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RESEARCH ARTICLE

The effect of gamma rays on quinoa plant and evaluation of promising genotypes under salinity stress

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ABSTRACT: There is no doubt that use of hybridization programs in the quinoa plant genotypes to induce genetic variation is difficult, however introducing the variations through mutation, to obtain promising genotypes, is much easier. In this research, quinoa seeds (Chipaya cv.) exposed to different doses of gamma rays and were cultivated in pots and open field under salinity stress. The results showed distinct differences at all studied traits in the native and mutant plants. Gamma ray's irradiation caused genetic variations that was categorized based on studied traits, tolerance indices, cluster analysis of protein and ISSR data, which led to obtaining two promising mutations during M2. It should be noted that 90 and 120 Gy revealed the highest effects in producing desirable genetic variations. Also, the data resulting from the evaluation of phenotypic traits and tolerance indices of plants were confirmed by the biochemical and molecular analysis results. This research is providing new insights of using molecular breeding program for quinoa improvement to produce new promising genotypes powerfully face environmental stress and potential aid in future food shortage disasters.

KEYWORDS: Mutations; grain yield; tolerance indices; breeding; ISSR

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) belongs to the Amaranthaceae family (Vega-Galvez et al., 2010) and is one of the most promising plants, as a cereal crop that contributes to bridging the food gap, especially in areas affected by salinity (Algosaibi et al., 2015). It has a great potential as a commercialized crop around the world because of its good nutritional composition, high protein content, and seeds containing 12-18% protein besides being gluten free, fiber, vitamins and minerals (Mastebroek et al., 2000). Salinity stress is the most abiotic forms on plants and affects about 800 million hectares of land, including 30% of high-yield farmland worldwide (Nguyen 2016). Gamma rays fall into the set of ionizing radiation and interact in cells with molecules to output free radicals. These radicals change important components in the cells of plants in cellular structure and metabolism and DNA (Wi et al., 2005). Induced mutations share in genetic diversity mainly by increasing DNA polymorphism. ISSRs used to estimate genetic diversity with biochemical as suitable markers for the identification of mutant plants (Rustikawati et al., 2012) in quinoa (Morillo et al., 2017).

The objective of this study is to identify the suitable techniques for quinoa breeding based on gamma

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radiation under salinity stress by investigating growth characteristics, genetic diversity of quinoa genotypes to identify powerful mutant with improved characteristics.

MATERIALS AND METHODS

Experimental stages, measurements and parameters of tolerance

Seeds of quinoa genotype (Chipaya cv.) were exposed to different doses of gamma rays; (0, 30, 60, 90, 120 and 150 Gy) (M0). The irradiation treatments were carried out in the farm of Soil and Water Research Department, Nuclear Research Centre, Inshas, Egypt. The effect of gamma irradiation on germination was observed where the data of germination (%) was used to determine the value of Lethal Dose 50 (LD50). During (M1), effect of gamma irradiation was determined based on; germination (%), shoot and root length in one-month-old plants according to (Cheema and Atta 2003) and chlorophyll content was determined after 5 weeks in both adult and juvenile leaves. At harvesting time, the evaluation done based on: the seeds of ripe inflorescence of M1 generation and the phenotype traits of first generation M1 to be cultivated in the second generation (M2). In the open field the experiment carried out in split plot design where two salinity levels (7000 ppm approximately and non-stress as normal condition) putted in main plot while genotypes putted in sub plot by comparison with untreated parent (Chipaya cv.) and each of them was planted in a secluded row (i.e. the parent and distinct mutations) to comparison between them.

Parameters of tolerance

Different salinity tolerance parameters calculated from the tested genotypes under non stress (Yn) and high stress (Ys) conditions as follow:

Salinity tolerance index (STI): according to Fernandez 1992, Yield injury % (YI) according to Blum et al., 1983, Superiority measure (SM) according to Lin and Binns 1988, Relative performance (RP) according to Abo-Elwafa and Bakheit 1999.

SDS-PAGE

The protein extraction technique employed was similar to the extraction technique described by Laemmli(1970). Bio-Rad gel documentation system (BIO-RAD-Gel-DocModel2000) employed for gel photography and documentation.

Table 1. List of ISSR primers and their nucleotide sequences.

