parameters of traits, such as heritabilities and genetic correlations are essential, hence the need to assess the genetic variability estimates of yield-related traits in Irish potato mutants

Study Design: At M1V1 generation, there was no replication of the mutant minitubers because each does not maintain the same genetical constitution after irradiation. In M1V2 and M1V3 generation the tubers were replicated 3 times in alpha lattice design.

Place and Duration of Study: Irradiation was done at the Plant Genetics and Breeding Laboratories (PGBL) at IAEA/FAO Seibersdorf, Vienna, Austria. After mutation induction, the mutant microtubers (consisting of Asante, Mpya and Sherekea) were transported to Kenya, University of Eldoret for establishment between April 2015 and March 2017.

Methodology: A total of 30 tubers each of the three potatoes was sent for irradiation. Two *in-vitro* radio-sensitivity tests were developed involving different tissues: Irradiation of *in vitro* nodal cuttings

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(without leaf) followed by *in vitro* shoot propagation and irradiation of *in vitro* nodal cuttings (with leaf) followed by direct *in vitro* micro-tuber production. After mutation induction, a total of 570 mutant microtubers (Asante 230, Mpya 160, Sherekea 180) were developed from the three potato varieties and was transported to Kenya, University of Eldoret for the establishment. The M1V1 microtubers were established in the greenhouse while M1V2 and M1V3 generations of mutants were planted at the at the University of Eldoret research field.

Results: It showed that the highest positive heritability percentage (H^2) estimates in Mpya and Sherekea mutants were in plant height with 81.51% and 87.7% respectively.

Conclusion: Tuber number exhibited high heritability estimates displaying that induced mutation was successful in the development of new potato genotypes which be used in future breeding programs.

Keywords: Potato; mutants; irradiation; variability; heritability; microtubers.

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, and 3]. Globally, the total estimated area under potato production is 19 million hectares with a total production of 381.7 million tonnes in 2014 [3]. In sub-Saharan Africa (SSA), the East and Central Africa region accounts for over 45 % of potato production and 52 % of the area harvested. Kenya is the fifth biggest producer of potato in SSA after Malawi, Rwanda, Ethiopia and South Africa [3].

Despite the importance of potato in Kenya, its production has not been achieved to its full potential with the national average yields of 18.5 tonnes per hectare (ton/ha) against an average crop potential of 40-60 ton/ha [3]. The low yields are due to the following constraints; inadequate supply and untimely availability of high quality certified seeds, low soil fertility, low yielding varieties, diseases and insect pests among others [4, 3]. About 1-2 % of the nationally certified seed potato requirement in Kenya is being met, and this negatively impacts the potato value chain [5,6].

The cultivated potato (*Solanum tuberosum* L.) is a tetraploid ($2n=4x=48$, 4 EBN (Endosperm Balance Number)) that exhibits complex tetrasomic inheritance patterns [7]. The crop is highly heterozygous and upon selfing suffers from inbreeding depression [7]. The level of heterozygosity is influenced by the four different alleles within a locus; the more diverse they are, the higher the heterozygosity and the greater the number of interlocus interactions, hence greater heterosis [8, 9, 10, 11]. Understanding the implications and complexities of tetrasomic

inheritance in the cultivated potato breeding is vital in enhancing efficiency in a breeding programme. Conventional potato breeding is cumbersome, takes a long time and less successful in crops like potato because of the heterozygous nature, making it an excellent crop to be improved by mutation. Knowledge of genetic parameters such as heritabilities and genetic correlations are also required to help guide an effective potato breeding strategy [12]. The study, therefore, explored the assessment of genotypic variability estimates of agronomic traits selection in potato mutants in Kenya.

2. MATERIALS AND METHODS

2.1 Irradiation of Plant Materials

Three commercial potato varieties namely; Asante, Kenya Mpya and Kenya Sherekea with various characteristics were obtained from Kisima farm [13]. A total of 30 tubers each of the three potatoes were sent to the Plant Genetics and Breeding Laboratories (PGBL) at IAEA/FAO Seibersdorf, Vienna, Austria, where they were grown at the greenhouse to initiate *in vitro* shoot cultures as described by Bado [14]. A radioactive cobalt-60 (Co^{60}) (gamma source) with a low dose rate of 2 grays per minute (Gy/min) were used to induce mutations. Two *in-vitro* radio-sensitivity tests were developed involving different tissues for irradiation of potato mutation induction as described by Bado [14] namely: 1) Irradiation of *in vitro* nodal cuttings (without leaf) followed by *in vitro* shoot propagation to dissolve chimaeras. A dose range of 0, 5, 10, 15, 20 and 30 Gy was used, and 2) Irradiation of *in vitro* nodal cuttings (with leaf) followed by direct *in vitro* micro-tuber production. The dose range used; 0, 3, 6, 9, 12, and 15 Gy.

