Research article

Rice growth, assimilate translocation, and grain quality in response to salinity under Mediterranean conditions

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Abstract: The effect of salinity on rice (Oryza sativa L.) cropping systems has been extensively studied in controlled experiments, but little is known about the performance of rice varieties to salt stress under field conditions. The study's purpose was to examine the effect of salinity on agronomic, physiological, and grain quality traits of indica and japonica rice varieties with different sensitivity to salt stress in a three-year (2010–2012) field study under Mediterranean conditions. Treatments consisted of two salinity levels, i.e., high salinity level (HSL, EC between 3.8 to 6.4 dS m⁻¹) and low salinity level (LSL, EC between 0.9 and 1.3 dS m⁻¹), and eight rice varieties arranged in a randomized complete block design in a split-plot arrangement. Rice growth showed an inconsistent response across varieties and years, demonstrating the importance of genotype and environment on rice response to salinity. The detrimental effect of salinity to rice growth was more evident when the weather conditions were favourable for rice production and salt stress was the main limiting factor for plant growth. Averaged across varieties, HSL prolonged the time to heading by 7 to 14 days compared to the LSL treatment. A considerable decrease of dry matter accumulation was observed in salt-sensitive varieties. In addition, salinity stress reduced the number of fertile tillers per plant up to 27% and the grain yield up to 50%. The contribution of pre-anthesis assimilates to grain yield was increased due to salinity in two of the three years of the experimentation. In tolerant varieties, no differences among salinity levels in grain weight were observed. Salinity had less effect on grain quality. Grain length was similar across salinity treatments, while there was no consistent correlation between salinity level and grain vitreosity. Findings provide suggestive salinity tolerance traits of the studied rice varieties under field
conditions that could be exploited for optimizing the performance of rice cropping systems in saline soils.

**Keywords:** dry matter; grain yield; growth; Indica; Japonica; rice cultivars

**Abbreviations:** HSL: high salinity level; LSL: low salinity level; EC: electrical conductivity; GY: grain yield; DMT: dry matter translocation; DMA: total aboveground dry matter at anthesis; DMMv: dry matter of vegetative parts at maturity; DMTE: dry matter translocation efficiency; CDMTG: contribution of the dry matter translocation to the grain yield.

1. **Introduction**

Crops are often exposed to various stresses that limit their growth, causing considerable reduction in the agricultural production around the world [1]. Soil salinity is one of the most common environmental stresses influencing plant growth [2]. Prevalence of soil salinity has serious implications for plant growth, ranging from reduced yields worldwide to total crop failure in most affected areas [3]. It has been estimated that 20% of the irrigated land (i.e., about 45 million ha), producing 33% of the food globally, is affected by salt stress [4]. In the European Union, soil salinity influences of around 10 million ha and is considered among the primary reasons for land desertification [5]. In Greece, almost 33% of about 0.5 Mha of irrigated land is affected by salinity, which has already diminished agricultural potential in affected areas, while in Spain the corresponding percentage is 3% [6,7].

Several factors, including human activities, are responsible for the salinization of the soil. In particular, the over-pumping of ground water to meet current needs of increased water resources usually results in seawater flow into the subsoil and the surrounding regions. In addition, soil drainage or quality of irrigation water may contribute to salt accumulations in the field [8]. To obtain sustainable yield from salt-affected soils, various management practices controlling salinity need to be adopted [9]. In the case of rice, the practice of flooding paddy fields reduces soil salinity, as the water layer on the soil surface prevents saline water ions from rising [10]. However, the success of rice cultivation during the drainage of soils with salinity problems is restricted by the salt sensitivity of plants until a sufficient salt concentration reduction has been achieved [11].

Crop tolerance to salinity is determined by the level of salinity in the soil surrounding roots causing yield losses. Decreased plant development occurs when salt concentration exceeds a critical value (the tolerance limit is usually indicated by electrical conductivity, EC), which is derived from the saturation extract of the soil at a temperature of 25 °C or from the exchangeable sodium content of the soil [12]. Rice is the primary crop in coasts and river deltas and is moderately tolerant to soil salinity, with NaCl being an essential salt causing the problem of salinity [13]. However, like most grain crops, rice is a glycophyte species, showing signs of pressure and decreased grain yield (GY) even at EC values less than 3.5 dS m⁻¹ [14]. In fact, a salinity value of 3.0 dS m⁻¹ is considered critical for rice, with a decrease in GY of 12% for each dS m⁻¹ beyond this point [15].

Although the effect of salinity on rice depends on the stage of plant growth [16], the development of rice plants may be affected by salt at any stage of their biological cycle. In particular, rice grains may fail or delay to germinate [17] and the crop establishment, the leaf area development,
as well as the seed set may be reduced because of soil salinity [18]. Increased soil salinity has been reported to reduce plant height [19] and increase kernel sterility [20]. In addition, biomass production is closely related to soil salinity [21], most likely because of the impact of salts on the rate of photosynthesis and leaf tissues development [22].

Grain yield is the result of several components that are seriously affected by soil salinity [23]. Water or soil salinity influences the number of panicles per unit area, the grain weight, the grains per panicle and the harvest index (HI) [24,25]. Grain yield losses may occur as a result of decreased growth of the vegetative parts and/or as a result of abnormalities in the reproductive process [26]. The effect of salinity was reported to be more pronounced during the reproductive period than during the vegetative period, and the response of rice plants may be more related to the osmotic part of the salt stress [27]. Nevertheless, the degree of the response depends on stress severity and climatic conditions, as well as on the degree of genotype tolerance [28,29]. Soil salinity usually occurs in field patches causing low and irregular plant density, and delayed plant development [30]. Since salinization of the soil is a dynamic process, the measurements of ions, which change over time, vary in the soil profile both horizontally and vertically [31], making it difficult to screen plant genotypes for salt tolerance under field conditions. Moreover, little is known about varietal differences that can be exploited to improve salinity tolerance, especially in rice cropping systems. Thus, additional knowledge on this topic may be instrumental in further understanding of salt tolerance mechanisms and can facilitate the selection of varieties with improved tolerance to salinity.