No.	Primer code	Primer sequence	No.	Primer	Primer sequence
1	44B	$(CA)_6$ AG	4	HB12	$(GTG)_3$ GC
2	HB-8	$(GA)_6$CC	5	HB-13	$(CT)_8$TG
3	HB-9	$(GT)_6$ CC	6	HB-15	$(CG)_8$TG

ISSR marker analysis

Young leaves of two weeks old M1 seedlings grown in pots were taken randomly for nucleic acid extraction in the Laboratory at 20 ^{O}C, using the DNA-easy Plant Mini kit (Qiagen, USA). Nano-drop2000C (Thermo Scientific) applied to measure nucleic acid quality and quantity tested in 1% agarose gel. Six anchored ISSR (Inter Simple Sequence Repeats) primers manufacture by (Life Technologies, Gaithersburg, Md.) were used for nucleic acid amplification as shown in Table (1). DNA thermo cycler (Biometra, Germany) Amplified for nucleic acid according to Sarwat (2012).

Experimental design and Statistical analysis

The experiments were carried out as split-plot in a randomized complete block design of two factors, where in the pots (M1) , the first factor was gamma rays doses and the second one was salinity levels while in the field (M2), the first factor was salinity levels and the second one was the genotypes with three replicates. Variance analysis was estimated for evaluated characters based on Steel & Torrie (1980) using MSTATC 17 program and GGEbiplot ver,7 (Yan 2001). The comparisons of means were estimated for studied traits based on least significant difference (LSD) method at 5% probability level of interaction between studied factors (doses of gamma rays, salinity levels and tested genotypes). Constructed dendrogram for both Protein and ISSR analysis was used based on combined analysis developed by NT-SYSPC software using UPGMA and Neighbor Joining (NJ) tree building methods.

RESULTS

The data in pots of M1 generation

The results showed in (Fig1 and Table 2) revealed a normal gradient in the significant differences between salinity levels. Noting that, under the same level of salinity there were clear significant differences among gamma radiation levels (M1) in the most of studied traits

Table 2 Means performance in pots of M1 generation using gamma ray's dosses under salinity conditions: Means in each column are not significant at 5% level.

Gamma rays (Gy)	Salinity levels (ds/m)	Germination (%)	Chloro-phyll (SPAD)	Shoot length (cm)	Root length (cm)	Grain weight /plant (g)	Protein (%)
N	control	98.67	56.50	50.33	16.93	8.03	11.30
	8	51.67	55.27	45.00	14.33	7.67	11.80
	16	34.33	52.77	42.67	7.500	5.20	12.10
30	control	98.33	45.00	57.67	17.00	10.13	12.50
	8	53.67	54.53	53.00	10.83	8.10	12.20
	16	36.33	53.57	48.67	10.67	7.37	12.50
60	control	98.33	59.00	60.33	14.33	13.30	12.65
	8	58.67	62.83	58.00	12.80	11.20	12.91
	16	41.00	56.23	53.33	11.27	8.93	13.25
90	control	92.33	58.40	42.00	18.33	11.27	12.88
	8	54.00	57.50	40.00	14.33	8.93	13.20
	16	39.00	54.77	38.00	12.83	7.37	13.40
120	control	81.67	42.63	37.67	16.00	6.57	13.00
	8	51.00	55.90	37.00	11.67	6.13	13.43
	16	35.00	56.33	36.67	11.50	5.63	13.80
150	control	55.00	43.73	35.67	20.67	5.27	13.30
	8	40.00	60.50	34.67	10.17	3.77	13.75
	16	29.67	63.28	31.67	7.33	3.00	14.30
LSD $_{(0.05)}$ of salinity (S)		42.40	0.75	0.91	0.35	0.23	0.21
LSD $_{(0.05)}$ of gamma rays (Gy)		n.s	1.06	1.29	0.50	0.33	0.29
LSD $_{(0.05)}$ of (S x Gy)		n.s	1.83	2.23	0.87	0.56	n.s

in addition to the non-numerical traits. Regarding to the wide range differences, it was evident from the data presented in Table 2 that they began to appear clearly when using gamma rays doses 90, 120 and 150 Gy and this was illustrated by the results through some of the characteristics like root and shoot length (cm), chlorophyll content and grain yield (g) compared with control (0 Gy) under the same salinity level.

