CHEMICAL CHANGES IN SOME NUTRITIONAL AND ANTI-NUTRITIONAL COMPOUNDS IN QUINOA GRAINS UNDER SALINE CONDITIONS AT RAS SUDR, SOUTH SINAI

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A field experiment was conducted for two consecutive seasons to study the effect of different concentrations of sodium azide (SA) and sodium nitroprusside (SNP) on some nutritional and anti-nutritional components in quinoa grains (Chipaya and Q37) under saline stress conditions in Ras Sudr, South Sinai. The results showed that quinoa was an excellent source of vitamins, essential and non-essential amino acids, and minerals, as well as some other medicinal compounds that are beneficial to health. It was found that the treatment with SA at a concentration of 0.04% had a positive effect on the decrease in the content of anti-nutritional compounds (saponins), and this was associated with a clear increase in grain yield of the Chipaya genotype under saline stress conditions. The same genotype recorded the highest rate of water-soluble vitamins (B1, B2, B9, and B12) as well as essential and non-essential amino acids when treated with SNP at a concentration of 0.75 mg/L. On the other hand, the genotype Q37 gave high values of essential and non-essential amino acids when treated with SA at a concentration of 0.04%. As for the mineral content of quinoa grains, it was found that the Chipaya genotype gave the highest manganese content when it was treated with SNP at a concentration of 0.25 mg/L. The highest content of zinc was recorded when it was treated with SA at 0.03%. The study also showed that, the same genotype recorded the highest iron content when it was treated with SA at a concentration of 0.01%. The use of molecular markers (ISSR-PCR) is crucial to identifying variation in genotypes, especially parameters that may lead to a change in genotypes. The study showed that the least polymorphism was detected by Q37 under salinity stress conditions and chemical treatments. This study confirms that the chemicals used (especially SA at a concentration of 0.04% and SNP at 0.75 mg/L), had a clear positive effect on increasing the content of nutritional components and decreasing the content of anti-nutritional contents in quinoa grains, and they did not cause a change in the genetic composition.

Continuing the study on these chemicals with higher concentrations is recommend to obtain genotypes with desirable characteristics in terms of production and chemical composition.

Keywords: quinoa, sodium nitroprusside, sodium azide, salinity, saponin, amino acids, ISSR, water soluble vitamins

INTRODUCTION

Global climate change represents a major constraint on the world food demand by affecting the conditions under which crops can be grown, particularly due to increasing soil salinity which is projected to affect more than 50% of all arable lands by the year 2050 (Vineis and Khan, 2012). Nowadays, adaptation of agriculture to soil salinity is an area of major scientific interest because salt stress may strongly affect plant productivity worldwide (Flowers and Colmer, 2015). Salinity is increasingly posing a significant risky to global food security since it can drastically lower crop yields and permanently destroy soil fertility. Additionally, climate change is now making this issue worse, particularly in drylands (Abidi et al., 2022). Limited water resources and hot, dry weather in arid and semi-arid locations lead to a salinity problem that negatively impacts agricultural development and productivity. Salt inhibits plant growth at low concentrations, and at larger quantities, it can lead to oxidative stress, which can result in plant death (Michael et al., 2004).

Quinoa (*Chenopodium quinoa*, Willd.) is a versatile plant that has lately been incorporated into the human diet as a source of protein substitute for animal products. Quinoa has significant concentrations of protein (12–18%), bioactive substances, essential amino acids, fatty acids, and minerals (Angeli et al., 2020). Quinoa is consumed in its seeds, leaves, and biomass, which is also used in animal feed. Additionally, its high saponin and colorant levels make it suitable for commercial and medicinal uses (Bazile et al., 2016). Due to its high nutritional content and ability to improve food security by resisting environmental restrictions like drought (Hirich et al., 2012 and Razzaghi et al., 2014), and salinity (Hirich et al., 2014 and Bouras et al., 2022), quinoa, a facultative halophyte, has recently attracted increasing interest worldwide. The halophytic crop quinoa has a high market value for its seed and has the potential to replace conventional crops (Chaganti and Ganjegunte, 2022). Among the various methods lately employed to tackle the salinity issues agricultural fields, pre-sowing seed treatment is a simple, low-cost, and risk method for dealing with salinity stress (Ramadan et al., 2019).

Chemical modification has been established as an effective approach for producing far more polymorphism. Living organisms have been reported to be mutagenic to sodium azide (SA) (Al-Qurainy and Khan, 2009). It is difficult to predict how SA may interact with cellular components such as DNA, proteins and membranes (Vwioko et al., 2019). Nitric oxide (NO), an

endogenous plant signaling molecule that is water and lipid soluble, has recently been studied for its ability to help plants tolerate salt stress (Mostofa et al., 2015). Due to its crucial function in stress tolerance brought on by oxidative stress in numerous processes of plant growth, development, metabolism, and cell death, NO has garnered a lot of interest (Manai et al., 2014). Additionally, NO mediates the metabolism of reactive oxygen species (ROS) and plant growth regulators, and it concentrates on how the NO participates in signal transduction and reactions to biotic and abiotic stimuli (Fan et al., 2007). NO, a signaling molecule present in all plants, is involved in a variety of physiological, biochemical, and developmental processes as well as plant responses to abiotic stress (Kolbert et al., 2021). NO transmits stress signals through plant tissue. Due to its antioxidant properties, NO exhibits anti-stress properties and have the capacity to stabilize membranes and cell walls. For crops including *Oryza sativa*, *Chenopodium quinoa*, *Glycine max* and *Medicago truncatula* to become more tolerant to stress, the exogenous administration of NO has been suggested as a viable strategy (Jabeen et al., 2021).

As being biochemical constituents, thiamin (B1), riboflavin (B2), niacin or niacinamide (B3), pantothenic acid (B5), pyridoxine or pyridoxal (B6), biotin (B7), folic acid (B9), and cobalamin comprise up the group of water-soluble vitamins recognized as the B vitamins (B12). B vitamins are crucial for cell metabolism and operate both together and separately in every cell. Vitamin B complex is the name given to supplements containing all eight (Lheureux et al., 2005 and Chawla and Kvarnberg, 2014). The healthy operation of living organisms depends on a diverse set of chemical substances known as vitamins. Usually, the B-group vitamins act as a reserve supply or a direct precursor to produce coenzymes. They must be received from our everyday food because mammals cannot synthesize them. Each specific group of B vitamins comprises of chemically unique substances with comparable biological function; these substances are referred to as "vitamins." Thiamine is an example of a simple vitamin that can exist in both free and bound forms. For example, thiamine can be chemically attached to proteins or carbohydrates. The ability of digestive enzymes to release bound forms and cofactors from food into forms that can be absorbed in the gastrointestinal tract determines how efficiently they are bioavailable. To accurately assess each vitamin's contribution to the overall nutritional content of food, proper quantification of each vitamin should be done because distinct B-group vitamins may differ in both their bioactivity and bioavailability (Gregory, 2012). Using repetition of ISSR (inter simple sequence repeats) markers are simple. It does not require knowledge of the DNA sequence and just requires a small amount of DNA. To use ISSR primers, a plant species must have a sufficient number and distribution of SSR motifs in its genome (Buhulikar et al., 2004).

The goal of the current study was to assess and contrast the effects of seed priming with sodium nitroprusside (SNP) a free radical (NO) donor and SA on quinoa genotype plants under salt stress in terms of growth, yield, and boosting nutritional features while reducing anti-nutritional components such saponins. Studying the polymorphism of both quinoa genotypes will be exhibited in this literature.

MATERIALS AND METHODS

Field experiments were conducted over two successive seasons (2020-2022) field experiments to determine the effect of chemical treatments on two quinoa genotypes nutritional and anti-nutritional components under salinity stress. They were cultivated at Ras Sudr experimental station, South Sinai governorate (an area of salinity stress) in Egypt. Two types of quinoa were investigated, Chipaya (obtained from Desert Research Center, Egypt) and Q37 (Chile). The grains of quinoa genotypes were cultivated in Ras Sudr at the rate of 3 kg fed^{-1} (7.5 kg ha^{-1}). The experiment design was in a split-plot design with three replicates. Grains were soaked in chemical treatments before cultivation, about 5 grains were put in each inferior in-depth 2.5 cm in the soil, then reduced to 2 plants in each inferior, quinoa was drip-irrigated at rate of 4 L in an hour for 1/2 h every 3 days, and fertilized regularly by fertilizers. All treatments received 37 kg P ha^{-1} and 150 kg K ha^{-1} as ordinary superphosphate of 68 g P kg^{-1} and K as potassium sulphate of 420 g K kg^{-1}. The chemical analysis of soil and water and mechanical dry sieving properties are represented in table (1), the soil is sandy in texture according to the same table according to Estefan et al. (2013).

