


RESEARCH

Open Access



# Individuation of the best agronomic practices for organic durum wheat cultivation in the Mediterranean environment: a multivariate approach

Federica Carucci<sup>1</sup>, Giuseppe Gatta<sup>2</sup>, Anna Gagliardi<sup>2</sup>, Simone Bregaglio<sup>3</sup> and Marcella Michela Giuliani<sup>2\*</sup> 

## Abstract

**Background** The main challenge of organic cereal systems is ensuring high yields and grain quality while maintaining pedo-environmental sustainability. Despite the potential benefits of organic farming systems, a debated limitation is their actual contribution to food security. Durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.], one of the most important staple food crops, is mainly grown in the Mediterranean environments, where farmers have to face profound inter-annual fluctuations in productions, especially under organic system, due to prolonged drought and heat spells. With the overarching objective of deriving practical indications to support organic wheat production in the Mediterranean region, we tested the effect of nitrogen and sulphur-based organic foliar fertilizers on two ancient and two modern durum wheat varieties grown in two seasons (2018–2019) characterized by different weather conditions. Moreover, we evaluated the effect of a foliar application of Selenium at booting on grain yield and quality.

**Results** Results from the Principal Component analysis revealed that seasonal weather and the varietal choice determined most of the variability of yield and quality traits, while Selenium application markedly affected the performance of organic durum wheat, especially in the milder season. The Cluster Analysis computed on the Principal Components revealed three groups, representative of (i) the modern variety, Marco Aurelio, grown in the driest season (average yield, low protein content), (ii) all varieties grown in 2018, with the addition of sodium selenate (high yield, high protein content), and (iii) the ancient variety, Cappelli, grown in both seasons (low yield, average protein content).

**Conclusions** This study evidenced that tailored agronomic practices are needed to sustain the organic durum wheat systems in the Mediterranean area. The promising beneficial effect of Selenium would deserve a dedicated research program, where additional experiments should further investigate its impact on organic durum wheat yield and quality. The multivariate approach permitted us to identify the most effective agronomic practices in relation to different environmental conditions; the outputs from this study are ready to be transferred to organic farmers aiming at improving the performance of durum wheat systems and at providing an effective contribution to food security.

**Keywords** Foliar fertilization, Organic farming, Nitrogen, Selenium, Sulfur, PCA, Cluster analysis

\*Correspondence:

Marcella Michela Giuliani  
marcella.giuliani@unifg.it

Full list of author information is available at the end of the article



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

## Introduction

According to the International Federation of Organic Agriculture Movements (IFOAM), organic agriculture is a production system that sustains the health of soils, ecosystems, and people, producing high-quality food without using mineral fertilizers, synthetic pesticides, animal drugs, and food additives that may have adverse health effects [1].

Some studies reported that, with certain crops and under certain growing conditions, organic systems come closer to matching conventional systems in terms of yields [2, 3]. On the contrary, in several studies, crop yield averages are reported to be from 8% to 25% lower in organic systems than in conventional ones [4–7]. Organic cereals have about 26% of yield reduction in organic farming [8] and this aspect is crucial since cereal is known to be one of the main food sources for world population [9]. Furthermore, climate change has, and will increasingly have, severe impacts for agricultural production and food security [10], with uneven effects depending on the geographical area. For example, the Mediterranean basin has been identified as one of the most vulnerable regions to climate changes globally [11], being highly affected by increasing water scarcity and drought.

Durum wheat [*Triticum turgidum* subsp. *durum* (Desf.) Husnot] is an important cereal crop feeding humanity [12]. It is mainly grown in the Mediterranean environments [13], where farmers must face profound inter-annual fluctuations in yield and quality due to prolonged drought and heat events [14]. Furthermore, in the organic farming, these environmental stresses, combined with the limited soil N availability of organic systems [15, 16], are detrimental to yield formation, as leaf water relations and photosynthetic activity are impaired, leading to reduced growth rates, shortened grain filling period, and lower grain weights [17].

Since the EU political framework is pushing towards a wider adoption of organic farming in the coming years [18], researchers need to provide farmers with innovative and sustainable agronomic strategies to stabilize organic durum wheat yield and quality to contribute to food security. Within the organic sector, there is a high interest in heritage varieties of wheat, and old wheat varieties are claimed to possess better characteristics than modern cultivars in several respects [19]. Moreover, in literature is often reported that the modern varieties are usually unsuitable for organic systems [20] which needed dedicated breeding programs. Other authors find it difficult to develop separate breeding programs for organic crops, considering also that many breeding goals are the same for organic and conventional grains [19]. Indeed, modern breeding approaches aim at obtaining cultivars capable of high yield under sustainable agricultural conditions

and adapting to climate change [9]. Relative to organic nitrogen management, even if it is well-known that splitting mineral N application in conventional agriculture increases fertilization efficiency [21–23], topdressing or foliar fertilizations are not commonly used in organic farming. The synergistic effect of sulfur (S) and organic N soil fertilization could also lead to higher yields and better quality in durum wheat [24]. Still, their contemporary use as organic foliar fertilizers in organic durum wheat is almost unexplored. Besides macronutrients, the European Commission Regulation (EC) No. 889/2008 allows using trace elements in fertilizer formulations for organic production. Selenium (Se) is not listed among eligible trace elements, although its beneficial effects on stress tolerance [25, 26] and its positive action on plant productivity and nutritional quality have been widely documented [27–30], also on wheat [31, 32]. However, its use as ingredient in foliar fertilizer formulations has not been proposed yet in organic systems [33].

We conducted a 2-year field experiment, where four durum wheat varieties were grown under alternative organic farming practices in the Mediterranean area to identify the most promising on yield and quality traits. We evaluated the effect of N and S foliar applications from organic sources in combination with Se on yield, grain protein concentration, plant N content, dry plant biomass, and harvest index using multivariate analyses. Our study provides the first scientific report on the effectiveness of Se as foliar fertilizer on organic durum wheat, giving quantitative figures to evaluate its potential inclusion among the eligible trace elements in the European Organic Production Regulation.

## Materials and methods

### Experimental setup

Experimental field trials were conducted in 2017–2018 and 2018–2019 (2018 and 2019 hereafter) at the Research Centre for Cereal and Industrial Crops (CREA-CI) in Foggia, Southern Italy (41°46'N, 16°54'E), as reported by Carucci et al. [33]. Two old (Old Saragolla and Cappelli) and two modern (Marco Aurelio and Nadif) durum wheat varieties were grown on clay soil (United States Department of Agriculture Classification, Washington, DC, USA) (Table 1) according to standard organic farming practices.

