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# D 1.8. Effects of saline water irrigation on protein quantity and quality analyzed

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## CNR-ISAFOM

| **Project co-funded by the European Commission within the Horizon 2020 Programme** | | |
|---|---|---|
| **Dissemination Level** | | |
| **PU** | Public | X |
| **PP** | Restricted to other programme participants (including the Commission Services) | |
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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

## 0. Summary

Generally, salinity affects negatively on crop yields, so the need to minimize the effects of salt stress on crop yield is urgent. This is further emphasized due to the increasing risk of climatic change. In Italy, the salt stress is principally due to seawater intrusion that causes a limitation for irrigation in avoiding salt accumulation in the soil as well as stress to irrigated crops during growth period under high evapotranspiration. Since the legumes grow in the period autumn-spring, the high autumn-winter precipitations, together with little spring rains, meet evapotranspiration requirements of these crops. On the contrary, the spring-summer crops have high evapotranspiration requirements due to high temperatures and low precipitations. A possible approach is the introduction of irrigated species capable of tolerating high soil salinities and species guaranteeing acceptable yields. Thus, quinoa and amaranth were considered suitable crops for the study.

Quinoa (*Chenopodium quinoa* Willd.) and grain amaranth (*Amaranthus Caudatus L.*) produce high quality protein seeds and their high tolerance to salinity, in respect to traditional crops, is well known. A multiannual field trial was planned to evaluate the effect of saline water and irrigation regime on yield, yield components and protein quality of these two crops, which could represent an important source of plant proteins in salt affected environments.

## 1. Introduction and objectives

Salinity is one of the adverse environmental factors affecting the growth of plants in the Mediterranean region (Gregory 2006; Lin et al. 2006). Salinization is increasing globally at a fast rate, affecting the average yields by up to a 50% decline for most major crop plants (Bray et al. 2000). Salinization may result in 30% loss of current agricultural land during the next 25 years, a loss that is expected to increase to 50% by 2050 due to the population growth (Altman, 1999; Ashraf, 1994). It is estimated, that salinization affects approximately 3.8 million hectares in Europe, which can be distinguished to primary salinization due to natural processes and secondary salinization introduced by human interventions, such as irrigation with saline water (Figure1).

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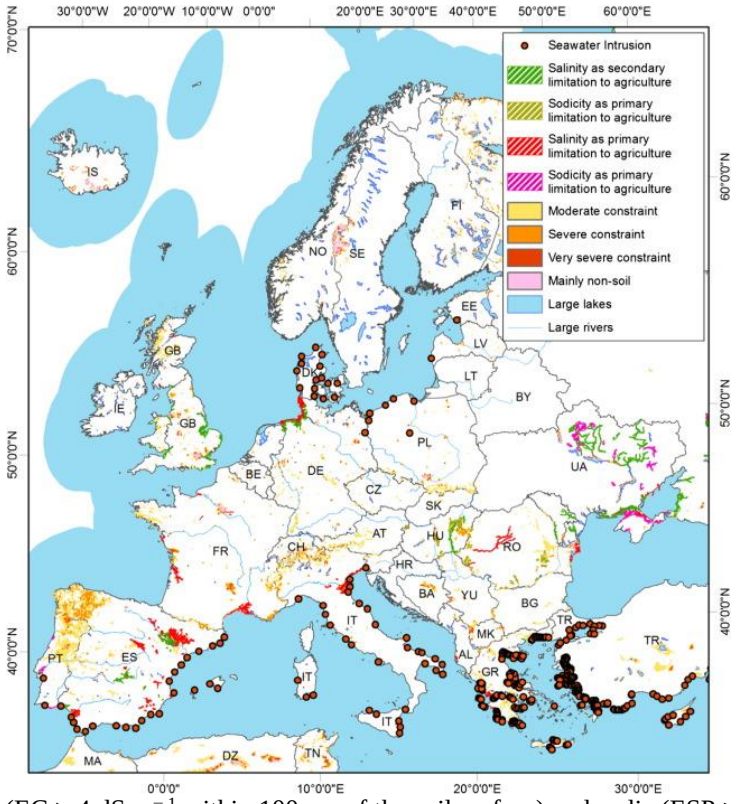


**Figure 1**. Saline (EC > 4 dS $m^{-1}$ within 100 cm of the soil surface) and sodic (ESP > 6% within 100 cm of the soil surface) soils that act as agricultural constraint and as primary and secondary limitations to agricultural use, and areas of seawater intrusion in Europe (Daliakopoulo et al. 2016).

