Effects of the application of a moderate alternate wetting and drying technique on the performance of different European varieties in Northern Italy rice system

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Abstract

Alternate wetting and drying (AWD) technique has been developed and evaluated on rice (*Oryza sativa*) systems in several countries worldwide for increasing water use efficiency and reducing negative effects of permanent flooding, like the increase in methane emissions and arsenic availability in soil. In this study, a paddy field experiment was carried out for two years to evaluate the application in Northern Italy rice area of a moderate AWD, i.e. only implemented during the vegetative phase of the crop and ponded water maintained thereafter, compared with Continuous Flooding (CF) system. The adaptability of 12 European commercial rice cultivars to AWD was investigated in terms of crop phenology, morphological traits, root production, nitrogen (N) uptake, yield, milled rice yield and microelement concentration in grains. Results showed substantial (40.7%) water saving probably favoured by the presence of a shallow water table. In these pedoclimatic conditions, very limited effects of a mild AWD on crop status and final productivity were recorded and the commercial cultivars did not display significant different adaptabilities to the water stress. Moreover, AWD decreased arsenic (As) concentration in grain but increased grain Cadmium (Cd) being the degree of such a response dependent upon the variety, suggesting that the genotype plays an important role in this aspect of adaptation to AWD.

Keywords: temperate rice, water-saving techniques, paddy field trial, rice phenotyping, cultivars adaptability

1. Introduction

Rice is an important crop in European and Mediterranean areas, where it is cultivated on a total of about 1.4 million ha (Source: FAOSTAT, 2017 data). In this area, the most important rice producing countries are Egypt (about 686 000 ha), Italy (234 000 ha) and Russian Federation (186 000 ha), followed by Turkey (110 000 ha) and Spain (108 000 ha). Due to the climate, EU rice production is limited to about 420·000 ha, almost totally located in Italy and Spain (82% of total EU rice area), and the rest in Portugal, Greece and Southern France. In Italy, rice production is mainly concentrated in the North-western Po valley, which represents the most northern area of rice cultivation in the Mediterranean region and has a very long tradition of irrigated rice due to the availability of water from spring precipitations and mountains runoff. However, such water availability is likely to decline under the projected climate change scenarios consisting in reduced rainfall and snow and increase in temperatures leading to more frequent and severe events of water scarcity. Introduced in this area in the 15th Century, its cultivation has developed alongside the establishment of a dense network of historic water channels (Pinto et al., 2002). The two most common water managements in Northern Italy are the traditional water sowing with continuous flooding and dry seeding with postponed flooding, with the latter developed in the last 20 years and now dominant (Zampieri et al., 2019). In both systems, paddy fields are flooded during most parts of the crop growth cycle, with some partial or total draining during cultivation for specific practices such as crop rooting, fertilization or herbicide spraying (Ferrero and Tinarelli, 2008). The permanent flooding technique consists in maintaining ponded water during the most part of the rice growing cycle - about 5 cm height during the vegetative phase and 10-15 cm from flowering to beginning of ripening – and has the main function of providing optimal temperature for rice growth, by reducing the fluctuation of temperatures and protecting rice from cold periods, and limiting weed growth (Kraehmer et al., 2017). This type of management leads to a huge water consumption at field scale compared with other crops, which is also very variable depending on several factors beside the irrigation method, as topographic gradient, groundwater depth and soil permeability (Mayer et al., 2019). In the Vercelli district, Zhao et al. (2015) reported a water use, in a coarse-silty soil, of about 1 600 mm for dry-seeding with postponed flooding, while, in the Pavese district, De Maria et al. (2016) calculated an irrigation requirement, for rice under continuous submergence in a sandy soil, ranging from 3 400 to more than 4 300 mm. Only about 550-650 mm of water is actually utilized for evapotranspiration, while a high fraction of water is reused into downstream paddy fields, discharged in channels, or lost into groundwater through deep percolation. In this area, water management for rice cultivation creates a unique agricultural landscape providing important habitats for waterbird species (King et al., 2010), but this agroecosystem is threatened by climate change, with increasing frequency of extreme drought events and water shortages, especially during the summer growing season (Bocchiola et al., 2015). Moreover, permanent flooding also causes several side effects and concerns due to greenhouse gas methane (CH_4) emissions (Meijide et al., 2017) and enhanced arsenic (As) availability in the soil with the risk of grain accumulation of this toxic element (Bouman et al., 2007). Therefore, there is growing pressure to evaluate the introduction of water-saving techniques for rice cropping in this region. Among them, Alternate wetting and drying (AWD) systems, have been developed and already adopted across several countries to reduce the water use for rice and reduce greenhouse gases emissions and As concentration into grain. In AWD, rice fields are allowed to dry naturally for one or more days after the disappearance of ponded water up to a given soil moisture content or water table depth and then re-flooded. Its effects on yield, CH_4 and nitrous oxide (N_2O) and heavy metal concentration in rice grains has been investigated in different rice areas (Linquist et al. 2015; Carrijo et al., 2017; Norton et al., 2017a; Yang et al., 2017). AWD can reduce rice yield compared with continuously submerged conditions due to sensitivity of rice to water stress, but it was found that results largely depend on the severity of the applied drying cycle. Maintenance of yield and plant production levels close to continuously flooded rice can be achieved so long as re-flooding occurs before plants become drought-stressed, and AWD can even improve yield by increasing the proportion of productive tillers (Price et al., 2013). Moreover, findings backed by results of both a single site comparison of nearly 300 cultivars (Norton et al., 2018) and a multi-site 22-cultivar comparison (Norton et al., 2017b) in Bangladesh suggested that there is not a different response to a mild AWD among varieties. It has also been shown that AWD can reduce soil availability of As and its concentration in rice grains, while cadmium (Cd) availability and uptake is favored under oxidizing conditions and may lead to high concentrations into grains which is a serious human health problem (Cattani et al., 2008). Considering water-saving techniques, the feasibility of intermittent irrigation, without ponded water, has been recently tested in the Northern Italy rice area in terms of yield, water productivity, greenhouse gases emissions and economic results (Hassen et al., 2017; Miniotti et al., 2016; Monaco et al., 2016; Peyron et al., 2016), but very few studies focus on AWD systems as a viable option for this region (Verhoeven et al., 2018). Rice breeding in Italy, driven by production and consumption market demand, gave rise to a wide germplasm of cultivars, in general belonging to *japonica* subspecies, selected under traditional water management and potentially showing a negative response to reduced water inputs. Investigations about rice cultivar adaptation to water stress conditions in Vercelli district (Volante et al. 2017) confirmed that most of the genotypes are favored under permanent flooding conditions, but, for several accessions, performances in limited water were similar to those obtained under permanent flooding. Similarly, Hassen et al. (2017) evaluated a panel of 90 Italian lines under intermittent irrigation maintained for the entire growing cycle in comparison with traditional flooding conditions and concluded that most of them were performing worst in a situation of water scarcity, even though lines with overlapping performances in the two water managements were highlighted.