Table (1). Chemical properties of the soil and irrigation water in Ras Sudr experimental station.

pH	EC ppm	Chemical properties							
		Cations (meq/L)				Anions (meq/L)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
Irrigation water (well)									
7.82	4557	10.8	7.15	53.6	0.35	-	5.30	39.1	26.8
Soil									
7.76	6195	4.6	3.2	88.3	0.67	-	4.95	65.7	26.1

1. Saponin Content

About, 10 g of defatted plant grains were refluxed with 80% ethanol for two days, then a concentrated solution of barium hydroxide was added on the filtrate and left overnight for complete precipitation, the precipitate was then filtered and washed several times with 80% alcohol and ashless filter paper was pre-weighed where the residue was put in a pre-weighed crucible and was entered to an oven till obtaining constant weight W1. Then the

crucible was transferd to a muffle furnace for complete ignition at 600ºC W2 Cheok et al. (2014). The calculation of saponin percent was according to the following formula:

$$\text{Total saponin \%} = \frac{\text{W1} - \text{W2}}{\text{plant wt X } 100}$$

2. Minerals Content

About 0.3 g from cleaned, polished and ground quinoa grains was dried overnight at 50°C in an oven. They were then processed using a sample mill. With the aid of an atomic-absorption spectrophotometer, minerals were analyzed as described in A.O.A.C (2010).

3. Water Soluble Vitamins Content

3.1. Extraction of water soluble vitamins from quinoa grains

The vitamin B group was isolated using 25 mL of H_2SO_4 (0.1N) solution by incubating 2 g of quinoa grains powder for 30 min at 121°C. The mixture was then chilled, its pH was raised to 4.5 using 2.5 M sodium acetate. Overnight storage of the preparation was done at 35°C. After filtration with a Whatman No. 4 filter, the liquid was filtered once more through a micropore filter (0.45 m) after being diluted with 50 mL of pure water (A.O.A.C., 1990).

3.2. HPLC conditions

High performance liquid chromatography (HPLC) analysis was carried out using an Agilent 1260 series. The separation was carried out using ZORBAX SB-C8 (4.6 mm x 150 mm i.d., 5 μm). The mobile phase consisted of water with 0.01% triflouroacetic acid (pH 2.9) (A) and methanol (B) at a flow rate 1.5 ml/min. The mobile phase was programed in case of water-soluble vitamins consecutively in a linear gradient as mentioned in the following table, and the injection volume was 5 uL (Marzougui et al., 2009). The multi-wavelength detector was monitored at 280 nm.

Time (min)	A (%)	B (%)	Flow (mL/min)
0	90	10	1.5
1	70	30	1.5
4	50	50	1.5
8	90	10	1.5
10	90	10	1.5

4. Combined Amino Acids Content

4.1. Extraction of amino acids

The hydrolyzed protein amino acids were determined according to the method described by Pellet and Young (1980) as follows:

Defatted powder of each plant (0.1 g) was digested with 10 ml of 6N HCl in a sealing tube. The mixture was hydrolyzed at 110°C for 24 h, then filtered and the hydrolyzed protein-amino acids were obtained by evaporation of the

hydrolyzate till dryness. The residue was washed with distilled water. The volume of the filtrate was adjusted to 100 ml using distilled water. The investigation of protein-amino acids was completed as in free amino acids.

4.2. Amino acid analyzer conditions

Column:	Hydrolysate column SYKAM (S_{4300}) – (4.6x150 mm) and its temperature was 57ºC.
Sample:	100 µl.
Buffer system:	Sodium acetate, buffer A (pH 3.45), buffer B (pH 10.85).
Flow rate:	0.25 ml /min for ninhydrin pump. 0.45 ml /min for quaternary pump.
Detection:	Ninhydrin was used for the detection of amino acids at λ 440 for proline and 570 nm for the other amino acids

5. Molecular Markers (ISSR-PCR Technique)

5.1. Extraction of DNA

Total DNA was extracted from fresh leaves using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions, marker (100-3000 bp).

5.2. ISSR-PCR reactions

Six ISSR primers were used in the detection of polymorphism (Table 2). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10 pcmol), 3 µl template DNA (10 ng) and 7 µl dH_2O, according to Ibrahim et al. (2019).

Table (2). Primers used and its sequence.

Primer Name	Sequence
ISSR-03	5'-ACACACACACACACACYT-3'
ISSR-04	5'-ACACACACACACACACYG-3'
ISSR-05	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR-11	5'-ACACACACACACACACYA-3'
ISSR-12	5'-ACACACACACACACACYC-3'

5.3. Thermocyling profile PCR

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 50 s, an annealing step at 45ºC for 50s, and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

5.4. Detection of the PCR products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1X TBE buffer

at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

5.5. Data analysis

For ISSR analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. Dice's similarity matrix coefficients were then calculated between genotypes using the unweight pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) according to Euclidean similarity index using the PAST software Version 1.91 (Hammer et al., 2001).

6. Statistical Analysis

Two–ways ANOVA was used. All treatments were arranged and analyzed as split plot design according to Snedecor and Cochran (1969) with three replicates, after testing the homogeneity of both seasons. Means of different treatments were compared using Duncan's multiple ranges at $P < 0.05$.

RESULTS AND DISCUSSION

1. Saponin and Yield

As steroidal glucosides or amphiphilic triterpenes, saponins named so because of their capacity to produce stable water-based foams due to their surface-active characteristics (Singh et al., 2003). The effect of chemical treatments on saponin content in two quinoa genotypes was presented at Table (3). The least content of saponin was detected by SA at 0.04% as a chemical treatment. Lower content of saponin gives quinoa variety a competitive edge in the Egyptian market, because the lower varieties of this anti-nutrient are preferred by the consumer. Concerning genotypes, Q37 achieved the lesser amount of saponin (6.88%). Interaction between genotypes and chemical treatments showed that the lowest content of saponin was recorded by Chipaya by SNP at 0.25 mg/L (5.113%), followed by SA at 0.04% when compared with Q37.

The maximum mean value of yield was detected by SA (0.04%). Concerning genotypes, Q37 exceeded Chipaya genotype in yield content and had lesser content of saponin. The interaction between quinoa genotypes and chemical treatments explored that, Chipaya gave highest content of yield (1508 kg/fad) was obtained by SA (0.04%) followed by Q37 by SNP at 0.25 mg/L (1203 kg/fad). These results are somewhat in agreement with Srivastava et al. (2011), where results show that SA appeared to be the most potent chemical modifying treatment for inducing micro-mutations in yield component traits at a dose of 0.02%. Also, Ramadan et al. (2019) reported the same results on sunflower yield by using SNP.

Table (3). Effect of sodium azide and sodium nitroprusside on saponin and yield of two quinoa genotypes subjected to salinity stress.

Chemical treatments		Saponin %			Yield (kg/fad)		
Type	Concentration	Chipaya	Q37	Mean	Chipaya	Q37	Mean
Control		6.817±0.081e	7.29±0.081c	7.052±0.057 D	680.83±7.781g	998.33±7.781b	839.58±5.502 D
SA (%)	0.01	9.830±0.081b	7.19±0.081c	8.510±0.057 B	747.11±7.781f	673.34±7.781g	710.23±5.502 F
	0.02	7.533±0.081c	8.27±0.081b	7.903±0.057 C	810.87±7.781c	826.67±7.781c	818.77±5.502 E
	0.03	5.627±0.081f	7.52±0.081c	6.575±0.057 E	619.42±7.781g	1096 ±7.781a	857.54±5.502 C
	0.04	5.343±0.081 f	6.27±0.081e	5.810±0.057 F	1508.00±7.781 a	1103±7.781a	1306.3±5.502 A
SNP (mg/L)	0.25	5.113±0.081 f	6.77±0.081e	5.942±0.057 F	598.36±7.781h	1203±7.781a	900.68±5.502 B
	0.50	16.120±0.081a	6.08±0.081d	11.100±0.057 A	681.03±7.781g	1025±7.781a	852.98±5.502 CD
	0.75	8.390±0.081b	5.60±0.081f	6.995±0.057 D	781.10±7.781f	530.07±7.781h	655.59±5.502 G
Mean		8.09±0.029A	6.88 ±0.029B		803.34 ±2.751B	931.912±2.751A	

The exogenous application of SNP greatly enhanced the canola yield and its constituents. The most effective SNP level was 50 M. Salinity stress drastically reduced all yield and its constituent parts. Meanwhile, application of SNP under salinity stress, particularly at 50 M, mitigated the negative impact of salinity on yield and its components (Farouk and Arafa, 2018). SNP as a nitric oxide donor is a small diffusible bioactive signaling molecule which plays several roles in plant growth, productivity under normal or stressed conditions such as saline stress (Ahmad et al., 2018 and Hanafy et al., 2018), scavenging ROS through the production of peroxynitrite ($ONOO^-$), which is less harmful than peroxides, SNP can reduce salt stress. It can also do this by boosting the activities of antioxidant enzymes and metabolites. Additionally, SNP application regulates proteins at the post-translational level required for cellular division and stimulates proton-pump activities within the tonoplast as a membrane separating a vacuole from the surrounding cytoplasm in a plant cell, increasing the K^+/Na^+ ratio (Sheokand et al., 2010).