The field experiment was arranged in a split–split plot design with three factors (variety, organic fertilization, Se application) and three replicates. The durum wheat variety was the main plot, the organic fertilization was the plot, and the selenium application was the sub-plot (10.2 m<sup>2</sup>). The fields chosen for experimental trials were homogeneous and without preceding crop (set-aside). The sowing dates were 1st December (2018) and 24th

**Table 1** Main soil physical and chemical properties of the experimental fields in 2018 and 2019

Soil properties	Unit	2018	2019
Sand	%	11.4	15.4
Silt	%	39.6	34.9
Clay	%	49	49.7
Total N (Kjeldhal method)	‰	1.3	1.1
Mineral N <sup>a</sup>	mg kg <sup>-1</sup>	15.9	19.2
Available P (Olsen method)	mg kg <sup>-1</sup>	62	68
Exchangeable K (Ammonium acetate method)	mg kg <sup>-1</sup>	422	450
Organic matter (Walkley–Black method)	%	2.5	2.6

<sup>a</sup> Mineral N was determined at 0.3 m soil depth in pre-sowing as the sum of nitrate and ammonium content [56]

November (2019). Sowing was performed at a seeding rate of 350 germinable seeds m<sup>-2</sup>. Four fertilization strategies were evaluated: (1) control (CTR), where 50 kg ha<sup>-1</sup> of dry blood meal was applied at sowing; (2) CTR, plus 45 kg ha<sup>-1</sup> of foliar S applied at flag leaf stage (BBCH 47, CTR+S); (3) CTR, plus 45 kg ha<sup>-1</sup> of foliar N applied at heading (BBCH 51, CTR+N); (4) CTR, plus N and S foliar application at flag leaf and heading stages, respectively (CTR+NS). The effect of Se application was evaluated by comparing Se0, without selenium application, and Se60, where one foliar application of sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>), at the rate of 60 g ha<sup>-1</sup> [34] was applied at booting stage (BBCH stage 41). Foliar fertilizers were applied with a hand-held knapsack sprayer. All agricultural practices were performed according to the organic practices commonly adopted by local farmers, following the European Council Regulation (EC) No. 834/2007. A weather station close to the experimental field recorded daily precipitation and temperature. In the 2018 and 2019 growing seasons, accumulated precipitations were 401 mm and 299 mm, and average temperatures were

13.5 °C and 11.7 °C, respectively. Figure 1 reports precipitation and temperature trends in the 2 years compared with the long-term average (2000–2017) (source: NASA POWER database [35]).

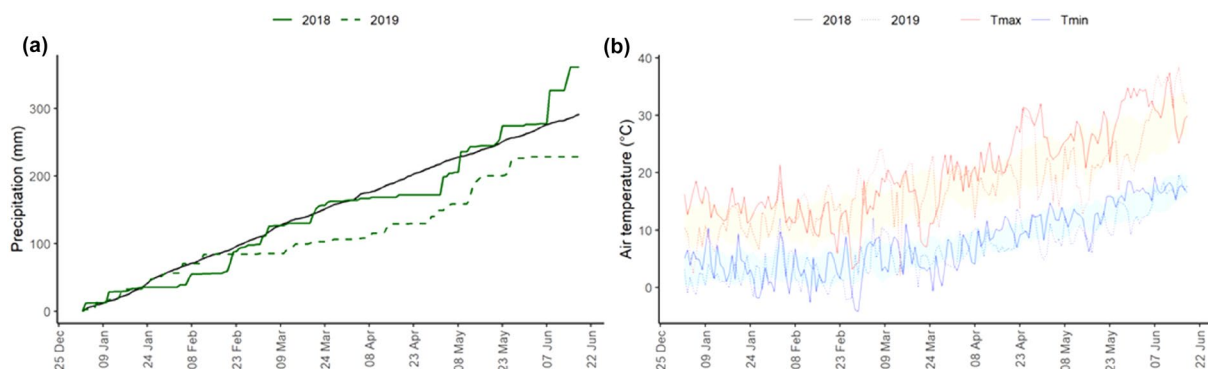
#### Determination of yield, grain protein concentration, plant N content, plant dry weight, and harvest index

At physiological maturity (BBCH stage 87), on 0.5 linear meters, plants were taken in two adjacent rows, cutting off the shoots at the crown level and separating them into straw and grain.

Plant dry weight was determined by oven drying the samples at 65 °C until constant weight. All samples were grounded using a Cyclotec Sample Mill 1093 (Foss Tecator, Hillerød, Denmark). N concentration in straw and grains was determined triplicate using Leco CHNS 628 Analyzer (Leco corporation, St. Joseph, Michigan); N content was computed as the product of dry weight and N concentration. Total plant N content was derived as the sum of N content of the straw and grain. Finally, Harvest Index (HI) was computed as the ratio of grain weight to aboveground dry matter [36]. At full maturity (11% humidity, on 29 and 18 June in 2018 and 2019), the crop was machine-harvested, and the yield was evaluated. Grain protein concentration (GPC, %) was determined on grain samples by near-infrared reflectance spectroscopy (Infratec 1229, Foss Tecator, Hillerød, Denmark).

#### Statistical analyses

Multivariate analyses were performed on the five durum wheat traits (i.e., yield, grain protein concentration, plant N content, plant dry weight, and harvest index), considering the four genotypes, the four organic fertilization, and the two Selenium applications over the 2 years as additional descriptors [37]. A correlation analysis followed by a Principal Component Analysis (PCA) was



**Fig. 1** Daily accumulated precipitation (mm) (a) and air temperature (°C) (b) in 2018 and 2019 (1st January–20th June). The black line (a)—average accumulated daily precipitations from 2000 to 2017. Shaded areas (b)—daily average mean  $\pm$  standard deviation for Tmax (orange) and Tmin (cyan) in 2000–2017

performed using all experimental traits (yield, grain protein concentration, plant dry weight, plant *N* content, and harvest index) as active quantitative variables. The variables were centered and scaled before the PCA through diagonalization of the correlation matrix and extraction of the associated eigenvectors and eigenvalues. All tested factors (growing season, variety, organic fertilizer, and Se application) were used as qualitative supplementary variables in the PCA, i.e., they did not contribute to the computation of Principal Components (PC). Their coordinates were calculated as the barycentre of the corresponding individuals in the PC space. We then applied a non-supervised Hierarchical Clustering on Principal Components (HCPC) using Euclidean distance and Ward’s criterion to identify groups of data showing similar behavior. The cluster’s mean of any experimental factor ( $\bar{X}_q$ ) was tested under the null hypothesis that the distribution of  $\bar{X}$  did not vary across clusters (Eq. 1):

$$u = \frac{\bar{X}_q - \bar{X}}{\sqrt{\frac{S^2}{n_q} \left( \frac{N-n_q}{N-1} \right)}} \tag{1}$$

where  $n_q$  is the number of experimental data in cluster  $q$ ,  $N$  is the total number of data, and  $S$  is the global standard