Therefore, in this area it is still necessary to test a suitable water-saving technique which could limit rice water stress thus maintaining rice yield. For this reason, a two-year field experiment was carried out in the agri-environmental conditions of the Northern Italian rice area with the aim of testing the feasibility of a mild AWD system, i.e. only implemented during the vegetative phase, and to evaluate the adaptation of a set of 12 representative European rice varieties to this system. The field experiment compared an AWD system with a dry-seeded system with postponed flooding and was part of a larger research project on the application of AWD in the European rice sector and adaptation of European cultivars, with parallel field experiments in Delta Ebro area (Spain) and Camargue (France).

2. Materials and Methods

2.1. Site description

The experiment was carried out in a paddy field of CREA-CI “Cascina Boraso” farm research station (45°19’24” N; 08°22’25” W) in the Vercelli district, that together with Novarese and Pavese districts, represents the most important rice production area in Italy and Europe. Vercelli lowland (133 m asl) is surrounded by the Morainic Amphitheatre of Ivrea and the Dora Baltea confluence (W), the Vercelli high plains (N, divided from southern ones by the “Spring Line”), the Po River (S) and Sesia River. The soils are alluvial, the climate is semi-continental Po Valley type, with cold and often foggy winters and hot and very sultry summers (annual temperature of 12.1°C) and two rainy periods in Spring and Autumn (precipitations of 923 mm per year). In this area, rice is the predominant crop, cultivated from April-May (sowing period) to September-October (harvest period). Paddies are generally continuously flooded with waters coming from a dense network of channels and this management replenishes the aquifers leading to the presence of very shallow ground water. In the CREA-CI research farm, there is a historical weather station for the region with a time series dating back to 1932 (Cat Berro et al., 2005), as well as one of the piezometers of the monitoring network of groundwater depth by the water-use association “Consorzio Ovest-Sesia”. The soil profile in the research station is classified as Anthraquic Eutrudept, coarse-loamy, mixed, nonacid, mesic and is very common in this plain area (Source: IPLA). It is a relatively ancient deposit that, in recent times, has been profoundly modified by agriculture with soil levelling and periodic flooding of paddy fields which has greatly modified the hydrological dynamics. The coarser alluvial deposits are in the deeper layers while the surface layers have mainly evolved on sandy deposits, with sub-acid reaction and low soil organic matter and cation exchange capacity (Table 1).

Table 1. Soil main physical and chemical characteristics

Horizon	**Unit**	**Ap1**[*]	**Ap2**
Depth	m	0-0.25	0.25-0.40
Sand	%	52.3 ±1.15	52.3 ±1.26
Silt	%	30.7 ±1.53	28.0 ±1.41
Clay	%	17.0 ±1.00	19.8 ±0.96
pH_{H2O}		5.93 ±0.15	6.60 ±0.14
TOC	%	1.37 ±0.10	0.63 ±0.15
TKN	%	0.14 ±0.01	0.07 ±0.02
Ca^{++}	ppm	1373 ±75.7	1645 ±80.6
K^{+}	ppm	145 ±9.24	87.3 ±4.79
P Olsen	ppm	37.3 ±8.50	20.0 ±8.12
Mg^{++}	ppm	164 ±3.46	185 ±2.00
Na^{+}	ppm	87.3 ±7.51	85.8 ±3.95
CEC	meq 100 g^{-1}	13.7 ±1.06	12.5 ±0.23

TKN: Total Kjeldhal N; TOC:Total Organic Carbon; CEC: Cation Exchange Capability.
Data are the mean of 4 measures ± S.D. [*]Horizons designation by USDA-Soil Survey Manual

2.2. Field trial preparation and management

The field trial (20 x 118 m) was carried out in a paddy field and arranged in a split-plot design with 4 blocks, with Water management **(**i.e. CF, Continuously Flooded, and AWD, Alternate Wetting and Drying) as main plots (27.3 x 7.6 m each) and rice Genotype as split-plots (1.6 x 6 m) and was repeated in two different years (2015 and 2017)**.** The panel of 12 varieties evaluated in the experiment covered all the European commercial classes: round (Centauro and Selenio from Italy; Gageron from France), medium (Vialone nano and Prometeo from Italy; J. Sendra and Gleva from Spain), long A (Baldo and Loto from Italy; Arelate from France), long B (Puntal from Spain; Gines from France). While rice varieties developed in Italy have been tested under different water managements in Volante et al. (2017) and in Hassen et al. (2017), varieties from Spain and from France were never been evaluated in this agrosystem before, except for Puntal which was also included in the panel assessed by Hassen et al., (2017). Field plots were established after plot sowing on the 6th of May 2015 and 10th of May 2017 and arranged as reported by Oliver et al. (2019). Briefly, after soil plowing and levelling in March, the fields were fertilized with a dry manure supplying 32.5 kgN ha^{-1} (10 April in 2015 and 26 April in 2017) and treated with glyphosate for weed control (30 April in 2015 and 4 May in 2017). Rice was then sown with a plot seeder on the 6th of May 2015 and 10th of May 2017, following the planned arrangement of varieties and treated in pre-emergence for weed control with Oxadiazon. Each main plot was then prepared by plowing its own perimeter for creating a soil bund surrounded by a small channel and leaving 3 m of uncultivated and pressed soil corridor between them. Each main plot had separate inlet and outlet for water supply and were connected by underground plastic pipes, positioned on different levels to allow overlapping. In 2015, each main plot was equipped with a Bazin Weir, measuring hourly the height of the water with a distance meter and applying the Bazin formula: $Q = 2/3\ C_q b\ (2g)^{1/2} h^{3/2}$, where Q is the water discharge (m/s), C_q is a run-off coefficient (equals to 0.60-0.62), b is the weir length (m), g is the constant of gravity (m/s^2) and h the height of water on the weir; usually $2/3\ C_q = 0.41$ is assumed (Mazza et al., 2016). In each AWD plot, two 15 cm-diameter plastic tubes were inserted vertically up to 50 cm depth for the measurement of the water table depth and 3 tensiometers per plot (60 cm 2710ARL series, Soil moisture Equipment Corp.) were placed for the soil water potential at 25 cm-depth. In 2015, a cone penetrometer (CP20 AgridryRimik PTY Ltd, Australia) was utilized for determining soil hardness in each plot at the end of the growing cycle (i.e. 128 days after sowing) to a depth of 600 mm as described by Norton et al. (2017a). Following common farm practices, CF was irrigated at the three leaves phase and then flooding conditions were maintained until the beginning of the ripening phase, with drying periods of about one week for fertilizer and herbicides spreading. AWD was irrigated cyclically from three leaves phase until heading, and then continuously flooded as CF to prevent water stress during flowering and maturation.