SDS-Protein electrophoresis of M1 generation

SDS- polyacrylamide gel electrophoresis was used in this study to investigate the effects of different treatments of gamma irradiation on the gene expression of the studied quinoa Chipaya genotype using different gamma irradiation (30, 60, 90, 120 and 150 Gy) compared with control in M1generation illustrated in (Fig 2). It is worth noted that the dose of 150 Gy was lethal dose. Nine bands were polymorphic (82 % polymorphism). On the other hand, there were many unique protein bands for irradiated plants under each dose which can be used as specific markers for each dose.

ISSR analysis of M1 generation

Six inter simple sequence repeat primers were applied to understand the effect of different radiation treatments on nucleic acid structural changes of M1 generation under salinity conditions as shown in Fig (3) and (Table 3). It is worth noted that the dose of 150 Gy was lethal with a complete breakage of DNA. The results revealed forty-five total bands, 26 polymorphic bands (57%).

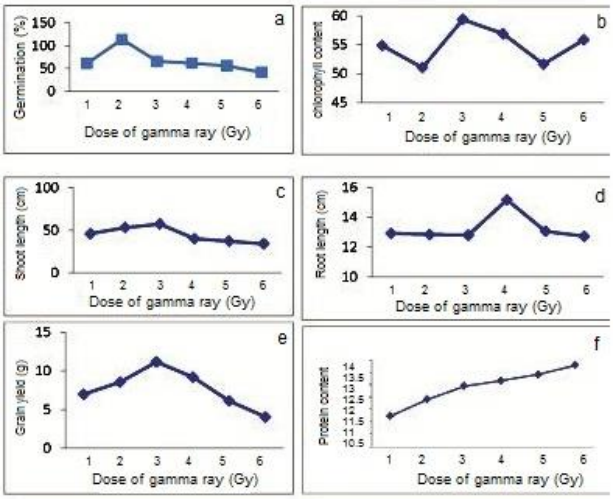

Figure 1. Effect of gamma rays on a) Germination (%), b) Chlorophyll content, c) Shoot length (cm), d) Root length (cm), e) Grain yield (g) and f) Protein content of quinoa genotype. (1) control; (2) 30 Gy; (3) 60 Gy; (4) 90 Gy and (5) 120 Gy.

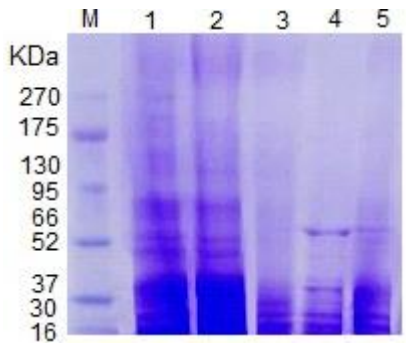

Figure 2. Protein profile using SDS-PAGE technique of Chipaya genotype as affected by different gamma rays on M1 generation: (M) Marker; (1) control; (2) 30 Gy; (3) 60 Gy; (4) 90 Gy and (5) 120 Gy.

Table 3. Total number of scorable bands, polymorphism % and a band size of ISSR markers obtained by six specific primers of M1 generation.

Primer code	Length range (pb)	Monomorphic bands	Polymorphic bands (with unique)	Unique bands	Total amplified Bands	Polymorphism (%)
44B	630-1060	1	5	5	6	83
HB-08	300-1720	2	6	6	8	75
HB-09	200-500	3	5	5	8	62
HB12	198-1200	4	4	4	8	50
HB-13	168-686	4	3	3	7	43
HB-15	300-1020	5	3	3	8	37
Total		19	26	26	45	57

44B15 and HB8 primers were highly polymorphic (83% and 75% polymorphism), respectively.

Combined analysis of M1 generation

The dendrogram of protein and ISSR banding patterns (M1) grouped the four doses of gamma rays individuals in one cluster (Fig 4). The first cluster was divided into two sub-clusters the first one contained the 120 Gy dose individuals and the second one had the other remained doses individuals. While, the control in was separated in another second cluster.

The data in open field of M2 generation

The data of the open field of (M2) showed clear significant differences among the five genotypes (the original cultivar Chipaya and the four selected mutated genotypes), where the highest value of plant height was recorded in the mutated genotype G4 and the lowest were recorded in the mutated genotype G5 and G3 compared to Chipaya cultivar (Table 4). In the same manner, the most important observed mutations in M2 generation showed in Fig (5).

