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Development of advanced mutant lines of barley with higher mineral concentrations through radiation-induced mutagenesis in Perú

Desarrollo de líneas mutantes avanzadas de cebada con mayores concentraciones de minerales a través de mutagénesis inducida por radiación en Perú

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Abstract

In Peru it is very important to increase food quality and production in the rural areas where a high poverty and malnutrition problems are found. Mutation induction is used to improve well adapted cultivars, by upgrading one or two characteristics, while retaining all its original attributes. *Hordeum vulgare* mutant lines were developed from the cultivar UNALM 96, following irradiation at 200 and 300 Gray. Mutant lines were selected in the M_8 generation with higher agronomic performance and nutritive quality. They were adapted to the highlands with grain yield within the range of 5, 100 and 8,731 kg/ha, over the value of the parental material (4,246 kg/ha) and showed improved contents of P (131 mg/g dry weight-DW), Zn (66 mg/g DW), Mn (55 mg/g DW), Fe (57 mg/g DW) and Cu (63 µg/g DW).

Keywords: barley, mutation, gamma irradiation, quality

Resumen

En Perú es muy importante aumentar la calidad de los alimentos y la producción en las zonas rurales donde se encuentran altos problemas de pobreza y malnutrición. La inducción de la mutación se utiliza para mejorar los cultivares bien adaptados, mediante la mejora de una o dos características, y conservando todos sus otros atributos. Las líneas mutantes de *Hordeum vulgare* se desarrollaron a partir del cultivar UNALM 96, después de la irradiación a 200 y 300 Gray. Las líneas mutantes se seleccionaron en la generación M8 con mayor rendimiento agronómico y calidad nutritiva. Se adaptaron a las tierras altas con un rendimiento de grano dentro del rango de 5, 100 y 8,731 kg/ha sobre el valor del material parental (4,246 kg/ha) y mostraron mejores contenidos de P (131 mg/g de peso seco-DW), Zn (66 mg/g DW), Mn (55 mg/g DW), Fe (57 mg/g DW) y Cu (63 µg/g DW).

Palabras clave: cebada, mutación, irradiación gamma, calidad

Introduction

Agriculture in the high Andean region of Peru is done under adverse conditions, with frequent drought and frost and in impoverished soils and mostly oriented to auto subsistence production. Above 3000 masl, the rural population uses mostly potatoes, barley, wheat, quinoa, beans and peas as staple food, and at lower altitudes maize, amaranth, among other crops. 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 amino acids and other nutritive compounds are provided by cereals (Jood and Singh, 2001; McKevith, 2004; Mahesh et al., 2010).

Barley (*Hordeum vulgare*) is an important introduced food crop in the highlands, and 70% of barley are used for human consumption as pearled grains, flakes and flour (Gomez-Pando et al., 2005). It could be a main source of minerals for low income rural population because it is a staple food in the Peruvian Andean region. It has agronomic value because its adaptability to marginal soils and climates. It has nutritious and medicinal value because it contributes with carbohydrates (~80%), proteins (6.9 to 25.0%), lipids (0.99 to 3.2%), minerals (~2 to 4%), fiber and other compounds to the diet (Kent, 1971; Newman and Mcguire, 1985; Ulrich, 2001). In terms of minerals, barley is a good source of potassium, magnesium and phosphorus, iron, sulfur, copper, zinc, manganese, chromium, selenium, iodine and molybdenum. It is considered an excellent source of vitamins B (Villacres, 1996) partly due to its content of soluble fiber (beta glucans), it protects the intestinal mucous membranes and reduces blood sugar levels (Fastnaught, 2002; Gordon, 2002; Finocchiaro et al., 2005).

National yield average of barley in Peru is relatively low, it is about 1,499.7 kg / ha (FAOSTAT, 2016). The production does not satisfy domestic demand so it is important to increase the quantity and quality of barley production in order to improve the nutrition and profitability of farmers in the Andean region.

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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 (Micke, 1998; Jain et al., 1998; Jamil and Khan, 2002; Muthusamy et al., 2003; Chopra, 2005; Gnanamurthy et al., 2012; Kurowska et al., 2012; Mehlo et al., 2013; Tshilenge-Lukanda et al., 2013; Oladosu et al., 2016, Raina et al., 2016, Gozukirmizi and Karlik, 2017). 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 impact the agronomic and quality performance. The present investigation was undertaken to induce useful mutations in barley using gamma irradiation.