Irrigation in AWD was managed in order to carry out a suitable system under real farm conditions and to reduce as much as possible rice water stress, which caused significant yield losses in previous experiment carried out in the same location (Hassen et al., 2017). This result was achieved by applying a simple plan and monitoring system connected to a common farm scheduling of operations (supplemental Table 1). For this reason, the first irrigation was planned at the same time as for CF, that is at the beginning of flooding at the three leave stage. A further irrigation was planned after fertilization and weed chemical treatment when CF is managed with a drying-fertilization-weed chemical treatment-reflooding cycle. Afterwards, the actual day of irrigation was planned when the soil water potential approximated the defined threshold in one out of four plots and rains were not expected in the following few days from local weather forecast. This protocol was chosen because of the normal time needed to plan water supply from the channel network to the fields in real farm conditions and to avoid rice water stress as much as possible. The irrigation consisted in providing water from the in-let in block one until it reached the closed out-let in block four, through the connecting pipes to each block, and then letting the water drain. This system avoids surface runoff and increases water infiltration. Moreover, the measurements with piezometers in addition to the soil water potential monitoring allowed an immediate verification of the dynamic of the surface groundwater depth. The alert system for irrigation scheduling was set at -30 KPa in 2015 and then reduced to -25 KPa in 2017 for avoiding early rice water stress symptoms detected in 2015 before the 3rd AWD cycle. Top-dress fertilization was carried out in both CF and AWD on 22 and 30 of June in 2015 and 2017, respectively, supplying 70 kg ha^{-1} of N as urea and 90 kg ha^{-1} of K_2O as KCl. Post-emergence, fungicide and insecticide treatments were the same for the entire field while time of harvest followed the maturity of each variety.

2.3. Crop phenotyping

Yield and yield components were evaluated at maturity with the measurements of the following parameters: the final number of panicles per square meter (PanicleNumber), the grain weight per Panicle (Grain/Panicle), the grain yield per square meter (GrainYield), the straw biomass production per square meter (StrawProd) and the harvest index (HarvestIndex). Specifically, the plant tillers were sampled at maturity in a 0.2 m^2 area for each plot, total productive panicles were counted, tillers were manually divided into grain, rachis and straw component and each sample dried in the oven at 65°C for 72 hours for dry matter weight determination. Roots were collected in each plot immediately after final biomass sampling, taking three soil-roots samples per plot using an auger (15 cm depth by 10 cm width) directly over the cut vegetation. Roots were then sorted, and fine root biomass (RootBiomass) was determined using the method described by Metcalfe et al. (2007). For the measurement of straw N uptake (StrawNuptake), grain N uptake (GrainNuptake) and total plant N uptake (TotalNuptake) parameters, dry samples of straw plus rachis, roots and rough rice were powderised in a ball mill and carbon and nitrogen concentration were determined using a NCS analyser (NA2500 Elemental Analyser; Carlo Erba Instruments Wigan, UK). Total milled rice yield was determined on a 100 g sample of rough rice at 13% of humidity from each plot with a SATAKE testing husking THU35A, which removed the husks from the grain, and calculated as percentage of dehulled divided by rough rice. Brown rice samples were then milled for Head Rice yield determination (HeadRice) with a grind rice milling machine and cleaned from broken grain fraction with a Satake testing rice grader; whole grain fraction was then weighed and expressed as percent of rough rice, excluding by visual inspection colour defected kernels (e.g. chalky, red). Rough rice samples were also analyzed by inductively coupled plasma – mass spectroscopy after microwave digestion in nitric acid and hydrogen peroxide as described in Norton et al. (2012) which provided data on multiple elements including those reported here, Mg, Zn, As and Cd. For phenology evaluation, DaysToFlowering, DaysToMaturity and FloweringToMaturity parameters were measured as in Volante et al. (2017): flowering and maturity days were counted from the date of sowing, when at least 50% of the plants showed at least 1/3 of the panicles or 2/3 of dried rachis, respectively, while FloweringtoMaturity was calculated as Maturity – Flowering. Plant morphology traits were: plant total height (TotalHeight), and panicular node height (NodeHeight) measured on the highest tiller of five representative plants for each plot, while panicle length (PanicleLenght) was calculated as difference between TotalHeight and NodeHeight. Data for leaf physiological traits were collected with the DUALEX 4 Scientific (Dx4, Force-A) chlorophyll meter developed for the simultaneous measurement of both leaf chlorophyll (Chl) and epidermal flavonoids (Flav), to assess the Nitrogen Balance Index (NBI= Chl/Flav), as a proxy of the plant nitrogen status (Goulas et al., 2004; Tremblay et al., 2012). According with references, measurements were carried out 7–10 days after the flowering date, a period during which the nitrogen status of the plant is stable. In each plot, three measurements were done on the adaxial and the abaxial faces of the panicle leaf of three representative plants; the 18 measurements were then averaged to obtain one Chl, Flav and NBI value per plot.

2.4. Statistical analyses

For all the statistical analyses, functions of the R environment (R Core Team, 2013) were applied. The Analysis of variance (ANOVA) was performed using the "lme" function to assess significance of Water management (W), Year (Y), Block (B), Genotype (G), Water management x Year (WxY), Genotype x Year (GxY), Water management x Genotype (WxG) interactions. In order to evaluate the possibility of inclusion of variance differences caused by the considered factors, the R "varIdent" function was also integrated in the analysis. To identify the best fitting model with or without different variances functions, the R "aov" function was applied and the model showing the lowest Bayesian Information Criterion (BIC) was selected. When a significant effect of a factor was detected, the differences among its levels were evaluated with a Bonferroni adjusted post-hoc test.

3. Results

3.1. Water management

Temperature and precipitation during 2015 and 2017 are reported in Figure 1. The average temperature during the rice growing period, from the 1st of May to the 30th of September was very similar in the two years (i.e. 22.8 and 22.7° C in 2015 and 2017, respectively), and higher than the period 2007-2014, which is used here as a reference (21.3° C). Total cumulative precipitation in the rice growing period was equal to 130.5 and 111.6 mm in 2015 and 2017, respectively, and it was much lower than those measured in the reference period (i.e. 358.6 mm). Moreover, shallow groundwater depths, in the rice growing period, were slightly different between the two years, with an average depth equal to -0.83 and -1.18 m in 2015 and 2017, respectively. The water was provided to the field 33 and 35 days after rice dry seeding (i.e. around 3 leaves unfolded stage) in both CF and AWD, in 2015 and 2017, respectively. In CF, the plots were then maintained flooded until day 115 in 2015 and 108 in 2017 (i.e. 50% of the plants between late milk and early dough stage), except for one drying period from day 44 to 54 in 2015 and from day 48 to 54 in 2017 for top-dress fertilization and herbicides spreading. In AWD treatment, after the first irrigation synchronized with the start of flooding in CF, plots were irrigated 3 times more in both 2015 (i.e. day 44, 63 and 77) and 2017 (i.e. day 41, 54 and 82), for a total of 4 AWD cycles. All AWD plots were permanently flooded as CF from rice flowering, i.e. on day 96 in 2015 and 89 in 2017 (i.e. 50% of the plants between beginning of panicle emergence and beginning of anthesis stage). The water managements lead to an irrigation water application of 2 511 for CF and 1 435 mm for AWD as assessed in 2015. AWD appears to cause a slight increase in the hardness of the paddy soil in the top 16 cm or so as measured at the end of the growing season in 2015 (supplementary material). The largest differences in soil hardness between the two water managements were found at 10 cm depth, where the penetration resistance was 1039 ± 60 KPa and 836 ± 60 KPa in AWD and CF, respectively.

