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# Identifying Some Morphological Features of Mutant Quinoa Plants (*Chenopodium quinoa* Willd.)

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## ABSTRACT

**Background:** This research was carried out to obtain seeds from M1 and M2 level mutant plants of Titicaca cultivar of quinoa and to make some distinctive morphological observations.

**Methods:** Sodium azide was applied to the Titicaca variety of quinoa in 2018 and planted in a random order on plots in the field to obtain seeds at M1 level. When the plants reached the maturity of the seed harvest, they were removed from the soil and the number of growing days, plant height, stem thickness, number of branches, biological yield, seed yield and harvest index were measured and calculated at the M2 generation study. The seeds obtained from the M1 generation were planted in the field. In the period when the plants had 3 leaves, total herbicide with imazamox active ingredient was applied to the green parts of the plants. Afterwards, resistant plants were determined and M2 seeds were produced. Then, some measurements were made by growing M2 seeds in vases under greenhouse conditions. Results from mutant plants were compared with control plants.

**Result:** According to the measurements made, M1 generation it was seen that the average number of branches was statistically less in mutant plants than in control plants. No statistically significant difference was found between the measurements obtained from control and mutant plants for other features. However, according to the measurements made in the M2 generation, the difference between the values of the control and mutant plants was found to be statistically significant.


**Key words:** *Chenopodium quinoa,* Herbicide resistance, Morphological features, Mutation.

## INTRODUCTION

Quinoa is a plant of South America origin. It belongs to *Chenopodiaceae* family. Quinoa have been cultivated for thousands of years at Andean region thanks to its rich seed and leaf (Pearsall, 1992). Adaptation capacity of quiona is well and it can be cultivated from sea level to 400 m altitude (Jacobsen, 2003). Resistance of the plant to hot, drought, frost and salinity is very strong and the seed of the plant has very high nutritional value.

Plants high quality protein contains essential amino acids like methionine, lysine, threonine (Bhargava *et al.* 2007; Comai *et al.* 2007). The seed has major nutrition for people who suffers from gluten sensitivity. Because it is lack of gluten. While existing from ground, development of the plant is slow but later it gets faster and it is highly yielding. Yield of the plant is between 800-2000 kg $ha^{-1}$ depending on variety and ecological condition. The nutrition value of the plant is very high. The rate of crude protein inside the plant is between 12-22% and the rate of digestion is between 63-69 %. Plant have high water binding capacity starch and swelling power than wheat and barley (Rana *et al.* 2019). Plant hay can be used for animal forage. Beside, the seed of the plant can be used to feed the animals with wingsome (Tan and Temel 2017, 2018).

FAD has announced 2003 year as quinoa year. After that interest and awareness to plant had been risen up. Quinoa is grown roughly 95 country around world (Thiam *et al.* 2021). The interest to plant in Turkey has been risen up and the plant has been started to be grown in a lot of city (Egritas *et al.* 2020). Quinoa can be grown up asan alternative plant in short season, in drough and in salinity regions thanks to its resistant to harsh climate and unfavourable soil. Cultivation of plant is difficult. Since there is no selective selective herbicide specific to quinoa, weed control is difficult in agricultural large areas. Quinoa is broadleaf plant and relative with (*Chenopodium album*) and *Amaranthus retroflexus*. And it is similar morphologically (Egritas *et al.* 2020). Competition with weed is low. Because plant growns slow when emerges from the ground. In pre-emergence period metamitron and propyzamide were applicated and in post emergence period propyzamide herbicedes were applicated and quinoa has shown resistancence to this herbicide but there needed many studies in this area (Díaz *et al.* 2015). In post emergence period herbicid applications caused to stress and deform stoma open close mechanism and it influences photosynthesis badly. Plants influenced negatively by fungus (Das *et al.* 2014). Yieldance and grownance of the plants

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decreasing (Ali *et al.* 2016). Because there are not a selective herbicide for quinoa and producing a new selective herbicide which is not harmful for quinoa and its nutrition content needs so time. It is necessary to develop new useful quinoa varieties. Herbicide resistant plants to be obtained as a result of mutation studies will facilitate the fight against weeds. Imidazolinone group herbicides fight weeds by blocking the acetohydroxyacid (AHAS) enzyme in the plant. This group of herbicides is effective aganist broad and weeds at low doses. It is less harmful to the environment and mammals. Although herbicide resistance studies have been carried out in quinoa, no resistant plants have been obtained yet. The aim of this study is to produce seeds at the M1 and M2 stage of the mutation study and to measure some characteristics of the plants.