Salinity produces osmotic and ionic effects on plat metabolism. The osmotic effect leads to dehydration of plant tissues, inhibition of water uptake and leaf development. Plants cope with osmotic effect by the mechanisms of osmotic adjustment, i.e., decreasing cellular osmotic potential. The ionic stress is caused by increase in salt ion concentration and can lead to leaf senescence, as well as inhibition of photosynthesis and protein synthesis. Plants can cope with increased ion concentrations either via salt ion exclusion from the cells, or via salt ion compartmentation in the intracellular compartments (accumulation in vacuoles). Both effects negatively affect crop yields, so the need to minimize the effects of salt stress on plant growth and crop yield is urgent. A possible approach is the introduction of species capable of tolerating high soil salinities and guaranteeing acceptable yields.

## 2. Activities for solving the task(s)

*Experimental site and climate*

The field experiments were carried out in Vitulazio (Caserta, Italy) at the experimental research station of CNR-ISAFoM (41°12' N and 14°20' E, 23 m above the sea level), during three growing seasons: 2015, 2016 and 2017. The climate is typically Mediterranean sub-humid, characterized by an average annual rainfall of 880 mm, mostly concentrated in autumn and winter months (October to March). The annual reference evapotranspiration (ET0 estimated by Penman–Monteith equation according to Allen et al. 1998) in the region is an average 1083 mm in the period 1976–2017.

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The main weather parameters for the study, including solar radiation, air temperature, relative humidity and precipitation, were obtained from a standard agro-meteorological station (iMetos ag, mod. IMT 280, Pessl Instruments, AT), which is located about 30 m from the experimental field. The soil is a clay-loam texture (clay, sand and silt; 46.1%, 30.2% and 33.7%, respectively) and defined as Mollic Haplaquept (USDA, 2006). Chemical and physical characteristics of the soil at the beginning of the experiments (April 2015) were the following: pH 8.05, Kjeldahl total N 1.81 g $kg^{-1}$, organic C 9.1 g $kg^{-1}$, electrical conductivity ECe = 0.23 dS $m^{-1}$, and bulk density 1.28 kg $dm^{-3}$. The volumetric soil water contents at field capacity was 0.38 $m^3$ $m^{-3}$, while the permanent wilting point was 0.13 $m^3m^{-3}$.

*Plant material*

The Danish quinoa variety "Vikinga" and one genotype of Amaranth "A14" received from the University of Copenhagen were sown (fig. 3). Vikinga is a variety characterized by a short growth cycle (3/4 months) and with low saponin content seeds (sweet variety). A14 is an accession of *Amaranthus Caudatus L.*, characterized by white seeds and short cycle length (4 months). Seeds of the two genotypes were sown after their germination had been tested (90% after 24 hours) in a Petri dish.

*Cultural practices, irrigation treatments and experimental design*

Quinoa and Amaranth were grown under two irrigation regimes. The first one is called I100, restitution of 100% of the water necessary to replenish to field capacity (F.C.) at 40 cm soil layer. The second one is I33, corresponding to restitution of 33% of full irrigation. For each irrigation level, a non-saline treatment was performed by irrigation with fresh water (100N and 33N), and a saline water treatment irrigated with a known salt concentration (100S and 33S).

Irrigation was carried out at fixed weekly intervals. Saline water is groundwater with dissolved sodium chloride (NaCl) together with calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium chloride ($MgCl_2$) and magnesium sulfate ($MgSO_4$) (Tab. 1).

**Table 1.** Added salts to groundwater

| | |
|---|---|
| NaCl *(mg l-1)* | 13380 |
| $CaCl_2$ *(mg l-1)* | 448 |
| $MgCl_2$ *(mg l-1)* | 1149 |
| $MgSO_4$ *(mg l-1)* | 1644 |
| KCl (mg l-1) | 339 |

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These salts are added to the groundwater in sufficient quantities to obtain the same stoichiometric balance as seawater. The conductivity achieved in the solution 1/1 (seawater/groundwater) is about 22 dS $m^{-1}$.

The water was pumped out of the tanks by two submerged pumps and filtered by two sand filters, used one for each tank, and then distributed through a system of pipes (figure 2). This consisted of two main supply pipes connected to a network of pipes. These pipes were placed along the plots, with drip lines at intervals of 0.5 m. The self-compensating drip emitters were placed every 0.3 m, with a nominal flow rate of 4 L $h^{-1}$ at the operating pressure of 0.1 MPa. The two tanks were used to keep the saline and non-saline treatments separate. Salt was dissolved in the tank used for saline water before every irrigation session.