The application of SA on crop is easy and inexpensive for improvement of agronomic traits. The development of an organic metabolite of the azide molecule mediates the biochemical effects. This metabolite penetrates the nucleus, interacts with DNA, and alters the genome at specific points, and this is concentration dependent (Khan et al., 2009). SA as a nitrogen gas generator (Gruszka et al., 2012), has been being used in various crops to improve their yield and quality traits and create resistance to them against biotic and abiotic stresses (Srivastava et al. 2011).

2. Minerals Content

Due to its ability to withstand severe conditions and the excellent nutritional value of its grains, quinoa, a facultative pseudocereal halophyte, has gained interest on a global scale (Nanduri et al., 2019). It is a pseudo grain with great nutritional value that has a remarkable balance of vital amino acids in addition to being rich in proteins, lipids, fiber, vitamins, and minerals. Moreover, quinoa has significant levels of phytochemicals that are good for your health, such as saponins, phytosterols, and phytoecdysteroids. It is well recognized that quinoa benefits human metabolic, cardiovascular, and digestive health significantly (Navruz-Varli and Sanlier, 2016). In Table (4), for the chemical treatments of quinoa plants under saline stress, the maximum content of Mn was detected by SA treatment at 0.04%. Notwithstanding, the content of Mg was significantly decreased by all used treatments. Furthermore, the highest Zn content was noticed by the application of SA at 0.03%. The highest content of Fe was determined by SA at 0.01% so that, SA had a positive effect on the most determined minerals in quinoa.

Concerning genotypes, Chipaya exceeded Q37 in Mn and Zn content. In contrast, Mg content in Q37 surpassed Chipaya. No significant effect was noticed between both genotypes in Fe content. For the interaction between genotypes and chemical treatments, the maximum content of Mn was done by

Table (4). Effect of sodium azide and sodium nitroprusside on some minerals of two quinoa genotypes subjected to salinity stress.

Chemical treatments		Minerals content (mg/g)					
		Manganese		Mean	Magnesium		Mean
Type	Concentration	Chipaya	Q37		Chipaya	Q37	
Control		0.036±0.005b	0.036±0.005b	0.037±0.003B	6.483±0.047b	9.357±0.047a	7.920±0.033A
SA (%)	0.01	0.038±0.005b	0.037±0.005b	0.038±0.003B	6.313±0.047b	4.340±0.047f	5.327±0.033E
	0.02	0.052±0.005a	0.052±0.005a	0.052±0.003A	4.667±0.047d	5.340±0.047e	5.003±0.033F
	0.03	0.046±0.005a	0.045±0.005a	0.045±0.003AB	5.097±0.047d	6.020±0.047b	5.558±0.033D
	0.04	0.056±0.005a	0.055±0.005a	0.056±0.003A	7.313±0.047a	5.680±0.047d	6.497±0.033B
SNP (mg/L)	0.25	0.063±0.005a	0.029±0.005c	0.046±0.003AB	1.420±0.047g	6.007±0.047b	3.713±0.033G
	0.50	0.060±0.005a	0.048±0.005a	0.054±0.003A	5.213±0.047c	5.340±0.047c	5.277±0.033E
	0.75	0.060±0.005a	0.049±0.005a	0.055±0.003A	5.333±0.047c	6.347±0.047b	5.840±0.033C
Mean		0.052±0.002A	0.044±0.002B		5.230±0.016B	6.054±0.016A	

		Zinc		Mean	Iron		Mean
		Chipaya	Q37		Chipaya	Q37	
Control		0.031±0.001e	0.023±0.001g	0.027±0.001F	3.464±0.012a	0.058±0.012i	1.761±0.008F
SA (%)	0.01	0.033±0.001c	0.035±0.001b	0.034±0.001C	2.997±0.012b	3.347±0.012a	3.172±0.008A
	0.02	0.034±0.001b	0.028±0.001e	0.031±0.001E	2.442±0.012d	3.308±0.012a	2.875±0.008B
	0.03	0.052±0.001a	0.031±0.001d	0.041±0.001A	2.741±0.012c	2.763±0.012c	2.752±0.008C
	0.04	0.033±0.001c	0.033±0.001c	0.033±0.001CD	2.380±0.012d	2.667±0.012b	2.524±0.008D
SNP (mg/L)	0.25	0.027±0.001f	0.044±0.001b	0.036±0.001B	1.638±0.012f	0.073±0.012h	0.856±0.008H
	0.50	0.031±0.001d	0.035±0.001b	0.033±0.001C	0.095±0.012g	2.134±0.012e	1.114±0.008G
	0.75	0.034±0.001b	0.034±0.001b	0.034±0.001C	1.838±0.012f	1.734±0.012e	1.786±0.008E
Mean		0.035±0.001A	0.033±0.001B		2.199±0.004A	2.010±0.004A	

Chipaya treated with SNP (0.25 mg/L). For Zn content, Chipaya recorded the maximum content of Zn by SA at 0.03%. The maximum content of iron was recorded by the same genotype in case of control followed by SA at 0.01%. Utilizing free radical nitric oxide (NO) donors like SNP has beneficial effects on plants by reducing salinity stress by enhancing the enzymatic activities of antioxidant enzymes and adjusting osmotic pressure, stimulates the production of the plant's antioxidant defense system. Through it significantly increased K and P content of the tested sunflower plants as reported by Ramadan et al. (2019). Similarly, Rasool et al. (2013) provided evidence that plants have well-regulated antioxidant machinery that can defend biomolecules from more damage in order to counteract the negative consequences of oxidative stress. Moreover, plants under salinity stress conditions adopted a different method of accumulating soluble proteins and carbohydrates to combat osmotic stress because cells' defense against oxidative damage through the suppression of lipid peroxidation and the scavenging of free radicals (Ahmad et al., 2016).

3. Water Soluble Vitamins

Vitamins profile separation by HPLC provided four vitamins identified with their standard due to the effect of chemical treatments like SA and SNP as a source of nitric oxide. SNP may help to mitigate the detrimental effects of salt stress on plant growth and development. There is little information available on the impact of SNP on water-soluble vitamins in quinoa plants grown in salinity conditions. However, SNP has been demonstrated in certain studies to increase the accumulation of various antioxidants in plants, which may help protect them from the oxidative damage brought on by salinity (Asgher et al., 2014 and Kausar et al., 2017).

Water-soluble vitamins such as B group have antioxidant properties; essential for the growth and development of plants, play important roles in plant growth and development. Some studies suggest that SNP may increase the accumulation of these vitamins in plants under stress conditions. The effect of SA on quinoa's water-soluble vitamins is not well studied. However, it is known that high salt concentrations can affect the bioavailability of some vitamins, leading to reduced absorption or destruction of vitamins in certain cases. Its effect on water-soluble vitamins in quinoa would likely depend on a variety of factors, including the concentration of SA, the amount of time it is in contact with the quinoa, and the specific water-soluble vitamins present. Overall, while more research is needed to fully understand the effects of SNP and SA on water-soluble vitamins in quinoa plants under salinity, it is possible that SNP may play a positive role in enhancing their accumulation and protecting the plants against oxidative stress.

The HPLC separated about four water soluble vitamins from quinoa seeds (vitamin B1, B2, B9 and B12) by µg/g as shown in Table (5) and Fig.

(1 and 2). Water-soluble vitamin B1, sometimes referred to as thiamine or thiamin, is crucial for the nervous system's health as well as the metabolism of carbohydrates. It supports healthy neuron and cardiac function and is crucial for converting glucose into energy. Serious health issues including beriberi and Wernicke-Korsakoff syndrome can result from thiamine deficiency. Whole grains, fortified cereals, legumes, nuts, lean meats, and fish are excellent dietary sources of thiamine (Rindi and Laforenza, 2019). Riboflavin, often known as vitamin B2, is a necessary ingredient for the body. It is essential to preserving overall health and wellbeing since it is important in the process of turning food into energy that the body can use. Keeping healthy skin, hair, and nails (https://ods.od.nih.gov/factsheets/Riboflavin-HealthProfessional/). The synthesis of red blood cells as well as the growth and function of healthy cells depend on folate (vitamin B9). The nutrient is necessary in the early stages of pregnancy to lower the chance of brain and spine birth abnormalities (https://www.mayoclinic.org/drugs-supplements-folate/art).

