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Yamuna Devi G
PG Student, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Thavaprakaash N
Associate Professor (Agronomy), Centre for Students Welfare, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Vanitha K
Assistant Professor (Crop Physiology), Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sangeetha SP
Assistant Professor (Agronomy), Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Thiyagarajan G
Assistant Professor (Soil and Water Conservation), Water Technology Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Mobeena S
Ph.D. Scholar, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Yamuna Devi G
PG Student, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Germination of quinoa seeds under different abiotic stress conditions

Yamuna Devi G, Thavaprakaash N, Vanitha K, Sangeetha SP, Thiyagarajan G and Mobeena S

Abstract
Moisture stress (drought and high moisture) as well as salt stress interfere on seed germination and further, the full development of several crops. The objective of this laboratory study was to evaluate the effect of water stress (high and low moisture) and salinity on the germination of quinoa seeds. The study was made at Department of Agronomy laboratory, Tamil Nadu Agricultural University, Coimbatore during January to March, 2022. The drought stress was examined using Polyethylene glycol (PEG) at six levels of water potential (0, -1 bars, -2 bars, -3 bars, -4 bars and -5 bars). The high moisture stress was made by adding seeds in test tube with 5 ml and 10 ml of distill water along with control. Salt stress was examined by using different salt concentration *viz.*, 20mM, 40mM, 60mM, 80mM, 100mM, 120mM, 140mM, 160mM, 180mM and 200Mm along with control (distill water). Germination characters such as germination percentage, germination index, mean daily germination, daily germination speed and germination rate were accounted after five days. This study results revealed that the germination was not affected upto 80mM, after that increase in salt concentration decreased the germination of quinoa seed. In PEG solution, germination was similar in -1 bar and -2 bar as same like control whereas increase in concentration of PEG solution beyond that declined the germination. Under both high moisture condition, the germination of seeds was affected. Hence, from the laboratory study it is concluded that quinoa was highly tolerant to salt stress (upto 80 mM NaCl), it can withstand drought condition (upto -2 bars) but it is sensitive to high moisture stress.

Keywords: Quinoa seeds, abiotic stress, salinity stress, drought stress, high moisture stress, germination percentage

1. Introduction

Quinoa (*Chenopodium quinoa* Wild.) is a traditional Andean seed crop that belongs to the Amaranthaceae family. It is an annual broad-leaved plant, 1-2m tall growing with deep penetrating roots and can be cultivated from sea level to an altitude of 3800 m above mean sea level. The grains enhanced nutritional properties, arising as a complement in human and animal diets, besides being used as a forage and cover crop (Strenske *et al*., 2017) [27]. The grain of quinoa is a pseudo-cereal with attractive nutritional properties increasingly getting attention because of its nutritional attributes has greatly increased its consumption in recent years.

Abiotic stress is the primary cause of crop losses decreasing yields by more than 50 per cent (Mittler, 2006) [19]. Water shortage is a serious problem affecting plant growth and yield. Developing and identifying drought-tolerant lines is a need due to prevalent weather conditions, understanding the functioning capacity of drought tolerant plants under water deficit conditions is inevitable (Bhardwaj and Yadav, 2012) [6]. Quinoa plant shows a remarkable tolerance to a wide range of abiotic stresses such as frost, salinity and drought, as well as the ability to grow on marginal soils (Maughan *et al*., 2009) [17]. The crop exhibits high yield potential under adverse climatic and soil conditions such as drought (Cocozza *et al*., 2012; Sun *et al*., 2014) [7, 28].

Salinity is a major environmental issue affecting crop production around the world. Up to 7.0 per cent of the total land surface is saline and about one-third of the world's irrigated land suffers from secondary induced salinization (Flowers and Yeo, 1995) [10]. Some varieties can grow in salt concentrations similar to those found in seawater (40 dS m^{-1}) and even higher (Jacobsen *et al*., 2001) [14], well above the threshold for any known crop species. The physiological mechanisms behind this remarkable abiotic stress tolerance remain unclear. Despite an apparent interest from agronomists moving toward the quinoa crop (Jensen *et al*.,