**Figure 2**. Tanks and sandy filters used for irrigation.

The irrigation started the day after sowing (DAS) on days 49, 12, and 56 for the growing seasons in 2015, 2016 and 2017, respectively. The rainfall and daily evapotranspiration distribution during the growing season affected the behavior and the response of crops to water deficiency. The more favorable rainfall patterns occurred in 2016 (Table 3), determined lower irrigation requirements in comparison to 2015. In 2017 the seasonal irrigation amount was lower than 2016 because the crop required lower numbers of irrigation supplies.

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**Figure 3.** Field experimental trials with quinoa and amaranth in Vitulazio.

A randomized complete block (RCB) design with two treatments (irrigation regime and water quality) per crops and three replicates per treatment was adopted. Each experimental plot consisted of 10 rows, 4 m in length.

*Soil measurements*

Runoff and capillary rise were assumed negligible due to Mollic Haplaquept soil features and the very deep soil water table in the area. Meanwhile the deep percolation below the root zone, caused by excessive precipitation and/or irrigation, was calculated as the surplus of water over the field capacity. The gravimetric method, based on the conventional oven-dry weight and multiplied by the bulk density (Qiu et al., 2001), was used during each growing season to measure the soil water content in each plot. The following depths were measured in each plot: 0-0.20, 0.20-0.40 and 0.40-0.60 m. Volumetric moisture measurements were carried out before and 24 hours after each watering, as well as after rainfall of 5 mm or more. Rainfall events with less than 5 mm within 24 hours was not considered of importance when estimating watering volume. Rainfall events during the irrigation session or before watering were considered effective for a maximum value equal to the capacity of the soil layer explored by the roots to retain water at the time of the event. Rain in the 24 hours following watering was not considered useful for the fully irrigated treatments, since the soil layer explored by the roots is already at field capacity.

The electrical conductivity of the soil was measured before the crop sowing, and at the end of the crop cycle. The soil samples were taken between the rows at the center of the plots and at the same depth of soil used to measure moisture, in order to evaluate the variations of this parameter in relation to the soil water content. The electrical conductivity of the soil (EC) was measured on an aqueous soil extract (ratio of water/soil = 2.5/1). The values were then related to those of the concentration in saturated paste (ECe) by using the equation [y = 3.693 x]. This

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helped to obtain the correlation between the values of conductivity of the aqueous extract and those of the saturated paste ($R^2$ = 0.98).

*Biomass, yield and seed quality*

At physiological maturity, the harvest was made by hand. The plants were cut at the base and laid out on tarp. The harvested plants were threshed using a stationary threshing machine mod. Cicoria Plot 2375.

The total yield, the 1000 seed weight and the above-ground biomass were determined on three plants per elementary plot. The harvest index (HI) was calculated as ratio between yield and total above-ground biomass. Furthermore, the seed samples for both species, for each harvest and for each studied treatment, were then chemically analysed to evaluate the principal qualitative components.

*Qualitative components*

Crude protein content was measured using the Kjieldahl method (International). Briefly, 2 g of sample were subjected to digestion at 450°C (PBI International mod. Mineral SIX) with 30 ml of 96% $H_2SO_4$, in presence of 7 g of $K_2SO_4$ and 0.7 g of $CuSO_4$. Digested were alkalinized with 45% NaOH and then subjected to steam distillation by using a distiller (Buchi mod. B-324). The condensed distillate was gathered in an Erlenmeyer flask containing 25 ml of $H_2SO_4$ 0.25 N. The sulphuric acid not neutralized by the ammonia present in the distillate was titled with 0.25 N NaOH in presence of an indicator methylene blue/methyl red mix. The ammonia rate, estimated on the difference in sulphuric acid, equivalents between those present before and after the ammonia distillate gathering. It was converted into protein using 6.25 as conversion factor.

Raw fat determination was carried out according to the AOAC, 920.85 method (International). Ten grams of sample were weighed in a Soxhlet extraction thimble. Three grams of anhydrous $Na_2SO_4$ were added, and absorbent cotton was used as a seal. Fats were extracted with hexane by using an automatic extractor (PBI International mod. Soxhraction). The hexane was first removed with vacuum-packed distillation and then in a stove at 105 °C for 1 hour. The extracted fat weight was compared to the initial 10 g of sample.

Ash content was determined according to the AOAC, 900.02 method on ashes (International). About 10 g of samples were weighed in a capsule previously calibrated at 550°C for 4 h and chilled in a silica gel dryer. Subsequently, the samples were burned on a little flame and then incubated overnight in muffle furnace (Heraeus mod. K1251F). Afterwards, ashes were chilled in a silica gel dryer and weighed soon after reaching room temperature. The ash rate was determined by the ratio between the remnant mass and the original sample mass.

