

# Screening old and modern wheat varieties for shading tolerance within a specialized poplar plantation for agroforestry farming systems implementation

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Abstract The sustainability of silvoarable agroforestry systems largely depends on the choice of suitable crop species and varieties for minimizing competitive interactions in the interaction zone with trees. Up to date crop intraspecific variability has been poorly investigated, and mainly in pot trials under artificial shading. Given this framework, a trial was conducted in field conditions in NE Italy to study the

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L. Furlan e-mail: lorenzo.furlan@venetoagricoltura.org response of 10 common wheat varieties cultivated in the 6-m narrow inter-row of a specialized 4-year old poplar plantation (AF, agroforestry) vs. full sun conditions (C, controls). Here it was demonstrated that large genetic variability exists in wheat, as grain yield reductions under shading ranged between 31 and 75% (average - 57%) depending on variety choice. Better tolerance was highlighted in old varieties (- 44% of yield in AF vs. C), that was associated to greater plasticity in delaying leaf senescence, and increased both leaf area index and SPAD (index of chlorophyll content), while achieving the highest grain protein contents (>17.3% DW). However, under shading modern wheat varieties still remained the highest yielding, particularly in bread-making varieties (469 g m<sup>-2</sup> on average), thanks to larger improvement of the leaf-toculm biomass ratio and better harvest index. We conclude that there is large scope for identifying wheat varieties suitable for agroforestry systems, while breeding programs can select for specific morphophysiological adaptation traits to low irradiance.

Keywords Silvoarable systems  $\cdot$  Leaf greenness  $\cdot$  Morpho-physiological traits  $\cdot$  Old and modern wheat varieties  $\cdot$  Yield components

# Introduction

Integrated land-use systems combining a woody vegetation of trees and/or shrubs with a lower storey

agricultural production, commonly referred as agroforestry farming systems, can benefit from the resulting ecological interactions (Mosquera-Losada et al. 2018; Burgess and Rosati 2018). Mixed farming systems had been largely applied in the temperate regions until the '70s, but later abandoned due to agriculture mechanization and intensification. As a consequence, agroforestry systems were progressively replaced by specialized monocropping systems without trees, especially in the fertile valley areas (Nair 2007; Nerlich et al. 2013; Seidel et al. 2021). However, in recent years, the stability of monocrops' production is being mined by climate change, in relation to increased temperature, drought, and flooding events (Brisson et al. 2010; Ray et al. 2012). In winter cereals, such as wheat, heat waves and drought in late spring are responsable for anticipated leaf senescence and shortening of the grain filling period, thus leading to considerable yield losses. Particularly within the Mediterranean area, the impact of climate change is expected to intensify in the short-term according with future forecasted climate change scenarios (Saadi et al. 2015; Moore and Lobell 2014; Trnka et al. 2014). Since the mean air temperature is foreseen to increase by at least 1.5 °C as global average in the next decades (IPCC 2021), the potential of current specialized farming systems to match the growing food demand is being questioned.

Within this framework, agroforestry systems are receiving a renewed attention for their potential to reduce vulnerability to climate shifts and improve resilience of farming systems (Burgess et al. 2022; Cardinael et al. 2021; Quandt et al. 2023). At present in Europe, silvoarable systems cover 358,000 ha, i.e. only~0.1% of the agricultural area (Nerlich et al. 2013), although they could be extended on a much larger land (Mosquera-Losada et al. 2018). Indeed, there is concern among farmers for worsening of crops' yield in the neighboring of trees, caused by resources competition, and for a more complex management of the agricultural system (Mosquera-Losada et al. 2018; Lakshmanakumar et al. 2013; Camilli et al. 2018). However, recent literature demonstrates higher overall productivity per land unit of well-conceived agroforestry systems, as highlighted by better Land Equivalent Ratio (LER) indexes as compared to separated tree and crop monocultures (Graves et al. 2008; Seserman 2018), and better profitability and sustainability (Luedeling et al. 2016).