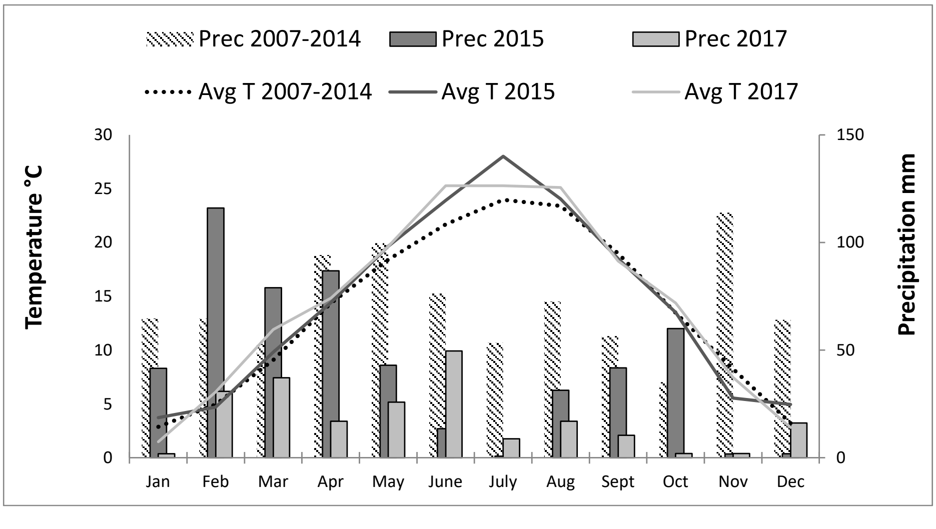

Figure 1. Monthly average temperatures (Avg T) and monthly precipitations (Prec) in 2015 and 2017 and average of 2007-2014 period measured at Cascina Boraso research station.

3.2. Yield and yield components

Water management effect was not significant for GrainYield, StrawProd, PanicleNumber and HeadRice, while it was significant for Grain/Panicle and HarvestIndex (Table 2). Compared with CF, the AWD treatment showed a significant decrease in Grain/Panicle (1.67 vs 1,85 g panicle^{-1}) and HarvestIndex (0.499 vs 0.516) (Figure 2). While Year and Water management x Year effects were not significant, a highly significant Year x Genotype interaction was detected for GrainYield as well as for PanicleNumber, StrawProd, HarvestIndex and HeadRice. Arelate, Gleva and Centauro exhibited the highest values of GrainYield in 2015 (936, 934 and 900 g m^{-2}, respectively) and Gleva in 2017 (1.159 g m^{-2}), while the lowest values were detected for Vialone nano in 2015 (729 g m^{-2}) and Baldo in 2017 (746 g m^{-2}). Arelate belonged to the first productivity group for HeadRice yield in both years (582 g m^{-2} on average) and Gleva showed the highest value in 2017 (671 g m^{-2}), while Loto, Vialone nano, and Baldo belonged to the less productive group in both years, with average values of 451, 432, 424 g m^{-2}, respectively. PanicleNumber and Grain/panicle traits were significantly and negatively correlated (R^2 = 0.747).

Table 2. Results of ANOVA for yield and yield components parameters.

	Grain Yield	**Straw Prod**	**Harvest Index**	**Head Rice**	**Panicle Number**	**Grain/ Panicle**
Water management	*ns*	*ns*	*0.0352*	*ns*	*ns*	*0.0488*
Year	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Genotype	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*
Block	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Water management x Year	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Year x Genotype	*0.0025*	*0.0023*	*<.0001*	*0.0077*	*<.0001*	*ns*
Water management x Genotype	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

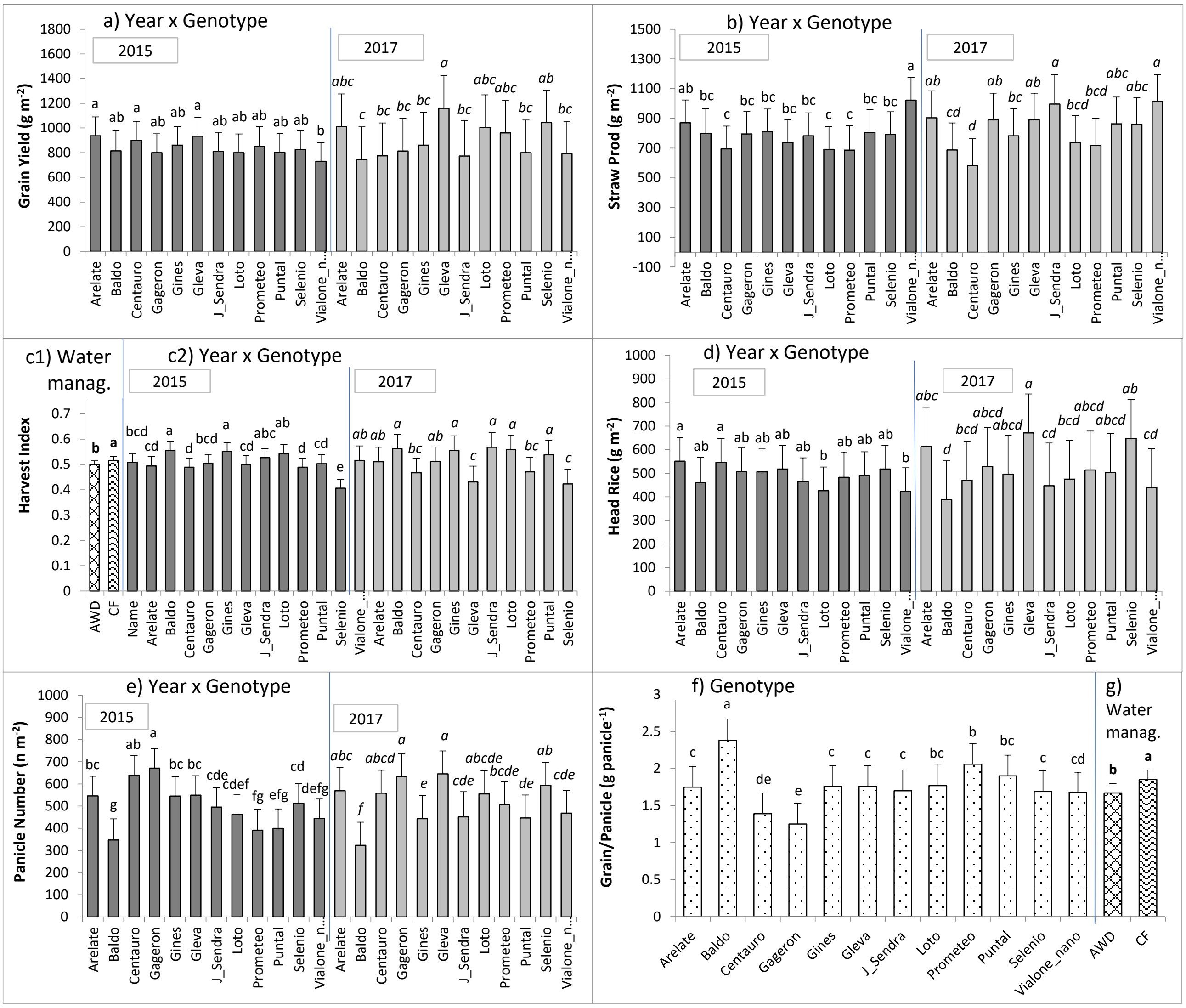

Figure 2. Yield and yield components parameters. Values are reported for effects highlighted as significant by ANOVA: Genotype x Year interaction (Figure a–e), Genotype (Figure f) and Water management (Figure g). Letters show significant difference for pF<0,05; error bars represent the upper confidence interval.