In Chipaya genotype, there were a marked increase in vitamin content by all chemical treatments, the highest content of vitamin B1 (1564 µg/g) was recorded in the treatment of SNP (0.75 mg/L), followed by 1186 µg/g recorded by SA at 0.02%. For vitamin B2, the highest content was determined by SA at 0.03% which provided 39.14 µg/g but declined in other treatments. In case of vitamin B9, the maximum content was noticed by SNP at 0.75 mg/L (376.57 µg/g), by two folds than control followed by SNP at 0.25 mg/L and SA at 0.04%. Vitamin B12 highest content was detected by SNP at 0.75 mg/L (843.51 µg/g) followed by SNP at 0.25 mg/L and SA at 0.01%. In Q37 genotype, the maximum content of B1 was detected by SA at 0.04% (789.75 µg/g). A marked increase was noticed in vitamin B12 and the highest content was introduced by SA at 0.02%, followed by SA at 0.01% (28.22 µg/g). Vitamin B9 maximum content was detected by SA at 0.02% (398.12 µg/g), followed by SNP at 0.25 mg/L (367.20 µg/g) and SNP at 0.75 mg/L (326.20 µg/g). For vitamin B12 content, the maximum value was detected by SA at 0.02% (697.39 µg/g) followed by SNP at 0.25 mg/L (666.35 µg/g). Hasanuzzaman et al. (2020) reported that **t**he increasing of water-soluble vitamins by SNP mangrove under salt stress may be due to the treatment of SNP that was found to increase the endogenous NO levels and reduce ion toxicity by improving the activities of antioxidant enzymes so that enhancing plant health.

Methionine is a necessary amino acid that acts as a building block for a number of significant constituents in plants, including several vitamins. Methionine itself is not a precursor to any of the B vitamins. Methionine biosynthesis pathway, a set of enzymatic activities that take place in plants to produce methionine, is responsible for this procedure. This pathway starts with the amino acid aspartate and involves multi-intermediate steps before generating methionine. Once biosynthesized, methionine can be used for

protein synthesis or can be converted into other important compounds such as S-adenosylmethionine (SAM), which is utilized in numerous biochemical processes in plants. While methionine is not a direct precursor to any of the B vitamins, it is involved in the synthesis of one of the precursors to vitamin B12. Specifically, SAM as a methyl donor compound, which is derived from methionine, is required for the conversion of cobalt into the form of cobalamin (vitamin B12) that can be consumed by plants (Roje, 2006).

4. Amino Acids

The biosynthesis of amino acids is a multistep process requiring the coordination of numerous enzymes and metabolic pathways. Knowing these pathways is essential for using genetic engineering and other biotechnological methods to increase agricultural yield and quality (Sánchez-López et al., 2014 and Rentsch and Hirner, 2016). Amino acids are the building blocks of proteins and are categorized into essential and non-essential based on their synthesis in the humans. The essential amino acids are synthesized only by plants, while non-essential amino acids are synthesized by both plants and humans (Tessari et al., 2016).

Quinoa is a food plant that is high in proteins. The value of proteins is determined by the fact that they have a balanced concentration of essential amino acids (Repo-Carrasco et al., 2003). In Q37 genotype with referring to Fig. (3) and Table (6), the maximum accumulation of essential and non-essential amino acids was detected by SA at 0.04%, followed by SNP at 0.25 mg/L. However, in Chipaya genotype as shown in Fig. (4) and Table (7), a fluctuation is happened where, the highest content of essential and non-essential amino acids was achieved by SNP at 0.75 mg/L, except methionine by SNP at 0.25 mg/L and histidine by SNP at 0.50 mg/L.

Table (5). Effect of sodium azide and sodium nitroprusside on water soluble vitamins two quinoa genotypes subjected to salinity stress.

Chemical treatments		Water soluble vitamins (µg/g)							
		Chipaya				Q37			
Type	Conc.	B1	B2	B9	B12	B1	B2	B9	B12
Control		458.92	38.20	171.52	608.11	547.08	10.49	22.48	485.38
SA (%)	0.01	1149.39	18.28	21.75	642.13	516.46	28.22	24.91	509.67
	0.02	1186.84	16.23	10.93	407.86	596.75	30.58	398.12	697.39
	0.03	973.42	39.14	5.69	334.66	614.91	17.76	10.00	661.31
	0.04	718.22	18.01	321.07	473.39	789.75	20.39	33.91	666.35
SNP (mg/L)	0.25	658.08	19.58	273.22	648.95	674.88	19.77	367.20	666.35
	0.50	1064.98	18.72	253.31	376.57	709.7	23.42	6.04	592.56
	0.75	1564.63	N.D.	376.57	843.51	537.88	16.37	326.57	432.22

N.D.: not detected and concentration is below the detection limit of the device

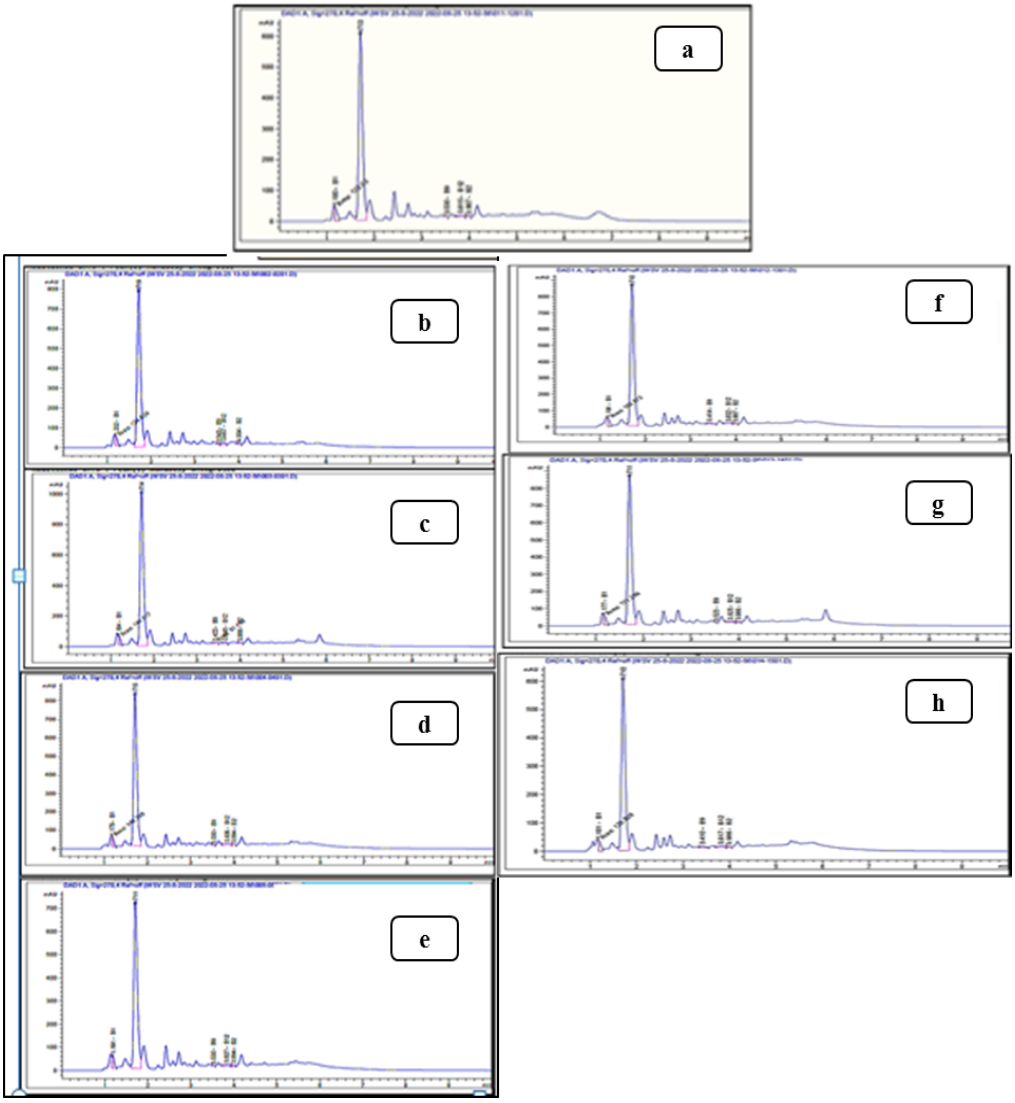

Fig. (1). Effect of chemical treatments on Q37 genotype water soluble vitamins; **a.** Control Q37, **b.** SA at 0.01%, **c.** SA at 0.02%, **d.** SA at 0.03%, **e.** SA at 0.04%, **f.** SNP at 0.25 mg/L, **g.** SNP at 0.50 mg/L and **h.** SNP at 0.75 mg/L.