Trees can also impact positively on crop yield stability through microclimate buffering (shelter and windbreak effects), soil water conservation and fertility enhancement (Torralba et al. 2016; McAdams et al. 2009). The agroforestry systems should be correctly designed, and tree management adequately managed in the short- and long-term. Essential tree characteristics are species choice, tree age, phenology and pruning management. These traits together with the plantation design, such as tree inter-row and intra-row, affect the competition for resources with the intercrops (Zhang et al. 2013; Dossa et al. 2008). With deciduous trees, one of the most important candidate intercrop is winter wheat, it being a C3 species and having limited overlap of the growing season with tree foliation of such trees (Jose et al. 2004; Pardon et al. 2018). In temperate climates, yield reduction of the understory wheat is commonly observed in the narrow interaction zone with trees, and solar radiation is recognized to be the main limiting resource (Eichhorn et al. 2006; Dufour et al. 2013; Artru et al. 2017; Burgess et al. 2005; Gill et al. 2009; Yang et al. 2020; Jose et al. 2004).

According to reduced solar radiation in tree-crop interaction, an essential aspect to be investigated is the genetic variability among wheat varieties in response to low irradiance (Ehret et al. 2015; Burgess et al. 2015). Modern wheat breeding has been carried out under full-sun conditions and it is unknown how modern varieties withstand shading conditions in comparison with abandoned old varieties. In this regard, there is much interest in screening shading tolerant wheats and identifying related morpho-physiological traits (Panozzo et al. 2020). According to Araus et al. (2008), it is necessary to evaluate primary traits that directly affect grain yield, such as biomass accumulation, plant height, and flowering time, and secondary traits such as phenology, senescence timing, and water status, which are related to crop stress tolerance. Up to date, the few wheat varietal screening trials for agroforestry have been carried out primarily under artificial shading, thus excluding soil competition.

Given this background, we carried out a screening trial on 10 common wheat genotypes, including modern and old local Italian varieties, within a 4-year old specialized poplar plantation in order to assess their shading tolerance in real agroforestry conditions. The study aimed at (i) quantifying the impact of trees on yield and grain protein content of wheat within the poplar plantation, as compared to full sun controls; (ii) to identify morpho-physiological parameters for shading tolerance in wheat to be used in future breeding programs for implementation of agroforestry systems; (iii) to verify whether differences for shading tolerance exist among old and modern wheat varieties, and among bread-making and biscuit-making varieties.

# Materials and methods

#### Experimental site and climatic conditions

The field trial was conducted during the 2020–2021 growing season at the "Sasse Rami" pilot and demonstrative farm of Veneto Agricoltura agency, located in Ceregnano (Rovigo, NE Italy, 45°03'N, 11°52'E, 12 m a.s.l.). The soil was clay-loamy, with 31% clay, 47% silt, 22% sand, 14.2 g kg<sup>-1</sup> organic matter, 0.86 g kg<sup>-1</sup> total N content, pH 8.0, available phosphorus 44 mg  $P_2O_5$  kg<sup>-1</sup>, and exchangeable potassium 88 mg  $K_2O$  kg<sup>-1</sup> (arable layer, beginning of the experiment). The local climate is subcontinental, with a mean monthly temperature ranging from 3.0 °C in January to 22.2 °C in June. The historical 30-year mean annual precipitation is 697 mm. During the trial, as recorded by the local meteorological station (ARPAV, Villadose, Rovigo Italy), the average monthly temperature was higher than the historical mean in December (6.0 °C vs. 3.8 °C), February (7.8 °C vs. 4.8 °C), and June (24.3 °C vs. 22.2 °C) (Fig. S1). During the wheat cycle, from October 2020 to June 2021, the site received 444 mm of precipitation vs. 577 mm of historical mean, with large heterogeneity in the monthly rainfall pattern (Fig. S1). Monthly precipitation was higher than the historical mean in October, December, January, and May, with the largest differences in December (106 mm vs. 56 mm).

# Experimental design

The trial was set up within a specialized poplar (*Populus*×*euroamericana*) plantation of 1.6 ha at the 4th vegetative growth, that has been planted in 2018 using 1-year old rods. The plantation consisted of 13 different clones of type HES (High Environmental

Sustainability), i.e., having high tolerance to the main fungal pathogens (*Venturia populina, Melampsora allii-populinaand, Marssonina brunnea*) and insects (i.e., *Phloeomyzus passerinii*), with nine trees each, replicated three times (overall n = 27). The tree rows are N-S oriented with a squared  $6 \times 6$  m planting design. The poplar clones surrounding the wheat plots were Mombello, I-214, Senna and Aleramo. During the wheat trial, trees reached 14 m of height at the beginning of April 2021, with 18 cm stem diameter at breast height, and 4.2 m canopy width.