2.2 Establishment of M1V1 and Coding of Surviving Putative Mutants in the Greenhouse

A total of 570 mutant microtubers (M1V1) were received from Seibersdorf laboratories, Vienna, Austria, 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 minitubers because each does not maintain the same genetical constitution after irradiation. Induced potato plants derived from minitubers were therefore assumed to be different. The coding of the M1V1 plants was developed based on the number of irradiated stakes that survived and was then advanced to the subsequent generations. This was done in sequence based on the dosage rate applied. The first letter in the name for each variety (A, M, and S for Asante, Mpwa and Sherekea respectively) was used to separate the specific mutants.

2.3 Planting of M1V2 and M1V3 Mutants in the Field

The tubers obtained at M1V1 were advanced to M1V2 and M1V3 generations of mutants by planting at the University of Eldoret research field.

Forty-eight (20 for Asante, 12 for Mpya and 16 for Sherekea) elite potato mutants and one standard check (Beauregard) for each of the three mutants were considered in this study for two generation cycles from the year 2015 to 2016.

2.4 Experimental Site

The experiment was carried out at the University of Eldoret (UoE) which is at an altitude of 2153 metres above sea level (masl), the latitude of 0°34'N and longitude 35°18'E. The average annual rainfall is 1295 mm with a bimodal distribution. The mean air temperature ranges from 15 to 28 °C. The soil type is rhodic ferralsol [15].

2.5 Experimental Design, Layout and Planting in Field Plots

In M1V2 and M1V3 generation five tubers per plot/mutant were planted replicated 3 times in alpha lattice design.

The linear model for alpha design was:

$$y_{ijtl} = \mu + g_i + r_j + \alpha_t + \alpha(r)_{jl} + \varepsilon_{ijtl}$$

Where;

y_{ijtl} = represent the observations,

μ = is the population mean,

g_i = the genotypic effects,

r_j = the resolvable replicate effects,

α_t = the latinized block effects,

$\alpha(r)_{jl}$ = the incomplete block effects within replicates and

ε_{ijtl} = the random errors.

The experimental area was divided into eight blocks where each block consisted of six plots. Each plot consisted of a mutant selection from a single variety and was planted in 1 × 0.6 meters (m) spacing. This setup was set in 3 replicates, and the distance between replicates was 3 m. All agronomic practices were carried out according to recommended practices [16]. The M1V2 generation was established in April to July 2015, and the M1V3 generation was planted between January to May 2016.

2.6 Data Collection

Data were collected on each of the mutant plants in M1V2 and M1V3 generation. Standard potato descriptors according to International Union for the Protection of New Varieties of Plants (UPOV) [17] were used to describe the potato mutant selections in M1V2 and confirmed in M1V3 as described in Table 1 above to determine the diversity of the selections. The M1V2 mutant plants were selected at harvesting of tubers based on the average tuber weight per plot. The M1V2 mutant plants that produced 25 % higher on average plant tuber weight compared with the control/parent were selected. Each of the selected individual mutant plants was labelled and advanced to M1V3 generation.

2.7 Statistical Analysis

The data on the effects of the different induction levels on the potato characters of the selected M1V2 and M1V3 generation were used to generate means for the two generations. Combined ANOVA was performed for the traits by SAS software. Differences in means were compared by Duncan multiple range tests (DMRT). Variance components were extracted from the expected mean squares (EMS) of main effects.

Table 1. Morphological descriptors used for scoring potato accessions

Descriptor	Characteristic	Score
Plant	Growth habit	1=very upright, 3=upright, 5=semi-upright, 7=spreading, 9=very spreading
	Stem number per plant (Average)	Counting
	Plant height per plant (Average)	in centimetres
Stem	Anthocyanin colouration	1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong
Leaf	Outline openness	1=closed, 3=intermediate, 5=open
	presence of secondary leaflets	1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong
	green colour	1=very light, 3=light, 5=medium, 7=dark, 9=very dark
	anthocyanin colouration on midrib of upper side	1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong
Flower corolla	Anthocyanin colouration	1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong
Inflorescence	Anthocyanin colouration on peduncle	1=absent or very weak, 3=weak, 5=medium, 7=strong, 9=very strong
Tuber	Weight per plant (Average)	in kilograms
	Number per plant (Average)	Counting

(Source: International Union for the Protection of New Varieties of Plants (UPOV) [17])

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by [18] as:

Genotypic coefficients of variation (GCV)

$$GVC = \frac{\sqrt{\delta_G^2}}{\mu} \times 100 \quad (1)$$

Phenotypic coefficients of variation (PCV)

$$PVC = \frac{\sqrt{\delta_P^2}}{\mu} \times 100 \quad (2)$$

Where μ is the grand mean value of the trait

Broad-sense heritability (H^2) in percentage was estimated in each character using variance components as described by [19].