Tolerance indices of salinity of grain yield and protein

The data of different salinity tolerance indices were calculated of grain yield and protein (%) per plant of mutant genotypes and Chipaya cultivar in open field (M2) under non salinity stress (Yn) and high salinity stress (Ys) conditions. The results showed in (Fig 6 and Table 5) indicated that the two mutants of 3 and 4 genotypes recorded the highest values for each grain yield per plant (indication of tolerance to salinity) based on salinity tolerance index (STI), superiority measure (SM) and relative performance (RP). Whereas, the highest rate of harm or called yield injury (YI) to the grain yield was recorded in mutagenic genotype 5, followed by Chipaya cultivar. On the other hand, the results in (table 4) confirmed the tolerance indices (5) the superiority of the genotype 5 following by Chipaya cultivar of protein in grain in M2. The previous result is reinforced by the measure of yield injury (YI), which confirms that the highest percentage of protein damage was recorded in genotypes 3 and 4, whereas the lowest level of protein influence was recorded for genotype 5 followed by Chipaya cultivar. The analysis of data using GGEbiblot indicates the distribution of genotypes in two different environments, confirming the superiority of the genotype 4 in grain yield and genotype 5 in protein ratio under salt stress conditions (Fig 6).

SDS-Protein electrophoresis of M2 generation

The results of SDS-PAGE of (M2) for the four ideal selected mutations of chosen genotype Chipaya at 90 and 120 Gy under salinity conditions revealed a total of 22 bands of which 14 bands were polymorphic (64 % polymorphism). On the other hand, there were eleven unique bands for the four mutations in M2 generation (Fig 7).

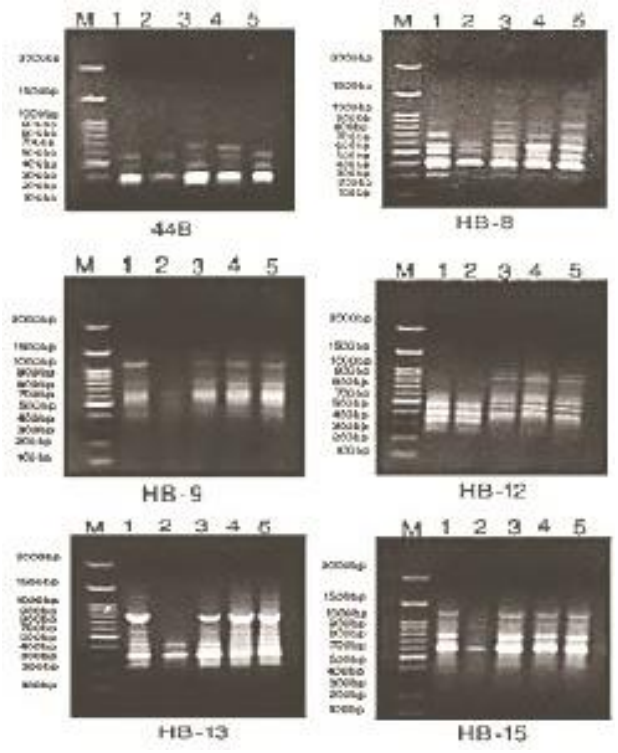

Figure 3. Genetic polymorphisms of Chipaya genotype as affected by different gamma rays on M1 generation as revealed by ISSR: (1) control; (2) 30 Gy; (3) 60 Gy; (4) 90 Gy and (5) 120 Gy.

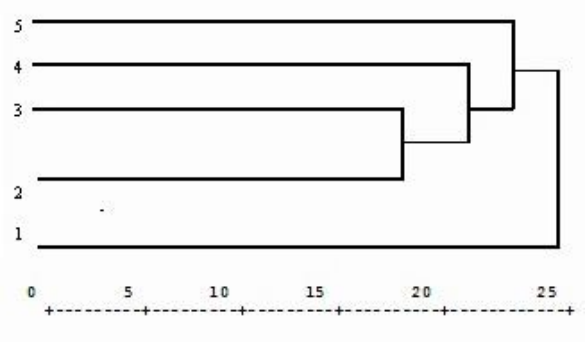

Figure 4. Clustering of Chipaya genotype affected by different gamma rays on M1 generation based on pooled protein and ISSR markers: (1) control; (2) 30 Gy; (3) 60 Gy; (4) 90 Gy and (5) 120 Gy.