Ten common wheat varieties were cultivated in the inter-row of the poplar plantation (AF, agroforestry treatment) and in a neighboring field without trees as control area (C, control treatment). The pool of wheat varieties studied here was chosen in order to consider a relatively large genetic variability, including both old local varieties (three varieties) preserved at the "Nazareno Strampelli" Institute of Genetic and Agricultural Research in Lonigo (Vicenza, Italy), and modern commercial wheat varieties (seven varieties), considering the most broadly cultivated in NE Italy. The main qualitative characteristics of the studied varieties are reported in Table S1. Four modern varieties are classified as for bread-making, two biscuitmaking and one hard. Based on the relatively low dough strength index W ( $< 100 \text{ J} \times 10^{-4}$ ), the old varieties can be used for low leavened oven productions, such as biscuits.

In both AF and C treatments, wheat was sown on 25 October 2020 with a plot seeder at a seed dose of 240 kg ha<sup>-1</sup>, with 15 cm apart rows. Soil was previously harrowed at 0.15 m depth, and fertilized with 32, 96 and 96 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively, as ternary fertilizer incorporated into the soil through a second harrowing. During the crop cycle, two N fertilizations with ammonium nitrate (57 kg N ha<sup>-1</sup> each) were applied, i.e. during tillering at mid-February and at the beginning of stem elongation at end-March for a total amount (including the dose at sowing) of 146 kg N ha<sup>-1</sup>. As recommended practice, wheat was protected against fungal pathogens by spraying Amistar® Xtra (1 L ha<sup>-1</sup>; a.i. azoxystrobin+ciproconazolo; Syngenta) at flowering stage (beginning of May). Wheat plots were 1.2-m wide and 3-m long  $(3.6 \text{ m}^2 \text{ each})$  with three replicates per variety (n=3), following a completely randomized block design (Fig. S2). In AF, wheat plots were set-up in the middle of the inter-row between two poplar lines, roughly in the center of the plantation. The uncultivated area at both sides of the plots was covered with natural grasses and regularly mowed during the wheat cycle. In controls, the wheat plots were set-up in a close field, at > 30 m distance from trees. Manual harvest of wheat took place on 29 June 2021 in  $1-m^2$  sampling areas for each 'replicate×variety×treatment' plot.

#### Microenvironmental parameters

Photosynthetic active radiation (PAR) reaching wheat during the crop cycle in AF and C treatments was provided hourly by two SQ-420X sensors (Apogee Instruments, Logan, UT, USA), placed one per treatment at 1-m height, from the sowing date of wheat until harvest time. The poplar plantation (AF) had high population density with even soil shading, and PAR was measured in the centre of the wheat plots.

Soil moisture was retrieved from core samples (4-cm  $\phi$ , 25 cm depth) taken from all the replicate × variety plots of both treatments on 28 April and 31 May 2021, between two wheat rows of the plots centre. Soil was collected in plastic bags, and weighted before and after oven-drying at 105 °C for 48 h for determining the water content (w/w).

#### Plant analysis

The normalized difference vegetation index (NDVI) was measured once a week from the end of April 2021 to wheat harvest by means of an active handheld Greenseeker spectrometer model RT100 (Ntech Industries, Ukiah, CA-USA) on the wheat canopy of all the replicate×variety plots of both AF and C treatments. While walking beside the plots and keeping the sensor head parallel to the ground at~30 cm of distance from the crop canopy, the equipment provides the reflectance at 660 nm (refRED) and 770 nm (refNIR) for the calculation of the vegetational index, as follows:

# $NVDI = \frac{RefNIR - RefRED}{RefNIR + RefRED}$

NDVI provides a measurement of combined soil coverage and canopy greenness, and varies from 0 to 1, the higher the index the better the canopy development and health. A greener vegetation is expected to provide higher NDVI values, as refNIR is supposed to be higher and refRED lower (Ali and Ibrahim 2020).