## MATERIALS AND METHODS

Study uses Titicaca variety of quinoa which is compatible for Eastern Anatolia region and early according to TÜBİTAK project (TOVAG-214O232) conducted in Erzurum and Iğdır conditions (Tan and Temel 2018).

This study has been conducted at Erzurum provience Atatürk University Plant-Production, Research and Application Center experimental area between 2018-2019 years. It include field, greenhouse and laboratory studies.

### Seed preparation

At this phase of study it has been used a protocol which is called imidazolinon (IMI) herbicide resistant wheat improvoment in USA (Newhouse *et al.,* 1992). Accoding to this protocol 400 g quinoa seed is put in tap water. Later it is kept for 20 minutes in 1.5 per cent sodium hipoklorit (NaClO) and it is washed 3 times with sterile water for sterilization. After that, seed is kept for 3 hours 1.5 mM sodyum azid solution and then seed is been dried up on the filter paper.

### M1 generation seeds to produce from mutation applicated seeds

At this phase of study mutated seed had planted in the Atatürk Universty Plant Production, Research and Application Center's testing area. At this phase, aim was to produce M1 seeds and to measure if they have same feature or not. Seed were planted between rows 35 cm, in row 15 cm in seedbed in may by hand (Tan and Yondem 2013). Fertilizer applicated 125 kg N $ha^{-1}$ and 80 kg $P_2O_5$ $ha^{-1}$ (Aguilar *et al.* 2003; Geren, 2015). One piece of phosphorus was applicated and two piece of nitrogen were applicated. Combating against to weed by hand. Plants were watered when it was needed. Plants has been harvested when seed was matured in september. All plants had been blended and seeds had been collected. At this phase 100 number plants were selected and measured for determine generally situation. Measurements were made from mutant plants as well as from control plants. One row mutant plant and one row control plant were planted to compare each other.

### Growing time (day)

The number of days to grow was determined by taking the number of days from sowing the seeds to the ripening period when the leaves of the plants turn yellow.

### Plant heigth (cm)

Determined by measuring the plants from the ground to the apex.

### Stem thickness (mm)

Calculated by measuring the plants from the soil above the second node.

### Branch number

Calculated by counting the branches from the first branch to the top of the plant from the soil.

### Biological yield (g $plant^{-1}$)

Calculated by cutting from the root collar when the plants are mature and measuring with a balance with a precision of 0.1 g.

### Seed yield (g $plant^{-1}$)

Calculated by taking seeds from mature plants and measuring them with a balance with an accuracy of 0.1 g.

### Harvest index (%)

For every plant determined seed yield and weight later calculated by this formula:

$$\text{Harvest index (\%)} = \frac{\text{Per plant seed weight}}{\text{Per plant totally weight}} \times 100$$

### 1000 seed weight (g)

Determined average 1000 seed weight by harvested seeds 4×100 seed be counted and be weighed

### M2 generation seeds to produce from herbicide applicated M1 plants

At this stage of the study, the seeds obtained from the M1 generation were planted in the Atatürk University Plant Production, Research and Application Center experimental area in May with 35 cm row spacing and 15 cm row spacing (Tan and Yondem 2013). Fertilizer 125 kg N and 80 kg $P_2O_5$ $ha^{-1}$ were also applied (Aguilar *et al.* 2003; Geren, 2015). Phosphorus was applied as one part and nitrogen was applied as two parts. Handle weed control. When the plants had 2-3 leaves, a total herbicide with 3 g a.i $ha^{-1}$ imazamox active ingredient was applied to their green parts. At this stage, 20000 plants were screened and herbicide resistant plants were determined. M2 seeds were produced from these plants.

Then, seeds of M2 and control plants were sown in vases under greenhouse conditions. The same measurements were made from mutant plants and control plants. Since the plants were grown in greenhouse conditions, some measurements such as seed yield, harvest index, 1000 seed weight could not be made. The measurements made are given as follows.

**Plant heigth (cm)**

Determined by measuring the plants from the ground to the apex.

**Root height (cm)**

Determined by measuring the plants from root end to root neck.