2000; Jacobsen *et al*., 2003) [16, 13], the underlying physiological mechanisms conferring this tolerance are still not understood. This is specifically true for salinity tolerance.
High moisture stress, which is also known as waterlogged, ponded, saturated or submerged soil, is one of the major stresses that significantly affects crop growth and ultimately leads to decreased yield and production of various crop plants (Normile, 2008) [22]. When there is unpredictable, erratic or irregular rainfall, it is typically caused by a climatic disparity (Bailey and Voesenek, 2008, 2010) [4]. This stress generally occurs in rainfed ecosystems when there is inadequate drainage. Globally, the issue of flooding seems to have a negative impact affecting nearly 13 per cent of total land area and nearly 10 per cent of agricultural land area (Cramer *et al*., 2011) [8]. With this, the overall yield loss in different crops caused by waterlogging ranges from 15 to 80 per cent which depends on the type of plant, type of soil and duration of stress. In this context, the present study is carried out to check the germination of quinoa seeds under different abiotic stress conditions (drought, flooding and salt stress).

2. Materials and Methods
The laboratory experiment was conducted in Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, India which is located in the western zone of Tamil Nadu. It is located at 11ºN latitude, 77 º E longitude and 426.7 m above mean sea level of altitude. The experiment follows completely randomized block design with five replication. The data were statistically analysed (Gomez and Gomez, 2010).

2.1 Salt stress
Quinoa seeds were washed with water then surface sterilized with 1% sodium hypochlorite for 2 min. for each rinse. Thirty seeds were placed in each 90 mm dia petri plate on a single sheet of filter paper and then petri plates were covered with lids to prevent the loss of moisture by evaporation. To examine the effect of salt stress on germination, eleven salt solutions *viz*., 0 (control/pure water), 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mM NaCl were used.

2.2 Drought stress
To examine the effect of drought stress on germination, polyethylene glycol (PEG) was used. PEG 6000 is usually used to induce water deficit due to its capacity of lowering water potential in medium. It helps to test the seeds within laboratory instead of adjusting soil water potential. Six PEG 6000 solutions with different water potential of 0, -1.0, -2.0, -3.0, -4.0 and -5.0 bars were prepared by dissolving 81, 123, 155, 182 and 206 g of PEG, respectively dissolved in 1000 ml of distilled water. Water potential (Ψ) was calculated by an equation that relates PEG concentration to water potential according to Michel (1983) [18].

$$\Psi = 1.29\,[PEG]\,[PEG]^2T - 140\,[PEG]^{2-4}\,[PEG].$$

Where, Ψ is the water potential of each treatment (bars), [PEG] is the concentration of PEG solution [g PEG (g $H_2O)^{-1}$] and T is the temperature (ºC).
Five ml of each concentration of PEG solution (distilled water) for control was added after every 2 days to each petriplate to maintain the required water potentials of each treatment concentrations.

2.3 High moisture stress
To study the effect of waterlogging on germination of quinoa, three treatments were used which included 10 ml, 5 ml of distilled water in the test tube and control (daily watering of 1ml testtube^{-1}). The seeds were immersed in the water in test tube for the germination.

2.4 Measurements
From the day of sowing, daily germinated seeds were counted upto 5 days and after that, the plants started wilting and no new seeds were germinated.
The following parameters were measured

$$\text{Germination percentage (GP)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed observed}} \times 100$$

$$\text{Mean daily germination (MDG)} = \frac{\text{Final emergence}}{30} \quad \text{(Jajarmi, 2008) [15]}$$

$$\text{Daily germination speed (DGS)} = \frac{1}{\text{MDG}} \quad \text{(Maguire, 1962)}$$

$$\text{Daily germination speed (DGS)} = \frac{1}{\text{MDG}} \quad \text{(Maguire, 1962)}$$

$$\text{Germination rate (GR)} = \frac{(n1\times t1) + (n2\times t2) + (n3\times t3) + \ldots + (ni\times ti)}{T} \quad \text{(Olmez et al., 2006) [23]}$$

$$\text{Germination index (GI)} = \frac{\text{Number of germinated seeds}}{\text{Days}}$$

Where, n-Number of days in which seed germinated, t-Number of germinated seeds in each counting day and T-Total number of germinated seed.

