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The Management Committee of Cost Action 814 wishes to dedicate this book to Mr. Peter Stamp, Mr. Armand Guckert and Mr. Klaus Pithan in appreciation of all their hard work and energy, not only in organising this Conference, but also throughout the duration of Cost Action 814. The success of the Action is evidence of their commitment to it.

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Agronomic and physiological response of quinoa (Chenopodium quinoa Willd.) to frost at three phenological stages

S. E. JACOBSEN[1], C. MONTEROS[2], J.L. CHRISTIANSEN[3] AND A. MUJICA[4]

[1] *International Potato Centre (CIP), Apartado 1558, Lima 12, Peru*
[2] *Instituto Nacional de Investigaciones Agropecuarias (INIAP), Apartado Postal 17-17-1362, Quito, Ecuador*
[3] *Dep. Of Agricultural Sciences, Royal Veterinary & Agricultural University, Thorvaldsensvej 40, DK-1871 FrederiksbergC, Denmark*
[4] *Proyecto Quinua CIP-DANIDA-UNAP, Universidad Nacional del Altiplano, Av. De. Ejercito 329, Puno, Peru*

SUMMARY

The capacity of frost resistance in quinoa is well known, but very little investigation has been made. Considering that quinoa is a valuable genetic resource, experiments were carried out to determine the response of quinoa to different intensities and durations of frost under different relative humidities, and to determine the variation of yield and content of sugar, proline, and protein related to frost, with the purpose of developing criteria for selection of frost-resistant varieties.

On the basis of results from greenhouse and phytotron experiments it was concluded that in the two true leaves-stage the cultivars from the altiplano of Peru tolerated temperatures of -8°C for 4 hours, while the Interandean valley cultivar tolerated -8°C only for 2 hours. At temperatures of -4°C and 60% relative humidity the percentage of dead plants was 56, while at the same temperature, but at 90% of relative humidity, there was only 25% dead plants. At -4°C, in two true leaves-stage, yields were diminished by 9.2% compared to the control without frost, while at the 12 true leaves and flowering-stage the yield reductions were 50.7 and 65.7%, respectively. An increased level of soluble sugars implied a greater tolerance to frost, resulting in improved yields.

Keywords : frost, physiology, quinoa

INTRODUCTION

The presence of frost is one of the principal limiting factors for agricultural production in the high Andean region, where one of the most important grain crops is quinoa

(*Chenopodium quinoa* Willd.), which generally is less affected by frost than most other crop species. Frost normally occurs in the Andean region between 12 p.m. and 6 a.m., with a duration from 1 to 6 hours (Grace, 1985; Capelo, 1993). The two most common types of frost are the radioactive and connective frosts, which are known as white and black frost, respectively. White frost, which causes relatively little damage in nature (Ruiz, 1995), occurs under a high relative humidity. With this type of frost water vapour is condensed and frozen on the leaf surface, a physical change which causes the release of heat and a slow cooling of the environment.

Black frost occurs when the air is dry and the dew temperature is not reached. Water vapour does not freeze; instead the water in the leaf tissue freezes. Air temperature falls rapidly because there is no atmospheric vapour to attenuate this phenomenon. When the sun begins to shine in the morning, the ice evaporates rapidly, causing necrotic spots in the foliage (Ruiz, 1995).

The present experiment had the objectives to: 1. Determine the damage in quinoa, caused by different intensities and duration of frost under different relative humidities, and 2. Determine the effects on seed yield and the content of sugar, proline, and protein, in two cultivars of quinoa, with different frost intensities at different phenological phases.

MATERIALS AND METHODS

The first study was carried out in greenhouse and phytotron conditions at the International Potato Centre (CIP), Lima, Peru. A Peruvian valley cultivar (Quillohuaman), and four cultivars from the altiplano of Peru (Wariponcho, LP-4B, Witulla, Ayara), were studied at different relative humidities (60 and 90%), temperatures (-2, -4, -8 °C) and frost duration (2, 4, 6 h). A random design was used, with three replications , and the data were analyzed as a factorial arrangement 5x3x3 and 2x2x2x3.

In the second study the same valley cultivar and one of the cultivars of the altiplano (Witulla) were used, in three phenological phases (two true leaves, 12 true leaves and anthesis), and three temperatures (-2, -4 and 19°C (control)). A random design with 24 treatments and 3 replicates was used, and the data were analyzed as a factorial arrangement 2x3x3.

The experimental units were made upof 8 plants (two pots with 4 plants in each), each pot containing 5 kg of soil. The soil was prepared with 2 parts of mineral soil, 1 part of compost and 1 part of sand. The soil humidity was kept at 75% of field capacity. Plant death and yield in kg ha^{-1} was calculated according to plant density, estimating 1,280 plants ha^{-1}.