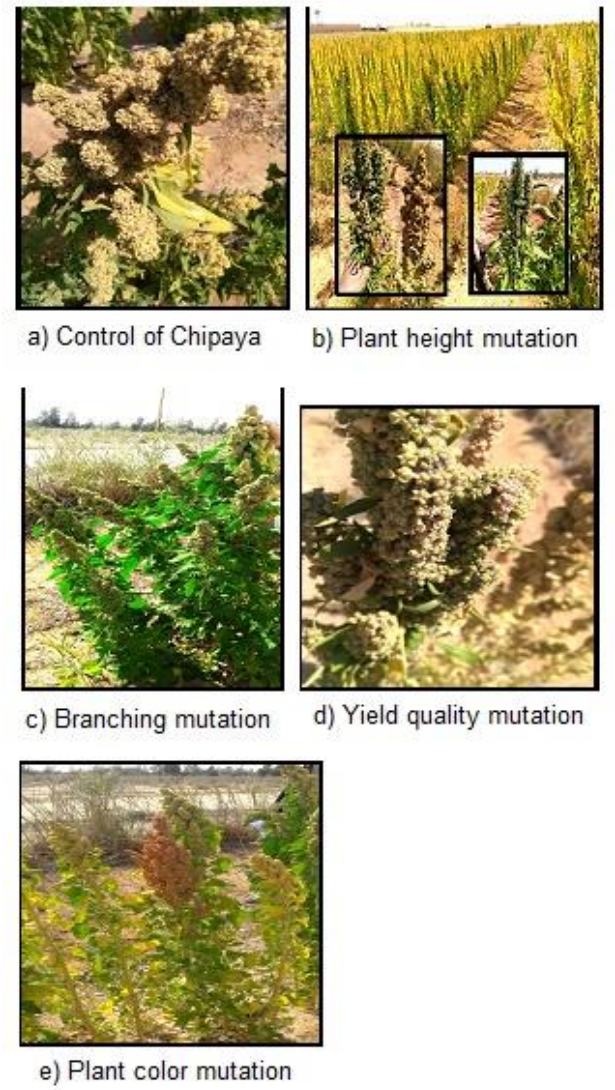

Figure 5. Plant abnormalities of Chipaya genotype affected by 90 and 120 Gy gamma rays in M2 generation:(a) control; (b) plant height mutant; (c) branching mutant; (d) grain yield mutant and (e) plant color mutant.

Table 4. Means performance in open field (M2) under two salinity levels of five Genotypes: Means in each column are not significant at 5% level.

Salinity (ppm)	Genotype	Plant height (cm)	Maturity date	Grain yield (g)	Protein (%)
Normal	1(Chipaya)	91.70	123.67	27.63	12.23
	G2	87.73	119.67	27.03	13.60
	G3	92.77	126.67	31.67	12.83
	G4	101.00	129.00	30.60	12.30
	G5	84.33	119.33	27.07	13.03
7000	1(Chipaya)	58.33	113.33	17.97	11.87
	G2	57.00	111.00	17.77	12.93
	G3	66.67	117.67	21.90	11.97
	G4	70.33	119.67	22.03	11.53
	G5	58.00	109.67	17.47	12.80
$LSD_{(0.05)}$ of salinity (S)		1.49	0.82	0.83	0.11
$LSD_{(0.05)}$ of genotypes (G)		2.36	1.29	1.31	0.17
$LSD_{(0.05)}$ of (S x G)		3.34	n.s	n.s	0.25