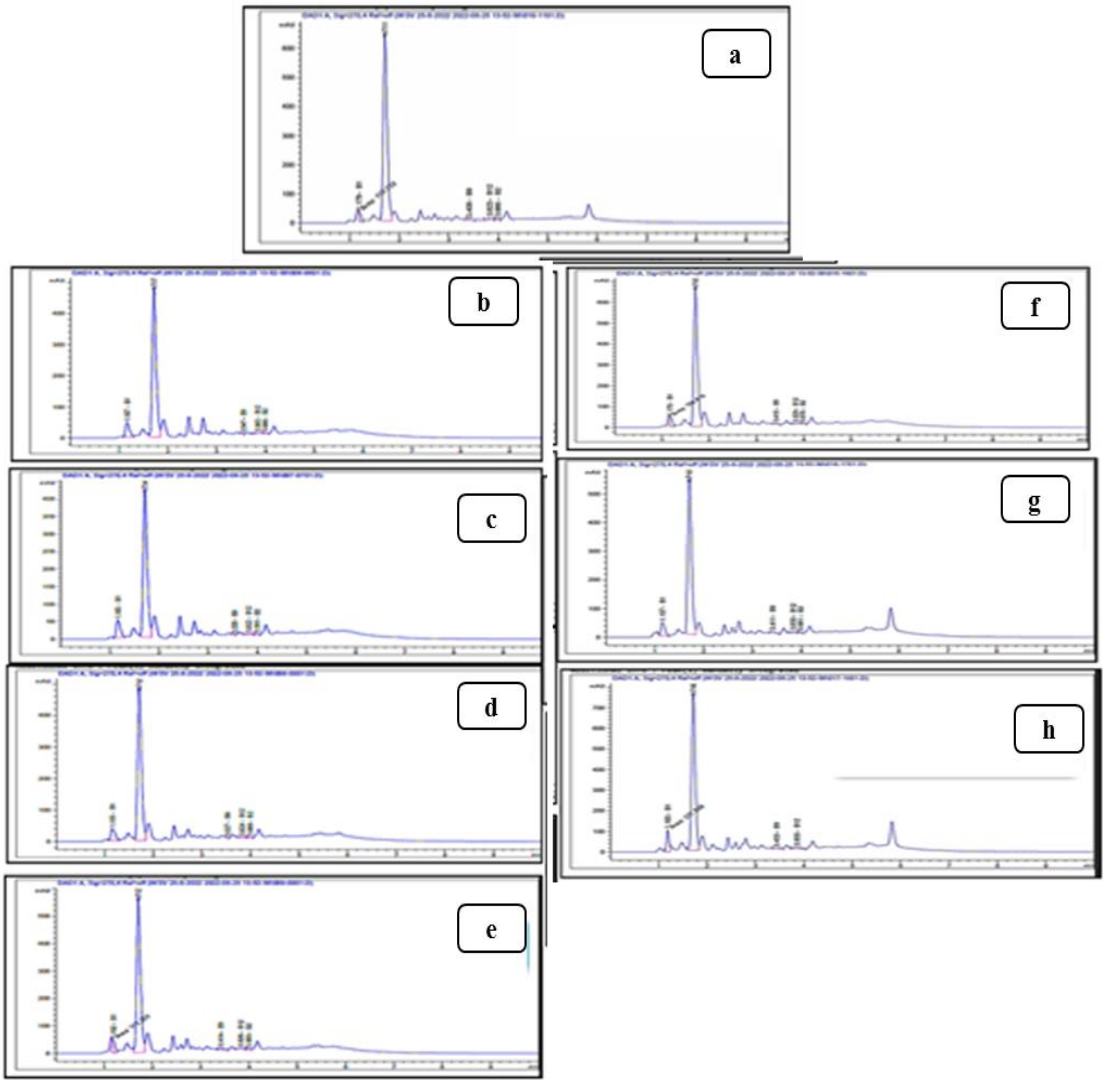

Fig. (2). Effect of chemical treatments on Chipaya genotype water soluble vitamins where; **a.** Control Chipaya, **b.** SA at 0.01%, **c.** SA at 0.02%, **d.** SA at 0.03%, **e.** SA at 0.04%, **f.** SNP at 0.25 mg/L, **g.** SNP at 0.50 mg/L and **h.** SNP at 0.75 mg/L.

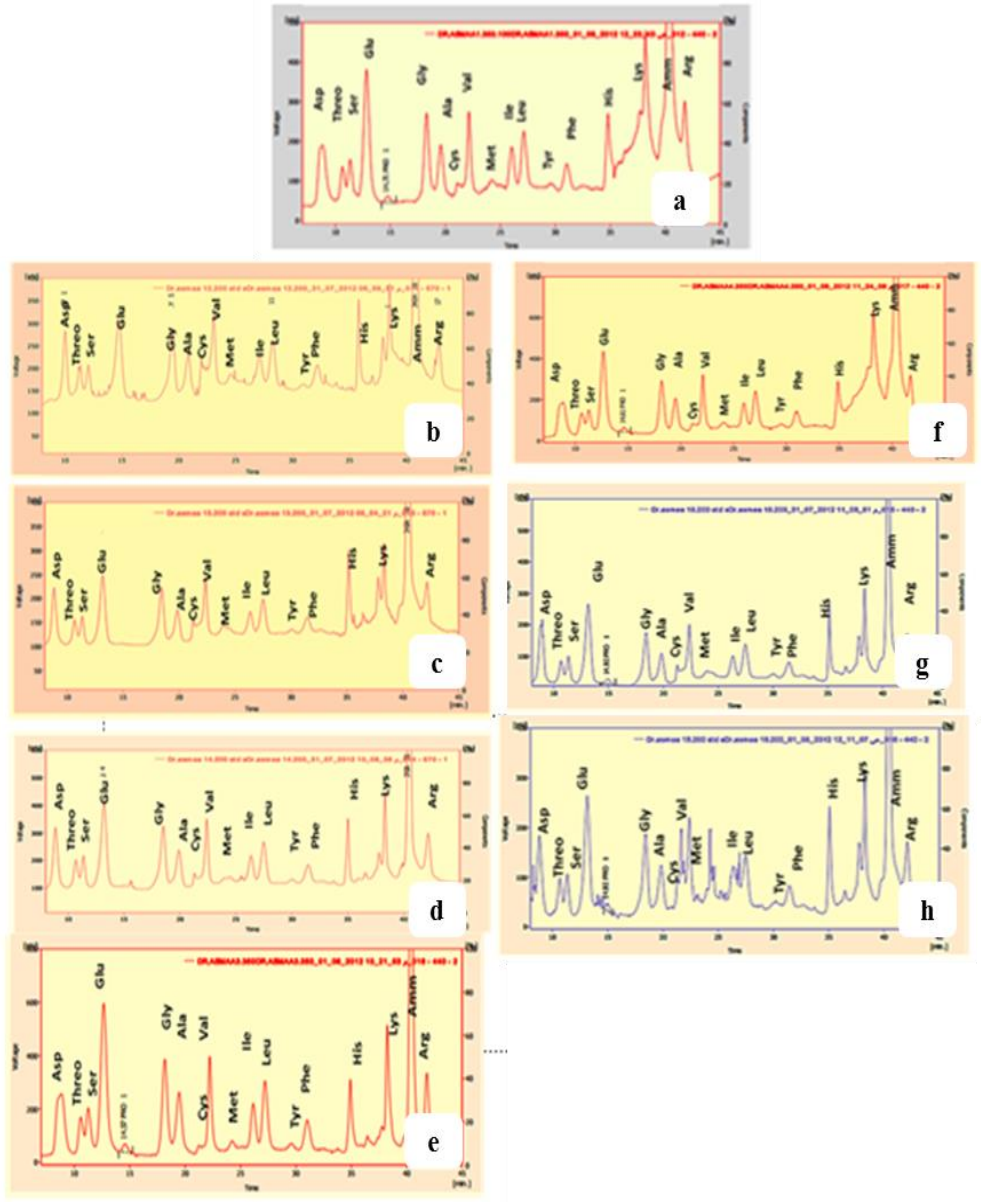

Fig. (3). Effect of chemical treatments on Q37 genotype amino acids where; **a.** Control Q37, **b.** SA at 0.01%, **c.** SA at 0.02%, **d.** SA at 0.03%, **e.** SA at 0.04%, **f.** SNP at 0.25 mg/L, **g.** SNP at 0.50 mg/L and **h.** SNP at 0.75 mg/L.

