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Developing Genetic Variability of Quinoa (*Chenopodium quinoa* Willd.) with Gamma Radiation for Use in Breeding Programs

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is a staple food produced mainly by small-scale subsistence farmers in Peru's highland. Dry seeds (cv. Pasankalla) were irradiated with doses of 150 Gy, 250 Gy and 350 Gy. In the M_1 generation, the germination process was delayed with increasing radiation dose; seedling height, root length and leaf development were most reduced at 250 Gy and at 350 Gy, no plants survived. In M_2, the maximum spectrum of chlorophyll mutations corresponded to 150 Gy and the maximum frequency to 250 Gy. The chlorine mutation was predominant, followed by xantha. Changes were registered for branch number, pedicel length, plant height, life-cycle duration, stem and foliage colour, and leaf morphology at the two doses, with improvements in plant type. More than one mutation per plant was found, especially at 250 Gy. In M_3, the same spectrum of mutations was observed, along with a valuable change in grain colour.

Keywords: *Chenopodiun quinoa*; Gamma Rays; Mutant

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is native crop of the Andes. Quinoa is cultivated in Peru by small farmers in plots below of 1 ha [1]. Its tolerance to drought, frost and saline soils makes it important in highland farming systems [2,3]. This annual crop belongs to the Chenopodiaceae (goosefoot) family. Quinoa has an exceptionally nutritious balance of protein, fat, oil and starch. Grains average 16% protein, higher than common cereals. Moreover, the protein is of unusually high quality, and is close to the FAO standard for human nutrition. Quinoa protein is high in the essential amino acids lysine, methionine and cystine, making it complementary to both other grains (deficient in lysine) and legumes (deficient in methionine and cystine) [4-6].

Quinoa is planted mostly in Puno to altitudes of over 3800 m. Due to the stressful climatic conditions and the technology in use there, productivity is low; for many years it ranged from 900 to 1100 kg/ha [1]. There are many ways to improve crop performance: one of them is genetic improvement via mutation induction. According to the IAEA database, there are more than 2500 mutant varieties of more than 170 different species [7]. There are many reports of improved morphological and physiological characteristics in cereals, grain legumes, oil seeds, fiber crops, vegetables and ornamentals following mutation induction, with gamma rays being the preferred agent, and plant type and yield are the traits most commonly reported [8-10]. Although the quality of many crops has been improved thru mutation induction [11], to the best of our knowledge, this technique has not been applied in quinoa to improve its agricultural performance in the marginal conditions of the Peruvian highlands but it was applied in barley and *Amaranthus* [12].

The Cereal and Native Grain Research Program at the National Agricultural University La Molina (Peru) is making efforts to improve the early cultivar Pasankalla-INIA, which has a growing period of 140 days; the plant can measure up to 120 cm in height; its panicle is light purple, and it has a glomerular inflorescence of intermediate density. Seeds have a reddish-brown pericarp and the episperm (seed coat) is lead-colored.

2. Materials and Methods

2.1. Plant Material

Two hundred grams of dry quinoa seeds with 12% of moisture of cv. Pasankalla were irradiated with gamma rays at the Peruvian Institute of Nuclear Energy (IPEN), at doses of 150, 250 and 350 Gy. Non-irradiated seeds were used as controls in each evaluation. Seeds have to be delivered for irradiation to a nuclear centre. Such centres have been established in most countries or at IAEA Headquarters in Vienna.

2.2. Percent of Germination in M_1 Generation

To establish percent of germination, 100 seeds per Petri dish (Ø: 10 cm, height: 2 cm) were placed on filter paper and covered with tissue paper. Each treatment included four Petri dishes (replicates) (for a total of 400 seeds per treatment). Distilled water (5 ml) was added at the beginning of the experiment. Every dish was then irrigated daily with 1 ml water to maintain moisture. The experiment was conducted under a photosynthetic photon flux density of 458 µmol/m^{2}s (12 h/day) at 20˚C. The germination percentage was recorded on days 3, 5, 7, 9, 11 and 15.