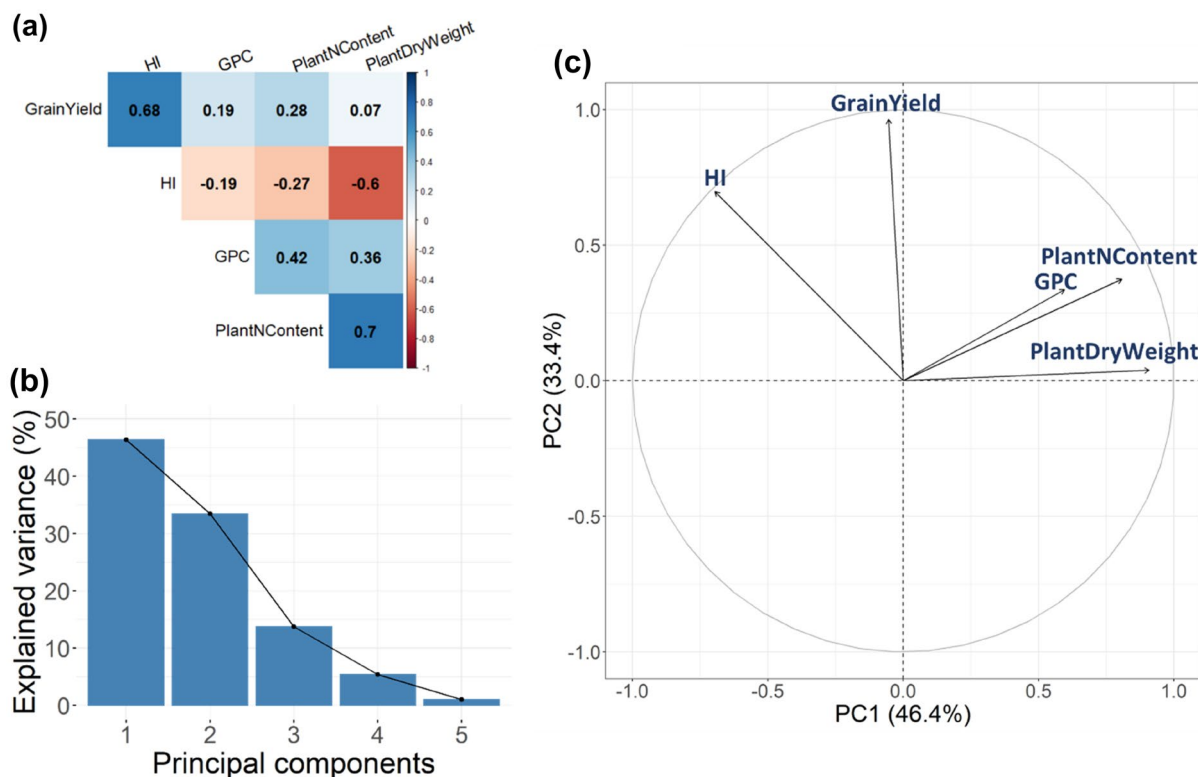
deviation. A  $\nu$  test was computed to characterize the clusters considering both active and supplementary variables under the null hypothesis (H0) that the cluster average did not differ from the overall average. The sign of the  $\nu$  test statistic indicates an under- (–) or over- (+) representation within the cluster. All statistical analyses were performed under the R 4.0.3 environment [38], FactoMineR package [39] for PCA and cluster analysis, and ggplot2 [40] package for boxplot analysis and graphical representations.

## Results

### Principal component analysis

All Pearson correlations among durum wheat traits were significant at  $p \leq 0.05$ , except for the correlation between yield and plant dry weight (Fig. 2a). Strongest positive correlations emerged between plant *N* content and plant dry weight (0.7), and between yield and HI (0.68), whereas plant dry weight was negatively correlated with HI (–0.6). GPC was positively correlated with plant *N* content (0.42), plant dry weight (0.36), and yield (0.19) and negatively with HI (–0.19).

The first two components, explaining 79.8% of the total variance, were retained in the analysis (Fig. 2b). Next, the characterization of PCs was performed by



**Fig. 2** Correlation matrix with Pearson’s  $r$  values (A), scree plot (B) of the principal component analysis, and biplot of variables (C) of durum wheat grain yield, grain protein concentration (GPC), plant *N* content (PlantNContent), plant dry weight (PlantDryWeight), and harvest index (HI)

**Table 2** Correlation coefficients between active quantitative variables, supplementary qualitative variables, and the first two Principal Components (PC), with indication of the explained variance

Variable	PC1	PC2
<i>Quantitative active variables</i>		
Yield	−0.05ns	0.96***
GPC	0.59***	0.34***
Plant N content	0.81***	0.37***
Plant dry weight	0.91***	0.03ns
HI	−0.70***	0.70***
<i>Qualitative supplementary variables</i>		
Growing season	0.40***	0.58***
Variety	0.49***	0.60***
Organic fertilizer	0.11ns	0.11ns
Selenium application	0.03ns	0.18***
Explained variance	46.4%	33.5%

ns, not significant

Significance codes: \*\*\* $p < 0.001$ 

calculating correlation coefficients with active and supplementary variables and the associated significance level (Table 2).

The first PC (PC1) explained 46.4% of the total variance; it was positively correlated with GPC, plant N content, and plant dry weight and negatively with HI, whereas its correlation with grain yield was not significant (Table 2). Thus, PC1 could be considered as a “qualitative factor”. The second PC (PC2) explained 33.4% of the total variance and was highly correlated with grain yield and HI, whereas GPC and plant N content showed weaker correlations with PC2, even if significant (Table 2). Thus, PC2 could be considered as a “quantitative factor”. Among the supplementary qualitative variables, growing season and variety were significantly and positively correlated with PC1 and PC2, while Se application was significantly and positively correlated with PC2 (Table 2).

Relative to PC1 (“qualitative factor”), positive barycenter’s coordinates were observed for 2018 and for the old variety Cappelli, which showed the highest positive coordinate, while 2019 data and the modern variety Marco Aurelio obtained significant negative coordinates (Table 3). On the “quantitative factor” PC2, significant positive values resulted for 2018 data and for the modern variety Marco Aurelio, which obtained the highest positive coordinate, and for Se60 plots (Table 3). Finally, the barycenter of Cappelli and Old Saragolla was placed on the negative side of PC2, along with 2019 and Se0 (Table 3).

**Table 3** Barycenter’s coordinates of the supplementary qualitative variable levels in the first two Principal Components (PC1, PC2)

Factor	Level	Coordinate	
		PC1	PC2
Growing season	2018	<b>0.60</b>	<b>0.75</b>
	2019	<b>−0.60</b>	<b>−0.75</b>
Variety	Cappelli	<b>1.23</b>	<b>−0.73</b>
	Old Saragolla	−0.19	<b>−0.49</b>
	Marco Aurelio	<b>−0.75</b>	<b>1.28</b>
	Nadif	−0.29	−0.07
Organic fertilizer	CTR	0.10	0.09
	CTR+N	0.23	−0.17
	CTR+S	−0.12	−0.13
	CTR+NS	−0.21	0.20
Selenium application	Se0	0.05	<b>−0.24</b>
	Se60	−0.05	<b>0.24</b>

Significant values are reported in bold

CTR, control; CTR+N, control plus N foliar application; CTR+S, control plus S foliar application; CTR+NS, control plus N and S foliar application; Se0, no selenium application; Se60, one foliar application of sodium selenate