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Starch was determined according to the American Association of Cereal Chemist (AACC) method (AACC 2001)76-13.

*Statistical analysis*

The data collected during three years of experimental work were analysed according to a RCB design. Each dependent variable (irrigation regime and irrigation quality) was first evaluated for normal distribution according to Kolmogorov–Smirnov test (Neter et al., 1996). Statistical analyses were performed through the GLM procedure of SAS/STAT. Duncan test at 0.05 probability level was used as mean separation test. Both were executed using SAS® University Edition.

Principal component analysis (PCA) using the correlation matrix was performed on seed quality parameters to explore relationships among variables and treatments, as well as to determine which traits were the most effective in discriminating between water regime and water quality. PCA outputs included treatment component scores and variable loadings to each selected component. The first two principal components (PC1 and PC2) were selected for the ordination analysis, and the correlation between the original traits and the respective PC was calculated. The PCs with eigenvalues greater than 1 were selected (Dunteman, 1989) and loadings greater than |0.6| indicate of significant correlations between the original variables and the extracted components (Matus et al., 1996). This analysis was carried out using the software package FactoMineR (Husson et al. 2014) in R studio software (R Core Team 2013).

# 3. Results

*Weather conditions*

The weather regime, in terms of precipitation (P), reference evapotranspiration (ETo), minimum and maximum temperatures (Tmin and Tmax) during each month are given in Table 2 for the three growing seasons as compared to the year historical means (1976-2017). Seasonal precipitation (Nov-Jun) was 437mm, 550mm and 220 mm in the first, second and third growing season, respectively. In comparison, the historical average is 377 mm.

After computing the deciles index (DI) designed by Gibbs and Maher (1967), the first (2015) and second (2016) growing seasons were classified as normal (DI = 9 and 7, respectively) whereas the third (2017) season was weak dry (DI = 4).

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**Table 2.** Weather parameters during the three seasons, compared to the long-run means

| | | March | April | May | June | July | August | September |
|---|---|---|---|---|---|---|---|---|
| **P (mm)** | **2015** | 145 | 77.8 | 51.6 | 38 | 19.6 | 56.2 | 49.2 |
| | **2016** | 160.8 | 55.2 | 95.2 | 47.6 | 27.8 | 4.2 | 158.8 |
| | **2017** | 55.4 | 9.6 | 16 | 9 | 19.4 | 0.2 | 110.8 |
| | **Long-run means** | 84.6 | 72.1 | 53.1 | 39.6 | 24.4 | 28.8 | 74.2 |
| **$ET_o$ (mm)** | **2015** | 66.3 | 85.5 | 124.9 | 150.0 | 166.4 | 137.7 | 112.0 |
| | **2016** | 57.6 | 88.0 | 109.5 | 127.0 | 155.1 | 147.6 | 89.0 |
| | **2017** | 75.0 | 94.1 | 129.6 | 158.8 | 169.2 | 167.5 | 95.0 |
| | **Long-run means** | 68.6 | 89.5 | 126.6 | 148.2 | 167.9 | 167.0 | 108.0 |
| **$T_{max}$ [°C]** | **2015** | 16.1 | 19.4 | 25.1 | 29.7 | 33.0 | 32.4 | 28.7 |
| | **2016** | 16.4 | 22.3 | 23.7 | 28.3 | 31.8 | 31.4 | 27.3 |
| | **2017** | 18.9 | 20.7 | 25.7 | 30.3 | 32.4 | 34.1 | 26.1 |
| | **Long-run means** | 15.5 | 18.5 | 23.2 | 27.3 | 30.1 | 30.4 | 26.7 |
| **$T_{min}$ [°C]** | **2015** | 7.3 | 8.2 | 13.1 | 16.5 | 20.6 | 19.7 | 16.6 |
| | **2016** | 6.0 | 9.2 | 11.7 | 16.4 | 18.9 | 18.8 | 16.6 |
| | **2017** | 6.1 | 8.2 | 12.3 | 17.6 | 18.3 | 19.6 | 15.0 |
| | **Long-run means** | 6.1 | 8.0 | 12.0 | 15.5 | 18.1 | 18.7 | 16.0 |

### *3.1 Quinoa*

Quinoa was sown on the following day of the year (DOY); day 111, 168 and 102 during years 2015, 2016 and 2017, respectively. The crop cycle length ranged from 110 to 117 days during the three experimental years (table 3). The difference in crop cycle length between the years was due to the different thermal sums. The ET0 demand during the crop cycle ranged from 466 mm (2016) to 531 mm (2015).