The objectives of this research were to examine the influence of salinity stress on various agronomic (e.g., plant height, tiller number, panicle length, GY, and kernel weight), physiological (e.g., assimilate translocation to grain) and grain quality (e.g., grain dimensions, total milling yield and grain vitreosity) characteristics of different rice varieties under Mediterranean conditions. This information is important to improve the management of rice cropping systems and to optimize their performance in saline soils.

2. Materials and methods

Field trials were conducted at the Experimental Station of the Plant Breeding and Genetics Resources Institute in Kalochori (40°33’ N, 23°00’ E, altitude 0 m), Thessaloniki, Greece during three growing seasons (2010, 2011, and 2012). The climate in the area is Mediterranean. The main weather characteristics (i.e., rainfall and temperature) during each growing season are given in Figure 1. The soil is classified as an Aquic Xerofluvents (silty loam) with a slightly alkaline pH (7.50) and low organic matter content (1.6%). Eight rice varieties, namely IR28, Alexandros (Indica type), Capataz, Chipka, Dimitra, Koral, Kulon, and Selenio (Japonica type), with different susceptibility to salinity were used. According to preliminary screening tests, the varieties IR28, Alexandros, Dimitra, and Selenio had been classified as susceptible and the varieties Koral, Kulon, Chipka, and Capataz had been classified as tolerant to salinity.

Seeds were sown in pots on 17 May 2010, 13 May 2011, and 17 May 2012. When seedlings reached the 5th to 6th leaf stage, they were transplanted by hand at their final positions in flooded fields on 28 June 2010, 24 June 2011, and 26 June 2012. After transplanting of seedlings, water height in the field remained uniform at about 5 to 10 cm until physiological maturity. The experimental area received annually 150 kg Nitrogen (N) ha⁻¹ (applied in three increments), 75 kg P₂O₅ ha⁻¹ and 75 kg K₂O ha⁻¹. The first increment of N (55 kg ha⁻¹) and all of the P and K were
applied prior to transplanting. The second N increment (60 kg ha\(^{-1}\)) was applied when plants were at the tillering stage and the final N increment (35 kg ha\(^{-1}\)) before the panicle initiation stage. Plants were grown under two salinity levels (i.e., low and high). In the low salinity level (LSL) treatment, common water management practices, including periodical water renewing for washing the salts from the field, were applied [32], while in the high salinity level (HSL) treatment no salts washing was performed. The EC in the paddies was measured every week and ranged between 0.9 and 1.3 dS m\(^{-1}\) in the LSL treatment and between 3.8 to 6.4 dS m\(^{-1}\) in the HSL treatment (Figure 2).

Treatments were arranged using the randomized complete block design in a split-plot arrangement, with three replications. The salinity levels were considered as the main-plots and the varieties as the sub-plots. Each plot composed of three rows of 2 m in length, with a distance between rows of 25 cm and between plants on the row of 10 cm. The time required for reaching the main phenological stages (i.e., heading and maturity) were recorded. In each plot, plant samples (cut at ground level) from an internal row of 1 m in length were collected at anthesis and maturity. Plant samples were divided into panicle and vegetative parts (i.e., leaf + stem) at anthesis, while panicles were further divided into grain and rachis at maturity. All plant samples were dried in an oven at a temperature of 70 °C until constant weight and then weighed. Grain yield (adjusted to 14% moisture) was determined on the basis of all the central rows of each plot that were hand-harvested and threshed with a machine (F. Walter-H. Wintersteiger, KG, Austria).

**Figure 1.** Minimum and maximum mean monthly temperature (°C) and monthly precipitation (mm) recorded during the growing seasons in 2010, 2011 and 2012.

The plant height and the number of productive tillers per plant were measured at maturity on a sample of 10 plants per plot. The individual grain weight was also determined at maturity using three samples of 100 grains per plot. Grains per panicle was determined on the basis of a sample consisted of 10 random plants from an internal row in each plot. Grain number per plant was calculated as the ratio of GY and the individual grain weight. The quality characteristics of rice grains, i.e., grain
length, the ratio of grain length/width, grain vitreosity, and total milling yield, were determined with the digital imaging system SeedCount™ SC4 (Seed Count Australasia, Condell Park, Australia).

![Figure 2. Electrical conductivity (EC) of paddies in low (LSL) and high (HSL) salinity levels during the growing period in 2010, 2011 and 2012.](image)

The following variables associated with the translocation of dry matter from leaves and stems to grain were determined following Ntanos and Koutroubas [33]:

1. \( \text{DMT} = \text{DMA} - \text{DMMv} \)
2. \( \text{DMTE} = (\text{DMA}/\text{DMT}) \times 100 \)
3. \( \text{CDMTG} = (\text{DMT}/\text{GY}) \times 100 \)

where DMT is dry matter translocation, DMA is total aboveground dry matter at anthesis, DMMv is the dry matter of vegetative parts at maturity, DMTE is dry matter translocation efficiency, CDMTG is the contribution of the dry matter translocation to the grain yield, and GY is grain yield.

Grain yield was expressed as a function of several yield components (i.e., number of tillers per plant, number of grains per panicle and individual grain weight), as well as a function of the final dry matter yield and the HI. In these expressions, the net contribution of each yield component to the variation among treatments in GY was determined using path analysis according to Moll et al. [34].

The statistical analysis of the data was done according to Steel and Torrie [35]. After testing the homogeneity of variances, an over years analysis of variance (ANOVA) was performed on data using the MSTAT-C (Michigan State University, East Lansing, USA) computer software. Since there were several year × treatment interactions, the data were eventually analyzed and reported separately for each year. The least significant difference (LSD) test was utilized for comparing treatment means. Linear correlation analysis was used to detect the relationships between variables.
3. Results

3.1. Meteorological data and crop phenology

The mean minimum and maximum monthly temperatures and the total precipitation recorded during each growing season are presented in Figure 1. There were differences in weather parameters among the three years. Despite the yearly differences in rainfall were smoothed out by the presence of a permanent water level in the field, the rainfall events during the growing season influenced the air temperature. In fact, the weather conditions were generally optimum for the rice development in 2011 (total seasonal rainfall 282.6 mm and mean seasonal maximum temperature 28.1 °C). On the other hand, the rainfall events during the experimentation in 2010 resulted in more fluctuating temperatures, while in 2012 higher temperatures were recorded, particularly during the anthesis stage and the grain filling period. Averaged across years, the emergence of seedlings occurred three to seven days after sowing, with no differences among varieties. The time from sowing to 50% heading was delayed in the HSL treatment in comparison to the LSL treatment. The over years mean time to 50% heading in the HSL treatment was delayed by 7 days (Koral, Dimitra, Chipka) to 14 days (IR28) compared with that observed in the LSL treatment (Figure 3).