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Materials and Methods

***Genetic materials*—**The experimental material was the barley commercial cultivar UNALM96.

***Management of mutant population*—**Barley dry seeds were gamma irradiated at doses of 200 and 300 Gray. Treated seeds of each dose along with an equal number of control (untreated) seeds were grown. All surviving plants were harvested individually to the M_2 generation and radio sensitivity was evaluated through germination, seedling survival, stem length and root length. The M_2 population was screened, the spectrum and frequency of mutations was established and chlorophyll mutations were identified using Gustaffson's classification (Gustaffson, 1947).

In the M_3 generation progeny, tests were conducted to determine the inheritance of changes or likely mutations in the M_2 generation. During the M_4 to M_8 generations, agronomic and quality characters were evaluated. Field control practices were based on standard managements for barley.

Determination of quality traits—

Determination of physical characteristics—Thousand kernel weight and test weight with the protocol of ICARDA (1988).

Determination of chemical characteristics—Grain protein content was made following the protocol of ASBC (1992).

Mineral grain content—Mg 24, P 131, S 34, K139, Zn 66, Ca 44, Mn 55, Fe 57 and Cu 63 was made by James Hutton Laboratories in the UK in Dundee, using ICP-MS – JAT technology.

Evaluation of agronomic traits—Yield (kg/ha), life cycle (days), plant height (cm) and disease response were recorded.

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 variation (CV).

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 parental material. Yield trials were conducted using a randomized block design with three replications

Results and Discussion

Somatic effects—In the M_1 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. These somatic effects were reported in other crops with different treatments and mutagens (Ciftci et al., 2006; Albokari et al., 2012; Scaldaferro et al., 2013; Arisha et al., 2014).

Chlorophyll mutation—Genetic differences in chlorophyll mutation have been observed. In a population of 342 958 plants, several types of chlorophyll mutants were identified. In six row spike plants, there were albino (0.09%), alboviridis (0.0003%), striata (1.7%), alboxantha (0.0005%), xanthalba (0.0011%), chlorine (0.15%), lutescens (0.005%), tigrina (0,25%) and xantha (0.03%). In two row spike plants, there were tigrina (0,006%) and chlorine (0.02%). In Figure 1, some of these mutants are presented.

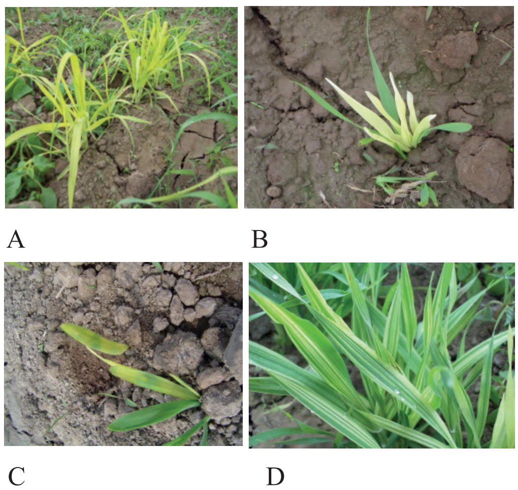

Figure 1. Chlorophyll mutation types in barley cv UNALM 96 irradiated with 200 Gy of gamma ray at M_2 generation (A: chlorine, B: albino, C: tigrina, D: striata). La Molina-Peru

Chlorophyll mutations are important markers for the evaluation of genetic effects of mutagenic treatments and have been reported in various crops (Gomez et al., 2009; Bhosale and Hallale, 2011; Ugorjiet al, 2012; Gnanamurthy et al., 2012; Tshilenge-Lukanda et al., 2013; Gomez-Pando and Eguiluz-de la Barra, 2013).

Morphological characteristics mutations—In the M_2 and following generations, a wide mutation spectrum was identified. The population that developed with the dose of 200 Gray was studied and the following modifications were found: stem color (1.68%), auricle leaf color (3.3%), spike morphology (4.78%), awn color (2%), awn –light curly (0.43%), awnleted (5%), elevated hood (0.18%), sessile hood (0.19%) and naked grain (0.70%). Some of these spike morphology mutations are presented in Figure 2.