Table (6). Effect of chemical treatments on Q37 genotypes amino acids content.

Chemical treatments		Amino acids composition (mg/g dry wt)																	
		Essential										Non-essential							
Type	Conc.	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Valine	Alanine	Glycine	Aspartic	Glutamic	Proline	Serine	Cysteine	Tyrosine	Arginine
Control		6.84	5.45	8.30	5.63	1.38	5.72	5.55	7.15	5.65	7.57	14.77	22.61	4.27	4.24	2.01	1.24	9.88
SA (%)	**0.01**	3.82	2.29	3.29	4.66	0.86	2.10	1.92	2.31	2.56	3.45	4.13	8.54	1.83	1.57	1.27	0.43	4.48
	0.02	3.77	1.41	2.46	1.99	1.13	1.48	1.40	3.22	1.75	2.50	4.14	6.81	1.18	1.37	1.09	0.36	6.00
	0.03	5.54	2.70	4.76	4.31	0.16	3.05	2.63	4.74	3.59	5.01	7.84	14.23	1.67	2.57	1.06	0.63	9.45
	0.04	9.12	9.06	14.35	8.61	1.57	10.09	7.86	10.06	10.39	12.68	23.02	36.55	8.21	6.33	0.52	2.10	15.95
SNP (mg/L)	**0.25**	6.96	6.57	10.72	4.91	1.50	7.17	5.93	8.54	6.53	8.65	16.67	26.76	5.42	4.52	2.15	2.06	11.79
	0.50	3.21	2.24	3.32	2.69	2.23	1.89	1.48	3.96	2.29	3.15	4.91	8.65	1.46	1.65	1.17	0.49	6.81
	0.75	3.84	1.30	2.19	2.46	1.76	1.71	1.42	2.19	2.09	3.06	2.79	8.23	1.18	1.56	1.18	0.57	6.55

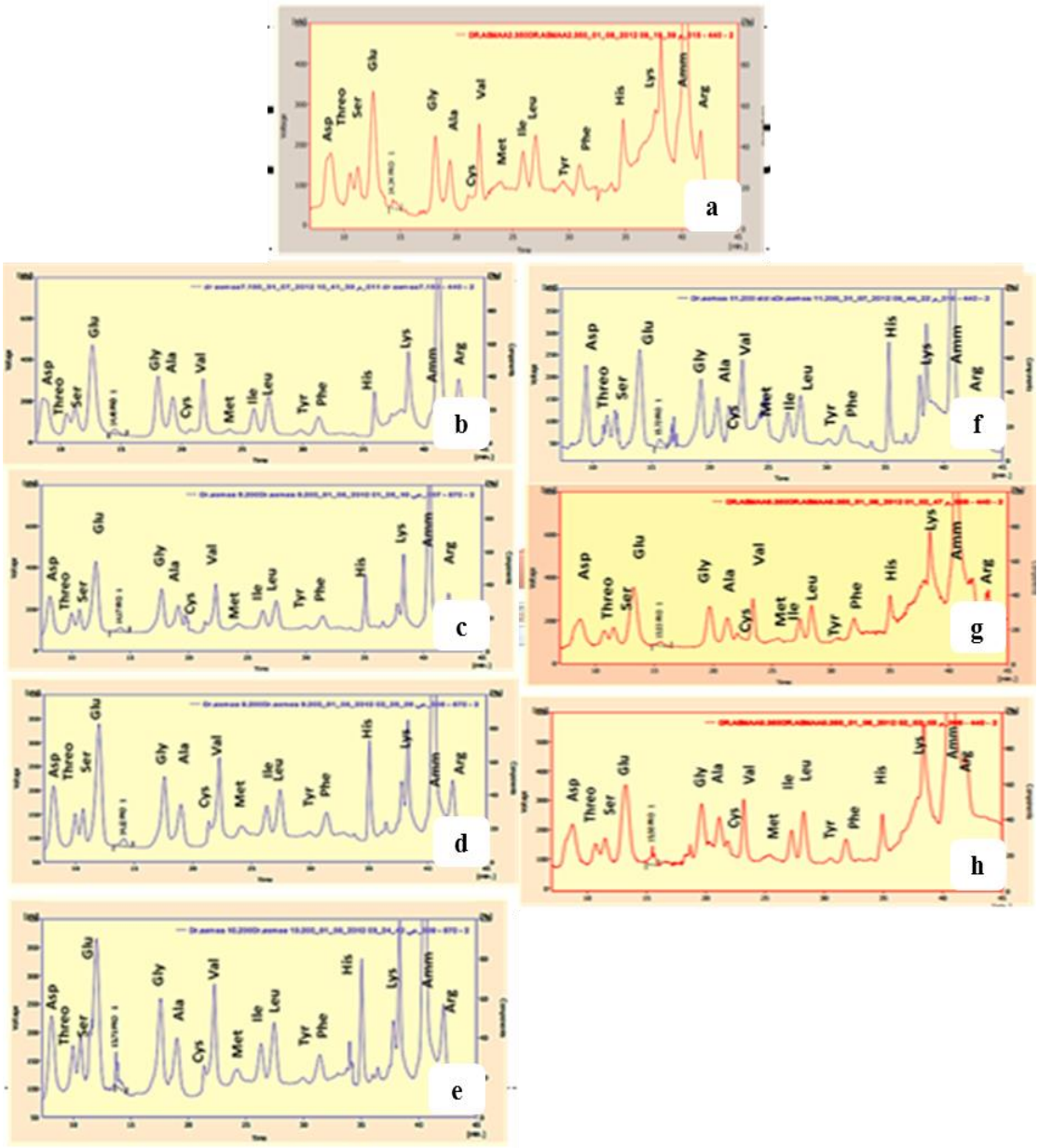

Fig. (4). Effect of chemical treatments on Chipaya genotype amino acids where; **a.** Control Chipaya, **b.** SA at 0.01%, **c.** SA at 0.02%, **d.** SA at 0.03%, **e.** SA at 0.04%, **f.** SNP at 0.25 mg/L, **g.** SNP at 0.50 mg/L and **h.** SNP at 0.75 mg/L.

Table (7). Effect of chemical treatments on Chipaya genotypes amino acids content.

Chemical treatments		Amino acids composition (mg/g dry wt)																
		Essential										Non-essential						
Type	Conc.	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Valine	Alanine	Glycine	Aspartic	Glutamic	Proline	Serine	Cysteine	Tyrosine	Arginine
Control		5.98	5.19	7.79	4.91	1.22	5.31	4.73	5.05	5.32	6.47	13.04	18.75	5.44	3.64	0.75	2.30	7.27
SA (%)	0.01	2.69	2.61	4.34	4.09	0.44	2.85	1.86	3.51	3.64	4.25	6.24	12.55	1.61	2.71	0.81	0.75	9.46
	0.02	3.76	2.36	4.00	3.60	0.86	2.57	2.25	4.33	2.26	3.84	6.21	12.34	2.04	2.25	1.00	0.63	8.25
	0.03	3.67	2.43	3.53	2.16	2.33	2.07	1.63	4.08	2.34	3.02	4.46	9.06	1.58	1.56	1.10	0.71	5.64
	0.04	3.78	2.54	3.77	2.78	2.45	1.96	2.00	4.21	2.64	3.65	4.99	10.33	2.87	1.96	1.11	0.65	6.64
SNP (mg/L)	0.25	4.58	2.46	3.89	5.64	5.08	1.67	2.01	4.49	2.67	3.33	4.79	8.30	1.75	1.81	3.67	0.65	4.36
	0.50	6.19	5.60	8.91	7.23	1.77	6.94	4.42	5.73	4.87	7.03	14.01	21.86	6.46	3.50	1.99	1.18	10.38
	0.75	5.27	6.10	9.44	8.77	1.74	7.37	4.82	6.52	6.04	7.59	16.24	21.06	10.96	3.71	4.47	1.74	11.87

Plants use antioxidants and osmoregulation systems to cope with salt stress (Gupta et al., 2021). To combat the negative consequences of salinity through a variety of actions against salinity-induced oxidative stress, signaling molecules as SNP sources of NO are added (Prakash et al., 2021). This study was compatible with Hajihashemi et al. (2022), who reported that SNP priming in quinoa can effectively combat salinity stress through improving the redox status of plants through increasing proline content.