3.3. Plant phenotyping: Phenology and morphology.

The results related with the growth cycle duration (Table 3) showed significant (P<0,05) Year, Genotype and Year x Genotype effects, while Water management, Water management x Year and Water management x Genotype were not significant. In more detail, a longer growing cycle was detected in 2015 compared with 2017 (total average of 155.9 vs 140,5 days from sowing to maturity and 93.1 vs 88,8 days to flowering, respectively: data not shown). J. Sendra and Vialone nano showed the longest total growth cycle in 2015 (175 and 173 days) and J. Sendra also in 2017 (166 days), while Loto the shortest in both years (138 and 123 days) (Figure 3). Moreover, the DaysToFlowering parameter was also affected by Block, with a higher value in the first block nearest the inlet channel compared with the farthest one (93.4 vs 87.9 days), while the other blocks showed intermediate values (91.8 and 90.7 days). With regards to the measured morphological parameters (Table 3), NodeHeight was significantly affected by Year, Genotype, Water management x Year and Year x Genotype, while PanicleLength was only significantly affected by Genotype. NodeHeight was higher in 2015 than in 2017 (77.7 vs 73.1 cm, respectively), and AWD in 2017 (69.4 cm) showed a lower value with respect to 2015-CF and 2015-AWD (78.0 and 74.4 cm, respectively) (data not shown). The tallest variety of the panel was Vialone nano and the shortest was Gleva, 125.3 and 65.7 cm of TotalHeight, respectively. Puntal, Prometeo and Vialone nano belonged to the group with the longest panicle (18.7 cm on average) and Gleva (12.1 cm) the shortest.

Table 3. Results of ANOVA for parameters related with crop phenology and morphology.

	Days to Flowering	**Flowering to Maturity**	**Days to Maturity**	**Total height**	**Node height**	**Panicle lenght**
Water management	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Year	*0.0065*	*<.0001*	*<.0001*	*0.0398*	*0.0179*	*ns*
Genotype	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*
Block	*0.026*	*ns*	*ns*	*ns*	*ns*	*ns*
Water management x Year	*ns*	*ns*	*ns*	*ns*	*0.0499*	*ns*
Year x Genotype	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*ns*
Water management x Genotype	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

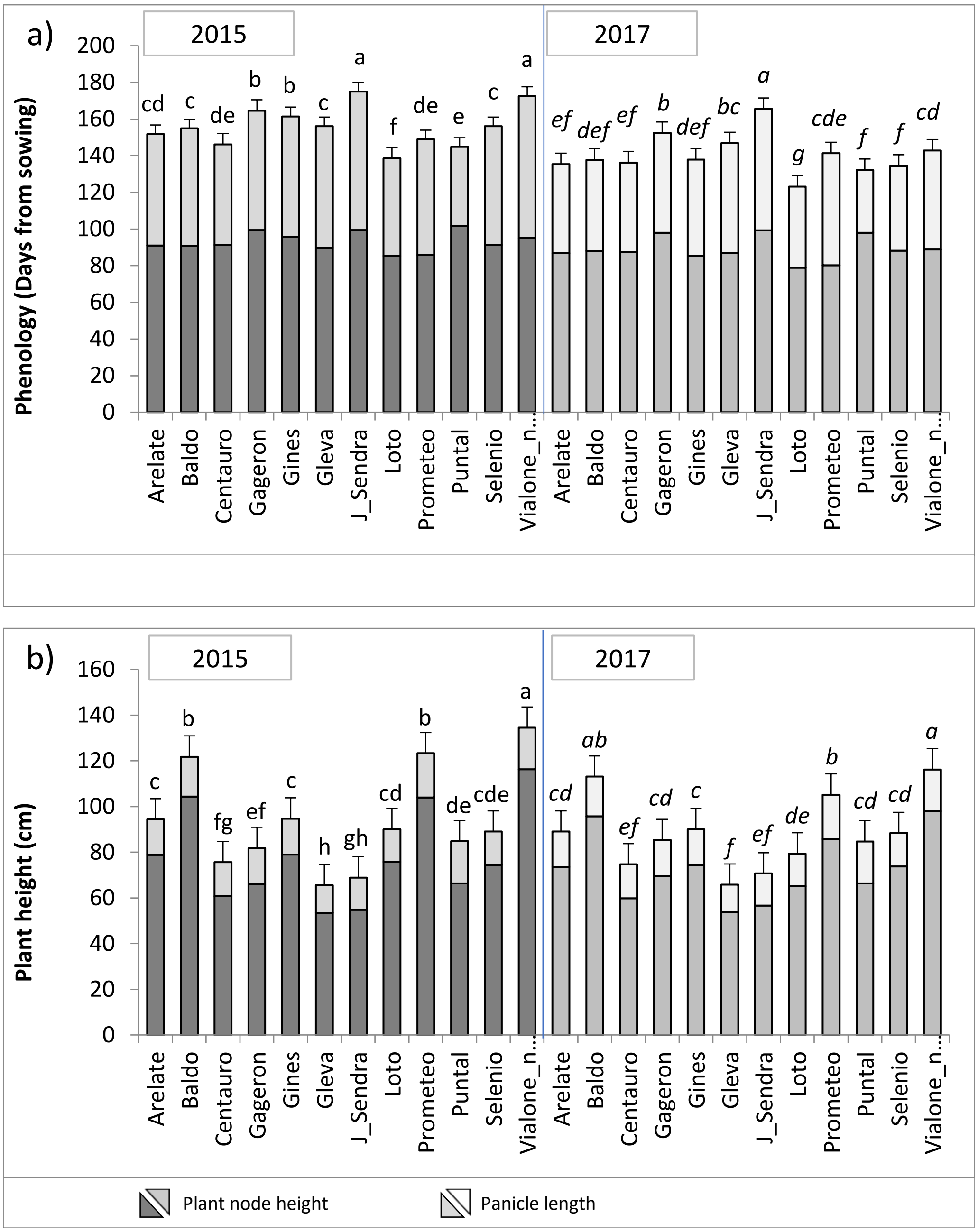

Figure 3. Phenology (a) and morphology (b) parameters. Values are reported for Genotype x Year effect. Letters refer to Days to maturity (a) and Total height (b), and show significant difference for pF<0,05. Error bars refer to Days to maturity (a) and Total height (b) and represent the upper confidence interval.