Figure 2. Spike morphology mutation types in barley cv UNALM 96 irradiated with 200 Gy of gamma ray at M_2 generation (A:6 row with different types of awn, B:two row with different types of awn. La Molina- Peru

Morphological mutations were reported for barley and other crops (Gomez *et al.,* 2009; Gnanamurthy*et al.*, 2012; Dyulgerova, B. 2012; Jagajanantham *et al*., 2013). Some of the morphological mutations had economic importance such as naked grains. Barley is used at the highland roasted so naked grains are more easily processed.

Agronomic traits—In the M_3 and following generations, plant height reduction (2.12%) and earliness (0.19%) were observed and the flowering periods were significantly reduced from 10 to 11 days compared to the control. Dockter and Hansson (2015) identified more than 1000 different short-culm barley mutants. In the M_8 generation, 64 lines were selected with higher agronomic performances adapted to the highlands with grain yield within the range of 5100 – 8731 kg/ha, compared with the parental material with 4246 kg/ha (Table 1).

Table 1.Grain yield of selected barley mutants of UNALM 96

Number of lines	Range of yield (kg/ha)
1	5250
10	5125 - 7731
11	5100 - 6863
37	5131 - 8731
1	5294
2	5106 - 5856
1	5675
1	5550
UNALM 96	4246

Improvement of agronomic characteristics using gamma irradiation has been reported in barley and in other crops (Gustafsson et al., 1971; Khan et al., 2005; Gomez et al., 2009; Mudibu et al., 2010; Muthusamy et al., 2011; Kurowska et al., 2012; Ugorji et al., 2012; Tshilenge-Lukanda et al., 2013; Gomez-Pando and Eguiluz-de la Barra, 2013; Udensiand Ntui, 2013; Gomez-Pando, 2014; Arisha et al., 2014; Badr et al., 2014, Obare et al., 2014).

Quality traits—Six hundreds and 65 advanced mutant lines in the M_8 generation from the variety UNALM 96, with different morphological and agronomic types of variation, were evaluated for their grain carbohydrate content and chemical characteristics, e.g., protein and mineral contents. Increase and decrease in all the characteristics evaluated were observed in the lines relative to the parental material. Almost all evaluated traits were improved and lines were selected with values of more than 20% over parentalal UNALM 96. For physical grain traits, 12 mutant lines were identified with improved thousand kernel weight within the range of 70.2 and 78.9 g which was greater than the value of parental material (56 g). For protein content, 21 mutant lines were identified within the range of 12.9 to 14.1%; the parental material had 10.5% protein content. For mineral components, we identified many mutant lines. For Mg24, 50 mutants within the range of 1.44 to 4.04 mg/g DW were selected (parent material had 1.167 mg/g of Mg 24 (mg/g DW)). For P131, 54 mutant lines ranging from 5.27 - 7.59 mg/g DW were superior to the parent with 3.980 mg/g DW. For S34, 63 mutant lines ranging from 1.61 – 2.05 mg/g DW of S34 were superior to the parent with 1.28 mg/g DW.

For Zn66, 67 mutant lines were selected ranging from 0.0574 to 0.0649 mg/g DW, which was superior to the parent with 0.0466 mg/g DW. For Ca 44, 32 mutant lines were selected ranging from 0.3976 to 0.4855 mg/g DW, which was superior to the parent with 0.3258 mg/g. For Mn 55, 67 mutant lines were selected ranging from 0.0193 – 0.0322 mg/g DW, more than the parent with 0.0158 mg/g DW. For Fe57, 16 mutant lines were selected ranging from 0.11 – 0.4761 mg/g DW, which is better than the parent with 0.0417 mg/g DW. For Cu63, 72 mutant lines were identified ranging from 0.0099 to 0.0210 µg /g DW, better than the parent with 0.0067 µg /g DW. Some mutant lines showed improved resistance to leaf rust, lodging and grain content of P-131 from 2.88 to 6.16 mg/g DW, Zn66 from 0.044 to 0.0586 mg/g DW, Mn55 from 0.0142 to 0.0382 mg/g DW, Fe57 from 0.0436 to 0.1396 mg/g DW and Cu63 from 0.0.006 to 0.0142 µg/g DW (Table 2).

