

Development of advanced mutant lines of native grains through radiation-induced mutagenesis in Peru

Abstract

In Peru is very important to increase food production in the rural areas where a high poverty and malnutrition problems are found. Mutation induction permits to improve adapted cultivars, by upgrading one or two characteristics, while retaining all its original attributes. Dry seeds of quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthuscaudatus*) were irradiated with gamma ray at doses of 150 and 250 Gray and 400 and 600 Gray; respectively. Selection of mutants with mutations in morphological, agronomic and quality traits were observed and selected, in both crops. In quinoa eight mutant lines were selected among them MQLM89-149 with higher yield equal to 4258.6 kg/ha, surpassing the control at 205.63% and MQLM89-42 with 14.7% grain protein, superior to the parent material with 12.3%. In kiwichanine mutant lines with 27 to 50% better yield potential than the parent material were selected.

Keywords: quinoa, kiwicha, mutations, gamma irradiation, genetic improvement

Volume I Issue 3 - 2017

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Received: October 30, 2017 | **Published:** December 28, 2017

Introduction

Agriculture in the high Andean region of Peru is usually made under adverse conditions, with frequent drought and frost Abiotic factors and in impoverished soils and mostly oriented to auto subsistence production. The diets consumed in the highland usually contain various sources of dietary protein, among them a mixture of cereals (barley, wheat and maize) with native grains (quinoa and amaranth). This mixture has a high biological value because cereals provide amino acids and other nutritive compounds that are deficient in native grains and vice versa. Quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthuscaudatus*) are native grains of the Andean region, very nutritious crops because of their content of all essential amino acids and their rich source of carbohydrate, lipids, minerals, vitamins, fiber and other biological health-promoting compounds.¹⁻⁶ The use of native grains is like leaf vegetable in the vegetative development stage and source of grains after harvesting. The native grains are a non-allergenic source of basic nutrients, as well as a food source for patients suffering from food intolerance (e.g. gluten enteropathy).⁷ These crops are well suited to marginal areas and are known to be tolerant to both drought and salinity. They have a potential to broaden the diversity of commercially grown crops and make an important contribution to food supplies in the near future when there will be water shortage or other Abiotic problems in Peru and the world due to climate change.

It is necessary to develop new varieties in these crops with better agronomic characteristics, conserving the valuable quality that they have. There are various plant-breeding tools for obtaining increased productivity and quality, e.g., mutation induction. Mutation breeding has been used for improving various crops.⁸⁻¹⁶ There are several reports of the use of mutations in improving underutilized native species.¹⁷⁻¹⁹ Mutations can improve agronomic traits and genetic quality while conserving valuable traits of existing varieties, including adaptation, quality etc. Mutations are random and the values of the mutations depend on changes in the morphology and physiology of plants that

affect the agronomic and quality performance. The objectives of the investigation were to induce genetic variation and the identification of useful mutations in quinoa and kiwicha using gamma irradiation.

Materials and methods

Genetic materials

The experimental material for the present study was commercial varieties of quinoaLM89 and kiwicha CICA-UNSAAC.

Methodology

Management of mutant population: Dry seeds of quinoa and kiwicha were irradiated with 150 and 250 Gray and 400 and 600 Gray doses, respectively. Seeds of each dose along with an equal amount of control (untreated) seeds were grown as M₁ generations and radio sensitivity was evaluated through germination, seedling survival, stem length and root length. All surviving M₁ plants were harvested individually to form the M₂ generation. The M₂ population was sown plant by row following the protocol established and during all the life cycle was observed to identify and select putative mutants. In the M₃ generation, progeny tests were conducted to determine the inheritance of changes or likely mutations in the M₂ generation. In M₄ to M₆ generation yield trials were established to study agronomic performance. The experimental materials studied in these experiments were cultivated using standard managements for native grains at commercial production. The areas were protected to avoid cross-pollination.

Evaluations: Agronomic and quality traits were evaluated following the protocol established for these types of evaluations.

General descriptive statistics

For each characteristic, data matrix was constructed using Microsoft Office 2007. The analysis started with basic descriptive statistics: mean standard deviation (SD) and coefficient of variance

(CV). Agronomic and quality traits evaluation was made with the statistical design random complete blocks with three replications. An analysis of variance was done on the variables measured. The F test was used to determine significant differences. The mean values for each cultivar were compared using Turkey's HSD Test for each agronomic and quality trait measured.

Selection of mutant lines for quantitative traits

To select the mutant lines, a range of theoretical values were established with values in most cases greater than or less than 10-20 percent from those mean values of the parent material and the selection were made at M₄ generation.