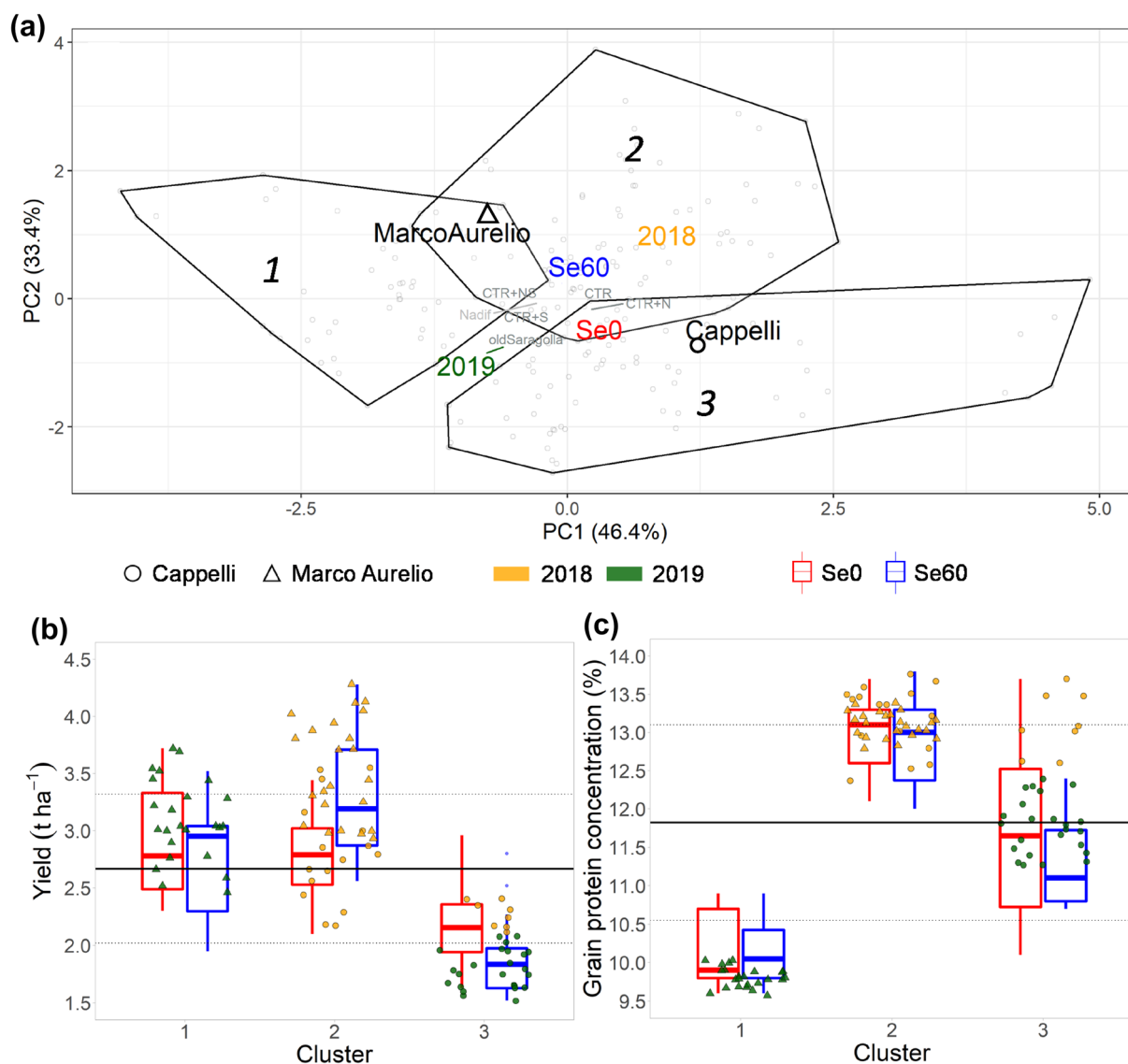
### Cluster analysis

Three clusters emerged from the hierarchical clustering performed on the extracted PCs (Fig. 3a). The clusters composition was characterized considering the representativeness of the qualitative and quantitative variables used in the PCA, using an alpha level  $\alpha = 0.05$  for all statistical tests. All quantitative variables significantly contributed to explaining the inter-cluster variance, with GPC ( $\eta^2 = 0.72$ ) and yield ( $\eta^2 = 0.62$ ) as the most relevant variables. Category frequency distributions within clusters for the qualitative variables highlighted that the growing season and the variety at  $p \leq 0.001$ , and Se application at  $p \leq 0.05$ , were significantly different from the overall frequency distribution according to  $\chi^2$  test, whereas organic fertilizer was not significant ( $p = 0.42$ ).

Cluster 1 (C1) was entirely composed of experimental data collected in 2019, and 55.8% of the data belonged to Marco Aurelio. Cappelli was absent from C1 (Table 4). This cluster was characterized by high HI, average grain yield, and low GPC (Table 5), and it was positioned on the negative side of the “qualitative factor” PC1 (Fig. 3a).

Cluster 2 (C2) grouped data from the 2018 growing season exclusively (Table 4). All varieties were equally represented in C2, with percentages ranging from 19.2% (Cappelli) to 28.9% (Marco Aurelio) (Table 4). A significant presence of the Se60 application was evident in C2, whereas Se0 data were significantly under-represented.

The values of all quantitative variables belonging to C2 were significantly higher than their average,



**Fig. 3** PCA biplot (a) with clusters delimitation (solid black lines and italics numbers); the barycenter of the supplementary variables most contributing to cluster variances are highlighted with colors (2018, orange; 2019, green; Se0, red; Se60, blu) and symbols (Cappelli, circle; Marco Aurelio, triangle). The other supplementary variables are reported in grey. Boxplots of distributions of yield (b) and grain protein concentration (c) resulting from the cluster analysis. Symbols and colors of boxplot charts reflect the visuals used in the PCA biplot

especially GPC and yield (Table 5). Data from C2 were mainly positioned on the positive side of both “qualitative” and “quantitative” factors PC1 and PC2 (Fig. 3a). Cluster 3 (C3) was the only cluster, where the two growing seasons were concurrently present, despite 80.3% of the data being collected in 2019. Nearly half of the data in C3 belonged to Cappelli, while Marco Aurelio was absent. Se0 treatment was over-represented (Table 4). This cluster was characterized by high plant dry weight, average GPC, and low HI and yield (Table 5), and it was positioned on the negative side of the “quantitative factor” PC1 (Fig. 3a).

Finally, focusing on Se application, a boxplot analysis was conducted using the distributions of yield and grain protein concentration, i.e., the key indicators of the value of durum wheat productions from a farmer’s perspective. In C1, Se application did not significantly affect yield ( $\bar{x}$  Se0 = 2.88 t ha<sup>-1</sup> with SD = 0.49 t ha<sup>-1</sup>;  $\bar{x}$  Se60 = 2.68 t ha<sup>-1</sup> with SD = 0.44 t ha<sup>-1</sup>) and GPC ( $\bar{x}$  Se0 = 10.2% with SD = 0.49%;  $\bar{x}$  Se60 = 10.1% with SD = 0.41%) (Fig. 3b, c). Conversely, in C2 Se application was determinant in increasing durum wheat yield, as Se60 treatment led to 3.32 t ha<sup>-1</sup> (SD = 0.51 t ha<sup>-1</sup>), which was 19.4% higher than the mean yield in Se0 ( $\bar{x}$  Se0 = 2.78 t ha<sup>-1</sup> with