**Table 3.** Dates of the main phenological stages, crop evapotranspiration and irrigation supply of quinoa crop grown during the three years of experimental work.

| **Quinoa** | **Year** | | |
|---|---|---|---|
| | **2015** | **2016** | **2017** |
| ***Phenological stages*** | | | |
| Sowing (DOY) | 111 | 168 | 102 |
| Harvesting (DOY) | 224 | 279-285 | 212 |
| Crop cycle (days) | 113 | 111-117 | 110 |
| Irrig. Start (DAS) | 49 | 27 | 61 |
| Last Irrig (DAS) | 84 | 68 | 91 |
| Irrigation time | 7 | 7 | 5 |
| ***Evapotranspiration, Rainfall and irrigation supply*** | | | |
| Cumulative ETo* (*mm*) | 531.2 | 466.2 | 488.7 |
| Rainfall (*mm*) | 170.8 | 230.8 | 47.8 |
| Seasonal irrigation supply (*mm*) | | | |
| I100N | 256.13 | 240.50 | 201.46 |
| I33N | 84.52 | 79.36 | 66.48 |
| I100S | 217.53 | 201.81 | 192.08 |
| I33S | 71.78 | 66.60 | 63.38 |

**Penman monteith*

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*Irrigation and Soil water content*

Irrigation was applied 7 times during 2016 and 2015, and 5 times in 2017. In 2015, 2016 and 2017, the amount of 256mm, 240mm and 201 mm of fresh water were respectively applied for the treatment 100N. 217mm, 201mm and 192 mm of saline water were similarly added for the treatment 100S. Obviously, the amounts of salt supplied by irrigation water to the soil were higher in the first year than in 2016 and 2017 because the seasonal irrigation supply in 2015 was higher. Differences in seasonal irrigation volume between the three years were also due to differences in evapotranspiration demand (table 3).

*Soil EC*

The development of electrical conductivity of the soil over time was monitored for the soil layers of 0-0.20m, 0.20-0.40m and 0.40-0.60 m. Table 4 reports the average ECe values (0-0.6 m) at the sowing stage and at end of crop cycle for the two applied saline irrigation treatments (33S and 100S) for each experimental year. The initial ECe value showed an increasing trend over the three years for both 100S and 33S treatment (Table 4). The final ECe value was significantly higher compared to the ECe initial values for each of the considered treatments. This was due to the winter rainfall that prevented salt accumulation in the first two layers of soil. In general, the surface layer is more subject to percolation caused by the autumn and spring rains, whereas the underlying layer presented a behavior that is more constant over time, with a tendency towards a gradual increase in ECe values.

**Table 4**. ECe values at beginning and end of crop cycle for quinoa and amaranth during three experimental years

| Date | Treatment | Amaranth Ece (dS $m^{-1}$) | | Quinoa Ece (dS $m^{-1}$) | |
|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final |
| 2015 | 100S | **1.26** | 5.66 | **1.81** | 5.80 |
| | 33S | 1.37 | 3.33 | 1.69 | 5.38 |
| 2016 | 100S | 1.87 | 8.88 | 1.76 | 9.10 |
| | 33S | 1.84 | 7.95 | 1.48 | 7,52 |
| 2017 | 100S | **2.66** | 8.19 | **2.62** | 9.30 |
| | 33S | 2.47 | 5.16 | 2.24 | 7.12 |

*Yield components*

The statistical analysis of main yield components (yield, dry biomass, harvest index) that were measured during the three experimental growing seasons did not present significant differences in results (Table 5). They were analysed for single effects irrigation level and saline treatment (water quality), and for interaction of irrigation treatment x saline treatment.

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**Table 5.** Statistical analysis of main yield component of quinoa from 2015 to 2017.