Genetic variability for quality characters can be induced successfully through mutations. Similar results were reported for barley (Muthusamy *et al.,* 2002; Gómez *et al.,* 2009; Dyulgerova, 2012; Mehlo *et al.,* 2013; Gómez, 2014), quinoa (Gomez-Pando, 2014) and other crops (Mehlo *et al*., 2013; Muthusamy *et al.,* 2002).

Table 2. Agronomic and quality characteristics of selected advanced mutant lines of barley from cultivar UNALM 96

Nº Origin M7 LM 09	Yield (Kg/ha)	P131 8 (mg/g DW)	Zn66 (mg/g DW)	Mn55 (mg/g DW)	Fe57 (mg/g DW)	Cu63 (ug/g DW)
CM6h-546	7075	3.93	0.0586	0.0187	0.062	0.0142
CM6h-718	6863	3.52	0.0555	0.0185	0.0671	0.0099
CM6h-658	6656	6.14	0.0516	0.0186	0.0629	0.0097
CM6h-722	6494	3.55	0.0543	0.0179	0.0622	0.0091
CM6h-721	6019	3.75	0.0513	0.0155	0.0548	0.0095
CM6h-542	8731	3.55	0.0535	0.0158	0.0436	0.0096
CM6h-717	6769	3.78	0.0581	0.0282	0.1396	0.0098
CM6h-26	6294	2.88	0.044	0.0142	0.0473	0.006
CM6h-543	7888	3.94	0.0556	0.0169	0.0541	0.0127
UNALM 96	4246	4.31	0.0479	0.0162	0.0813	0.0076

Weight of 1000 seeds (g) and test weight (kg/hl^{-1}) were improved and are related to the kernel plumpness that are associated with endosperm size and starch content. In general there were values of TKW over and below the value of the parental material (56.8 g). For this character, 12 lines with higher value were selected (65 to 78.9 g). The mass of a thousand grains is a good indicator of quality of malting and feed barley; the amount of malt extract, is positively correlated with grain size (Madić *et al.,* 2005; Madić *et al.*, 2006). Significant breeding progress was observed for 1000-grain weight (Ferrio *et al., 2004).* This trait was enhanced in 106 spring barley accessions of Slovak and former Czechoslovakia (Žáková *et al.,* 2006). Grain Test Weight is strongly related to the degree of grain quality (grain size, shape, amount of starch, etc.), the parental material had an average value of 66 kg/hl and naked mutant lines with higher values for this characteristic were identified within the range of 71.1 to 80.3 kg/hl. It mainly depended on the degree of filling, and shape and grain moisture (Madić *et al.,* 2005; Madić *et al.,* 2006). It is also an indirect indicator of malt extract and a variety with malting quality must have a value >66 kg/hl.

The parental material UNALM 96 had 10.5% protein content and a group of mutant lines with values over and below the value of the parental material were identified. Globally there is evidence of improvement in food and malting barley quality (Swanston *et al.,* 2002; Tamm, 2003). The use of barley is determined mainly by the protein. From the point of view of the brewery, barley should have a high content of starch as a source of fermentable sugars and protein content between 10 and 12% for good enzymatic activity. From a nutritional standpoint, the barley must have a good carbohydrate and high protein content, and other nutrients such as minerals (Emebiri et al, 2003; Emebiri et al, 2004; Emebiri et al, 2005; Iqbalet al., 2007). Crops can be enriched with micronutrients using plant breeding and/ or transgenic strategies, because micronutrient enrichment traits exist within their genomes that can be modified for substantially increasing micronutrient levels without negatively impacting crop productivity (Welchand Graham, 2004). Gregorio *et al.* (2000), White and Broadley (2009) and Mulualem (2015) indicated that the increment of bioavailable mineral elements in food crops through plant breeding is one important way to contribute to the solution of mineral deficiencies in human population and that can be combined with agronomic characteristics improvement also. Velu *et al*. (2012) and Velu *et al*. (2014) mentioned that is very important to consider the association between micronutrients and agronomic traits such as grain yield, plant height, grain size and quality traits.

Conclusions

The dose applied of 200 and 300 Gray induced genetic variability for several studied traits when compared to the parental material. In addition, high doses of gamma irradiation in barley (300 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 barley. These improved mutant lines are free of the regulatory restrictions imposed on genetically modified organism. In some advanced mutant lines the change was in one or two desired characters without changing the rest of the genotype.

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