![Figure 3. Time after sowing (days) for the 50% heading of rice grown under low (LSL) and high (HSL) salinity levels. Values are means of three years of experimentation and three replications (n = 9). Vertical bars represent the standard errors of the means. Within each variety, bars with the same letter are not significantly different (P < 0.05) according to LSD test.](image-url)
Table 1. Agronomic and grain quality traits of rice as affected by the salinity treatment (LSL, low salinity level; HSL, high salinity level) and the variety in 2010.

| Salinity treatment | Variety     | Grain yield (g plant⁻¹) | Number of tillers (plant⁻¹) | 1000 grain weight (g) | Number of grains per panicle (plant⁻¹) | Total dry matter at anthesis (g plant⁻¹) | Total dry matter at maturity (g plant⁻¹) | Harvest index | Plant height (cm) | Panicle length (mm) | Grain length (mm) | Length/width ratio | Total milling yield (%) | Grain vitreosity (%) |
|-------------------|-------------|--------------------------|----------------------------|-----------------------|----------------------------------------|------------------------------------------|------------------------------------------|--------------|-------------------|----------------------|---------------------|---------------------|-----------------------|-------------------|
| LSL               | IR28        | 15.5                     | 6.6                        | 25.4                  | 92.0                                   | 17.2                                     | 23.0                                     | 0.673         | 82.5              | 20.4                 | 6.65                | 2.87                | 77.3                  | 91.9              |
|                   | Koral       | 9.3                      | 4.7                        | 29.7                  | 65.3                                   | 20.7                                     | 23.3                                     | 0.400         | 107.4             | 18.3                 | 6.62                | 2.40                | 77.2                  | 92.9              |
|                   | Kulon       | 9.9                      | 4.9                        | 29.9                  | 66.3                                   | 16.2                                     | 18.3                                     | 0.540         | 75.8              | 13.7                 | 6.81                | 2.46                | 78.9                  | 88.1              |
|                   | Alexandros  | 9.9                      | 6.2                        | 24.3                  | 66.3                                   | 18.4                                     | 21.0                                     | 0.473         | 76.3              | 18.4                 | 6.88                | 3.22                | 76.5                  | 95.1              |
|                   | Dimitra     | 11.4                     | 7.2                        | 27.7                  | 58.7                                   | 18.0                                     | 23.0                                     | 0.497         | 85.5              | 15.5                 | 6.60                | 2.47                | 76.9                  | 96.2              |
|                   | Chipka      | 6.8                      | 4.9                        | 22.4                  | 62.7                                   | 16.3                                     | 16.6                                     | 0.410         | 92.1              | 15.3                 | 5.73                | 1.75                | 71.6                  | 45.1              |
|                   | Selenio     | 8.8                      | 6.1                        | 24.9                  | 58.7                                   | 18.4                                     | 19.1                                     | 0.463         | 82.3              | 18.0                 | 5.31                | 1.83                | 77.6                  | 99.7              |
|                   | Capataz     | 8.9                      | 8.7                        | 33.0                  | 30.7                                   | 21.3                                     | 24.5                                     | 0.363         | 92.6              | 16.7                 | 6.35                | 2.01                | 81.3                  | 35.3              |
|                   | Mean        | 10.1                     | 6.2                        | 27.2                  | 62.6                                   | 18.3                                     | 21.1                                     | 0.478         | 86.8              | 17.0                 | 6.37                | 2.38                | 77.2                  | 80.5              |
| HSL               | IR28        | 11.4                     | 8.3                        | 22.4                  | 61.7                                   | 18.8                                     | 23.2                                     | 0.500         | 81.2              | 19.7                 | 6.77                | 3.06                | 76.3                  | 87.0              |
|                   | Koral       | 6.9                      | 5.1                        | 28.8                  | 46.0                                   | 18.9                                     | 19.7                                     | 0.347         | 103.1             | 18.2                 | 6.82                | 2.59                | 72.3                  | 98.7              |
|                   | Kulon       | 8.5                      | 5.5                        | 29.7                  | 51.7                                   | 16.3                                     | 17.2                                     | 0.487         | 75.2              | 14.3                 | 6.83                | 2.43                | 70.1                  | 95.5              |
|                   | Alexandros  | 8.9                      | 6.3                        | 24.3                  | 58.7                                   | 18.7                                     | 19.5                                     | 0.450         | 76.9              | 19.6                 | 7.10                | 3.22                | 78.4                  | 87.1              |
|                   | Dimitra     | 7.2                      | 6.0                        | 27.0                  | 44.0                                   | 19.1                                     | 17.4                                     | 0.413         | 80.8              | 15.4                 | 6.79                | 2.59                | 78.2                  | 94.2              |
|                   | Chipka      | 5.2                      | 4.9                        | 29.9                  | 35.7                                   | 14.8                                     | 13.4                                     | 0.390         | 87.3              | 14.9                 | 6.01                | 1.89                | 77.5                  | 20.2              |
|                   | Selenio     | 6.1                      | 6.5                        | 18.4                  | 50.7                                   | 15.5                                     | 15.7                                     | 0.390         | 79.8              | 17.2                 | 5.46                | 1.90                | 72.0                  | 98.5              |
|                   | Capataz     | 6.6                      | 7.9                        | 26.7                  | 31.0                                   | 16.7                                     | 18.7                                     | 0.350         | 85.8              | 15.8                 | 6.45                | 2.16                | 68.4                  | 65.4              |
|                   | Mean        | 7.6                      | 6.3                        | 25.9                  | 47.4                                   | 17.3                                     | 18.1                                     | 0.416         | 83.8              | 16.9                 | 6.53                | 2.48                | 74.2                  | 80.8              |
|                   | LSD0.05†    | 2.4                      | 0.6                        | 0.1                   | 14.1                                   | 1.0                                      | 0.2                                      | 0.104         | 3.5               | 1.6                  | 0.17                | 0.06                | 0.7                   | 0.7               |
|                   | LSD0.05‡    | 3.1                      | 1.5                        | 0.3                   | 14.0                                   | 2.3                                      | 5.8                                      | 0.059         | 5.2               | 1.7                  | 0.12                | 0.06                | 3.7                   | 1.9               |
|                   | LSD0.05§    | 3.6                      | 1.4                        | 0.3                   | 18.1                                   | 2.3                                      | 5.5                                      | 0.108         | 5.7               | 2.1                  | 0.19                | 0.08                | 3.5                   | 1.8               |