RESULTS AND DISCUSSION

There were highly significant differences for the studied variables: cultivar, phenological phase, relative humidity, duration and intensity of frost.

The risk of cellular tissue death by ice formation and dehydration of the tissue increases with longer frost periods and lower temperatures. In the phenological phase of two true leaves, the five cultivars were not damaged when they were exposed to temperatures of -2 and -4 °C of 2, 4 and 6 hours duration (no dead plants), while at temperatures of -8 °C the proportion of dead plants was 5.0, 11.7 and 21.7%, for 2, 4 and 6 hours of frost exposure, respectively (Table 1). This is in accordance with Limache (1992), who indicated that in the phenological phase of six true leaves, 16 ecotypes had a damage rate from 2.7 to 26.5%.

TABLE 1. NUMBER AND PERCENTAGE OF DEAD PLANTS UNDER DIFFERENT COMBINATIONS OF TEMPERATURE AND DURATION IN TWO TRUE LEAF STAGE
THE LETTERS A-D INDICATE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P< 0.01)

Temp. °C	Duration (hours)	Dead plants (number)	Dead plants (%)	
-2	2	0.000	0.000	D
	4	0.000	0.000	D
	6	0.000	0.000	D
-4	2	0.000	0.000	D
	4	0.000	0.000	D
	6	0.000	0.000	D
-8	2	0.408	5.000	C
	4	0.911	11.66	B
	6	1.911	21.66	A

The susceptibility to frost depended on cultivar, indicating that the genetic constitution plays a role for the level of frost resistance. In the phenological phase of two true leaves, the cultivars from the altiplano tolerated temperatures of -8 °C for 4 hours, with a rate of dead plants from 4 to 13%, while the valley cultivar at the same temperature, -8 °C, reached the same level after only 2 hours (Fig. 1).

The rate of damage and plant death, which must be caused by extracellular ice formation and dehydration of the tissue, is seen in Table 2. At a temperature of -4 °C and 60% relative humidity, the rate of dead plants was 56%, while at the same temperature, but with high relative humidity the rate of dead plants was only 25%. With 60% relative humidity and 6 hours duration, the rate of dead plants was 46%, while with 90% relative humidity and the same duration, the rate was 27% (Table 2).

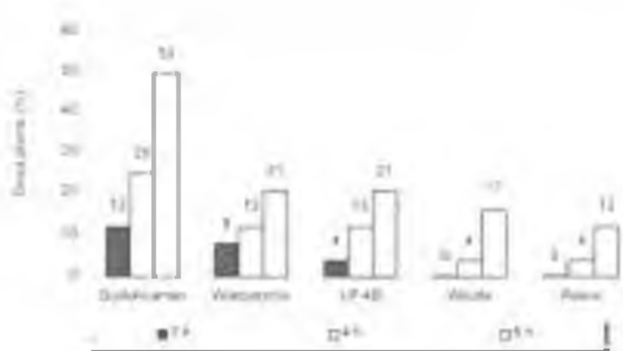

FIGURE 1. PERCENTAGE OF DEAD PLANTS IN FIVE CULTIVARS EXPOSED TO -8 °C OF DIFFERENT DURATION

TABLE 2. DEAD PLANTS WITH DIFFERENT COMBINATIONS OF RELATIVE HUMIDITY, TEMPERATURE AND FROST DURATION
THE LETTERS A-E INDICATE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS (P<0.01)

Rel. hum. %	Temp. °C	Dead plants (number)	Dead plants (%)	
[illegible]	-2	[illegible]	11.286	C
[illegible]	-4	[illegible]	46.250	A
[illegible]	-2	1.444	[illegible]	D
[illegible]	-4	[illegible]	21.000	B

Rel. hum. %	Duration hours	Dead plants (number)	Dead plants (%)	
[illegible]	2	1.417	[illegible]	D
[illegible]	4	[illegible]	10.342	B
[illegible]	6	[illegible]	15.811	A
[illegible]	2	1.167	[illegible]	E
[illegible]	4	[illegible]	[illegible]	D
[illegible]	6	1.167	[illegible]	C

The phenological stage seems to be important, as plants at two true leaves-stage were only little affected by temperatures of -4 °C, with a significant seed yield decrease of 9.2% compared to the control grown at 19 °C, while yield of plants exposed to frost of -4°C at the 12 true leaves-stage and at anthesis decreased 50.7 and 65.7%, respectively (Fig. 2). This result is in accordance with results of Canahua and Rea (1979), who found that quinoa in the cotyledoneous leaf-stage, and in two and five true leaves stage, behaved as tolerant with no damage by low temperatures, while frost during bud formation and anthesis seriously affected the quinoa. Limache (1992) concluded that quinoa resists frost without major damage before the bud formation phase, but is very susceptible to frost during anthesis. Monteros and Jacobsen (1999) showed that quinoa was most tolerant to frost in the 12 leaf stage, and most frost sensitive in anthesis.