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2} \quad (3)$$

The expected gain or genetic advance with one cycle of selection, assuming the selection intensity of 25 %, as described by [20].

$$GA = (K)(\delta_P)(H^2) \quad (4)$$

Genetic advance in percentage of the mean (GAM) was calculated to compare the extent of

predicted genetic advance of different traits under selection, using the following formula:

$$GAM = \left(\frac{GA}{\mu}\right) \times 100 \quad (5)$$

3. RESULTS AND DISCUSSION

The association between phenotypic and genotypic values is important in predicting the outcome of selection in a collection of genotypes. For all the traits studied in all the 3 potato mutants, the estimates of the phenotypic coefficient of variation (PCV) were higher than the corresponding genotypic coefficient of variation (GCV) (Table 2). This indicates that these characters were influenced by the environment or generation and phenotype selection alone on the basis of these traits can be effective for improvement. The results on high PCV values compared GCV values agreed with [21] in sweet potatoes and those of [22] in linseed (*Linum usitatissimum* L.) genotypes.

Heritability estimates has been classified as low (below 30%), medium (30-60%) and high (above 60%) [23]. In the present study, the characters studied expressed all the heritability estimates from low to high ranging from 0.0 to 100.0 percent (Table 3). The disparity inheritability is not only a property of a trait but also of the

mutant population, generation and the genotypes circumstances which indicates that the induced variability in mutant population can be fixed by selection. High heritability estimates of more than 60% was reported in tuber number (Asante) for plant height, stem and tuber number in Mpya mutants and only plant height in Sherekea mutants. Similar findings on high heritability have been reported in different crops in various traits studied by different authors [24- 21, 28].

Table 2. Genetic estimate for various agronomic traits in a selected population in Asante, Mpya and Sherekea potato mutants

Mutants	Source of variation	DF	PH	PSN	PTN	PTW
Asante	Blocks	2	1491**	54.7***	362.2***	183.8***
	Genotypes	19	1164***	11.4***	76.7*	11.8*
	Error	38	199	3.9	126.4	55.5
Mpya	Blocks	2	0.01**	10.89*	9430***	60.5***
	Genotypes	11	526*	6.18*	204.3*	2.1*
	Error	22	125.2	0.33	95.1	0.38
Sherekea	Blocks	2	52.5	0.01	152.5	183.8***
	Genotypes	15	963.1***	9***	84.6*	24*
	Error	30	322.4	1.9	55.7	7
Combined	Blocks	2	2439***	632**	2322.5***	572.6***
	Genotypes	47	9163.1***	29***	84.6**	24***
	Error	94	322.4	13	142.5	257

*=significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$. Degree of freedom (DF), Growth habit (GH), Stem anthocyanin colouration (SAC), Leaf Outline openness (LOO), Leaf presence of secondary leaflets (LPSL), Leaf green colour (LGC), Flower corolla anthocyanin colouration (FCAC), Plant height (PH), Plant stem number (PSN), Plant Tuber number (PTN), Plant Tuber weight (PTW), Combined (All the 48 mutants: Asante, mpya and Sherekea).

Table 3. Estimates of Genetic parameters for various agronomic traits in Asante, Mpya and Sherekea potato mutants