Source of variation

| Salinity Level (S) | * | NS | ** | * | NS | ** | NS | NS | NS | NS | * | ** | NS |
| Variety (V)        | ** | ** | ** | * | NS | ** | ** | ** | NS | ** | NS | ** | NS |
| S × V              | NS | NS | ** | NS | NS | ** | NS | NS | NS | NS | ** | ** | NS |
| CV (%)             | 21.1 | 14.0 | 0.6 | 15.3 | 7.8 | 17.8 | 7.9 | 3.6 | 6.0 | 1.1 | 1.4 | 3.0 | 5.8 |

Note: † Least significance difference for comparing salinity treatment means averaged across varieties; ‡ Least significance difference for comparing variety means in the same salinity treatment; § Least significance difference for comparing two variety means in different salinity treatment; *, ** Significant at 0.05 and 0.01 probability level, respectively; NS, Non-significant; CV, coefficient of variation.
| Salinity treatment | Variety | Grain yield (g plant\(^{-1}\)) | Number of tillers plant\(^{-1}\) | 1000 grain weight (g) | Number of grains panicle\(^{-1}\) | Total matter dry at anthesis (g plant\(^{-1}\)) | Total dry matter at maturity (g plant\(^{-1}\)) | Harvest index | Plant height (cm) | Panicle length (cm) | Grain length (mm) | Length/width ratio | Total milling yield (%) | Grain vitreosity (%) |
|-------------------|---------|-------------------------------|-------------------------------|----------------------|-----------------------------|---------------------------------|-----------------------------------|-------------|----------------|-------------------|-----------------|-----------------|-------------------|-------------------|
| LSL               | IR28    | 26.6                          | 11.4                          | 25.8                | 91.3                        | 17.6                            | 38.0                              | 0.700       | 75.1            | 20.4              | 6.63            | 2.84            | 80.7              | 96.6              |
|                   | Koral   | 19.7                          | 7.3                           | 33.0                | 84.0                        | 20.1                            | 34.5                              | 0.570       | 89.8            | 17.3              | 6.50            | 2.34            | 88.4              | 96.9              |
|                   | Kulon   | 18.6                          | 6.3                           | 30.2                | 97.7                        | 17.7                            | 26.6                              | 0.697       | 68.8            | 13.8              | 6.83            | 2.40            | 89.9              | 88.9              |
|                   | Alexandros | 18.8                     | 7.5                           | 27.3                | 93.0                        | 16.4                            | 28.0                              | 0.670       | 68.3            | 18.8              | 7.28            | 3.20            | 84.8              | 96.3              |
|                   | Dimitra | 20.0                          | 6.8                           | 31.2                | 95.7                        | 16.5                            | 30.4                              | 0.660       | 72.9            | 14.8              | 6.56            | 2.43            | 87.2              | 96.2              |
|                   | Chipka  | 20.6                          | 7.0                           | 32.9                | 90.7                        | 17.4                            | 33.8                              | 0.610       | 73.2            | 14.6              | 5.74            | 1.74            | 87.3              | 47.2              |
|                   | Selenio | 16.0                          | 6.9                           | 25.3                | 95.3                        | 17.4                            | 25.1                              | 0.633       | 69.7            | 15.9              | 5.33            | 1.79            | 86.7              | 99.6              |
|                   | Capataz | 29.3                          | 12.5                          | 35.2                | 66.7                        | 20.5                            | 47.1                              | 0.623       | 77.4            | 16.7              | 6.20            | 1.92            | 87.7              | 38.6              |
|                   | Mean    | 21.2                          | 8.2                           | 30.1                | 89.3                        | 17.9                            | 33.0                              | 0.645       | 74.4            | 16.5              | 6.38            | 2.33            | 86.5              | 82.6              |
| HSL               | IR28    | 17.9                          | 11.9                          | 23.2                | 68.7                        | 18.1                            | 28.8                              | 0.623       | 76.6            | 19.7              | 6.57            | 2.95            | 77.3              | 99.5              |
|                   | Koral   | 8.6                           | 5.5                           | 29.3                | 58.7                        | 20.5                            | 16.9                              | 0.510       | 88.7            | 16.7              | 6.88            | 2.55            | 80.1              | 98.6              |
|                   | Kulon   | 9.8                           | 5.7                           | 27.8                | 64.3                        | 16.5                            | 16.3                              | 0.607       | 66.7            | 13.9              | 6.84            | 2.47            | 86.5              | 99.4              |
|                   | Alexandros | 12.2                     | 5.8                           | 25.4                | 81.7                        | 18.1                            | 18.7                              | 0.640       | 74.2            | 18.7              | 7.06            | 3.24            | 80.0              | 77.6              |
|                   | Dimitra | 7.4                           | 7.0                           | 27.9                | 40.0                        | 18.4                            | 13.5                              | 0.553       | 72.1            | 14.8              | 6.77            | 2.58            | 80.6              | 92.3              |
|                   | Chipka  | 9.4                           | 6.3                           | 23.4                | 67.0                        | 14.5                            | 15.7                              | 0.607       | 63.9            | 18.3              | 5.99            | 1.82            | 83.6              | 19.4              |
|                   | Selenio | 8.9                           | 6.3                           | 21.8                | 67.3                        | 16.3                            | 13.1                              | 0.683       | 72.0            | 16.3              | 5.41            | 1.83            | 84.3              | 97.9              |
|                   | Capataz | 10.7                          | 9.5                           | 29.2                | 38.7                        | 16.4                            | 22.9                              | 0.470       | 79.4            | 14.4              | 6.49            | 2.07            | 84.6              | 43.2              |
|                   | Mean    | 10.6                          | 7.2                           | 26.0                | 60.8                        | 17.4                            | 18.2                              | 0.587       | 74.2            | 16.6              | 6.50            | 2.44            | 82.1              | 78.5              |
|                   | LSD\(_{0.05}\)\(\dagger\) | 1.0                        | 1.3                           | 1.6                | 3.5                         | 2.7                            | 5.7                               | 0.111       | 2.5             | 0.3               | 0.12            | 0.07            | 2.5               | 3.0               |
|                   | LSD\(_{0.05}\)\(\ddagger\) | 3.8                        | 2.7                           | 2.6                | 30.8                        | 1.9                            | 5.1                               | 0.094       | 6.2             | 1.9               | 0.18            | 0.08            | 2.4               | 4.0               |
|                   | LSD\(_{0.05}\)§ | 3.7                        | 2.8                           | 2.8                | 29.0                        | 3.0                            | 7.0                               | 0.131       | 6.2             | 1.8               | 0.20            | 0.10            | 3.1               | 4.5               |