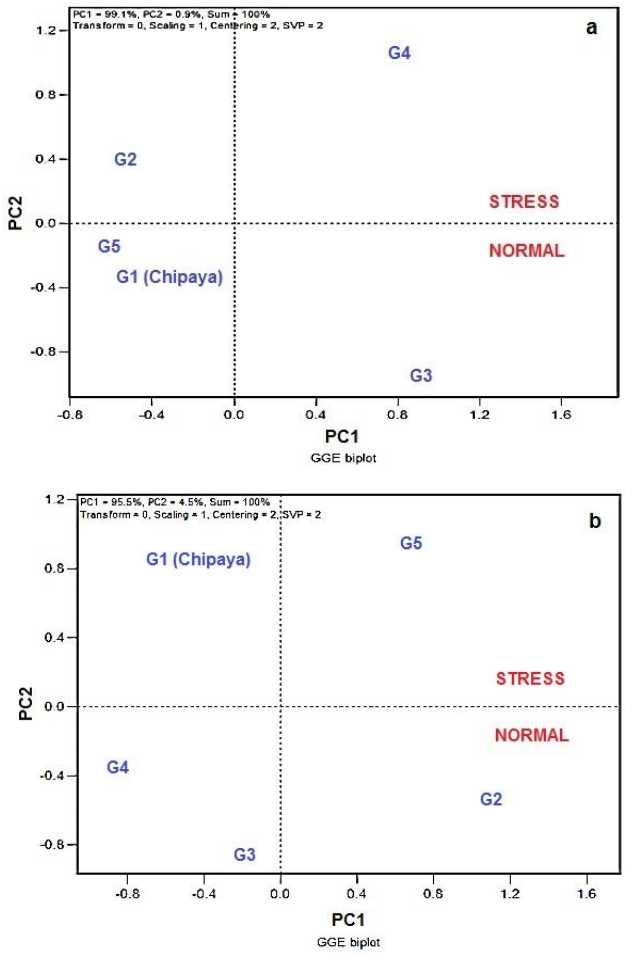

Figure 6. Distribution of tested genotypes under non salinity and salinity stress conditions based on grain yield (a) and protein content (b).

Table 5. Tolerance indices of tested quinoa genotypes under stress and non-stress of grain/ plant and protein content: (STI) salinity tolerance index; (SM) superiority measure; (RP) relative performance and YI = yield injury.

Genotype	STI		SM		RP		YI (%)	
	Grain weight/ plant	Protein	Grain weight/ plant	Protein	Grain weight/ plant	Protein	Grain weight/ plant	Protein
Chipaya(G1)	0.599	0.886	0.650	0.970	0.964	1.016	34.980	2.992
G2	0.579	1.074	0.657	0.951	0.974	0.996	34.277	4.904
G3	0.836	0.937	0.692	0.933	1.025	0.977	30.843	6.748
G4	0.813	0.866	0.720	0.938	1.067	0.982	27.997	6.236
G5	0.570	1.018	0.645	0.982	0.957	1.029	35.468	1.788
Mean	0.680	0.9600	0.673	0.955	0.998	0.999	32.713	4.534

Table 6. Total number of scorable bands, polymorphism % and a band size of ISSR markers obtained by six specific primers of M2 generation.

Primer code	Length range(bp)	Monomorphic bands	Polymorphic bands (with unique)	Unique bands	Total amplified Bands	Polymorphism (%)
44B	100-1200	1	13	4	14	92
HB-08	200-1600	1	14	9	15	93
HB-09	700-2000	1	9	4	10	90
HB12	800-3000	1	9	7	10	90
HB-13	100-2000	2	9	3	12	75
HB-15	450-3000	9	7	6	15	46
Total		15	57	33	68	84

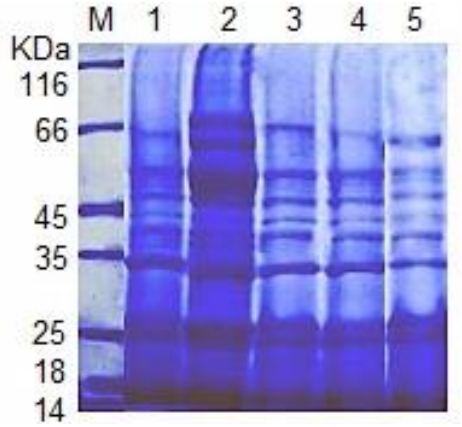

Figure 7. Protein profile of M2 generation using SDS-PAGE of Chipaya genotype affected by 90 and 120 Gy gamma rays: (1) control; (2) plant height mutant; (3) branching mutant; (4) grain yield mutant and (5) plant colour mutant.