3.4. Root biomass, N uptake and physiological parameters.

RootBiomass, GrainNuptake and StrawNuptake were not significantly affected by Water management (Table 4). RootBiomass was significantly affected by Year, with 2017 average value higher than 2015 (573 vs 346 g m^{-2}, respectively), GrainNuptake and StrawNuptake were significantly affected by Genotype, and StrawNuptake also by Water management x Genotype. In more detail, the highest GrainNuptake was measured for Arelate (116 kgN ha^{-1}) and the lowest for Vialone nano and J.Sendra (77.0 and 76.6 kgN ha^{-1}), these two having the highest StrawNuptake considering only the Genotype factor (53.1 and 53.8 kgN ha^{-1}, respectively) (Figure 4). Puntal showed a higher StrawNuptake in CF than in AWD, which were equal to 61.7 and 34.2 kgN ha^{-1}, respectively, and correspond to an average 88±30 kgN ha^{-1} of GrainNuptake (data not shown). TotalNuptake was not affected by any factor and was on average equal to 137 kg N ha^{-1}. NitrogenBalanceIndex, Chlorophyll and Flavonoids contents, as assessed by leaf light absorbance, were not affected by Water management while highly significantly affected by Year and Genotype. Flavonoids was also affected by Water management x Year, Year x Genotype and Water management x Genotype. Year 2017 showed higher values than 2015 for Chlorophyll (30.0 vs 27.7 µg cm^{-2}) and Nitrogen Index (11.1 vs 9.2); for Flavonoids, 2015 average was higher than 2017 (1.48 vs 1.36µg cm^{-2}) and AWD was higher than CF in 2017 (1.39 vs 1.34 µg cm^{-2}).

Table 4. Results of ANOVA for parameters related with root biomass, N uptake and crop physiology.

	Root Biomass	**Straw N uptake**	**Grain N uptake**	**Total N uptake**	**Chlorop hyll**	**Flavonoi ds**	**Nitrogen Index**
Water management	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Year	*0.0001*	*ns*	*ns*	*ns*	*0.0066*	*<.0001*	*0.0001*
Genotype	*ns*	*<.0001*	*0.0016*	*ns*	*<.0001*	*<.0001*	*<.0001*
Block	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Water management x Year	*ns*	*ns*	*ns*	*ns*	*ns*	*0.019*	*ns*
Year x Genotype	*ns*	*ns*	*ns*	*ns*	*ns*	*0.014*	*ns*
Water management x Genotype	*ns*	*0.0221*	*ns*	*ns*	*ns*	*<.0001*	*ns*

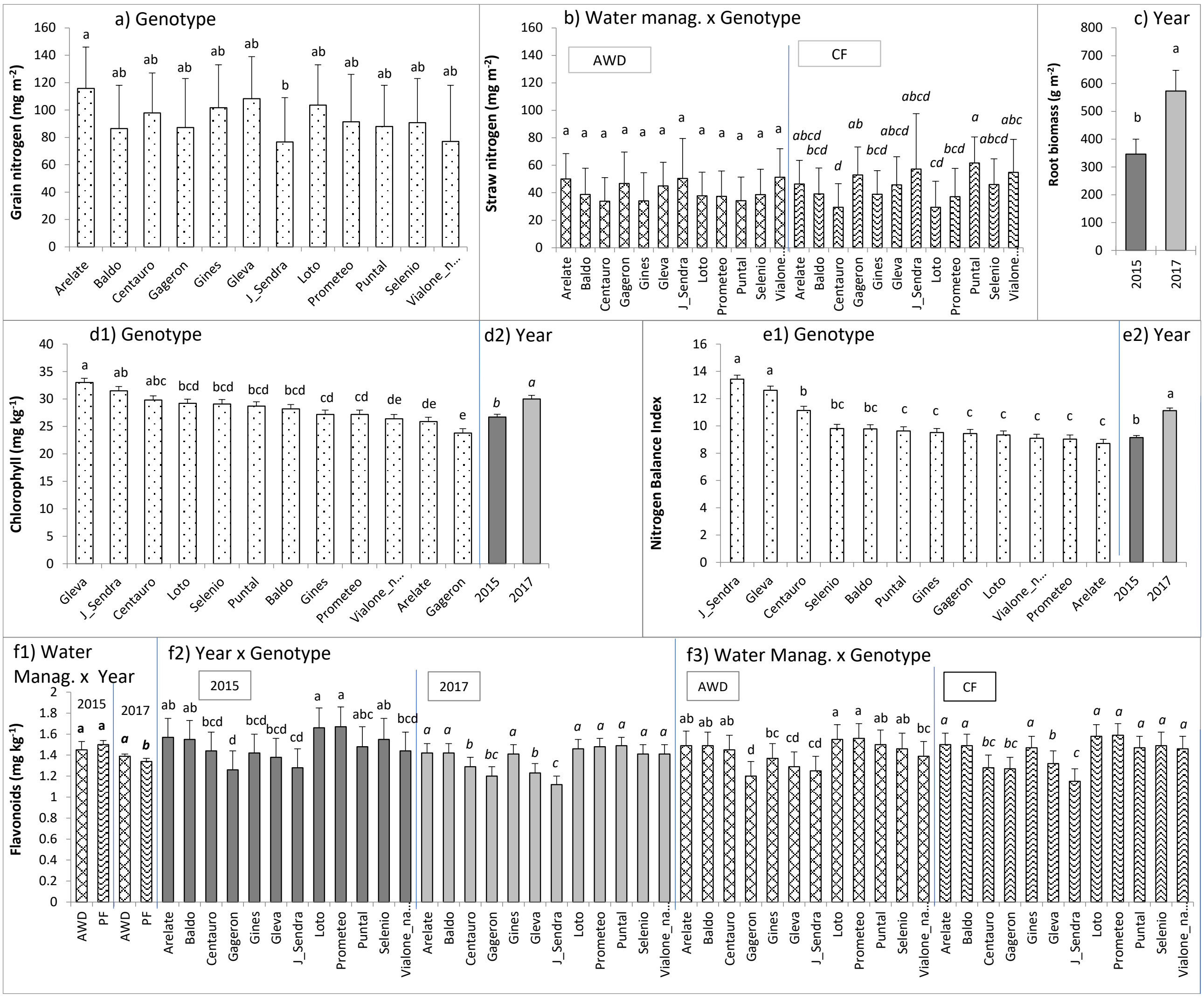

Figure 4. Grain nitrogen (a), Straw nitrogen (b), Root biomass (c) and physiological (d-f) parameters. Values are reported for effects highlighted as significant by ANOVA: Genotype (a, d1, e1), Year (c, d2, e2), Water management x Year (f1), Year x Genotype (f2) and Water management x Genotype (b, f3). Letters show significant difference for pF<0,05; error bars represent the upper confidence interval.