2.3. Physiological Damage in M_1 Generation

To determine the physiological damage or somatic effects caused by the irradiation, seedling height and root length were measured in 1-month-old plants using the "sandwich blotter" technique [17]. Thirty seeds from each treatment were used in this experiment in four replicates (for a total 120 seeds per treatment). The level of somatic effects after mutagenic treatment can be evaluated on the basis of various parameters, including delay in seed germination; level of disturbances in the cell cycle; reduced seedling emergence; reduced seedling and plant growth; appearance of chlorophyll defects; and reduced fertility and plant survival. The term "reduced" indicates change in expression of a particular character in relation to the control, usually the parent cultivar or the breeding line whose seeds were treated with a mutagen [14]. The rest of the seeds from each treatment were sown in beds under field conditions following the protocol described for quinoa crop in Peru [15]. The formation of cotyledonal and true leaves and percent of survival were evaluated. Individual heads or inflorescences of the M_1 generation were harvested. Data were analyzed using a completely randomized design.

2.4. Evaluation of M_2 Generation

The M_2 population was cultivated in the field, one inflorescence per row. The following traits were evaluated: chlorophyll mutations, life-cycle duration, plant height, and morphological characteristics. Putative mutants were identified and harvested separately as individual plants. This generation was cultivated following the protocol described for different crops under isolated field conditions [14].

2.5. Evaluation of M_3 Generation

The selected putative M_2 mutants were cultivated as row/plant (in the field, one plant per row) for progeny determination and homozygosity testing. This generation was cultivated following the protocol described for different crops under field conditions planted in a row or rows depending on the amount of seed [14].

2.6. General Experimental Scheme

Figure 1 shows the experimental scheme followed in this research.

2.7. Data Analysis

The Statistical Package for Social Sciences (Version 17.0 for Windows, SPSS Inc.) was used to perform ANOVA and Tukey tests ($p \leq 0.05$).

3. Results and Discussion

3.1. Analysis of M_1 Generation

Germination of the control, non-irradiated seeds was normal, reaching 100% at 7 days. In contrast, the germination process was slow in the irradiated seeds, becoming slower as the dose increased (**Table 1**). At 15 days, germination of all treated seeds had reached 99%. The effect of the radiation was evident only during the germination process.

Radiosensitivity was also evaluated by looking for physiological damage in the developing seedlings. Statistical differences were found by One-Way ANOVA and Tukey tests in root length and seedling height among the controls and all evaluated treatments (150, 250 and 350 Gy) (**Table 1**).

Under field conditions, using a scale that reflects the phenological stages of quinoa development during vegetative growth [16], differences were observed among the treated plants and controls (**Table 1**). The control plants

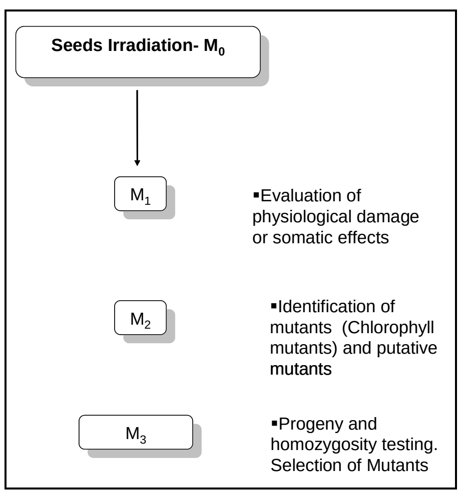

Figure 1. Experimental scheme followed in this research.

Table 1. Germination and early growth of quinoa (*Chenopodium quinoa* Willd.) cv. Pasankalla following gamma-irradiation treatment.

Gamma ray dose (Gy)		0	150	250	350
Germination (%)[1]	3 days	53 a	30 b	25 c	20 d
	5 days	78 a	55 b	49 c	41 d
	7 days	100 a	71 b	63 c	52 d
	9 days	100 a	84 b	77 c	68 d
	11 days	100 a	99 a	84 b	79 c
	15 days	100 a	99 a	99 a	99 a
Root length at 30 days of germination (cm)		7.23 a	4.58 b	4.07 b	2.80 c
Seedling height at 30 days of germination (cm)		3.81 a	3.05 b	2.73 bc	2.55 c
Days required for leaf formation[2]	Cotyledonal leaves	10 b	12 b	15 a	15 a
	1st pair of true leaves	8 c	14 b	18 a	True leaves were not formed in this treatment.
	2nd pair of true leaves	14 c	19 b	25 a	
	3rd pair of true leaves	18 c	27 b	30 a	
	4th pair of true leaves	22 c	30 b	34 a	
Plant survival at 30 days of field growth (%)[1]		80 a	53 b	28 c	0 d