Results

Evaluation of somatic effects

In the M₁ generation, general reductions of germination, survival, length of seedlings height, length of seedlings root and fertility with the increment of the doses of gamma ray were observed in quinoa and kiwicha. These somatic effects were reported in other crops with different treatments and mutagens.²⁰⁻²³

Chlorophyll mutation

In quinoa and kiwichano chlorophyll mutants were observed in this research work. However in kiwicha cv Selección Ancash, a xhanta type chlorophyll mutation was reported with a frequency of 0.002% with 400 gray dose and in cv "SelecciónHuacho" albino and xantha chlorophyll mutation types were found in a treatment with 400 gray with a frequency of 0.008%.^{20,24}

Morphological characteristics mutations

Quinoa: In the M₂ generation with 150 Gray dose, the following mutation spectrum and frequency were observed: stem color(0.06%), stem branching (0.204%), stem streak color (0.20%), leaf axil color (0.046%), leaf spots (0.46%), leaf shape-inflorescence (0.014%), leaf shape- plant (0.008%), leaf tooth (0.029%), inflorescence shape (0.009%), inflorescence color (0.17%). In the M₃ the same spectrum of mutations were founded and 63 mutant lines were selected after a treatment of terminal drought stress in the milk grain phenology stage.

Kiwicha: In the M₂, mutations morphological and physiological characteristics with the following average mutation frequencies were observed for 400 Gray dose: stem pubescence (0.33%), stem color(0.89%), stem branching (0.207%), stem streak color (0.07%), leaf pubescence (0.27%), leaf color (0.26%), leaf spots (0.46%), leaf shape (0.19%), leaf margins (0.42%), pigmented veins (0.37%), pigmented petiole (0.54%), inflorescence shape (0.11%), inflorescence density (0.19%), inflorescence position (0.035%), inflorescence color (0.035%), plant height reduction (0.09%), plant height increase (0.15%) and earliness (0.024%). In the M₃ 343 putative mutants selected in the M₂ were studied and the progeny test gave the following spectrum and frequency of mutations: stem color (0.3.6%), stem branching (0.99%), stem streaks color (5.2%), leaf color (2.4%), leaf spots (3.2%), leaf shape (2.33%), leaf margins (2.7%), inflorescence shape (1.5%), inflorescence density (3.11%), inflorescence position (2.37%), inflorescence color (2.8%); there was increased frequency of all mutant characteristics in this generation. New mutants were observed in the M₃ with the following frequency: spiny bract (0.23%), grain color (0.14%) and waxy grain (0.29%). Some of the mutations observed in kiwicha are presented in Figure

1. Morphological mutations were reported for quinoa and kiwicha^{19,24} and for other crops.¹¹⁻²⁷



Figure 1 Morphological mutation types in kiwicha (*Amaranthuscaudatus*) cv CICA USAC irradiated with 400 Gy at M₃ generation. (A) narrow leaf shape mutation, (B) leaf color mutation, (C) inflorescence and leaf color mutation, (D) Life Cycle mutation. La Molina-Peru.

Agronomic traits

Quinoa: In the M₃, plant height was reduced with 0.028% frequency. In the experiment made with M₆ mutant lines statistical differences were observed for grain yield (kg/ha), maturity (days) and plant height (cm) for the phenotypes. Differences among mutant lines and the original cultivar were observed for all the traits evaluated. MQLM89-149 reached the highest yield equal to 4567 kg/ha higher than the original cultivar that yielded 1345 kg/ha. Mutant line MQLM89-134 stood out for its precocity reaching maturity at 93 days compared with the original cultivar that completed the maturity at 117 days. Mutant line MQLM89-155 was selected for reduced height equal to 135 cm lower than the original cultivar with 145 cm (Table 1).

Kiwicha: Plant height reduction (3.1%) and plant height increase (0.38%) and earliness (0.26%) were observed in the M₃ and following generation. The agronomic evaluation of mutant lines from M₄ to M₆, in the highland of Peru resulted in the selection of nine mutant lines with 27 to 50% better yield than the parent material. Among mutant lines and the original cultivar, grain yield, maturity days and plant height were also found to be statistically different (Table 2). The following valuable mutants were selected: CICA-108 selected by its highest yield equal to 1867.7 kg/ha higher than the original cultivar that yielded 1216.7 kg/ha. CICA-123 was identified as the earliest with 158 days to maturity considering that the original cultivar matured at 190 days. CICA-36 was selected for its lower plant height equal to 124 cm lower than the original cultivar with 138 cm. Improvement of agronomic characteristics using gamma irradiation has been reported in quinoa and kiwicha^{19,24} and in other crops.¹⁷⁻³⁰

Quality traits

Quinoa: Significant differences among mutant lines and the original cultivar were noted for all the traits evaluated (Table 3). Considering the values of the evaluated traits a group of mutant lines were selected

such as MQLM89-for higher 1000 grains weight equal to 3.5 g; MQLM89- for better protein content in the grain equal to 13.4% and MQLM89-for lower Saponin content in the grains equal to 0.45%. The original cultivar reached the following values for 1000 grains weight, protein content in the grain and saponin content in the grains equal to 3.01 g, 12.3% and 0.7%; respectively.