3.5. Element contents

Rough grain analysis highlighted Water management factor significant for Zn, As and Cd, Water management x Year interaction for Mg and Water management x Genotype for As and Cd. Moreover, Year, Genotype and Year x Genotype factors were highly significant for all considered elements (Table 5). In more detail, a higher GrainAs was detected in CF than in AWD (0.236 vs 0.147 mg kg^{-}

^{1}) and a higher GrainCd in AWD than in CF (0.135 vs 0.075 mg kg^{-1}) (Figure 5). These treatment differences varied among varieties. In CF treatment, J. Sendra, Gageron and Gines showed the lowest GrainAs content, (0.198, 0.183 and 0.181 mg kg^{-1}, respectively), and Selenio and Prometeo the highest (0.292 and 0.290 mg kg^{-1}, respectively). In the AWD treatment, J. Sendra and Gines showed the lowest GrainCd values (0.097 and 0.091 mg kg^{-1}, respectively) and Baldo, Vialone nano and Prometeo the highest (0.175, 0.171 and 0.165mg kg^{-1}, respectively). The grain concentration of As and Cd were also affected by the year, with 2015 values higher than 2017 for both elements (i.e. 0.235 vs 0.148 mg of As kg^{-1} and 0.129 vs 0.081 mg of Cd kg^{-1} in the two years). Mg content was higher in 2017 than 2015 (1107 vs 907 mg kg^{-1}), but year difference was emphasized in the AWD treatment (data not shown). Water management caused a slightly higher Zn content in AWD than CF, with values equal to 21.5 and 20.0 mg kg^{-1}, respectively.

Table 5. Results of ANOVA for rough grain elements.

	Grain Mg	**Grain Zn**	**GrainAs**	**GrainCd**
Water management	*ns*	*0.0418*	*<.0001*	*0.0005*
Year	*<.0001*	*<.0001*	*<.0001*	*0.0014*
Genotype	*<.0001*	*<.0001*	*<.0001*	*<.0001*
Block	*ns*	*ns*	*ns*	*ns*
Water management x Year	*0.019*	*ns*	*ns*	*ns*
Year x Genotype	*0.0082*	*0.0119*	*0.0035*	*0.0051*
Water management x Genotype	*ns*	*ns*	*<.0001*	*<.0001*

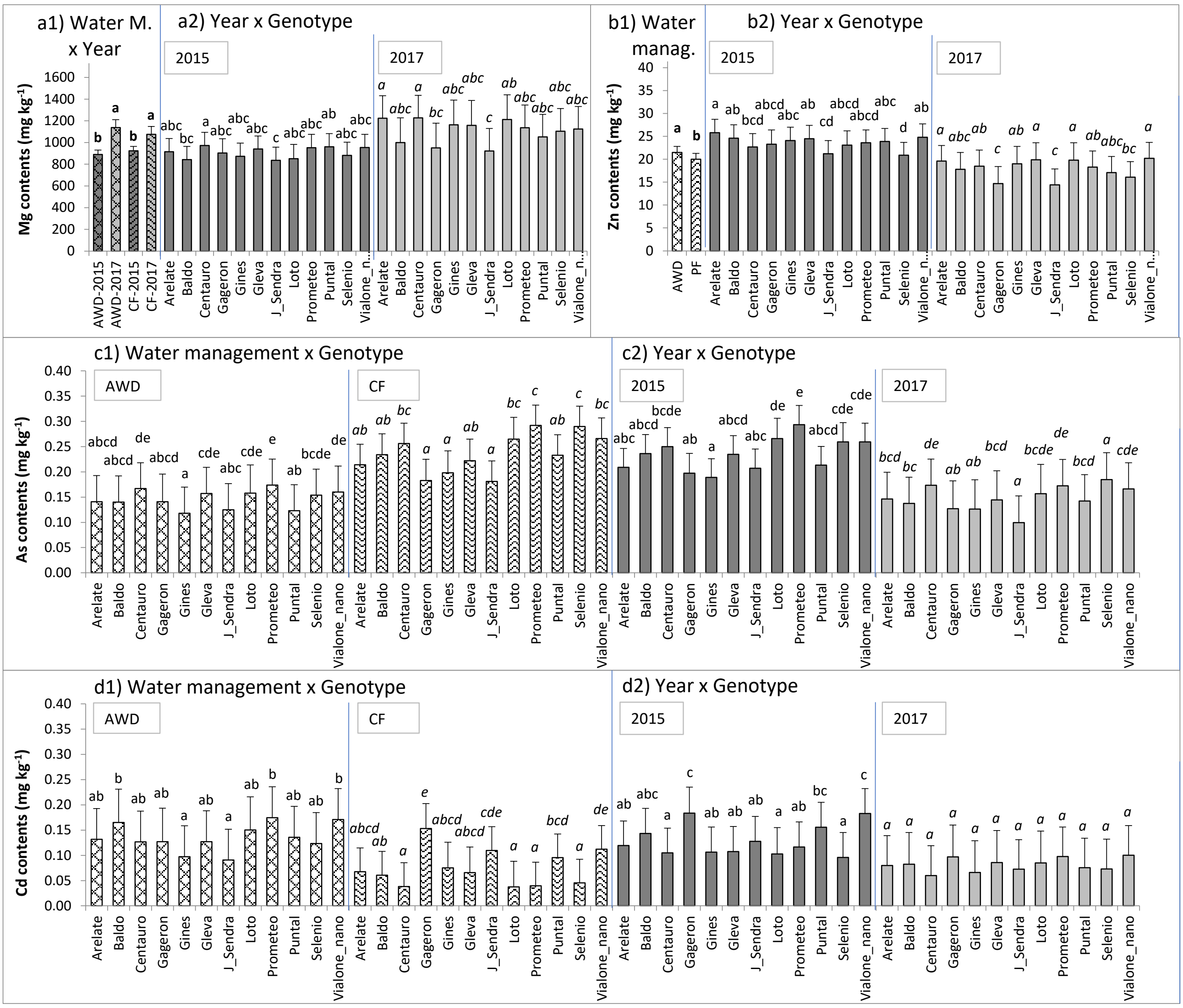

Figure 5. Elements contents in rough grain. Values are reported for effects highlighted as significant by ANOVA: Water management x Year (a1), Water management x Genotype (c1, d1) and Year x Genotype (a2, b2, c2, d2). Letters show significant difference for pF<0.05; error bars represent the upper confidence interval.