Source of variation
- **: Significant at 0.01 probability level;
- NS: Non-significant
- §: Least significance difference for comparing two variety means in different salinity treatment
- \(\dagger\): Least significance difference for comparing salinity treatment means averaged across varieties
- \(\ddagger\): Least significance difference for comparing variety means in the same salinity treatment
- \(\ddagger\): Least significance difference for comparing two variety means in different salinity treatment

Note: *: Significant at 0.05 and 0.01 probability level, respectively; NS, Non-significant; CV, coefficient of variation.
Table 3. Agronomic and grain quality traits of rice as affected by the salinity treatment (LSL, low salinity level; HSL, high salinity level) and the variety in 2012.

| Salinity treatment | Variety | Grain yield (g plant⁻¹) | Number of tillers plant⁻¹ | 1000 grain weight (g) | Number of grains panicle⁻¹ | Total dry matter at anthesis (g plant⁻¹) | Total dry matter at maturity (g plant⁻¹) | Harvest index | Plant height (cm) | Panicle length (cm) | Grain length (mm) | Length/width ratio | Total milling yield (%) | Grain vitreosity (%) |
|-------------------|---------|-------------------------|---------------------------|-----------------------|---------------------------|------------------------------------------|------------------------------------------|--------------|------------------|---------------------|---------------------|----------------------|------------------------|-----------------------|
| LSL               | IR28    | 11.7                    | 9.2                       | 23.9                  | 53.3                      | 17.5                                    | 21.7                                     | 0.540        | 93.8            | 20.5                | 6.63                | 2.91                 | 77.0                   | 87.3                  |
|                   | Koral   | 9.2                     | 4.9                       | 30.0                  | 63.0                      | 17.7                                    | 19.2                                     | 0.477        | 103.2           | 16.9                | 6.68                | 2.42                 | 81.9                   | 88.8                  |
|                   | Kulon   | 13.3                    | 7.8                       | 30.4                  | 56.7                      | 17.7                                    | 23.6                                     | 0.563        | 80.4            | 14.6                | 6.81                | 2.43                 | 80.7                   | 87.4                  |
|                   | Alexandros | 9.6                  | 7.0                       | 22.4                  | 62.0                      | 16.1                                    | 17.2                                     | 0.553        | 72.7            | 15.4                | 6.88                | 3.22                 | 78.6                   | 93.9                  |
|                   | Dimitra | 11.0                    | 8.1                       | 27.3                  | 49.7                      | 17.3                                    | 19.1                                     | 0.577        | 85.2            | 12.6                | 6.64                | 2.51                 | 80.5                   | 96.2                  |
|                   | Chipka  | 8.7                     | 4.5                       | 28.6                  | 67.0                      | 16.6                                    | 16.7                                     | 0.523        | 105.5           | 15.1                | 5.74                | 1.77                 | 83.2                   | 43.1                  |
|                   | Selenio | 8.4                     | 7.5                       | 22.1                  | 50.0                      | 18.1                                    | 14.8                                     | 0.560        | 84.0            | 14.4                | 5.30                | 1.84                 | 81.1                   | 99.7                  |
|                   | Capataz | 9.1                     | 9.5                       | 33.6                  | 28.3                      | 20.4                                    | 19.4                                     | 0.467        | 89.3            | 11.4                | 6.37                | 2.01                 | 82.3                   | 32.1                  |
|                   | Mean    | 10.1                    | 7.3                       | 27.3                  | 53.8                      | 17.7                                    | 19.0                                     | 0.533        | 89.3            | 15.1                | 6.38                | 2.39                 | 80.7                   | 78.6                  |
| HSL               | IR28    | 9.8                     | 8.6                       | 23.5                  | 48.3                      | 18.5                                    | 19.7                                     | 0.497        | 82.9            | 18.1                | 6.98                | 3.04                 | 76.7                   | 86.1                  |
|                   | Koral   | 8.2                     | 3.6                       | 30.2                  | 75.0                      | 19.0                                    | 15.6                                     | 0.517        | 102.3           | 15.0                | 7.00                | 2.55                 | 81.2                   | 98.5                  |
|                   | Kulon   | 9.5                     | 4.7                       | 28.2                  | 71.3                      | 16.1                                    | 15.7                                     | 0.603        | 71.5            | 11.3                | 6.89                | 2.42                 | 82.4                   | 90.1                  |
|                   | Alexandros | 8.7                  | 6.2                       | 24.3                  | 57.7                      | 19.0                                    | 17.3                                     | 0.507        | 72.4            | 15.8                | 7.18                | 3.19                 | 78.6                   | 91.7                  |
|                   | Dimitra | 9.8                     | 5.2                       | 28.0                  | 68.3                      | 16.9                                    | 17.7                                     | 0.567        | 84.2            | 12.6                | 6.89                | 2.59                 | 79.2                   | 91.7                  |
|                   | Chipka  | 8.0                     | 3.4                       | 30.9                  | 75.0                      | 14.3                                    | 16.0                                     | 0.510        | 98.7            | 16.5                | 5.97                | 1.84                 | 82.8                   | 19.4                  |
|                   | Selenio | 6.3                     | 4.2                       | 24.2                  | 61.7                      | 14.9                                    | 13.1                                     | 0.500        | 77.5            | 13.4                | 5.46                | 1.84                 | 81.4                   | 99.8                  |
|                   | Capataz | 5.7                     | 6.5                       | 31.4                  | 28.3                      | 16.7                                    | 14.1                                     | 0.407        | 86.3            | 12.9                | 6.42                | 2.19                 | 79.7                   | 83.8                  |
|                   | Mean    | 8.2                     | 5.3                       | 27.6                  | 60.7                      | 16.9                                    | 16.2                                     | 0.513        | 84.5            | 14.5                | 6.60                | 2.46                 | 80.3                   | 82.7                  |
|                   | LSD₀₀.₀₅ | 1.4                    | 0.3                       | 0.5                   | 10.1                      | 0.2                                    | 5.3                                      | 0.169        | 7.5             | 1.9                 | 0.05                | 0.06                 | 3.1                    | 3.7                   |
|                   | LSD₀₀.₀₅ | 1.4                    | 0.3                       | 0.5                   | 10.1                      | 0.2                                    | 5.3                                      | 0.169        | 7.5             | 1.9                 | 0.05                | 0.06                 | 3.1                    | 3.7                   |
|                   | LSD₀₀.₀₅ | 1.4                    | 0.3                       | 0.5                   | 10.1                      | 0.2                                    | 5.3                                      | 0.169        | 7.5             | 1.9                 | 0.05                | 0.06                 | 3.1                    | 3.7                   |
|                   | LSD₀₀.₀₅ | 1.4                    | 0.3                       | 0.5                   | 10.1                      | 0.2                                    | 5.3                                      | 0.169        | 7.5             | 1.9                 | 0.05                | 0.06                 | 3.1                    | 3.7                   |