**Table 4** Within-cluster distributions (Mod.Cla),  $v$  test, and  $p$  value of supplementary qualitative variables

	Cluster 1			Cluster 2			Cluster 3		
	Mod.Cla (%)	$v$ test	$p$ value	Mod.Cla (%)	$v$ test	$p$ value	Mod.Cla (%)	$v$ test	$p$ value
<i>Growing season</i>									
2018	0.0	-8.2	<0.001	100.0	13.4	<0.001	19.7	-6.2	<0.001
2019	100.0	8.2	<0.001	0.0	-13.4	<0.001	80.3	6.2	<0.001
<i>Variety</i>									
Cappelli	0.0	-5.0	<0.001	19.3	-1.6	0.1	48.5	5.3	<0.001
Old Saragolla	16.3	-1.5	0.1	25.3	0.1	0.9	30.3	1.2	0.2
Marco Aurelio	55.8	5.0	<0.001	28.9	1.1	0.3	0.0	-6.6	<0.001
Nadif	27.9	0.5	0.6	26.5	0.4	0.7	21.2	-0.9	0.4
<i>Organic fertilizer</i>									
CTR	20.9	-0.7	0.5	24.1	-0.3	0.8	28.8	0.9	0.4
CTR+N	16.3	-1.5	0.1	25.3	0.1	0.9	30.3	1.2	0.2
CTR+S	27.9	0.5	0.6	25.3	0.1	0.9	22.7	-0.5	0.6
CTR+NS	34.9	1.6	0.1	25.3	0.1	0.9	18.2	-1.6	0.1
<i>Se application</i>									
Se0	44.2	-0.9	0.4	42.2	-1.9	0.04	63.6	2.7	0.01
Se60	55.8	0.9	0.4	57.8	1.9	0.04	36.4	-2.7	0.01

CTR, control; CTR + N, control plus N foliar application; CTR + S, control plus S foliar application; CTR + NS, control plus N and S foliar application; Se0, no selenium application; Se60, one foliar application of sodium selenite

**Table 5**  $v$  test, mean in the cluster, overall mean, standard deviation (SD) in the cluster, overall standard deviation, and  $p$  value of the active quantitative variables

	$v$ test	Mean	Overall mean	SD	Overall SD	$p$ value
<i>Cluster 1</i>						
Yield, t ha <sup>-1</sup>	1.7	2.8	2.7	0.5	0.7	0.1
GPC, %	-9.8	10.2	11.8	0.4	1.3	<0.001
Plant dry weight, t ha <sup>-1</sup>	-6.8	6.7	8.5	1.1	2.0	<0.001
Plant N content, kg ha <sup>-1</sup>	-5.4	75.2	93.1	16.7	24.7	<0.001
HI	7.6	0.4	0.3	0.1	0.1	<0.001
<i>Cluster 2</i>						
Yield, t ha <sup>-1</sup>	7.8	3.1	2.7	0.5	0.7	<0.001
GPC, %	10.4	12.9	11.8	0.5	1.3	<0.001
Plant dry weight, t ha <sup>-1</sup>	1.7	8.8	8.5	1.3	2.0	0.1
Plant N content, kg ha <sup>-1</sup>	3.9	101.2	93.1	21.1	24.7	<0.001
HI	3.3	0.4	0.3	0.1	0.1	0.0
<i>Cluster 3</i>						
Yield, t ha <sup>-1</sup>	-9.7	2.0	2.7	0.3	0.7	<0.001
GPC, %	-2.3	11.5	11.8	0.9	1.3	0.02
Plant dry weight, t ha <sup>-1</sup>	4.2	9.4	8.5	2.4	2.0	<0.001
Plant N content, kg ha <sup>-1</sup>	0.6	94.7	93.1	27.1	24.7	0.5
HI	-10.1	0.2	0.3	0.1	0.1	<0.001

GPC, grain protein concentration; HI, harvest index

SD = 0.38 t ha<sup>-1</sup>) (Fig. 3b). The effect of Se application on GPC was negligible (Fig. 3c).

Finally, the effect of Se application in cluster 3 led to 13% decrease in mean yield ( $\bar{x}$ Se0 = 2.13 t ha<sup>-1</sup> with

SD = 0.31 t ha<sup>-1</sup>;  $\bar{x}$ Se60 = 1.88 t ha<sup>-1</sup> with SD = 0.31 t ha<sup>-1</sup>) (Fig. 3b) and a slight reduction in average GPC ( $\bar{x}$ Se0 = 11.7% with SD = 1.08%;  $\bar{x}$ Se60 = 11.3% with SD = 0.57%) (Fig. 3c).

## Discussion

In this study we combine Principal Component Analysis (PCA) and Cluster Analysis to give an analytic workflow capable to synthesize experimental evidence and current knowledge on organic wheat systems in semi-arid environments, entailing traditional and modern varieties, alternative foliar fertilization strategies and the addition of Selenium as bio-stimulant to plant metabolism to improve yield and quality response.

The occurrence of drought stress will likely be even more impacting in the coming years in the Mediterranean area [41], leading to a reduction of crop yield on major crops, with a negative impact on food security [10]. Wheat is one of the most important crops affecting global food security and is known as the source of food for more than 50% of the world's population. Since it often is a rainfed crop, prolonged period of water scarcity conditions severely compromises its grain yield [9]. In our field experiment, particularly harsh conditions occurred in 2019, which was characterized by very low precipitations, 299 mm, compared with 401 mm in 2018. The field data from 2019 obtained negative coordinates on both 'qualitative' and 'quantitative' PCA factors (PC1 and PC2, respectively) and grouped together in Cluster 1. However, Cluster 1 was mainly positioned on the positive side of the 'quantitative' PC2 factor, showing a slightly higher yield level than the overall mean due to the higher yield potential of Marco Aurelio. This result highlights that the choice of the variety Marco Aurelio has buffered the negative impact of water scarcity on quantitative parameters, such as yield and HI. Marco Aurelio is a modern variety released in 2010, recently approved for use in organic farming [42], and is among the highest yielding varieties. Thus, this result does not comply with the hypothesis that varieties that perform well under conventional farming may not perform well under organic management [20] and confirmed the assumption that modern varieties derive from breeding programs that aim to both satisfy food demand and support sustainable agricultural productivity for adaptation to climate change [9]. Besides, Marco Aurelio is also characterized by high variability in GPC. This latter aspect was confirmed by the negative coordinates obtained by Cluster 1 on the 'qualitative' PC1 factor, highlighting the detrimental impact of drought stress on GPC on this modern variety [43]. On the contrary, despite Cluster 3 grouped 80.3% of the data from the drier growing season, this Cluster was mainly positioned on the positive side of the 'qualitative' PC1 factor, showing a significative higher GPC value than the overall mean. This behavior can be attributed to the positive effect of Cappelli, the most represented variety in Cluster 3, on the qualitative traits. Indeed, Cappelli is an old variety (year of release 1915), selected from individual

lines from Italian, Syrian–Palestinian, and North African landraces [44], characterized by high stability levels of protein, dietary fiber, and antioxidants [45] also under water stress condition. Our results suggest that the varietal choice in organic durum wheat systems can be considered the most crucial agronomical factor, especially under water scarcity conditions like those foreseen in the coming years. Moreover, the varietal choice in organic durum wheat systems could reflect a different farmer's attitude. The modern variety Marco Aurelio is the right choice when high yield is sought. On the contrary, the old variety Cappelli seems to be the most feasible alternative when seeking stability in grain protein concentration, even accepting lower yields.