3. Results and Discussion
The germination process is a critical one in the plant life cycle, depending on the genetics of each species and the environmental conditions to which the seeds are exposed. In arid and semi-arid regions, with recurrent adversities such as salinity and water deficiency, the water absorption by the seed during the germination process is hampered by the negativity of the soil matrix potential (Santos *et al*., 2016). In this view, evaluation of the germination under conditions of water deficiency, secondary water deficiency and high moisture conditions are substantial since they may be related to crop sensitivity or tolerance at subsequent development stages.

3.1 Salt stress
The germination percentage of quinoa seeds as a function of time under salt concentration is shown in Fig. 1. The germination percentage was decreased as salt stress levels (NaCl concentrations) increased. Higher germination percentage (91.0%) was obtained after 5 days in control which was comparable at 20 to 80 mM of NaCl concentration (88.0-90.7%). There was no drop in germination power (50%) in any of the levels, upto 140 mM NaCl. The germination threshold of NaCl concentrations was found to be 140 mM. About 30 to 36 per cent of germination was observed even at higher salt concentrations (160 to 200 mM of NaCl). Table 1 shown that the germination index, mean daily germination, daily germination speed and germination rate of quinoa under salt stress. Except DGS, all these parameters were declined significantly with increasing the NaCl concentration.

Higher concentrations of Na salts are more harmful and deleterious effects on quinoa seeds. The decrease in germination under salt stress may be due to the some constraints including salt content which could have affected the water absorption, disturbed the mobilization of reserves and there by disrupt the structure or affect the protein in germinating embryos. Dodd and Donovan (1995) also expressed similar opinion on the germination process due to salts. Quinoa is considered as a crop with higher resistance to NaCl than many cereals and vegetable crops. Being a pseudo-cereal, grown in adverse condition, naturally have resistance to many abiotic stresses including salts. Peterson *et al*. (2015) also documented the resistivity of quinoa with higher level of NaCl over many crops. Quinoa can withstand medium to high salinity levels (100-750 mM) (Orsini *et al*., 2011) [24], which is higher salt concentration than sea water (Adolf *et al*., 2013).

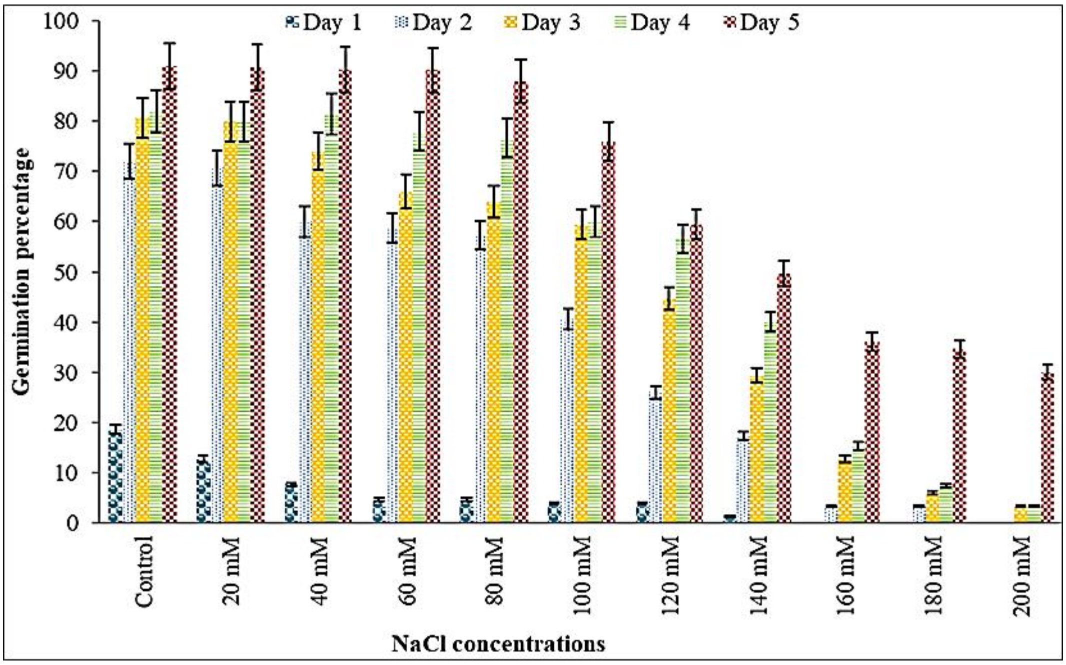


Fig 1: Quinoa germination under salt stress (bars represent the standard deviation)