ISSR analysis of M2

Six ISSR primers were used to investigate the effect of chosen treatment of gamma irradiation after (M0 and M1 generation) on DNA structural changes of M2 generation mutations of Chipaya under salinity conditions as shown in Fig (8) and (Table 6). Sixty-eight total bands, fifteen monomorphic and 57 polymorphic distinct bands (84%polymorphism) were revealed by the six ISSR primers. The results showed that HB8 and 44B primers were highly polymorphic (93% and 92% polymorphism respectively) which produced wide base pair length that ranged from 200 to 1600 and 100 to 1200bp, respectively.

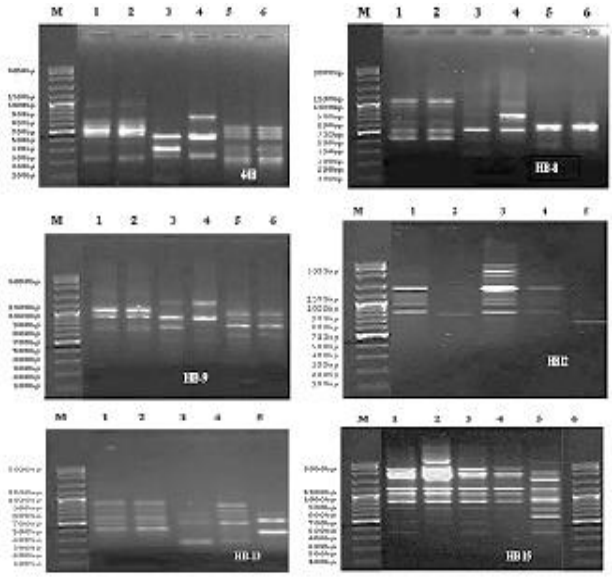

Figure 8. Genetic polymorphism of Chipaya genotype affected by 90 and 120 Gy gamma rays on M2 generation revealed by ISSR analysis: (M)100 bp DNA ladder; (1) control; (2) plant height mutant; (3) branching mutant; (4) grain yield mutant and (5) plant colour mutant.

Combined analysis of M2 generation

The dendrogram grouped the four mutations of 90 and 120 Gy in one cluster (Fig 9). The first cluster was divided into two sub-clusters the first one contained the different plant color mutation and the second one had the grain yield, branching and plant height mutations. However, the control was separated in another cluster.

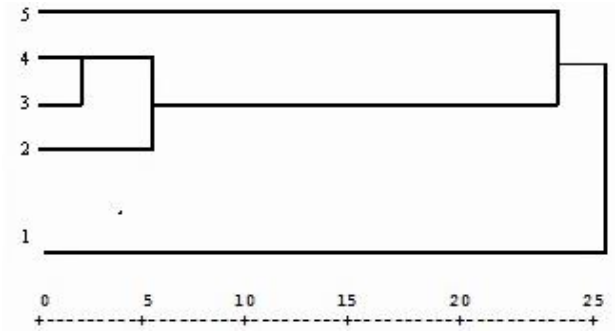

Figure 9. Clustering of Chipaya genotype as affected by 90 and 120 Gy gamma rays on M2 generation based on pooled protein and ISSR markers: (1) control; (2) plant height mutant; (3) branching mutant; (4) grain yield mutant and (5) plant colour mutant.

DISCUSSION

Evaluation of M1 and M2 generations

Looking at the average performance, there were clear significant differences between data recorded from growing plants using different radiation doses and salinity levels. On the other hand, when neutralizing the level of salinity, the results also differ with the different radiation doses used, and this indicated the possibility of mutations.

The previous results are consistent with (Maluszynsski et al., 2009) who stated that, the degree of somatic effects after using mutagenic treatment can be determined according to different growth and vegetative parameters.

Tolerance parameters of salinity stress

When discussing the results tolerance indices can be relied upon to distinguish between genotypes (four mutants and its parent) based on weight of grain yield and protein percentage, and it is not necessary of genotypes to go in the same direction. On the other hand, the graphs are reliable in dividing the genotypes in M2 generation according to environmental conditions (salinity and non-salinity stress), according to grain yield and protein percentage. According to tolerance indices, Majidi et al. (2011) mentioned that the best criterion for determining genotypes that are more resistant to moisture stress conditions compared to non-stress field environments (normal conditions) with a higher yield is the stress tolerance index (STI), geometric average productivity (GMP) and means productivity (MP).