Cluster 2 showed the best quantitative and qualitative performance, since it included all data from the 2018, the milder growing season. Selenium application was selected as a determinant contributor to Cluster 2, where it was associated with about 20% yield increase, consistently on all varieties. To date, Selenium is not listed among eligible microelements in organic agriculture by the European Commission Regulation (EC) No. 889/2008. The rationale for including foliar Selenium application in our experimental trial relies on scientific evidence of its beneficial effects on plant stress tolerance [25, 46]. Our results agree with several authors who reported increases in grain yield grown under conventional agronomic systems after selenate foliar applications [27, 28, 30, 32], even if other authors did not report any significant effect [47–49]. On the contrary, the absence of beneficial effects of Selenium in the drier growing season disagree with studies conducted under conventional agronomic systems, in which late foliar applications of microelements demonstrated to enhance wheat growth parameters under drought stress only [50]. To date, the effect of foliar applications of micronutrients is still controversial [51] and requires further experimental insights and a careful case-by-case evaluation. Any deviation from the correct ratio of elements may lead to antagonism phenomena determining impairment of absorption and transport [52]. The decisive yield increase obtained in response to Selenium applications in our experiment claims for a more articulated research program. Alternative solutions, doses, and timing of applications have to be tested to evaluate the inclusion of Selenium in commercial formulations for organic agriculture.

Finally, we tested the effect of organic *N*- and *S*-based foliar fertilization on durum wheat for the first time, even if at a low *N* concentration in the solutions (4% of total *N*). Our choice was driven by the evidence that foliar *N* applications at heading demonstrated to be effective in improving wheat nutrition [21, 23], being leaves more efficient than roots at absorbing nutrients at late



development stages [53, 54]. However, the foliar organic fertilization did not significantly contribute to explaining the clusters' difference considering frequency distribution. These results suggest the need for further investigations to develop more effective organic foliar fertilizer formulations, particularly with increased *N* concentration. Moreover, recent trends in fertilizer costs, along with their scarcity on the international market, are shrinking crop yields and food security [55]. This situation and the need to foster the sustainability of the agricultural farming practices sector must push organic fertilizers as an alternative to massive mineral fertilizers.

### Conclusion and future studies

The debate regarding the role of organic agriculture remains open, particularly when related to food security and climate change [8]. We do agree with the idea that the conventional and organic systems do not have to necessarily be considered competing entities with each other nor necessarily be compared in terms of productivity [8]. However, considering the objective set by the European Commission to reach at least 25% of agricultural land in organic farming by 2030, it is crucial to investigate agronomic strategies capable of improving the productive response of organic systems and, therefore, their contribution to food security. This study provides practical agronomic information based on experimental evidence to support organic farmers in advancing their practices to sustain durum wheat yield and quality in the Mediterranean. We tested the effects of the main alternatives in the hands of farmers, from the varietal choice (two ancient and two modern wheat varieties) up to the possible foliar applications of nutrients. We then added Selenium to evaluate its possible bio-stimulant effect. This micro-nutrient, still not listed as an eligible nutrient in organic legislation, demonstrated its efficacy in the milder season. The analytic workflow based on multivariate statistical techniques proposed here permitted us to identify the most promising combination of agronomic practices according to different environmental conditions. Further experiments are needed to shed more light on these complex cropping systems, also considering the consequences of the adoption of agronomic management practices on the socio-economic and environmental sustainability.

### Abbreviations

ANOVA	Analysis of variance
N	Nitrogen
S	Sulfur
Se	Selenium
CTR	Organic fertilization with 50 kg ha <sup>-1</sup> of dry blood meal applied at sowing

CTR + S	Organic fertilization with 50 kg ha <sup>-1</sup> of dry blood meal applied at sowing and 45 kg ha <sup>-1</sup> of foliar <i>S</i> applied at flag leaf stage
CTR + N	Organic fertilization with 50 kg ha <sup>-1</sup> of dry blood meal applied at sowing and 45 kg ha <sup>-1</sup> of foliar <i>N</i> applied at heading
CTR + NS	Organic fertilization with 50 kg ha <sup>-1</sup> of dry blood meal applied at sowing, plus 45 kg ha <sup>-1</sup> of foliar <i>N</i> applied at heading and 45 kg ha <sup>-1</sup> of foliar <i>S</i> applied at flag leaf stage
Se0	No selenium foliar application
Se60	Foliar application of sodium selenate (Na <sub>2</sub> SeO <sub>4</sub> ) at the rate of 60 g ha <sup>-1</sup>
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
HI	Harvest index
GPC	Grain protein concentration
PCA	Principal component analysis
PC	Principal component
HCPC	Hierarchical clustering on principal components

### Acknowledgements

This research was part of project SOFT (Smart Organic Farming Techniques) financed under the PSR Puglia 2014–2020 funds, Measure 16—Cooperation, Submeasure 16.2—Support for pilot projects and the development of new products, practices, processes, and technologies (CUP B79J20000080009).

### Author contributions

Conceptualization, MMG. Methodology, MMG, GG and FC. Validation, MMG, GG, and SB. Formal analysis, FC, GG, MMG, and SB. Investigation, FC and AG. Writing—original draft preparation, FC. Writing—review and editing, MMG, GG, and SB. Visualization, AG and FC, and SB. Supervision, MMG, and GG. Project administration, MMG. All authors have read and agreed to the published version of the manuscript.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Availability of data and materials

The data sets used and/or analyzed during the current study will be available from the authors on reasonable request.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

#### Author details

<sup>1</sup>Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy. <sup>2</sup>Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, 71122 Foggia, FG, Italy. <sup>3</sup>Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), 40128 Bologna, BO, Italy.

Received: 5 August 2022 Accepted: 7 March 2023

Published online: 19 May 2023

### References

1. IFOAM; 2008. <https://www.ifoam.bio/why-organic/organic-landmarks/definition-organic#:~:text=in%20Vignola%2C%20Italy,-Organic%20Agriculture%20is%20a%20production%20system%20that%20sustains%20the%20health,of%20inputs%20with%20adverse%20effects>. Accessed on 23 Dec 2021.