**Seed yield (g plant$^{-1}$)**

Calculated by taking seeds from mature plants and measuring them with a balance with an accuracy of 0.1 g.

**Root weight (g)**

Calculated by taking the plants root cutting root neck. Them with a balance with an accuracy of 0.1 g.

**Stem thickness (mm)**

Calculated by measuring the plants from the soil above the second node.

**Branch number**

Calculated by counting the branches from the first branch to the top of the plant from the soil.

## RESULTS AND DISCUSSION

Sodium azide was applicated to the seeds as a mutagen. It was then planted in the field to produce M1 seeds. 100 of the obtained mutant and control plants were taken and measurements were made.

M2 seeds produced from M1 lines were planted in the field. When the plants had 2-3 leaves, total herbicide with imazamox active ingredient was applied to the green parts of the plants at 3 g a.i/da. Herbicide-resistant plants were identified and M2 seeds were produced. M2 and control seeds were sown in pots under greenhouse conditions. The same measurements were made from mutant plants and control plants. The averages obtained were compared with the T test.

**Growing time (day)**

According to statistical analyse at the M1 generation difference between mutant plants and control plants are insignifcant. That is to say, statistically there is not much difference between them. It can be seen in Table 1. Growth process of mutant plantsis 106,8 days and it is 108,2 days for control plants. There are also different studies about this issue. Chickpea applicated diffirent EMS doses and gamma ray, growing time compared between mutant plants and control plants there are diffirent results (Dinkar *et al.* 2020). Cemen grass applicated chemical mutegen and growing time changing according to mutajen doses (Basu *et al.* 2008). Broad bean applicated diffirent chemical mutajen and doses there are diffirent effects on growing time (Laskar *et al.* 2014). Chickpea applicated EMS and application has reduced the number of breeding days (Singh *et al.* 2015). In conclusion, different growing time effected mutation applications according to our working results and other working results. the average of mutant and control plants are the same. It is thought that this may be due to the fact that the mutation affects the plant genome randomly or does not affect the relevant gene region (Ul-Allah *et al.* 2019).

**Plant height**

According to statistical analyse, at the M1 phase diffirence between mutant plants and control plants are insignificant. Result of the study can be seen in the Table 1. According to this result mutant plants shows height average low compared with control plants height everage. But this different is statictically insignificant. There is a difference between mutant plants and control plants according to M2 Phase. According to these results, mutant plants have higher mean height than control plants. The difference between the means was statistically significant at the 5% level. There are different result about mutation applications on the plants height (Ul-Allah *et al.* 2019). Cotton plants applicated mutation and measured M1 generation plant height there arent much different between control plants height and mutant plants height (Ahmed *et al.* 2020) wheat applicated chemical mutagen finded mutant plants height higher than control plants. Chrysanthenum applicated diffrents EMS doses which is found mutant plants height higher than control plants (Chen *et al.* 2020). Sesame (*Sesanum indicum* L.) reported that the application of gamma rays and EMS mutagen in sesame plant significantly decreased the plant height depending on the dose (Sheeba *et al.* 2005). Tomatoes aplicated sodium azide and highest plants height is control plants and plants height decrease depend on mutagen doses (Adamu *et al.* 2007); Efe *et al.* (2017). Hungarian vetch applicated different gamma ray and found different result dependenting onvariety (Ibukun *et al.* 2019). Kenaf (*Hibiscus cannabinus*) plants four varieties used applicated different sodium azide doses. According to result plants height. It differs according to the dose and type (Rysbekova *et al.* 2020). Millet

**Table 1:** Control and applicated quinoa plants height, stem thickness, branch number, biological yield, seed yield, harvest index and 1000 seed weight results.

| | Grown time (day) | Plant height (cm) | Stem thickness (cm) | Branch number | Biological yield (g plant$^{-1}$) | Seed yield (g plant$^{-1}$) | Harvest index (%) | 1000 seed weight (g) |
|---|---|---|---|---|---|---|---|---|
| Control | 108.2 | 77.0 | 1.38 | 14.0 | 183.4 | 43.9 | 23.95 | 270 |
| Application | 106.8 | 74.7 | 1.25 | 9.6 | 171.8 | 44.1 | 25.91 | 271.3 |
| t-test | ns | ns | ns | * | ns | ns | ns | ns |

*: 5% different significant.

ns: Different insignificant statistically.