Molecular analysis

Induction of mutation, genetic variation and molecular characterization of certain characters are the basics foundations of mutation breeding. Molecular markers have contributed efficiently in genetic identification, screening and evaluation mutation of certain characters. Evaluation of these biological changes is very important as they occur through such biochemical changes. Analysis of protein profiles using SDS-PAGE showed that the doses 90 and 120 Gy were the most effective among different treatments of gamma irradiation on the gene expression of (M1) under salinity conditions. However, the existence of unique bands for each dose and the highest were revealed by 120 Gy that gave important clues in understanding the origin of different mutations for important yield characters as (Vanhoudt et al., 2010) noted that higher gamma doses, the more cross-linked were products of the degraded protein molecules and unique bands occur with various nucleic acid rearrangements via; addition, deletion and translocation. All four mutations of 120 Gy gamma ray of (M2) were due to DNA disorder and rearrangement in nucleotide sequences by transition mutations in growth, grain yield, protein and vegetative responsible genes, or it may be caused by mutation in the biosynthetic pathway of structural or regulatory genes may cause a change in flower color (Abd El. Hamid et al., 2014). On the other hand, there were eleven new unique bands for the four mutations in M2 generation could be used as specific molecular markers. This perfectly proofed that gamma rays have induced changes in sequence and gene expression leading to change in mutant genotypes. Clustering indicated promising mutations in grain yield and protein content (Taheri et al., 2013).

CONCLUSION

The results of our experiments indicated that, certain doses of gamma radiation caused morphological and genetic variations in tested quinoa genotypes under different salinity treatments. The results point out to the importance of assessing valuable traits through the evidence of salinity tolerance in relation to crop return output measurements under salinity conditions using GGE biplot program. Our results showed that SDS-PAGE and ISSR techniques are reliable in detection genetic polymorphism among gamma radiation treated individuals and conformed morphological data.

CONFLICT OF INTEREST

No conflict of interest is found among the research authors.

AUTHORS CONTRIBUTION

AB and RK designed and oversee this research. AB and EK supervised phenotypic data collection and fieldwork. AB performed the statistical analyses of data. AB, RK and EK wrote and edited the manuscript. All co-authors reviewed the final version and approved the manuscript before submission.

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تاثیر پرتوتابی گاما بر گیاه کینوآ و ارزیابی ژنوتیپ‌های نویدبخش در شرایط تنش شوری

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چکیده

ایجاد تغییرات ژنتیکی در ژنوتیپ‌های کینوآ با برنامه‌های هیبریداسیون برای دستیابی به ژنوتیپ‌های برتر مشکل می‌باشد. با اینحال از طریق موتاسیون وارد کردن تغییرات ژنتیکی و بدست آوردن ژنوتیپ‌های نوید بخش بسیار آسان‌تر می‌باشد. برای این منظور، بذرهای کینوآ (رقم چیپایا) در معرض دزهای مختلف پرتو گاما قرار گرفت و در شرایط تنش شوری در گلدان و در مزرعه کشت شدند. نتایج تفاوت‌های روشنی در صفات مورد مطالعه میان ژنوتیپ‌های مادری و موتانت نشان داد. تغییرات ژنتیکی ایجاد شده با پرتو گاما با استفاده ازشاخص‌های تحمل، تجزیه و تحلیل خوشه‌ای برای پروتئین‌ها و نشانگرهای ISSR ارزیابی شد که منجر به به دست آمدن دو موتانت نویدبخش در نسل دوم شد. دزهای ۹۰ و ۱۲۰ گری بالاترین تاثیر را داشتند. داده‌های حاصل از ارزیابی صفات فنوتیپی و شاخص‌های تحمل با نتایج حاصل از تست‌های بیوشیمیایی و آنالیزهای مولکولی تایید شد. این تحقیق چشم‌اندازهای جدیدی برای برنامه‌های اصلاح مولکولی کینوآ و تولید ژنوتیپ‌های نویدبخش با توانایی مواجهه با تنش‌های محیطی و با پتانسیل کمک به فائق آمدن بر کمبود مواد غذایی فراهم می‌کند.

کلمات کلیدی: جهش، عملکرد دانه، شاخص‌های تحمل، اصلاح، ISSR