Table 1: Quinoa germination under salt stress

Concentration	GI	MDG	DGS	GR
Control	36.08	3.03	0.330	7.22
20 mM	33.84	3.02	0.331	6.77
40 mM	30.22	3.01	0.332	6.04
60 mM	28.06	3.01	0.333	5.61
80 mM	27.43	2.93	0.341	5.49
100 mM	22.29	2.53	0.395	4.46
120 mM	17.38	1.98	0.506	3.48
140 mM	11.91	1.66	0.604	2.38
160 mM	5.08	1.20	0.833	1.02
180 mM	3.73	1.16	0.865	0.75
200 mM	2.38	1.00	1.000	0.48
S.Ed.	0.30	0.03	0.006	0.05
CD (0.05)	0.61	0.061	0.012	0.11

3.2 Drought stress

For screening of drought tolerance in crop, the germination test with high osmotic potential solution is key laboratory technique (Ahmadizadeh, 2013) [2]. PEG helps to induce water deficit stress to a seedling stage of crop (Muscolo *et al*., 2014) [21]. The germination percentage of quinoa seeds as a function of time under six water potentials is shown in Fig. 2. The germination percentage was decreased as water stress levels i.e., PEG concentrations increased. On 5 DAS, compared to the control, almost 78 to 80 per cent of germination is noticed at -1 and -2 bars and it was declined to 25-27 per cent germination at -3 and -4 bars. There was no or very poor germination (less than 10%) is expressed the concentration of PEG at -5 bars. The germination index, mean daily germination, daily germination speed and germination rate of quinoa under PEG was given in Table 2. It shown declining of values with increasing the concentration. It is obvious in any crop, drought stress influence on the germination of seeds. However, quinoa crop shown its resistivity on germination due to drought stress upto -2 bar level and beyond that it also affected. It was clear from the present study that water stress at the germination stage could result in the delay, decrease or even complete prevention of germination. Moral *et al.* (2015) [20] also found similar kind of results which supports the present study.

Table 2: Quinoa germination under PEG

Concentration	GI	MDG	DGS	GR
Control	39.43	2.87	0.349	7.89
-1 bar	30.55	2.67	0.375	6.11
-2 bar	26.54	2.6	0.385	5.31
-3 bar	12.09	0.92	1.084	2.42
-4 bar	10.22	0.83	1.200	2.04
-5 bar	3.58	0.31	3.214	0.72
SEd	0.13	0.04	0.019	0.03
CD (0.05)	0.27	0.08	0.039	0.06

3.3 High moisture stress

The germination percentage of quinoa seeds as a function of time under high moisture stress is shown in Fig. 3. Control recorded higher germination percentage compared to 5 cm and 10 cm water depth of flooding stress. Due to the high moisture stress (5 cm and 10 cm water depth), during the second and third day itself all the seeds are germinated but afterwards all the germinated and ungerminated seeds got decayed. This may be due to low availability of oxygen in the root zone leads to rooting of roots and shoots. Table 3 gives the germination index, mean daily germination, daily germination speed and germination rate which shows these parameters were declined with increasing the level of water. Parra *et al*. (2019) [25] reported that the modification of soil water by rain or irrigation, affect the quinoa growth, development and production. Due to rain flooding, decreased production of tropical quinoa was noticed (Jacobsen *et al*., 2007) [12].