Table 1 Agronomic performance of selected quinoa (*Chenopodium quinoa* Willd) mutants developed from La Molina 89 using gamma rays irradiation.

Line	Yield (kg/ha)	Maturity (days)	Plant height (cm)
MQLM89-149	4258.6a	96def	158 bc
MQLM89-131	3940.1abc	96def	182 a
MQLM89-82	4108.3ab	95ef	158 bc
MQLM89-14	4225a	98cde	155c
MQLM89-146	3933.6abc	100c	155c
MQLM89-150	3743.5bcd	10c	162b
MQLM89-153	3851.6abc	98 cde	152c
MQLM89-134	4240.1a	93f	141d
MQLM89-137	3358.6d	108b	155c
MQLM89-52	3544.8cd	95ef	153c
MQLM89-155	3406.5 d	95ef	137d
MQLM89-152	4099 ab	96def	152c
MQLM89-92	3867.2abc	99cd	155c
QLM89 (O.M.)	1392.5e	117a	153c

Means within a column with the same letter are not significantly different (Tukey $p > 0.05$, $n=3$). General Mean=3712kg/ha and Mean Standard Error= 83kg/ha. General Mean=99.1 days and Mean Standard Error= 0.62 days. General Mean= 154.9 cm and Mean Standard Error= 1.29cm.

Table 2 Agronomic performance of selected kiwicha mutants of CICA

Line	Yield (kg/ha)	Maturity (days)	Plant height (cm)
CICA - 108	1867.7a	185b	131de
CICA - 145	1860.9a	187ab	134bcd
CICA - 133	1805.8a	158c	129e
CICA - 146	1760.9a	188ab	134bcd
CICA - 109	1610.4b	188ab	131de
CICA - 117	1600b	190a	132cde
CICA - 36	1588.5b	188ab	124f
CICA - 89	1587b	187ab	135abc
CICA - 54	1555.7b	187ab	137ab
CICA-UNSAC (OC)	1216.7c	190a	138a

Means within a column with the same letter are not significantly different (Tukey $p > 0.05$, $n=3$). General Mean= 1645.4kg/ha and Mean Standard Error= 22.7kg/ha. General Mean=184.8 days and Mean Standard Error= 0.63 days. General Mean= 132.5 cm and Mean Standard Error= 0.72cm.

Table 3 Quality characteristics of selected advanced mutant lines of quinoa (*Chenopodium quinoa* Willd) mutants developed from La Molina 89 using gamma rays irradiation

Name	1000 grain weight (g)	Grain protein %	Grain saponine %
MQLM89-149	2.7e	10.6g	1.03b
MQLM89-131	3.1cd	11.8f	0.94c
MQLM89-82	2.9de	12.3e	0.68e
MQLM89-14	3.2bc	12.4de	0.96c
MQLM89-146	2.7e	12.7cd	1.1a
MQLM89-150	2.9de	12.6cde	0.71de
MQLM89-153	3.3abc	12.9bc	0.68e
MQLM89-134	3.5a	13.2ab	0.75d
MQLM89-52	3.4ab	12.6cde	0.72de
MQLM89-155	2.9de	12.4de	0.45g
MQLM89-152	2.9de	13.4a	0.72de
MQLM89-92	3.2bc	13.2ab	0.55f
QLM89-10 (OC)	3.1cd	12.3e	0.7e

Means within a column with the same letter are not significantly different (Tukey $p > 0.05$, $n=3$). General Mean= 3.06 g and Mean Standard Error= 0.53 g. General Mean=12.5% and Mean Standard Error= 0.073%. General Mean= 0.77% and Mean Standard Error= 0.009%.

Kiwicha: Mutations of grain color were identified and are associated with commercial value. Color is linked with pigment concentration that can be related to some antioxidant product.^{5,31} Genetic variability for quality characters can be induced successfully through mutations. Similar results were reported quinoa¹⁹ and other crops.^{9,13} Mutation induction can play a major role in the development of new cultivars, of many under-utilized species, cultivated in marginal growing conditions of the world. The improved mutant lines are free of the regulatory restrictions imposed on genetically modified organism, which is very important for the organic production in the highland of Peru.

Conclusion

The dose applied for quinoa (150 and 250 Gray) and for kiwicha (400 and 600 Gray) induced genetic variability for several traits when compared to the parent material. In addition, high doses of gamma irradiation in quinoa (250 Gray) and in kiwicha (600 Gray) caused severe somatic effects. It was possible to identify improved or novel phenotypes that can be used as cultivars or exploited as source of desirable characters in conventional breeding programs of quinoa and kiwicha.

Acknowledgements

The authors would like to thank the IAEA and IUC/VLIR for providing funding for these Research Projects through IAEA's Coordinated Research Project (CRP) Project: RLA/5/068 "Yield improvement and potential commercial value of Economic Importance Crops, RLA/5/063".

Conflict of interest

The author declares no conflict of interest.

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