6. Genetic Stability by ISSR PCR

The ISSR DNA fingerprinting technology demonstrates the genetic stability. This approach was used in this study to evaluate the genetic stability of the two quinoa genotypes (Q37 and Chipaya), which underwent chemical treatments and salinity stress. In previous literatures like, Mahdi et al. (2021 a and b) and Mahdi (2022) used ISSR technique for determining genomic stability of DNA. In this accord, a list of 6 primers was used in this technique and the amplified fragments were generated and exhibited in Table (8, 9, 10 and 11) and Fig. (5, 6, 7 and 8). The used primers produced distinct and scorable loci in size. The highest number of bands was produced by ISSR-4 and ISSR-5 for both genotypes. The referred abbreviations on gels were as following: Q: control Q37, Q1: SA at 0.01%, Q2: SA at 0.02%, Q3: SA at 0.03%, Q4: SA at 0.04%, Q5: SNP at 0.25 mg/L, Q6: SNP at 0.50 mg/L, Q7: SNP at 0.75 mg/L. C: control Chipaya, C1: SA at 0.01%, C2: SA at 0.02%, C3: SA at 0.03%, C4: SA at 0.04%, C5: SNP at 0.25 mg/L, C6: SNP at 0.50 mg/L, C7: SNP at 0.75 mg/L. For both genotypes, the used six primers were, ISSR-3 (1250 – 180 bp), ISSR-4 (870 – 150 bp), ISSR-5 (990 – 160 bp), ISSR-10 (870 – 170 bp), ISSR- 11 (600 – 200 bp) and ISSR – 12 (810 – 170 bp).

Considering the Q37 genotype, Table (8) and Fig. (7) show that, there was 35 monomorphic bands, 27 polymorphic without unique, 6 unique bands, 33 polymorphic with unique and 68 total bands. The highest polymorphism percent was 69% recorded by ISSR-4 but the lowest polymorphism percentage was 22% and average polymorphism percentage for this genotype was 46.83%. The six unique bands were due to the reaction of the chemical treatments or salinity stress effect on the genetic material of the quinoa genotypes. They were as follows: ISSR-3 gave 230 bp when treated with Q7, ISSR-4 released 730 and 870 bp by Q1 and 150 bp by Q3. ISSR-11 gave 230 and 260 bp by Q7.

As in the case of Chipaya genotype (Table 10) and Fig. (8), there were 33 monomorphic bands, 32 polymorphic without unique bands, 7 unique bands, 38 polymorphic with unique bands, and a total of 70 polymorphic bands. The ISSR-4 achieved the highest polymorphism percentage (77%), whereas the ISSR-11 had the lowest polymorphism percentage (33%). In this genotype, the average polymorphism percentage was 51.5%. The ISSR-3 identified the two different, distinctive bands (220 bp by C3 and 390 bp by C4). With C1, C4, and C7, the ISSR-4 primer produced 800 bp, 160 bp, and

240 bp, respectively. Via using C5, ISSR-10 produced 320 bp and ISSR-11 gave 710 bp amplicon.

The molecular distance between treated quinoa genotypes (Q37 and Chipaya) based on cluster analysis with chemical treatments. The dendrogram was used for both genotypes to express genetic stability in terms of the highest percentage that was close to the control percentage resulting in a substantially comparable result. Chipaya displayed the highest value of similarity to control exhibited by the application of Q7, which was linked to highest water soluble vitamins content amino acids content, followed by other treatments according to Table (9 and 11) and Fig. (5 and 6). Q37 displayed the highest value of similarity to control exhibited by the application of C2 followed by other treatments. The ISSR profile showed a shift, showing that the chemical treatments were responsible for dose- and type-dependent genetic variation. It is possible to refer to the ISSR profile's presence of new DNA bands and absence of normal ones as a mutation, which is likely the result of DNA damage or rearrangements brought on by chemically produced genetic variation.

One of the most common chemical modification agents in agricultural plants is SA, a chemical mutagen. An organic metabolite of the azide molecule is produced because of the mutation. This metabolite penetrates the nucleus, interacts with the DNA, and modifies the genome in a point-like manner. Further information regarding the effects of time, pH, temperature, seed immersion, and different concentrations is needed to improve the effectiveness and efficiency of SA, particularly the metabolite (Khan et al., 2009). To improve agronomic features, SA can be applied to crops readily and inexpensively (Lal et al., 2009).

Table (8). Effect of chemical treatments on DNA behavior of Q37 genotype as ISSR PCR.

Primers	Bands range (kbp)	Monomorphic bands	Polymorphic without unique bands	Unique bands	Polymorphic with unique bands	Total bands	Polymorphism %	Frequency
ISSR- 3	1250-180	7	4	1	5	12	42	0.76
ISSR- 4	870 - 150	4	6	3	9	13	69	0.63
ISSR- 5	990 - 160	5	8	0	8	13	62	0.72
ISSR- 10	870 - 170	7	4	0	4	11	36	0.83
ISSR- 11	600 - 200	5	3	2	5	10	50	0.69
ISSR- 12	810 - 170	7	2	0	2	9	22	0.90
Total bands		**35**	**27**	**6**	**33**	**68**	**Average: 46.83**	

Table (9). Dendogram and similarity matrix between chemical treatments and Q37 genotype.

MW	Q	Q1	Q2	Q3	Q4	Q5	Q6	Q7
Q	100							
Q1	86	100						
Q2	85	92	100					
Q3	85	81	83	100				
Q4	88	90	91	85	100			
Q5	87	89	91	78	89	100		
Q6	87	87	87	81	87	94	100	
Q7	89	84	84	82	82	88	88	100

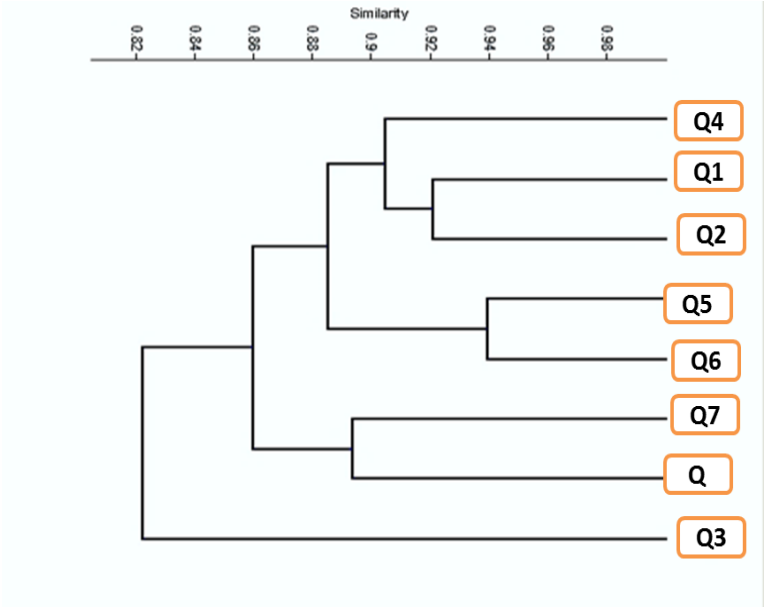

Fig. (5). Similarity matrix between chemical treatments on Q37 genotype.

Table (10). Effect of chemical treatments on DNA behavior of Chipaya genotype as ISSR PCR.

Primers	Bands range (kbp)	Monomorphic bands	Polymorphic without unique bands	Unique bands	Polymorphic with unique bands	Total bands	Polymorphism %	Frequency
ISSR-3	1250-180	6	6	2	6	12	50	0.71
ISSR-4	870-150	3	7	3	10	13	77	0.59
ISSR-5	990-160	7	6	0	7	14	46	0.76
ISSR-10	870-170	5	6	1	7	12	58	0.75
ISSR-11	600-200	6	2	1	3	9	33	0.75
ISSR-12	810-170	6	5	0	5	11	45	0.75
Total bands		**33**	**32**	**7**	**38**	**70**	**Average: 51.5**	

Table (11). Dendogram and similarity matrix between chemical treatments and Chipaya genotype**.**

MW	C	C1	C2	C3	C4	C5	C6	C7
C	100							
C1	84	100						
C2	86	85	100					
C3	86	88	87	100				
C4	82	88	80	86	100			
C5	82	88	77	81	88	100		
C6	84	81	76	84	83	90	100	
C7	83	80	80	83	80	86	87	100

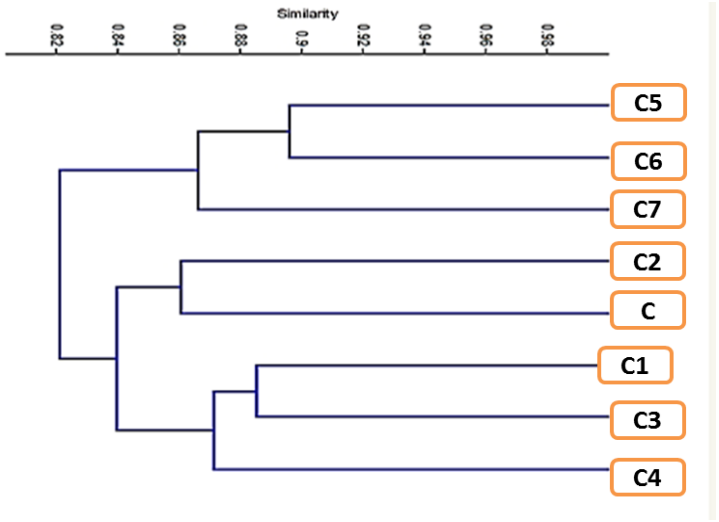

Fig. (6). Similarity matrix between chemical treatments on Chipaya genotype.