2. Denison RF, Bryant DC, Kearney TE. Crop yields over the first nine years of LTRAS, a long-term comparison of field crop systems in a Mediterranean climate. *Field Crop Res.* 2004;86:267–77. <https://doi.org/10.1016/j.agee.2011.05.020>.
3. Pimentel D, Hepperly P, Hanson J, Douds D, Seidel R. Environmental, energetic, and economic comparisons of organic and conventional farming systems. *Bioscience.* 2005;55:573. [https://doi.org/10.1641/0006-3568\(2005\)055\[0573:EEAECO\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0573:EEAECO]2.0.CO;2).
4. Gabriel D, Sait SM, Kunin WE, Benton TG. Food production vs. biodiversity: comparing organic and conventional agriculture. *J Appl Ecol.* 2013;50:355–364. <https://doi.org/10.1111/1365-2664.12035>.
5. Kirchmann H, Bergström L, Kätterer T, Andrén O, Andersson R. Can organic crop production feed the world? In: Kirchmann H, Bergström L, editors. *Organic crop production—ambitions and limitations*. Dordrecht: Springer; 2008. <https://doi.org/10.1007/978-1-4020-9316-6>.
6. Seufert V, Ramankutty N, Foley AJ. Comparing the yields of organic and conventional agriculture. *Nature.* 2012;485:229–32. <https://doi.org/10.1038/nature11069>.
7. Tuomisto HL, Hodge ID, Riordan P, Macdonald DW. Does organic farming reduce environmental impacts? A meta-analysis of European research. *J Environ Manag.* 2012;112:309–20. <https://doi.org/10.1016/j.jenvman.2012.08.018>.
8. Crespo-Herrera LA, Ortiz R. Plant breeding for organic agriculture: something new? *Agric Food Secur.* 2015;4:25. <https://doi.org/10.1186/s40066-015-0045-1>.
9. Munaweera TIK, Jayawardana NU, Rajaratnam R, Dissanayake N. Modern plant biotechnology as a strategy in addressing climate change and attaining food security. *Agric Food Secur.* 2022;11:26. <https://doi.org/10.1186/s40066-022-00369-2>.
10. Muluneh MG. Impact of climate change on biodiversity and food security: a global perspective—a review article. *Agric Food Secur.* 2021;10(1):1–25. <https://doi.org/10.1186/s40066-021-00318-5>.
11. IPCC; 2014. AR5 Climate Change 2014: Mitigation of Climate Change. <https://www.ipcc.ch/report/ar5/wg3/>. Accessed on 19 Sept 2021.
12. Awika JM. Major cereal grains production and use around the world. In: *Advances in cereal science: implications to food processing and health promotion*. New York: American Chemical Society; 2011. p. 1–13. <https://doi.org/10.1021/bk-2011-1089.ch001>.
13. Martínez-Moreno F, Ammar K, Solís I. Global changes in cultivated area and breeding activities of durum wheat from 1800 to date: a historical review. *Agronomy.* 2022;12(5):1135.
14. Tappi M, Nardone G, Santeramo F. On the relationships among durum wheat yields and weather conditions: evidence from Apulia region, Southern Italy. *Forthcoming in: BAE-Bio-based and Applied Economics*; 2022.
15. Bhardwaj AK, Rajwar D, Yadav RK, Chaudhari SK, Sharma DK. Nitrogen availability and use efficiency in wheat crop as influenced by the organic-input quality under major integrated nutrient management systems. *Front Plant Sci.* 2021;12:752. <https://doi.org/10.3389/fpls.2021.634448>.
16. Mazzoncini M, Antichi D, Tavarini S, Silvestri N, Lazzeri L, D'Avino L. Effect of defatted oilseed meals applied as organic fertilizers on vegetable crop production and environmental impact. *Ind Crop Prod.* 2015;75:54–64. <https://doi.org/10.1016/j.indcrop.2015.04.061>.
17. Mobasser HR, Mohammadi GN, Heidari H, Abad S, Rigi K. Effect of application elements, water stress and variety on nutrients of grain wheat in Zahak region, Iran. *J J Bio Env Sci.* 2014;5(1):105–10.
18. Meredith S, Lampkin N, Schmid O. *Organic action plans: development, implementation and evaluation*. 2nd ed. Brussels: IFOAM EU; 2018.
19. Løes AK, Frøseth RB, Dieseth JA, Skaret J, Lindö C. What should organic farmers grow: heritage or modern spring wheat cultivars? *Org Agric.* 2020;10:93–108. <https://doi.org/10.1007/s13165-020-00301-7>.
20. Murphy KM, Campbell KG, Lyon SR, Jones SS. Evidence of varietal adaptation to organic farming systems. *Field Crop Res.* 2007;102:172–7. <https://doi.org/10.1016/j.fcr.2007.03.011>.
21. Blandino M, Vaccino P, Reyneri A. Late-season nitrogen increases improver common and durum wheat quality. *Agron J.* 2015;107(2):680–90. <https://doi.org/10.2134/agronj14.040>.
22. Carucci F, Gatta G, Gagliardi A, De Vita P, Giuliani MM. Strobilurin effects on nitrogen use efficiency for the yield and protein in durum wheat grown under rainfed mediterranean conditions. *Agron.* 2020;10(10):1508. <https://doi.org/10.3390/agronomy10101508>.
23. Yu ZHAO, Wang JW, Chen LP, Fu YY, Zhu HC, Feng HK, Xu XG, Li ZH. An entirely new approach based on remote sensing data to calculate the nitrogen nutrition index of winter wheat. *J Integr Agric.* 2021;20(9):2535–51. [https://doi.org/10.1016/S2095-3119\(20\)63379-2](https://doi.org/10.1016/S2095-3119(20)63379-2).
24. Rossini F, Provenzano ME, Sestili F, Ruggeri R. Synergistic effect of sulfur and nitrogen in the organic and mineral fertilization of durum wheat: grain yield and quality traits in the Mediterranean environment. *Agron.* 2018;8(9):189. <https://doi.org/10.3390/agronomy8090189>.
25. Germ M, Stibilj V. Krefit I Metabolic importance of selenium for plants. *Eur J Plant Sci Biotechnol.* 2007;1:91–7.
26. Yao X, Chu J, Wang G. Effects of selenium on wheat seedlings under drought stress. *Biol Trace Elem Res.* 2009;130(3):283–90. <https://doi.org/10.1007/s12011-009-8328-7>.
27. Lara TS, de Lima Lessa JH, de Souza KRD, Corguinha APB, Martins FAD, Lopes G, Guilherme LRG. Selenium biofortification of wheat grain via foliar application and its effect on plant metabolism. *J Food Compos Anal.* 2019;81:10–8. <https://doi.org/10.1016/j.jfca.2019.05.002>.
28. Li GL, Gao HM. Effects of spraying Na<sub>2</sub>SeO<sub>3</sub> on wheat yield. *Chin Agri Sci Bull.* 2009;25:253–5.
29. Lyons GH, Genc Y, Soole K, Stangoulis JCR, Liu F, Graham RD. Selenium increases seed production in Brassica. *Plant Soil.* 2009;318(1):73–80. <https://doi.org/10.1007/s11104-008-9818-7>.
30. Nawaz F, Ashraf MY, Ahmad R, Waraich EA, Shabbir RN. Selenium (Se) regulates seedling growth in wheat under drought stress. *Adv Clin Chem.* 2014;2014:1–7. <https://doi.org/10.1155/2014/143567>.
31. Carucci F, Moreno-Martín G, Madrid-Albarrán Y, Gatta G, De Vita P, Giuliani MM. Selenium agronomic biofortification of durum wheat fertilized with organic products: se content and speciation in grain. *Agronomy.* 2022;12(10):2492. <https://doi.org/10.3390/agronomy12102492>.
32. Ducsay L, Ložek O, Marček M, Varényiová M, Hozlár P, Lošák T. Possibility of selenium biofortification of winter wheat grain. *Plant Soil Environ.* 2016;62(8):379–83. <https://doi.org/10.17221/324/2016-PSE>.
33. Carucci F, Gatta G, Gagliardi A, De Vita P, Bregaglio S, Giuliani MM. Agronomic strategies to improve N efficiency indices in organic durum wheat grown in Mediterranean area. *Plants.* 2021;10(11):2444. <https://doi.org/10.3390/plants10112444>.
34. De Vita P, Platani C, Fragasso M, Ficco DBM, Colecchia SA, Del Nobile MA, Padalino L, Di Gennaro S, Petrozza A. Selenium-enriched durum wheat improves the nutritional profile of pasta without altering its organoleptic properties. *Food Chem.* 2017;214:374–82. <https://doi.org/10.1016/j.foodchem.2016.07.015>.
35. Stackhouse PW Jr, Zhang T, Westberg D, Barnett AJ, Bristow T, Macpherson B, Hoell JM. POWER release 8 (with GIS applications) methodology (data parameters, sources, and validation) documentation date May 1, 2018 (all previous versions are obsolete) (data version 8.0.1). NASA. Retrieved from [https://power.larc.nasa.gov/documents/POWER\\_Data\\_v8\\_methodology.pdf](https://power.larc.nasa.gov/documents/POWER_Data_v8_methodology.pdf).
36. Pradhan S, Babar MA, Robbins K, Bai G, Mason RE, Khan J, Shahi D, Avci M, Guo J, Maksud Hossain M, Bernardo A. Understanding the genetic basis of spike fertility to improve grain number, harvest index, and grain yield in wheat under high temperature stress environments. *Front Plant Sci.* 2019;10:1481. <https://doi.org/10.3389/fpls.2019.01481>.
37. Mongiano G, Titone P, Tamborini L, Pilu R, Bregaglio S. Evolutionary trends and phylogenetic association of key morphological traits in the Italian rice varietal landscape. *Sci Rep.* 2018;8(1):1–12. <https://doi.org/10.1038/s41598-018-31909-1>.
38. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020. <https://www.R-project.org/>. Accessed on 3 Sept 2021.
39. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. *J Stat Softw.* 2008. <https://doi.org/10.18637/jss.v025.i01>.
40. Wickham H. *Elegant graphics for data analysis*. Media. 2009;35(211):10–1007.
41. Raymond C, Horton RM, Zscheischler J, Martius O, AghaKouchak A, Balch J, White K. Understanding and managing connected extreme events. *Nat Clim Change.* 2020;10(7):611–21. <https://doi.org/10.1038/s41558-020-0790-4>.
42. Trebbi G, Negri L, Bosi S, Dinelli G, Cozzo R, Marotti I. Evaluation of equisetum arvense (horsetail macerate) as a copper substitute for pathogen management in field-grown organic tomato and durum wheat