| | | Quinoa | | | | | |
|---|---|---|---|---|---|---|---|
| **Year** | **Source of Variation** | **Biomass (g, Plant$^{-1}$)** | | **Yield (g,Plant$^{-1}$)** | | **HI (%)** | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | NS | | NS | | NS | |
| | | I_100 | 13.79±5.42 | | 0.89±0.50 | | 6.61±2.95 |
| | | I_33 | 13.43±6.16 | | 0.81±0.40 | | 6.33±2.37 |
| **2015** | Water quality (Wq) | NS | | NS | | NS | |
| | | N | 13.04±5.72 | | 0.91±0.52 | | 7.02±2.82 |
| | | S | 14.14±5.87 | | 0.80±0.38 | | 5.93±2.39 |
| | Wr*Wq | NS | | NS | | NS | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | NS | | NS | | NS | |
| | | I_100 | 3.20±1.02 | | 0.35±0.13 | | 7.59±3.34 |
| | | I_33 | 3.05±0.93 | | 0.30±0.19 | | 7.88±2.80 |
| **2016** | Water quality (Wq) | NS | | NS | | NS | |
| | | N | 3.29±0.92 | | 0.37±0.14 | | 8.13±3.95 |
| | | S | 2.95±1.00 | | 0.29±0.18 | | 7.30±1.51 |
| | Wr*Wq | NS | | NS | | NS | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | NS | 16.10±9.40 | NS | 0.87±0.54 | NS | 7.03±5.24 |
| | | I_100 | 14.14±7.54 | | 0.73±0.30 | | 6.40±1.84 |
| | | I_33 | | | | | |
| **2017** | Water quality (Wq) | NS | 12.64±6.28 | NS | 0.74±0.29 | NS | 8.19±5.02 |
| | | N | 17.60±9.67 | | 0.87±0.55 | | 5.54±2.51 |
| | | S | | | | | |
| | Wr*Wq | NS | | NS | | NS | |

**, **, *** indicate respectively differences at P≤0.05, P≤0.01 and P≤0.001. NS indicates no significant difference.*
*"Means followed by the different letter in each column are significantly different according to the LSD test (P=.05)"*

*Quality*

Table 6 shows the average chemical composition of quinoa seeds deriving from the harvests made in 2017. They were prepared for the study and differentiated according to treatment. No significant differences were recorded for each parameter analysed. The average protein content of quinoa seeds was about 15% of seed weight. The principal component analysis (figure 4) showed that quinoa and amaranth recorded similar values in terms of protein content. Amaranth seeds showed higher starch concentration respect to quinoa seeds. Furthermore, quinoa seeds have more fat and ashes in respect to grain amaranth.

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**Table 6**: Seed quality parameters as affected by water quality and water supply on Quinoa.

| Source of variation | Starch | Ashes | Total Protein | Fat |
|---|---|---|---|---|
| Water quality (WQ) | ns | ns | ns | ns |
| Fresh water | 49.00±2.36 | 4.37±0.62 | 14.97±0.92 | 3.70±0.43 |
| Salinity | 49.00±2.21 | 4.53±0.95 | 15.30±0.70 | 3.52±1.40 |
| Water supply (WS) | ns | ns | ns | ns |
| I100 | 47.63±1.56 | 4.47±0.73 | 15.13±0.50 | 3.55±0.78 |
| I33 | 50.37±1.87 | 4.43±0.87 | 15.13±1.08 | 3.66±1.25 |
| WQ x WS | ns | ns | ns | ns |

NS= Not significant.
Means followed by different letter in each column are significantly different according to LSD test (P=0.05).