Source of variation

| Salinity Level (S) | * | ** | NS | NS | * | NS | NS | NS | NS | ** | * | NS | * |
|-------------------|---|----|----|----|---|----|----|----|----|----|---|----|---|
| Variety (V)       | **| ** | ** | ** | **| ** | ** | ** | ** | ** | **| ** | **|
| S × V             | NS | ** | ** | NS | **| NS | NS | NS | * | ** | NS | ** | **|
| CV (%)            | 16.7| 10.6| 3.4| 16.7| 7.1| 14.6| 8.2| 4.2| 11.4| 1.2| 1.7| 1.9| 14.8|

Note: † Least significance difference for comparing salinity treatment means averaged across varieties; § Least significance difference for comparing two variety means in different salinity treatment; *, ** Significant at 0.05 and 0.01 probability level, respectively; NS, Non-significant; CV, coefficient of variation.
3.2. Dry matter accumulation, partitioning and translocation

Total aboveground dry matter at anthesis stage was, on average, comparable across the three years. The corresponding value at maturity was higher in 2011 than in the other two years. Averaged across varieties, the HSL tended to decrease dry matter at anthesis and maturity stages compared with the LSL treatment; however, the differences among treatments were significant at maturity in 2010 and 2011 (P < 0.01, Tables 1 and 2) and only at anthesis in 2012 (P < 0.01, Table 3). In particular, total dry matter at anthesis in 2012 was lower for the stressed plants, resulting in an average reduction of 4.5% across varieties relative to that achieved in the non-stressed plants. An exception to this trend was observed for the variety Alexandros, which accumulated 15.3% more dry matter in the HSL treatment than in the LSL treatment. Moreover, plants under salinity stress tended to have lower plant height than those grown under no salinity stress, with the differences being significant (P < 0.05) for the varieties Capataz (in 2010), Chipka (in 2011) as well as for IR28 and Kulon (in 2012) (Tables 1–3).

![Figure 4](image_url)

**Figure 4.** Response of (a) dry matter accumulation at maturity (DMA), (b) dry matter translocation (DMT), (c), dry matter translocation efficiency (DMTE), and (d) contribution of pre-anthesis assimilates to grain yield (CDMTG) of rice as affected by the salinity (LSL, low salinity level; HSL, high salinity level) and the year of the experimentation. Each bar represents a mean value derived from eight varieties and three replications (n = 24). Vertical bars represent the standard errors of the means. Within each year, bars with the same letter are not significantly different (P < 0.05) according to LSD test.

Plants in the HSL treatment had, on average, higher DMT than those in the LSL treatment in 2011 and 2012, while a reverse trend was observed in 2010 (Figure 4). Regarding the DMTE, the trend was similar to that of DMT, but the differences between treatments were not significant in any year. By contrast, the CDMTG had higher values under salinity stress in all years. The HI ranged from 0.45 (in 2010) to 0.62 (in 2011) and was not affected by the salinity level (Tables 1–3). The
contribution of HI to the variation among treatments in GY was low (12.5%) compared with that of biomass production (87.5%) in 2011 (Table 4). The corresponding values were closer in 2010 (51.9% vs. 48.1%) and in 2012 (31.7% vs. 68.3%).

Table 4. Contribution of the component trait to the resulting trait for rice grown in low salinity level (LSL) and high salinity level (HSL).

| Resulting trait                  | Component trait                  | $\sum_{i}x_{i}y_{i}(\sum_{i}x_{i}^{2})^{-1}$ | LSL   | HSL   |
|----------------------------------|----------------------------------|---------------------------------------------|-------|-------|
| $Y_{1}$ log grain yield (g plant$^{-1}$) | $X_{1}$ log tillers plant$^{-1}$ | 0.761                                       | 0.460 |       |
|                                  | $X_{2}$ log grains panicle$^{-1}$ | 0.108                                       | 0.681 |       |
|                                  | $X_{3}$ log grain weight (g)    | 0.131                                       | -0.142|       |
| $Y_{2}$ log grain yield (g plant$^{-1}$) | $X_{4}$ log biomass (g plant$^{-1}$) | 0.698                                       | 0.667 |       |
|                                  | $X_{5}$ log HI (g g$^{-1}$)     | 0.302                                       | 0.333 |       |

3.3. Grain yield and yield components

Salinity reduced GY and the degree of the reduction depended both on the variety and the year. Averaged across varieties, the reduction of GY due to salinity was 25% in 2010 (Table 1), 50% in 2011 (Table 2) and 19% in 2012 (Table 3). When the HSL and the LSL treatments were compared, the percentage of yield reduction due to salinity stress was lower for the varieties Alexandros (10%) and Kulon (14%) in 2010, IR28 (33%) and Alexandros (35%) in 2011, and Chipka (8%) and Alexandros (9%) in 2012.