plants applicated sodium azide according to result plants height. It differs according to the dose and type (Abdulkareem *et al.* 2017). Various tomatoes variety applicated different sodium azide doses according to result plant height different depent on doses and variety. There are a study that aims to quantitative variability in mungbean induced by chemical mutagens according to this study results at the M1 and M2 generations plant height decrease depend of mutagen doses. Highest plant height measured from control plants (Khan *et al.* 2006). There are a study aims to mutagenic induction of variability and selection in M2 generation of selected ricebean (*Vigna umbellata* Thunb.) cultivars of manipur according to this study results at M2 generations highest plant height measured control plants (Devi *et al.* 2006). According to our study and others results mutation applications various effect on the plants height. This result is related to the measured generation. There is also general agreement that mutation effects can be observed at M2 and M3 stages (Roychowdhury *et al.* 2012).

**Stem thickness**

Results in Table 1 study aims mutation aplications that effects on the stem thickness. At the M1 generation different between control plants and applicated plants is insignificant. At the M2 generation different between control plants and applicated plants is significant. According to these results, the mean stem thickness of mutant plants is lower than the mean of control plants. The difference between the means was found to be statistically significant at the 1% level. Plant stem thickness varies depending on mutagent effects on the plants genome, there are high and thin stem plants as well as short broad stem plants (Hussein *et al.* 2017). The aim the study is to carry out to determine the mutagenic effect of sodium azide ($NaN_3$) on M2 generation *Brassica napus* L. (Dunkled variety), the average stem thickness of mutant plants was found to be lower than the average of control plants (Akhtar *et al.* 2017). Canola applicated different doses sodium azide and highest plant stem were founded from control plants. According to results stem thickness decrease depent on consantraion (Nizamani *et al.* 2020). It has been reported that the plant stem thickness differs according to the variety and dose in the mutation performed by chemical mutagen in the okra plant. In this study, it can be said that the difference between M1 and M2 results is due to recessive mutations. For this reason, it can be said that the effects of mutation may occur with recessive homozygous genetic structure in M2 generation.

**Branch number**

Study aims mutation effects on the branch number and according to M1 result between different statistically 5% in degree is insignificant. According to Table 1 control plants branch number are more than mutant plants. However, according to M2 results, the difference between the average number of mutant and control plant branches was found to be statistically significant by 5%. According to Table 2, the average number of branches of mutant plants is higher than that of control plants. Bolbhatve *et al.* (2012) *Macrotyloma uniflorum*a applicated varios gamma (100-400 Gy) and EMS (0.2 - 0.5%) according to result control plants branch number more than mutant plants. Chrysanthenum applicated varius chemical mutajen and according to result mutation application decreased branch number (Ghormade *et al.* 2020). According to our study result mutant plants branch number smaller than control plants branch number. This results can effect from measured generation. plants mutations effects can be seen at M2 and M3 generation (Roychowdhury *et al.* 2012).

**Biological yield**

According to results, difference between mutant plants and control plants biyological yield isinsignificant. Mutant plants biyological yield are less than control plants biyological yield in seen Table 1. (Akhtar, 2014) Tomatoes (*Lycopersicon esculentum* L.) applicated varios EMS (Ethyl Methane Sulphonate) doses and Gamma ray. According to the results of the study, the EMS chemical mutagen is not effective at low doses. Difference between control plant and mutant plants biyological yield is insignificant but mutant plants biyological yield is a little low. According to others studies plants morphologically can decrease because of mutation application.

**Seed yield**

Mutation application on the seed yield effects are insignificant. It can be seen in Table 1. Mutant plants of Durum wheat Gediz-75 variety compared at M6 and M7 phases in terms of yield and quality between control and mutant plants differents insignificant (Sakin *et al.* 2005).

Expressed different doses of sodium azide doses and ethyl methane sulphonate were applied to chickpeas plant (Barshile, 2016). Accordingly, mutation application decreased seed yield. Roychowdhury *et al.* (2012) expressed EMS mutagen was applied to mungbean plant. Accordingly, the lowest seed yield was obtained from control plants. It seen in our study and others mutaiton applications effects on the seed yield various. In the our study difference between control and mutant plants insignificant. It is because of mutation application ineffective on the related seed yield or reses if mutations.