The leaf chlorophyll content of wheat was measured periodically, at heading (28 April 2021), flowering (13 May) and during grain filling (31 May). It was measured indirectly as SPAD units, through a Soil Plant Analysis Development SPAD-502 chlorophyll meter (Konica-Minolta, Hong Kong) (Hoel and Solhaug 1998) on the last fully expanded leaf of three tagged plants randomly chosen in each plot/replicate. Two measurements were taken in each plant, one at one-third and one at two-thirds of the leaf length, that were averaged.

Some morphological traits of wheat were determined on plants collected from 0.1 m long row for each plot at the two times of SPAD measurements, i.e., flowering (13 May) and during grain filling (31 May). Plant height (from coleoptile to spike apex) was measured on the main culm of three randomly chosen plants in each sample. On the same samples, the leaf area index (LAI) and culm area index (CAI) were measured by means of a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, Nebraska), allowing the LAIto-CAI ratio to be calculated. After oven-drying (105 °C for 48 h), leaf and culm dry weights (DW) and their ratio were also calculated.

#### Grain yield and quality

Grain yield of wheat varieties was assessed in AF and C treatments at maturity by collecting and threshing  $1\text{-m}^2$  sampling area from all plots (replicate × variety). The harvest index (HI) was calculated as grain-to-total shoot DW measured after oven-drying at 105 °C for 48 h. Further measured parameters (n=3) were the thousand grain weight (TGW) after seed counting and weighting, and the testing weight through the GAC 500XT equipment (Dichey-John, IL-USA). The quality parameters of wheat grain, i.e. the grain protein content and wet gluten content (% DW) were estimated by Near-InfraRed Spectroscopy (NIRS) using the Infratec-1241 equipment (Foss Analytical, Hillerød, Denmark).

#### Statistical analysis

The data of all the parameters for each 'variety×treatment' combination were subjected to ANOVA using R Studio software ver. 1.4 [RStudio Public Benefit Corporation (PBC), Boston, MA, USA]. Separation of means was made at  $P \le 0.05$  by the Tukey's HSD test.

Principal component analysis (PCA) and factorial discriminant analysis (Multigroup Discriminant Analysis, MDA) with Wilks' lambda and Pillai's trace tests (Podani 2007) were carried out with MS Excel XLSTAT (Addinsoft, Paris, France) to describe the response of wheat to shading in agroforestry (AF) and full-sun (C) conditions, in terms of vegetational parameters, aboveground biomass, leaf and culm area indexes and grain yield. Before analysis, multivariate data normality was verified by the Shapiro test using R 3.0.1 software (Ihaka and Gentleman 1996), and data were standardized by subtracting the mean and dividing by the standard deviation within each variable.

#### Results

#### Microclimatic conditions

In AF, photosynthetic active radiation (PAR) was reduced by 42% as compared to the open field control (C) as an average of the whole wheat growing cycle, from sowing to harvest, with progressively greater reductions starting from the beginning of April along with leaf sprouting of the poplars while wheat was at the stem elongation stage.

A regards the soil moisture in the top 25 cm layer, it was assessed in two distinct growth phases of wheat. i.e. heading (April 28) and grain filling (May 31). At heading, the average soil moisture was 15%w/w in the C treatment, with some variations within the range 13.8%–15.7% depending on the genotype considered (Table S2), while in the AF treatment, with trees at about half of complete foliation, the average soil moisture was 14.2% w/w (- 0.8% vs. C; p < 0.05), with some variations among varieties within the range 12.3%-15.4%. In plots of wheat var. LG Ayrton, Guà 113 and Piave soil moisture was significantly lower in AF than in C. On the opposite, at the end of May, when trees had completed the foliation from about 10 days, a slightly higher soil moisture content was revealed in the AF treatment (15.1% vs. 14.8%; p>0.05) (Table S2). At this time, the difference in soil moisture between AF and C ranged between -2.6% up to +2.0% depending on the wheat variety considered.