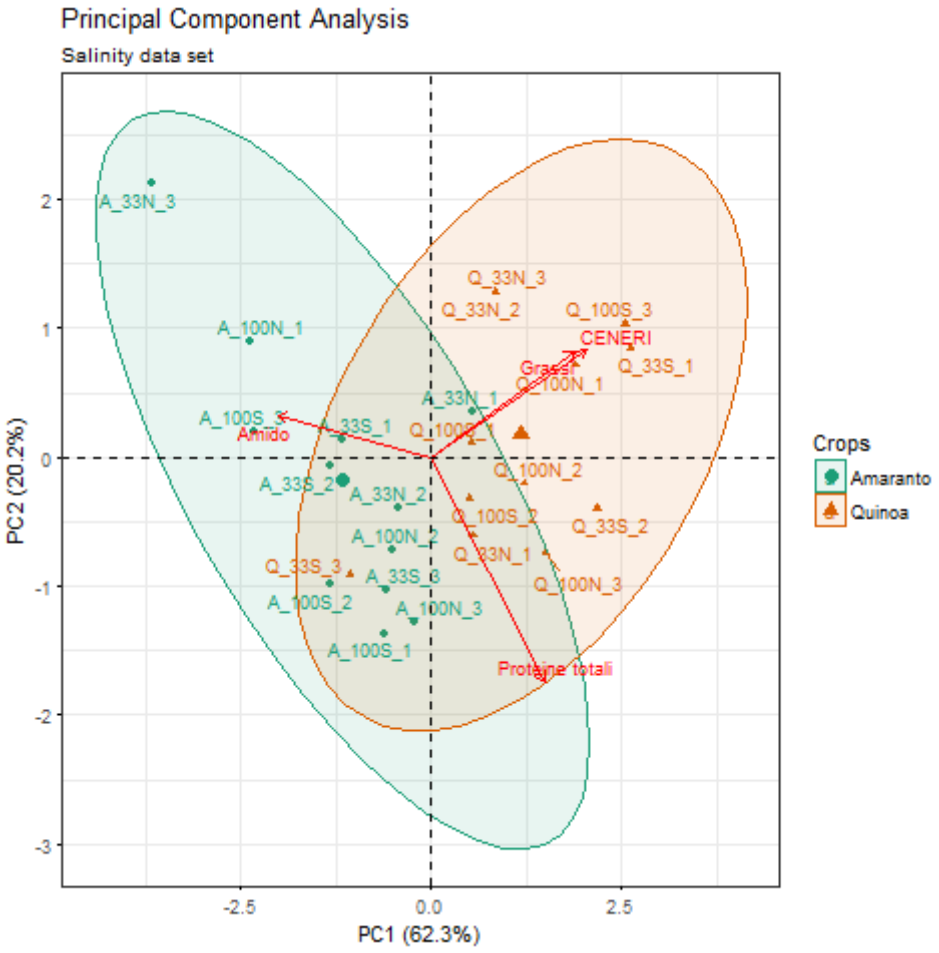


**Figure 4.** Principal component analysis of main qualitative traits of quinoa and amaranth seeds harvested in 2017.

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### *3.2 Amaranth*

Amaranth was sown at DOY on days 111, 147 and 107 respectively in years 2015, 2016 and 2017. The crop cycle length ranged from days 120 to 144 during the three experimental years (table 7). The ET0 demand during the crop cycle ranged from 565 mm (2016) to 659 mm (2017).

**Table 7**: Dates of main phenological stages, crop evapotranspiration and irrigation supply of amaranth crop grown during three years of experimental work

| **Amaranth** | **Year** | | |
|---|---|---|---|
| | **2015** | **2016** | **2017** |
| ***Phenological stages*** | | | |
| Sowing (DOY) | 111 | 147 | 107 |
| Harvesting (DOY) | 231 - 238 - 243 | 288 - 291 | 241- 242 – 243 – 247 - 248 |
| Crop cycle (days) | 120-132 | 141-144 | 134-141 |
| Irrig. Start (DAS) | 49 | 12 | 56 |
| Last Irrig (DAS) | 93 | 96 | 100 |
| Irrigation time | 7 | 7 | 7 |
| ***Evapotranspiration, Rainfall and irrigation supply*** | | | |
| Cumulative ETo*(*mm*) | 608 | 565 | 659 |
| Rainfall (*mm*) | 192 | 364 | 63.2 |
| Seasonal irrigation supply (*mm*) | | | |
| I100N | 264.14 | 312.46 | 300.94 |
| I33N | 87.17 | 103.11 | 99.31 |
| I100S | 233.43 | 209.14 | 220.30 |
| I33S | 77.03 | 69.02 | 72.70 |

*Penman monteith

*Irrigation and Soil water content*

In each experimental year, 7 irrigations were carried out with a total amount of 264 mm, 312 mm and 300 mm, respectively, of fresh water for the treatment 100N. Similarly, irrigations of saline water with a total of 233 mm, 209 mm and 220 mm, respectively, were carried out for the treatment 100S during the three years of experiments. The total amount of applied irrigation was higher for no saline treatments in respect to the saline treatments, probably due to the different transpiration rates caused by different plant development.

*Soil EC*

The average ECe values (0-0.6 m) at sowing indicated of an increasing trend over the three years for both 100S and 33S treatments, similar to with the quinoa trial. The values ranged for treatment 100S from 1.26 to 2.66 dS $m^{-1}$ during sowing, and from 5.66 to 8.19 during harvest.

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*Yield components*

Significant difference in yield and harvest index values were recorded in 2015 (table 8). The values recorded were P≤0.001 for water quality effect and at P≤0.05 for data of dry biomass in the interaction (water quality and irrigation regimes). In both cases, the N treatments resulted in higher values in respect to the S treatments.

In 2017, significant differences were recorded in biomass values for water regimes as well as for yield and HI values for water quality. No differences were recorded in 2016 for each of the analysed yield components.