In each row, results with the same *letter* are not statistically different (One-Way ANOVA, Tukey, $p > 0.05$). [1]For statistical analyses only, percentages were transformed according to $y' = 2 \arcsin((y/100)^{0.5})$. [2]For statistical analyses only, the numbers of days required for leaf formation were transformed according to $y' = y^{0.5}$.

and the plants from the 150 and 250 Gy treatments advanced from germination to vegetative growth, but the 350 Gy-treated plants did not. In this latter treatment, the plants did not form leaves and none of them survived. Number of days to leaf development increased with increasing dosage of gamma rays. We observed 80% survival in the control, 53% survival in the 150 Gy treatment and 28% survival in the 250 Gy treatment.

3.2. Analysis of M_2 Generation

Table 2 presents the frequency and spectrum of chlorophyll mutations: the maximum spectrum corresponded to 150 Gy and the maximum frequency to 250 Gy. The chlorina mutation was predominant, followed by the xantha mutation. The frequency of chlorophyll mutations increased with increasing dosage. Leaf pigment mutations were observed at 150 Gy, as a change in cotyledon leaf color from green to pink, at a frequency of 0.086% (**Table 2**).

Table 2 also presents the entire spectrum of morphological and physiological mutations observed among the irradiated plants. Changes were registered for branch number and pedicel length, stem and foliage color, plant height and life cycle (both reduced), leaf morphology (spoon type) and plant type (improved).

In the 150 Gy treatment, the highest frequencies corresponded to vigorous plant (growth) and branching (**Table 2**). In the 250 Gy treatment, higher frequencies were found for plants with cream-colored grains and light green foliage. A similar frequency spectrum was found at each dose, but there were more mutations per plant at the higher doses. Some of the possible mutant plants did not give seeds.

3.3. Analysis of M_3 Generation

The changes in morphological and physiological characteristics identified in the M_2 generation, in general, were observed in the M_3 generation, confirming the mutations (**Table 3**). The light green foliage observed in M_2; almost phosphorescent, was not observed at M_3 generation. The most valuable changes were those of grain color on the pericarp from reddish-brown to cream and on the episperm (seed coat) from lead to cream and plant height reduction.

The response observed in quinoa to gamma ray irradiation treatments showed similar somatic effects to that reported to other crops at M_1 generation. The root length and seedling height of generation M_1 decreased proportionally to dosage amount (**Table 1**), as reported for other crops [14,17] and the identification of irradiation dosage limit of 350 Gy for cv. Pasankalla [18]. One peculiar response of quinoa to the damage of irradiation is the longer time for true leaf formation and the absence of them at high dose finishing with the death of the seedlings.

In similar way, radiation genetic effects were observed in generation M_2 and M_3. Mutation of different characters were founded (**Tables 2** and **3**) as reported in other crops [9,19,20]. In quinoa at seedling stages chlorophyll and anthocyanin mutations were observed as early indicator

Table 2. Spectrum and frequency of mutations in M_2 generation of quinoa (*Chenopodium quinoa* Willd.) cv. Pasankalla following gamma-irradiation treatment at doses of 150 and 250 Gy.

150 Gy (total number of plants studied = 55,758)			250 Gy (total number of plants studied = 10,372)		
	No. of mutants	Mutation frequency (%)		No. of mutants	Mutation frequency (%)
Chlorophyll and leaf pigment mutations					
Xantha	20	0.036	Chlorina	10	0.096
Chlorina	21	0.037			
Leaf color: anthocyanin (pink)	48	0.086			
One characteristic modified by gamma-irradiation					
Improved plant type	48	0.086	Improved plant type	6	0.058
Reduced plant height	1	0.002	Earliness	1	0.010
Earliness	29	0.052	Light-green foliage	2	0.020
Branching habit	47	0.085	Grain colour	50	0.480
Light-green foliage	25	0.045			
Lateness	2	0.004			
Increased flower pedicel	1	0.002			
Purple stem	1	0.002			
Two characteristics modified by gamma-irradiation					
Improved plant type and earliness	11	0.020	Improved plant type and reduced plant height	2	0.019
Improved plant type and stem with purple streaks	3	0.005	Improved plant type and branching habit	6	0.058
Stem with purple streaks and spoon-type leaves	5	0.009	Improved plant type and light-green foliage	9	0.086
Improved plant type and increased plant height	1	0.002	Improved plant type and earliness	9	0.086
Improved plant type and increased flower pedicel	1	0.002	Reduced plant height and earliness	2	0.019
Improved plant type and branching habit	6	0.011	Reduced plant height and light-green foliage	4	0.038
Improved plant type and light-green foliage	10	0.018	Branching habit and light-green foliage	58	0.557
Reduced plant height and earliness	2	0.004	Improved plant type and compact inflorescence	1	0.010
Reduced plant height and light-green foliage	8	0.001	Branching habit and earliness	1	0.010
Branching habit and purple stem	1	0.002	Branching habit and lateness	1	0.010
Branching habit and increased plant height	4	0.007			
Branching habit and reduced plant height	9	0.016			
Branching habit and light-green foliage	10	0.018			
Earliness and light-green foliage	2	0.004			
Three characteristics modified by gamma-irradiation					
Branching habit, increased plant height and stem with purple streaks	1	0.002	Branching habit, light-green foliage and lateness	3	0.029