**Harvest index**

Difference harvest index of between mutant and control plants are statistically insignificant. It can be seen in Table 1 and said between mutant and control plant harvest index difference is insignificant dependent on biological and seed yields. Cotton plants applicated gamma ray, EMS and SA and mutant plants harvest index more than control plants (Muthusamy *et al.* 2011). It is known resesif of 95% mutation on the genoma. Mutation effects a bit unobservable M1 generation because of it has resesif genes. It can be observed M2 and M3 generations (Roychowdhury *et al.* 2012).

**1000 seed weight**

Difference between mutant and control plants 1000 seed weight is statically insignificant. There are similar results;

**Table 2:** Control and applicated quinoa plants height, root length, seeding weight, root weight, stem thickness, branch number results.

| | Plant height (cm) | Root length (cm) | Seeding weight (g) | Root weight (g) | Stem thickness (mm) | Branch number |
|---|---|---|---|---|---|---|
| Control | 15.4 | 7.5 | 5.59 | 0.23 | 3.7 | 14.5 |
| Application | 16.5 | 5.7 | 4.81 | 0.17 | 3.48 | 15.5 |
| t-test | * | ** | * | * | * | * |

* 5% different significant. **1% different significant.

(Sheikh, 2012) wheat applicated various sodium azide doses and between mutant and control plants 1000 seed weight difference statistically insignificant. Rice applicated various chemical mutagen and doses there arent diffrence between control and mutant plants 1000 seed weight (Dewi, 2016). But pepper applicated ethyl methane sulphonate (EMS) and diethyl sulphate (DES) and there are difference between control plants and mutant plants 1000 seed weight. Lentil applicated chemical mutagen methyl methanesulphonate (MMS) and expressed mutation applcations at low doses (0.1, 0.2 and 0.3 mM) increased 1000 seed weight (Khursheed and Khan 2014). There arent difference between control and mutant plants because of reses if heredty or mutation application ineffect on the related genome 1000 seed weight.

### Root length

The difference between the root length averages of mutant and control plants was found to be statistically significant at 1%. According to Table 2, root length averages of mutant plants are lower than control plants. Adamu *et al.* (2007) In tomatoes treated with sodium azide, the highest root length was obtained from the control plant and the root length decreased depending on the mutagen doses (El-Nashar, 2012). Sodium azide (SA) and diethyl sulphate (DES) were applied to *Calendula officinalis* L. (Cv. Calypso Yellow) plant and according to the measurements obtained from the M2 generation, the average root length decreased depending on the chemical mutagen doses. The highest root length was measured in control plants, while the lowest root length was measured from high mutagen dose applications. According to the results of the study conducted for lethal dose determination in Malaysian rice (cv. MR219) treated with Ethyl Methane Sulfonate (EMS), root length decreased due to EMS doses (Talebi *et al.* 2012). In the study on the biological susceptibility and variability of lentil (Lens culinaris Medik) to chemomutagens. In M1 and M2 generations, plant root length decreased depending on the mutagen dose. The highest root length was measured in control plants (Wani, 2021).

### Root weight

The difference between the root weight averages of mutant and control plants was found to be statistically significant at 5%. According to Table 2, root weight averages of mutant plants are lower than control plants. There are some studies; the aim of the study is carried out to determine the mutagenic effects of sodium azide on growth and yield characteristics of wheat (*Triticum aestivum* L. em. Thell.), it was reported that increasing mutangen doses had a decreasing effect on plant root weight Srivastava *et al.* (2011).

### Seedling weight

The difference in seedling weight between mutant and control plants was statistically significant at 5%. As can be seen in Table 2, the average seedling weights of mutant plants are lower than the control plants. In a study conducted to determine the effects of canola (*Brassica napus* L.) on seed viability, germination and seedling growth from chemical mutagens, it was reported that mutation application reduced seedling weight Emrani *et al.* (2011).

## CONCLUSION

This study aims to produce M1 and M2 generations seeds as well as measured some morphological feature results compared with T test which obtained from control and mutant plants. According to results M1 generation difference between obtained control and mutant plants is statistically insignificant. However, the difference between the measurements obtained from control and mutant plants in the M2 generation is statistically significant. It is thought that this situation may be caused by recessive mutations. Effects of recessive mutations on heterozygous gene structure cannot be observed. However, recessive mutation effects can be observed in homozygous gene structure. Generally, as a result of mutation applications, high rate of recessive mutations occur.

**Conflict of interest:** None.

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