Table 3: Quinoa germination under high moisture stress

Concentration	GI	MDG	DGS	GR
Control	34.96	2.94	0.34	6.99
5 cm water depth	27.00	0.11	9.00	5.4
10 cm water depth	23.34	0.11	9.00	4.67
S.Ed.	0.30	0.03	0.10	0.05
CD (0.05)	0.65	0.06	0.21	0.11

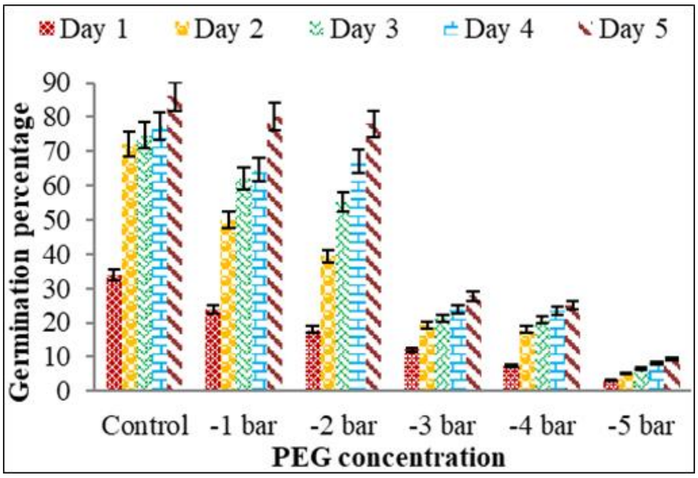


Fig 2: Germination percentage of quinoa under drought (bars represent standard deviation)

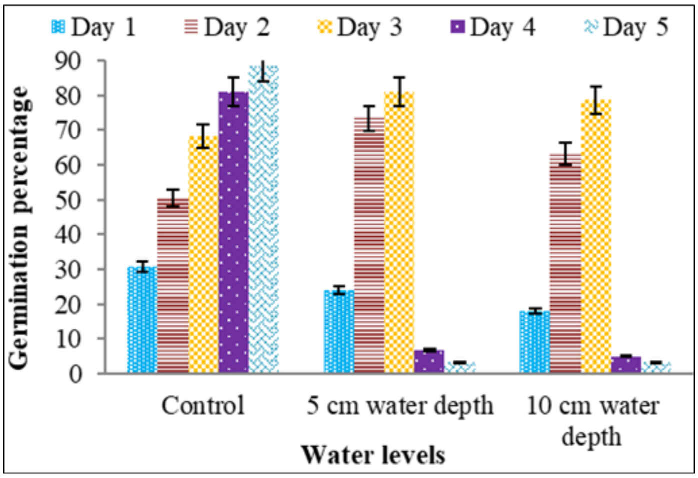
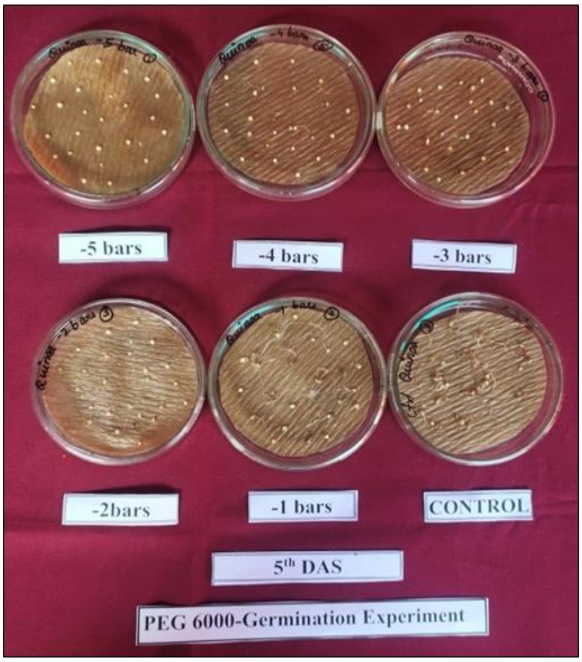


Fig 3: Germination percentage of quinoa under water logging (bars represent standard deviation)

Quinoa germination under salt stress

Quinoa germination under PEG-6000

Salt stress



Drought stress

High moisture stress

Conclusion

From this investigation it concluded that quinoa can germinate in high salinity condition (upto 80Mm NaCl) and can withstand medium drought condition (-2 bar). But, quinoa is sensitive to high moisture stress.

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