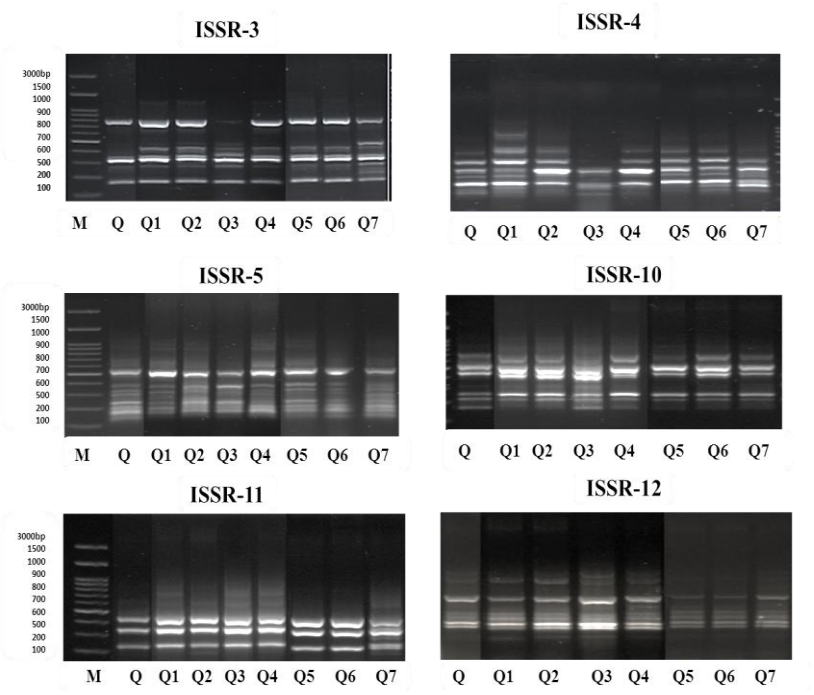

Fig. (7). ISSR PCR of DNA subjected to chemical treatments on Q37 genotype.

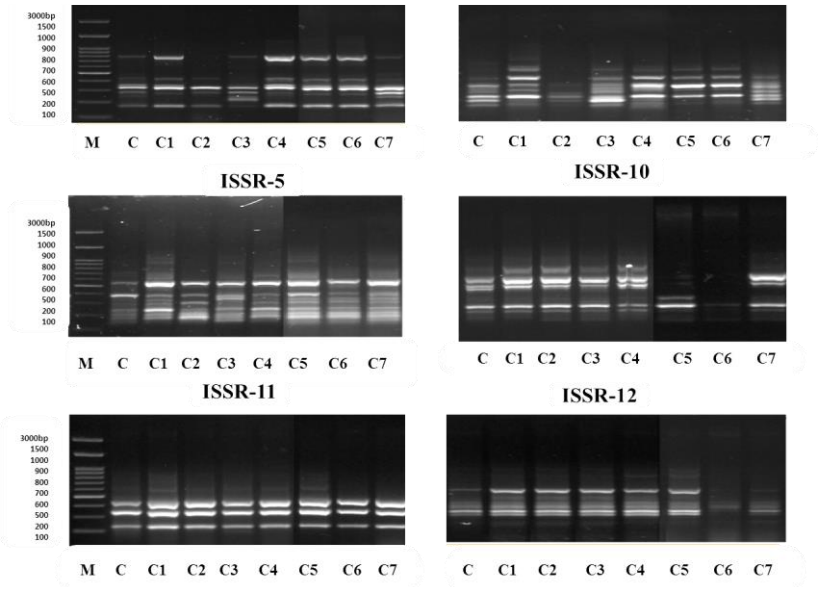

Fig. (8). ISSR PCR of DNA subjected to chemical treatments on Chipaya genotype.

CONCLUSION

It can be concluded that, Chipaya exceeded Q37 in some biochemical characteristics which were bound to some changes in DNA and increasing yield. Q37 exceeded Chipaya in the content of essential and non-essential amino acids by the application of SA at 0.04% under salinity stress. It might be concluded that this study was effective in improving the nutrition value and yield of quinoa at the new reclaimed desert lands.

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التغيرات الكيميائية في بعض مركبات التغذية والمضادة للتغذية في حبوب الكينوا تحت ظروف الملوحة برأس سدر، جنوب سيناء

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تم إجراء تجربة حقلية لمدة موسمين متتابعين لدراسة تأثير تركيزات مختلفة من أزيدات الصوديوم ونيتروبروسيد الصوديوم على بعض مركبات التغذية والمضادة للتغذية في حبوب الكينوا (Chipaya، Q37) تحت ظروف الإجهاد الملحي برأس سدر، جنوب سيناء. وقد أظهرت النتائج أن حبوب الكينوا كانت مصدرًا ممتازًا للفيتامينات والأحماض الأمينية الأساسية والغير أساسية والعناصر وكذلك بعض المركبات الطبية الأخرى المفيدة للصحة. وقد وجد أن المعاملة بأزيدات الصوديوم بتركيز ٠.٠٤٪ كان لها تأثير إيجابي على انخفاض محتوى المركبات المضادة للتغذية (الصابونين) وكان هذا مرتبطًا بزيادة واضحة في محصول الحبوب للتركيب الوراثي Chipaya تحت ظروف الإجهاد الملحي. وقد سجل نفس التركيب الوراثي أعلى معدل للفيتامينات القابلة للذوبان في الماء (B1, B2, B9, B12) وكذلك الأحماض الأمينية الأساسية والغير أساسية عند معاملته بنيتروبروسيد الصوديوم بتركيز ٠.٧٥ ملجم / لتر. ومن ناحية أخرى، فقد أعطى التركيب الوراثي Q37 قيم مرتفعة من الأحماض الأمينية الأساسية وغير الأساسية وذلك عند معاملته بأزيدات الصوديوم بتركيز ٠.٠٤٪. وبالنسبة لمحتوى حبوب الكينوا من العناصر، فقد وجد أن التركيب الوراثي Chipaya أعطى أعلى محتوى من المنجنيز عند معاملته بنيتروبروسيد الصوديوم بتركيز ٠.٢٥ ملجم / لتر. كما سجل أعلى محتوى من الزنك عند معاملته بأزيدات الصوديوم بتركيز ٠.٠٣٪. كما أظهرت الدراسة أن نفس التركيب الوراثي سجل أعلى محتوى من الحديد عند معاملته بأزيدات الصوديوم بتركيز ٠.٠١٪. ويعد استخدام الكاشفات الجزيئية (تقنية ISSR-PCR) أمرًا بالغ الأهمية لتحديد الاختلاف في التركيب الجيني وبخاصة المعاملات التي قد تؤدي لحدوث تغير في التركيب الوراثي. فقد أظهرت الدراسة أن أقل نسبة تعدد الأشكال تم الكشف عنها بواسطة Q37 تحت ظروف الملوحة والمعاملات الكيميائية المطبقة. وتؤكد الدراسة على أن المواد الكيميائية المستخدمة (وبخاصة أزيدات الصوديوم بتركيز ٠.٠٤٪، ونيتروبروسيد الصوديوم بتركيز ٠.٧٥ملجم / لتر كان لها تأثير إيجابي واضح على زيادة محتوى مركبات التغذية وانخفاض محتوى المركبات المضادة للتغذية في حبوب الكينوا كما أنها لم تؤدي لحدوث تغير في التركيب الجيني. ونوصى باستمرار الدراسة على تلك المواد الكيميائية مع استخدام تركيزات أعلى من السابقة للحصول على تراكيب وراثية ذات صفات مرغوبة من حيث الإنتاج والمحتوى الكيميائي.