Mutants	Traits	Mean±SE	Range	S ² _g	S ² _p	PCV	GCV	H ² (%)	GA	GAM (%)
Asante	PH	88.3±8.14	46 to 124	307.50	328.13	20.51	6.53	2.51	80.24	20.51
	SN	4.88±1.14	1 to 9	0.85	1.85	27.87	9.16	2.70	29.70	27.87
	TN	40.5±6.49	6 to 77	-23.03	6.08	9.31	5.52	82.30	81.91	6.09
	TW	21.76±4.30	5.1 to 44.2	3.38	8.63	13.01	11.09	53.33	85.54	13.01
Mpya	PH	82.1±10.91	46 to 124	10.88	71.19	10.28	0.12	81.51	85.59	10.28
	SN	3.5±1.04	1 to 9	0.66	1.10	29.98	2.86	65.61	81.76	29.98
	TN	26.4±8.25	6 to 77	-9.28	20.90	17.32	0.38	61.24	39.16	17.32
	TW	3.48±0.84	5.1 to 46.2	0.29	0.41	18.34	2.87	31.04	18.61	18.34
Sherekea	PH	93.5±6.39	43 to 135	-18.08	111.35	11.29	0.11	87.70	64.32	11.29
	SN	6.3±0.79	3 to 10	1.77	2.01	22.51	1.59	49.29	87.96	22.51
	TN	48.6±16.79	34 to 124	-8.28	6.44	5.22	0.21	66.60	63.88	5.22
	TW	34.48±0.86	6.8 to 44.2	1.75	3.88	43.94	2.23	69.35	80.58	43.94
Combined	PH	88.3±8.14	43 to 135	257.50	138.13	12.51	4.53	62.51	82.27	24.54
	SN	4.88±1.14	3 to 10	1.25	1.45	24.82	6.64	52.70	42.75	32.72
	TN	40.5±6.49	6 to 77	-15.03	9.08	12.31	3.51	64.30	73.15	16.86
	TW	21.76±4.30	5.1 to 46.2	2.58	6.63	23.01	8.09	63.43	65.54	23.01

Standard error (SE), Genotypic variance (S²_g), Phenotypic variance (S²_p), Plant height (PH), Stem number (SN), Tuber number (TN), Tuber weight (TW) Combined (All the 48 mutants: Asante, mpya and Sherekea).

High heritability could be due to the high contribution of genotypic component hence heritability alone is not a reliable parameter to predict effective selection. High heritability for plant height has been reported in various crops such as wheat [25], spring wheat [26] and sorghum [28]. Mishra [29], reported moderate heritability in potato in plant height (54%) and high heritability estimates in dry weight of tubers, marketable tuber yield per plot, total tuber yield per plot and fresh weight of tubers per plant. The disparity in heritability results in potato plant height could be because heritability is a not merely a property of character but also of the population, environment and the circumstances to which the genotypes are subjected to. Concerning genetic advance, a higher percentage of more than 40% were observed in all the yield traits except stem number in Asante and tuber number and weight in Mpya mutants. The high heritability accompanied by high genetic advance signifies the predominance of additive gene effects that are substantively contributing towards the manifestation of these parameters.

All the three mutant populations showed significant genotypic variation on the tuber yield components such plant height, tuber number, stem number and tuber weight. The existence of variability in the mutant populations can be attributed to the effect of the induced mutation. Similar findings have been reported in mung bean for quantitative traits which showed that mutagenic treatments could alter mean values and create additional genetic variability [30]. The varietal differences have also been reported with respect to mutagen sensitivity in various crops such as *Lens culinaris* [24], *Arachi hypogyea* [31] and *Ipomoea batatas* [32]. The sensitivity of an organism (plant) depends upon the type of mutagen employed, plant genetic makeup, DNA amount and replication time in the initial stages beside physical factors such as moisture, oxygen pH, and temperature [33-36,14].

Highest estimates of genetic advance as percentage of the mean of more than 20% were obtained for most characters studied. However, the low genetic advance as per cent of the mean of less than 20% was also observed for the characters; plant height (Mpya and Sherekea), tuber number and tuber weight (Asante and Mpya), tuber number (Sherekea and the combined mutants). The low heritability indicates the existence of non- additive gene effects. The high genetic advance of the mean, high

genotypic coefficient of variation along with high heritability gives important information regarding selection advance of each parameter and therefore helps the breeder to predict the rate of improvement that can be achieved in different characters [37].

For successful genetic improvement of a crop, high heritability together with high genetic advance is helpful in assessing the nature of gene action and positive effects of selection. Gaul and Hesemann [38] reported that high genetic gain estimates can be obtained by comparing each generation of the mutant population or line with the best lines selected from the control population. High heritability and high genetic advance in genotype effects have also been reported by in sorghum by different authors [39,40,28]. Low genetic advance with moderate heritability observed tuber weight shows that its most probably governed by non-additive gene action and the presence of intra and interallelic interactions in the appearance of such character [41].

4. CONCLUSION

The present study indicates that the potato mutants showed diverse genetic variability estimates. It was observed that the tuber number exhibited high heritability estimates within the mutant populations. The induced mutation was successful in the development of new potato genotypes which could be used in future breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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