**Table 8** Statistical analysis of main yield component of amaranth from 2015 to 2017.

| | | Amaranth | | | | | |
|---|---|---|---|---|---|---|---|
| **Year** | **Source of Variation** | **Biomass (g .Plant$^{-1}$)** | | **Yield (g.Plant$^{-1}$)** | | **HI (%)** | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | NS | | NS | | NS | |
| | | I_100 | 10.41±3.77 | | 0.69±0.37 | | 7.91±4.63 |
| | | I_33 | 10.25±3.34 | | 0.74±0.42 | | 7.75±3.51 |
| **2015** | Water quality (Wq) | NS | | **** | | **** | |
| | | N | 11.26±2.71 | | 0.98±0.35 a | | 10.09±3.77 a |
| | | S | 9.45±3.99 | | 0.50±0.27 b | | 5.56±2.94 b |
| | Wr*Wq | * | | NS | | NS | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | NS | | NS | | NS | |
| | | I_100 | 20.83±7.70 | | 1.44±0.61 | | 7.61±3.70 |
| | | I_33 | 20.83±7.55 | | 1.31±0.54 | | 7.40±4.17 |
| **2016** | Water quality (Wq) | NS | | NS | | NS | |
| | | N | 22.92±6.08 | | 1.48±0.56 | | 7.06±3.55 |
| | | S | 18.22±8.48 | | 1.24±0.58 | | 8.07±4.31 |
| | Wr*Wq | NS | | NS | | NS | |
| | | Pr. | Means | Pr. | Means | Pr. | Means |
| | Water regime (Wr) | * | | NS | | NS | |
| | | I_100 | 103.56±25.14 a | | 8.87±4.19 | | 7.80±2.84 |
| | | I_33 | 82.10±23.80 b | | 7.09±3.07 | | 8.34±2.30 |
| **2017** | Water quality (Wq) | NS | | ** | | *** | |
| | | N | 95.10±23.84 | | 9.63±4.07 a | | 9.49±2.49 a |
| | | S | 89.30±29.19 | | 6.34±2.54 b | | 6.65±1.74 b |
| | Wr*Wq | NS | | NS | | NS | |

*, **, *** indicate respectively differences at P≤0.05, P≤0.01 and P≤0.001 ; NS indicates not significant difference.

"Means followed by the different letter in each column are significantly different according to the LSD test (P=.05)"

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*Quality*

No significant differences were recorded for the quality parameters analyzed. The average protein content of amaranth seeds was about 15% of seed weight. Great part of amaranth seeds was composed by starch (Table 9).

**Table 9**: Seed quality parameters as affected by water quality and water supply on amaranth.

| Source of variation | Starch | Ashes | Total Protein | Fat |
|---|---|---|---|---|
| Water quality (WQ) | ns | ns | ns | ns |
| Fresh water | 53.02±3.89 | 2.18±0.24 | 14.60±1.25 | 2.48±0.61 |
| Salinity | 54.17±3.08 | 2.16±0,06 | 14.80±1.18 | 2.54±0.25 |
| Water supply (WS) | ns | ns | ns | ns |
| I100 | 53.82±4.23 | 2.18±0.09 | 14.75±1.46 | 2.44±0.31 |
| I33 | 53.37±2.72 | 2.16±0.23 | 14.66±0.81 | 2.61±0.56 |
| WQ x WS | ns | ns | ns | ns |

NS Not significant.
Means followed by different letter in each column are significantly different according to LSD test (P=0.05)

# 4. Conclusion and next steps

During this study, the effect of salinity was evaluated on two high protein quality crops of quinoa and amaranth. The field trials carried out from 2015 to 2017 in Italy showed that both cultivars were very tolerant to high levels of salinity. In fact, the seed yields for both crops did not vary significantly when comparing the saline to non-saline treatments for a $P \leq 0.05$. Differences were recorded only for amaranth seed yield at $P \leq 0.001$ in 2015 and at $P \leq 0.01$ in 2017.

These results suggest of good adaptation potential and a high degree of flexibility for both quinoa and amaranth production. Since both crops have indicated of high tolerance and resistance to salt stress, both amaranth and quinoa represent a valid source of high quality protein to be cultivated in European marginal areas. Marginal areas are usually affected either by primary or secondary salinization problems, and crops surviving in these areas require a high resistance to salt stress. The data received in this study will be further used as a basis for extending, developing and deepening our knowledge and other deliverables in the project, such as the D1.11 (Effects of abiotic stresses on the selected crops grown under European conditions defined).

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## 5. Delays and difficulties

Since the total protein content in the seeds did not vary between different treatments and no difference was found in the weight of the seeds collected, due to the high cost of the analysis on amino-acidic composition, it was assumed that there were no changes in the amino acid composition of both crops.

## 6. Impact and outreach

The data collection of this trial and other relevant information from this study could be used to sum up the results from all work packages of the project to help gain optimal selection of species and cultivars. Taking also into account that these two crops can be cultivated in the marginal lands under saline and drought conditions, the positive impact could be high on utilization of degraded soils and on the agricultural production in Europe. The results of this study can therefore help gain information of the quality and sustainability of the seed crops and their production potential in Europe, particularly in marginal lands.

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