4. Discussion

A panel of 12 representative European varieties were evaluated at field scale for their potential adaptation to a mild AWD system, consisting in avoiding permanent flooding conditions while limiting water stress from sowing to flowering, and then maintaining ponded water as in the continuously flooding system. About 1 435 mm of water irrigation was estimated in this experiment in AWD which is in the range calculated by Mayer et al. (2019) for AWD in the Northern Italian rice area (i.e. from 964 to 1 620 mm depending on groundwater table depth) and very similar to the average at field scale for the entire irrigation district (i.e. 1·394 mm). In Mayer’s study, AWD system was applied for the total rice growing cycle with short irrigation cycles (7 days). Considering our CF reference system, with dry seeding and about 1 month of delay for permanent flooding, Zhao et al. (2015) reported an average water consumption of 1 602 mm and a range of 1·037-2·167 mm measured in Vercelli at field scale in a silty soil, which is consistent with our estimate of 2·510 mm in a sandy-loam soil with reduced infiltration due to the applied soil tillage. Moreover, Carrijo et al. (2017) reported an average water saving of 23.4% with mild AWD compared with CF in a meta-analysis study. A water use reduction of 40.7% was assessed in our experiment at field scale, but this higher estimate could be the result of a shallow water table depth which allowed to have only 4 AWD cycles during the vegetative phase, each lasting on average 15.8 days in 2015 and 13.5 days in 2017.

AWD caused a slight (approx. 25%) but highly significant increase in penetration resistance in at least the top 20 cm of the soil which was maximal at 10 cm depth. This is consistent with increases in the top 12 cm observed after AWD in Bangladesh (Norton et al. 2017a) and demonstrates that AWD alters the soil physical properties in a plastic way. This was confirmed in experiments with small cores by Fang et al. (2018) and is driven by shrinkage-induced consolidation caused by the drying stress (Yoshida & Hallett 2008). It is not likely that this increase in penetration would greatly impact root growth since resistances of higher than 1.5 MPa are considered inhibitory in most soils (Bengough 1997).

The applied AWD system in this experiment did not cause any significant effect on paddy and head rice yield, also considering the interaction with variety and year factors, although a general tendency of a slight decrease in yield due to AWD with respect with CF was detected (842±69 vs 891±69 g m^{-2}) and confirmed by the evidence of a reduction in grain weight per panicle and harvest index in AWD. The result was probably due to the application of a mild AWD system aimed at minimizing yield losses while allowing water saving and the creation of soil aerobic conditions with expected positive effects on CH_4 emissions reduction and As bioavailability. This result agrees with those found in the above-mentioned meta-analysis (Carrijo et al., 2017**)**, which reported that when AWD is not carried out for the whole season, there was no yield reduction, with AWD relative yield of −0.5%, while when AWD is practiced throughout the whole season, yield decreases of 8.1% are observed. No effect of AWD on yield and yield components was found considering each variety separately as Water management x Genotype was not significant for any parameter of this group. Norton et al. (2017b) found no interaction between water management and cultivar in a field experiment with 22 rice *indica* when yield and yield components were considered, a result also confirmed in nearly 300 cultivars (Norton et al., 2018). Our results seem to confirm that, in a mild AWD system, characteristics of differential adaptation to water stress of diverse rice varieties, as those highlighted under more severe water stress (Volante et al., 2017; Hassen et al. 2017), have been very little or not expressed. Averaged across irrigation systems, there were highly significant differences among the 12 *japonica* cultivars on yield traits (grain, straw yield and harvest index) and it is worth highlighting that two varieties that are widely grown in different rice regions (i.e Gleva – medium grain - in Spain and Arelate - long A grain- in France) showed a very good performance in terms of both paddy and head rice production in the North Italy rice agroecosystem. The evidence of limited effects caused by AWD in this experiment was also confirmed by phenology and root parameters and pre-harvest measurements. Growth cycle duration, chlorophyll content, Nitrogen Index and roots were not influenced by water management but only by year, genotype, and year x genotype interaction. Only panicular node height was also affected by year x water management interaction, and flavonoids contents by year x water management and water management x genotype. These two parameters, together with grain per panicle weight and harvest index, seem to be the most sensitive parameters to water management. We can hypothesize that a moderate water stress, imposed only during the pre-flowering phases, influences the development of the non-productive part of the plant indirectly by increasing or mitigating the effects of environmental conditions (i. e. year variation of temperatures and precipitation trend), while the differences are lost in the late phase when optimal water conditions are restored (no effect on total height and panicle length). The same is true for the flavonoid synthesis which is a known stress response, to an extent which is variable depending on the variety (Buer et al. 2010; Fini et al., 2011; Isshiki et al. 2014; Rayee et al., 2020). Similar considerations apply to straw N uptake which showed water management x genotype effect, while grain N concentrations were only affected by genotype; in this case an effect of water treatment was again observed on a trait mainly connected with the pre-reproductive phase, while translocation and grain N content primarily showed a genetic control. Beyrouty et al (1997) also found reduced N uptake with water deficit stress prior to panicle differentiation in Puntal. With regards to the effects of water management on Mg, Zn, As and Cd accumulation in rough grain, all considered elements were affected, either alone (i.e. Zn) or in combination with Year (i.e. Mg) or Genotype (i.e. As and Cd). As expected, AWD management caused a reduction in As accumulation into grains and an increase in Cd contents compared with CF. The scale of average AWD impact on lowering grain arsenic reported here (i.e. average of 38%) is rather high compared to the range, depending on years, sites and cultivars, of 14-26%, 0-16% and 15-18% reductions reported for *indica* rice in Bangladesh by Norton et al. (2017a), Norton et al. (2017b) and Norton et al. (2019), respectively. The impact of AWD on substantially increasing grain cadmium by about 80% is also consistent with the Bangladesh studies cited above, where, for example 43% increases are reported in 22 cultivars (Norton et al., 2017b) and 49% and 108% increases are reported in nearly 300 cultivars in two different years (Norton et al., 2021). Cattani et al. (2008), reported an increase of 80-160% in Cd content into brown rice in Italy comparing flooded and dry conditions and a range of 0.05-0.14 mg kg^{-1} of Cd into grain under flooding, which is consistent with 0.075 mg kg^{-1} of Cd measured in our experiment in CF. Moreover, it is very important to highlight the varying genotype responsiveness to AWD in terms of As and Cd accumulation as it was possible to differentiate among variety responses to water management. In more detail, J. Sendra and Gines appeared more suitable for AWD for the lower Cd contents, and J. Sendra and Gageron in CF for the lower As concentration. A different response of varieties to AWD was also found by Norton et al. (2017b) for Cd but not for As. Considering the results on Italian varieties, Somella et al. (2013) reported an average total As concentration of 0.28 mg kg^{-1} for Vialone Nano grain from market outlets, similar to those measured in our experiment (i.e. 0.27 mg kg^{-1} in CF).

5. Conclusions

The results of this field experiment showed that, under pedoclimatic conditions and rice farming systems of Northern Italy rice area, it is possible to apply a mild AWD technique, maintained only during the vegetative phase, with very limited effects on crop status and final productivity. The safety of this mild AWD is appliable to a panel of 12 representative European rice cultivars which showed comparable adaptabilities among them in terms of both yield and yield components. Therefore, varieties developed in other European regions represent a valuable option for cultivation under both water managements. Our study confirms the significant effect of AWD on element concentration in grain, that is the decline in As but increase in Cd concentration with varying degrees of response among the studied cultivars. These findings have important implications in terms of food security.

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