- cultivations. *Agriculture*. 2021;11(1):5. <https://doi.org/10.3390/agriculture11010005>.
43. Gagliardi A, Carucci F, Masci S, Flagella Z, Gatta G, Giuliani MM. Effects of genotype, growing season and nitrogen level on gluten protein assembly of durum wheat grown under Mediterranean conditions. *Agron J*. 2020;10(5):755. <https://doi.org/10.3390/agronomy10050755>.
  44. Rizza F, Ghashghaie J, Meyer S, Matteu L, Mastrangelo AM, Badeck FW. Constitutive differences in water use efficiency between two durum wheat cultivars. *Field Crops Res*. 2012;125:49–60. <https://doi.org/10.1016/j.fcr.2011.09.001>.
  45. Dinelli G, Marotti I, Di Silvestro R, Bosi S, Bregola V, Accorsi M, Catizon, P. Agronomic, nutritional and nutraceutical aspects of durum wheat (*Triticum durum* Desf.) cultivars under low input agricultural management. *Ital J Agron*. 2013;8(2):e12. <https://doi.org/10.4081/ija.2013.e12>.
  46. Terry N, Zayed AM, de Souza MP, Tarun AS. Selenium in higher plants. *Annu Rev Plant Phys*. 2000;51:401–32. <https://doi.org/10.1146/annurev.arplant.51.1.401>.
  47. Broadley MR, Alcock J, Alford J, Cartwright P, Foot I, Fairweather-Tait SJ, Hart D, Hurst R, Knott P, McGrath SP, Meacham MC, Norman K, Mowat H, Scott P, Stroud JL, Tovey M, Tucker M, Hart DJ, Tucker M, White PJ, Young SD, Zhao FJ. Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. *Plant Soil*. 2010;332(1):5–18. <https://doi.org/10.1007/s11104-009-0234-4>.
  48. Grant CA, Buckley WT, Wu R. Effect of selenium fertilizer source and rate on grain yield and selenium and cadmium concentration of durum wheat. *Can J Plant Sci*. 2007;87(4):703–8. <https://doi.org/10.4141/CJPS06060>.
  49. Tang YX, Wang HM, Yang JF, Lv YH. Studies on the selenium content and selenium enriched technique of winter wheat in Hebei Province. *J Triticeae Crops*. 2011;31(2):347–51. <https://doi.org/10.1017/S0021859616000836>.
  50. Karim MR, Zhang YQ, Zhao RR, Chen XP, Zhang FS, Zou CQ. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. *J Plant Nutr Soil Sci*. 2012;175(1):142–51. <https://doi.org/10.1002/jpln.201100141>.
  51. Wojtkowiak K, Stepień A. Nutritive value of spelt (*Triticum aestivum* ssp. spelta L.) as influenced by the foliar application of copper, zinc and manganese. *Zemdirbyste*. 2015;102(4):389–96. <https://doi.org/10.13080/z-a.2015.102.049>.
  52. Rietra RP, Heinen M, Dimkpa CO, Bindraban PS. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Commun Soil Sci Plant Anal*. 2017;48(16):1895–920. <https://doi.org/10.1080/00103624.2017.1407429>.
  53. Uscola M, Villar-Salvador P, Oliet J, Warren CR. Foliar absorption and root translocation of nitrogen from different chemical forms in seedlings of two Mediterranean trees. *Environ Exp Bot*. 2014;104:34–43. <https://doi.org/10.1016/j.envexpbot.2014.03.004>.
  54. Visioli G, Bonas U, Dal Cortivo C, Pasini G, Marmiroli N, Mosca G, Vamerali T. Variations in yield and gluten proteins in durum wheat varieties under late-season foliar versus soil application of nitrogen fertilizer in a northern Mediterranean environment. *J Sci Food Agric*. 2018;98(6):2360–9. <https://doi.org/10.1002/jsfa.8727>.
  55. Benton TG, Froggatt A, Wellesley L, Schröder P. The Ukraine war and threats to food and energy security. Environment and Society Programme, Chatham House; 2022. <https://www.chathamhouse.org/sites/default/files/2022-04/2022-04-12-ukraine-war-threats-food-energy-security-benton-et-al.pdf>. Accessed 15 Apr 2022.
  56. Keeney DR, Nelson DW. Inorganic forms of nitrogen. In: *Methods of soil analysis*. Madison, Wisconsin: American Society of Agronomy; 1982. p. 643–98.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