The number of the reproductive tillers per plant was reduced significantly by salinity only in 2012 (Table 3); the magnitude of the reduction was 44% in Selenio, 40% in Kulon, 36% in Dimitra, 32% in Capataz, 27% in Koral and 24% in Chipka. In both salinity levels, the number of tillers per plant was positively correlated with GY ($r = 0.77$, $P < 0.01$ in LSL and $r = 0.69$, $P < 0.01$ in HSL) and with final dry matter yield ($r = 0.74$, $P < 0.01$ in LSL and $r = 0.84$, $P < 0.01$ in HSL).

The number of grains per panicle was significantly affected by salinity in 2010 (Table 1) and in 2011 (Table 2). The percentage of reduction was, on average, 24% and 32%, respectively. The response of grains per panicle to salinity was not similar across varieties. The variety Alexandros had the lowest reduction due to salinity stress (11% in 2010 and 12% in 2011), while the highest reductions were observed in varieties IR28 (33%), Koral (30%) and Chipka (43%) in 2010 and in varieties Kulon (34%), Dimitra (58%), Selenio (29%) and Capataz (42%) in 2011.

Salinity resulted in lower grain yield in 2010 (Table 1) and in 2011 (Table 2) compared with the non-stressed plants, while in 2012 the grain weight was similar across treatments (Table 3). The over varieties mean reduction of grain weight because of salinity was 5% in 2010 and 14% in 2011. The varieties Alexandros and Kulon in both years and the variety IR28 in 2011 had no significant differences between the salinity levels, while the variety Chipka in 2010 had higher grain weight under salinity stress than under non-stressed conditions.

In the absence of salinity, GY was correlated with the number of tillers per plant ($r = 0.59$, $P < 0.01$), the number of grains panicle$^{-1}$ ($r = 0.66$, $P < 0.01$), the grain weight ($r = 0.39$, $P < 0.01$), the HI ($r = 0.74$, $P < 0.01$), and the final dry matter yield ($r = 0.94$, $P < 0.01$). In addition, under salinity stress GY was significantly correlated with the number of tillers per plant ($r = 0.55$, $P < 0.01$), the
grain weight \((r = 0.61, P < 0.01)\), the final dry matter yield \((r = 0.79, P < 0.01)\), and the HI \((r = 0.55, P < 0.01)\).

In the HSL treatment, the variation in GY among varieties was primarily due to the variability of the number of grains per panicle rather than to that of the number of tillers per plant (Table 4). On the contrary, in the LSL treatment the contribution of the tiller number per plant to the GY variation among varieties was much higher compared with that of the grains number per panicle. Moreover, most of the variation among varieties in GY was due to the differences in total dry matter yield (69.8% in LSL and 66.7% in HSL) rather than to the differences in HI (30.2% in LSL and 33.3% in HSL) (Table 4).

### 3.4. Grain quality traits

Grain length, a component of the appearance quality of rice, was significantly affected by variety in 2010 (Table 1), by variety and variety × salinity interaction in 2011 (Table 2), and by variety, salinity level, and their interaction in 2012 (Table 3). In all years and levels of salinity, grain length was the longest in variety Alexandros and the shortest in Selenio. Compared with plants grown under LSL, HSL treatment resulted in longer grains in 2012. Except for the varieties Kulon and Capataz, the grain length was increased significantly when the salinity level increased. The ratio of grain length and width tended to increase in plants under salinity stress compared with that obtained in the non-stressed plants. However, the response of grain length-to-width to the salinity level was not consisted across varieties. No differences between salinity levels in grain length-to-width were observed for the varieties Kulon, Alexandros and Selenio in all years, for Chipka in 2011 and 2012, and for Dimitra in 2012 (Tables 1–3).

The over varieties mean total milling yield in the HSL treatment was lower than that obtained in the LSL treatment in 2010 (Table 1) and 2011 (Table 2), while was not affected by the salinity level in 2012 (Table 3). The response of varieties regarding the total milling yield to salinity level was not consistent across years. In 2010, the total milling yield of the varieties IR28, Alexandros and Dimitra were not affected by salinity level. However, salinity stress reduced the total milling yield of the varieties Koral, Kulon, Selenio and Capataz and increased total milling yield of the variety Chipka. On the contrary, in 2011 the salinity level did not affect the total milling yield of the varieties Kulon, Selenio and Capataz, while the HSL treatment reduced the total milling yield of the other varieties.

No differences between the salinity treatments were observed for the over varieties mean grain vitreosity in 2010. Grain vitreosity of plants grown in the HSL treatment was reduced in 2011 and increased in 2012 compared with those grown in the LSL treatment, while no differences between treatments were observed in 2010. The response of individual varieties to salinity was not consistent across years of the experimentation (Tables 2 and 3).

### 4. Discussion

Salinity is an important abiotic stress which limits crop production. Several studies, the majority of which have been conducted under controlled environmental conditions, have shown the negative effects of salinity on rice crop [27,36,37]. The present three-year field study revealed considerable influence of salinity stress on the agronomic performance, as well as on several physiological and
grain quality traits of rice. However, the response of plants was not consistent across varieties and/or years, demonstrating the considerable effect of genotype and environment on rice response to salinity.

Salinity stress had a remarkable effect on phenological development of rice. In fact, the HSL treatment prolonged the time to heading by 7 to 14 days compared with the LSL treatment. Similarly, Fraga et al. [38] found that the use of salt water for irrigation purposes prolonged the life cycle of rice by 17 days. Furthermore, Castillo et al. [27] reported that the higher the EC, the greater the elongation of the biological cycle of the plant. On the contrary, in cereal crops other than rice, such as wheat, the duration of spikelet primordium initiation was shortened due to salinity [39].