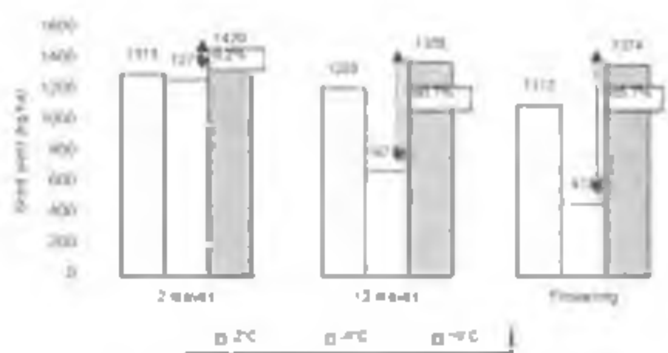

FIGURE 2. SEED YIELD (KG/HA) AT -2, -4 AND 19°C IN THREE PHENOLOGICAL PHASES
THE BAR AND THE ARROWS REPRESENT THE DIFFERENCE BETWEEN YIELD AT -4°C COMPARED TO THE CONTROL

A temperature of -4°C seriously affected the valley cultivar, which yielded 0.6 t ha^{-1}, representing a yield decrease of 56.2% compared with the control, not exposed to frost. Cultivar Witulla of the altiplano was less affected, yielding 1.0 t ha^{-1}, corresponding to a 26.7% decrease compared to the control (Fig. 3).

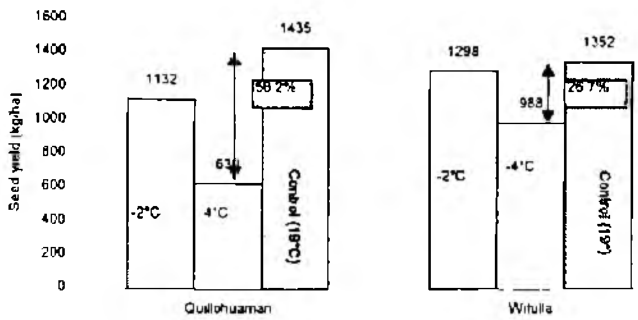

FIGURE 3. SEED YIELD (KG HA^{-1}) OF TWO CULTIVARS OF QUINOA EXPOSED TO -2, -4 AND 19°C. THE BAR AND THE ARROWS REPRESENT THE DIFFERENCE BETWEEN YIELD AT -4°C COMPARED TO THE CONTROL.

The variation in frost tolerance between the two cultivars of quinoa may be due to the content of soluble sugars. The cultivar of the altiplano (Witulla) presented higher content of soluble sugars than the valley cultivar (Quillohuman). At the 12 true leaf-stage Witulla had a sugar content of 3,215 mg g^{-1} dry weight, while Quillohuaman had 2,853 mg g^{-1} dry weight (Table 3). Lancer (1995) and Stone (1993), cited by Hardy (1996), mention that the frost tolerance may be related to high content of solutes, which are responsible for protection and support of the cellular structures under frost stress. Exposed to frost, the two cultivars accumulate soluble sugars, and it is seen that at –4 °C Witulla and Quillohuman had higher content of sugar (3,310 and 3,096 mg/g dry weight), respectively), than at 19°C (2,648 and 2,381 mg g^{-1} dry weight), respectively.

TABLE 3. CONTENT OF SOLUBLE SUGARS, WITH DIFFERENT COMBINATIONS OF PHASE AND TEMPERATURE, IN TWO CULTIVARS OF QUINOA.

Phenological phase	Cultivar	Soluble sugar (mg/g dry weight)	Temp. °C	Cultivar	Soluble sugar (mg/g dry weight)
12 true leaves	Quilloh.	2.853 B	-4	Quillohuaman	3.096 B
	Witulla	3.215 A		Witulla	3.310 A
Flowering	Quilloh.	2.624 C	19	Quillohuaman	2.381 D
	Witulla	2.743 BC		Witulla	2.648 C

CONCLUSION

The content of soluble sugars was positively correlated to yield (r=0.688), for which reason it is concluded that the level of soluble sugar may be utilized as indicator of frost tolerance.

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