Continued

Branching habit, light-green foliage and reduced plant height	1	0.002	Improved plant type, branching habit and lateness	2	0.019
			Improved plant type, branching habit and earliness	1	0.010
			Improved plant type, branching habit and light-green foliage	4	0.038
			Improved plant type, earliness and reduced plant height	2	0.020
			Improved plant type, compact inflorescence and light-green foliage	1	0.010
			Improved plant type, reduced plant height and light-green foliage	1	0.010
			Improved plant type, earliness and branching habit	5	0.048
			Branching habit, earliness and light-green foliage	2	0.019
Four characteristics modified by gamma-irradiation					
			Improved plant type, branching habit, earliness and light-green foliage	1	0.010
			Branching habit, light-green foliage, lateness and reduced plant height	2	0.019

Table 3. Spectrum and percentage of plants showing mutation in M_3 generation of quinoa (*Chenopodium quinoa* Willd.) cv. Pasankalla following gamma-irradiation treatment at doses of 150 and 250 Gy.

M_2-Mutation type	M_3-Mutation type	% of mutation type in M_3
One characteristic		
Improved plant type	Improved plant type	57
Earliness	Earliness	100
Grain color	Grain color	100
Two characteristics		
Improved plant type and reduced plant height	Improved plant type and reduced plant height	25
Improved plant type and branching habit	Improved plant type and branching habit	100
Improved plant type and earliness	Improved plant type and earliness	100
Reduced plant height and earliness	Reduced plant height and earliness	37.5
Improved plant type and compact inflorescence	Improved plant type and compact inflorescence	100
Branching habit and earliness	Branching habit and earliness	100
Branching habit and lateness	Branching habit and lateness	100
Three characteristics		
Improved plant type, branching habit and lateness	Improved plant type, branching habit and lateness	100
Improved plant type, branching habit and earliness	Improved plant type, branching habit and earliness	100
Improved plant type, earliness and reduced plant height	Improved plant type, earliness and reduced plant height	35.5
Improved plant type, earliness and branching habit	Improved plant type, earliness and branching habit	100

of mutation occurrence, similar to sesame, where four types of chlorophyll mutations have been reported: xantha, chlorina, striate and xantha viridis [21].

4. Conclusions

It is important to highlight valuable agronomic changes observed in this population thru mutation, considering that this crop was neglected by many decades and a minimum genetic improvement work was made until now. The farmers are still using land races with very long cycle and very high height plants [22], being these characters negative limiting factors for the introduction of quinoa to modern agricultural systems. Identified mutants with reduced life cycle, could be beneficial considering that some of the actual cultivars growing have long-cycle reaching over 7 months in the field with the flowering and grain maturity time under adverse weather conditions (drought and frost) that significantly reduce the performance. In similar way the identified mutants with reduction in plant height will be very useful considering that will reduce the high tendency to lodging and could improve the yield in similar way to that achieved in wheat [23-25].

The mutation in grain color of quinoa will be very important for some industrial use. This change also was reported in wheat, where light-colored grain mutants have been obtained from Pusa Lerma [26], Tonari 71 [27] and Sonora 64 [9] and were very appreciated by the end users.

Other important aspect observed in this study was that cv. Pasankalla explodes when roasted, similar to popcorn. This feature is retained in the mutants, with the added value of a final product that has a more appealing commercial appearance. The selected mutants will be evaluated for their agronomic performance in the next generations.

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