The effect of salinity on plant growth, as it was assessed by the DMA, was also evident in the present study. Plants grown under salinity stress generally tended to accumulate lower dry matter in the aboveground parts at both samplings (i.e., anthesis and maturity) compared with the non-stressed plants. A different response was observed for the variety Alexandros, which showed similar DMA across salinity treatments. This is probably since Alexandros is a local variety, well adapted in conditions in which the experiments were conducted. Thus, despite that Alexandros was found to be susceptible in the preliminary tests, it showed salinity tolerance traits under field conditions, such as increased early DMA [40]. Besides, it is well known that moderately tolerant genotypes may show susceptibility in greenhouse test [41]. In addition, the response of rice to salinity in the present study was also affected by the environmental conditions and was more evident when the air temperature was favorable for rice production (e.g., in 2011), and therefore salt stress was the major limiting factor for plant growth.

Grain yield is a complex characteristic resulting from a series of physiological and biochemical processes that occur during the crop cycle [42]. It may be expressed in terms of several yield components, such as the number of fertile tillers, the number of grains per panicle and the individual grain weight. In some cases, the HSL treatment induced a reduction up to 27% in the number of fertile tillers per plant. Similarly, several studies have shown high rates of decrease in the number of tillers due to salinity, ranging from 34.9% to 51.4%, compared with plants grown in a non-saline environment [43,44]. However, the variety Alexandros exhibited again a salinity tolerance performance, with no significant reduction in the number of tillers across salinity levels in either year (Tables 1–3). One explanation for this response may be that high tillering ability is considered a plant adaptation mechanism [19], since the salts are allocated to higher shoot dry weight and, hence, their toxic effect is decreased [24]. Overall, the losses in rice GY due to salinity in the present study (19 to 50%) were comparable to those reported in other studies [45,46].

Several physiological characteristics, such as biomass production and partitioning of assimilates within plants, have been identified as critical determinants of seed yield in different crop species [47,48]. In the present study, the variation in GY among varieties was mainly explained by the differences in rice biomass at maturity. Moreover, different yield components were identified as decisive for the formation of GY in each salinity treatment. Indeed, in plants grown under salinity stress the differences in number of grains per panicle accounted for most of the variation in GY (68.1%). On the contrary, in non-stressed plants the variation in GY (76.1%) was primarily due to the differences in number of tillers per plant. However, the negative correlation detected between the number of grains per panicle and the number of fertile tillers suggests a compensatory relationship between these two traits in accordance with previous reports [49].

The HSL treatment tended to enhance the DMT to grains, probably due to the suppression of plant photosynthesis during the later growth stages compared with the LSL treatment. These results
agree with previous studies, which have reported that the DMT increased in rice plants grown under abiotic stresses [50, 51]. Concerning biotic stresses, Koutroubas et al. [47] revealed that rice plants grown under high disease intensity caused by the blast fungus (*Magnaporthe grisea* (Hebert) Barr, anamorph: *Pyricularia oryzae* Cavara) translocated less pre-anthesis assimilates to the grains than plants grown under low disease intensity. In the present study, the high DMT was followed by increased DMTE, indicating that the ability of rice plants to translocate assimilates to grain (i.e., translocation efficiency) was an important factor determining the net amount of DMT. The DMT contributed up to 72% to the GY of the non-stressed plants and even more in plants under salinity stress, indicating the importance of pre-anthesis redistribution within plants for GY formation of rice under Mediterranean conditions. This value is considerable higher than the value of 42% reported for rice in the same area [33]. The discrepancy between these studies may be due to the different varieties used or the different weather conditions prevailed during the experimentation.

Overall, in the varieties less affected by salinity, both the tillering ability and the early dry matter accumulation were maintained almost unchanged across salinity levels. This adaptation supported the translocation of assimilates from the vegetative parts to the grain during the filling period, thus reducing the loss of grain yield due to the presence of salt. These findings provide suggestive tolerance traits that can be used to improve the efficiency of rice growing systems in saline soils.

Rice quality is very important for all those involved in the process of production, processing, handling and consumption of rice, as it affects not only the nutritional, but also the commercial value of the seeds [52]. In the present study, the traits of grain quality were less influenced by the salinity level, in contrary to what was observed for the agronomic traits. Furthermore, the effect of salinity on grain quality was not consistent throughout the years of the experimentation. Except for 2012, grain length was similar across treatments and remained constant, even when the salinity level was increased. This was probably an adaptive response of rice to salt stress for compensating the reduction of GY. Enhanced caryopsis dimensions under salt stress have been reported by Thitisaksakul et al. [53]. On the contrary, Denis et al. [54] reported a reduction of grain size when rice plants were grown in high salt concentration.

The enhanced grain transparency, which is expressed by the percentage of vitreosity of the white grain, is one of the desirable traits of rice grain [55]. No constant association between salinity stress and grain vitreosity was observed in the present study. In addition, grain vitreosity varied greatly among years of the experimentation, demonstrating the decisive influence of the environmental conditions in the presence of pearl in the grain. Indeed, it is well known that grain vitreosity is genetically regulated, but it is also influenced by the fluctuations of the night air temperature during grain filling [56]. Grain vitreosity has been reported to be negatively correlated with the length and length-to-width ratio of the grains, and positively with their width [52]. No constant associations between grain vitreosity and grain dimensions were found in the present study.

5. Conclusions

The present study confirmed the importance of varietal choice as one of the most essential practices to limit the effect of salinity on rice. The influence of salinity was more pronounced on agronomic traits, such as heading time and grain yield, than on grain quality traits. Salinity stress prolonged the time to heading and reduced the grain yield mainly by reducing the number of grains.
per panicle. Under salinity stress, an increased translocation of pre-anthesis assimilates to the grains was observed. In terms of grain quality, grain length was generally similar across salinity treatments, while grain vitreosity was primarily determined by the environment. Findings provide additional knowledge on rice response to salinity under field conditions that could lead to better exploitation of saline soils for the production of this crop, minimizing economic losses for rice growers. This is particularly important for the Mediterranean region, where flooded rice is commonly cultivated in river deltas and coastal areas with a status of elevated soil salinity.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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