#### Leaf greenness

At the beginning of the measurement period at wheat heading (end April), the average NDVI value was significantly higher in C as compared to AF (0.73 vs. 0.63;  $p \le 0.05$ ). However, starting from mid-May, when poplars completed the foliation, NDVI increased sharply in AF, thus reaching higher values as compared to full sun conditions, and allowing for a superior dynamics in the rest of the wheat cycle. In particular, during maturation in June, shading in AF brought to slower NDVI decline and retarded leaf senescence compared to C. On June 10, NDVI was 0.75 in AF and 0.63 in C ( $p \le 0.05$ ) (Fig. 1). Regarding varietal comparison, different NDVI dynamics were registered from heading to maturity, as the studied genotypes generally showed higher NDVI in AF vs. C during the last part of the cycle, although not significantly for LG Ayrton, Solehio and Vivendo. This indicates a prolonged stay green in agroforestry as compared to full sun conditions. This effect was statistically significant in half of the varieties (5 out of 10), and most evident in the old varieties (Canove, Guà 113, Piave) as compared to the modern ones. On June 10, NDVI values were averagely 40% higher in AF vs. C in old varieties, and 11% in modern ones  $(p \le 0.05)$  (Fig. S3).



Fig. 1 Dynamics of Normalized Difference Vegetation Index (NDVI) (means $\pm$ S.E.; n=30) from heading to maturity of common wheat in agroforestry (AF) and control (C) treatments, as average of 10 varieties. Asterisks indicate significant differences between treatments for each date according to the Tukey's HSD test (p  $\leq$  0.05)

The measurement of the leaf chlorophyll content on the flag leaf highlighted larger and significant differences between the two farming systems (Table 1). At wheat flowering (May 13), SPAD was averagely higher by 9% in AF vs. C (50.3 vs. 46.2), with the largest differences in the three old varieties and in the modern var. Vivendo (bread-making) and Rebelde (hard wheat), which SPAD was enhanced by > 12% in AF vs. C ( $p \le 0.05$ ). During grain filling on May 31, the differences between the two treatments increased further as compared with the previous measurement date, with SPAD being 11% higher in AF vs. C (50.8 vs. 45.9; p < 0.05). This effect was more evident in the old var. Guà 113 and Canove, and in the modern varieties Nemo, Rebelde and Vivendo, which enhanced leaf chlorophyll content by at least 16% in AF vs. C  $(p \le 0.05)$  (Table 1).

# Plant morphology

The morphological parameters at wheat flowering (May 13) showed significant variations in the two treatments (Tables 2, 3 and Fig. 2). In the full sun treatment (C), plants were averagely 79 cm heigh, with significant smaller plants in modern varieties (71 cm) vs. old varieties (100 cm). Plant height was averagely increased by 14% in AF as compared to C, with large variability depending on variety choice (Table 3). The greatest increment under shading (AF)

**Table 2** Morphological traits (mean  $\pm$  S.E.; n=3) of common wheat at flowering (May 13) in agroforestry (AF) and control (C) treatments as average of 10 varieties. DW: dry weight. For each parameter, the percentage variation of AF treatment vs. C is reported, and asterisks indicate significant differences between treatments according to the Tukey's HSD test (p  $\leq 0.05$ )

Parameter	С	AF	% var./C
Plant height (cm)	79.3 (±1.6)	90.3 (±3.3)	+14% (*)
Aboveground bio- mass (g DW m <sup>-2</sup> )	1279 (±80)	1339 (±192)	+5%
Leaf/Culm DW ratio	$0.28 (\pm 0.01)$	$0.35 (\pm 0.02)$	+24% (*)
LAI	$4.5 (\pm 0.3)$	$5.4(\pm 0.7)$	+19%
LAI/CAI	$2.25 (\pm 0.12)$	$2.32 (\pm 0.11)$	+3%

occurred in the old var. Canove and Guà 113, which increased their height by >40% (p  $\le 0.05$ ), while small not significant variations, both increases and decreases, were observed in the modern varieties, with the exception of var. Bagou, it having 14% significant enhanced height in AF.

At the same time, the aboveground dry biomass (DW) showed averagely a slight increment of 5% in AF vs. C (~1340 g DW m<sup>-2</sup>), despite large variations in some varieties. A reduction of shoot biomass in response to shading was revealed in the modern var. Arkeos, LG Ayrton and Nemo, i.e. by>30% as compared to full sun conditions. A marked increase

**Table 1** Chlorophyll content of the flag leaf measured as SPAD (Soil Plant Analysis Development) units (means  $\pm$  S.E.; n=3) atflowering (May 13) and grain filling (May 31) stages in ten common wheat varieties in agroforestry (AF) and control (C) treatments

Varietal group	Variety name	13 May			31 May		
		C	AF	% var./C	С	AF	% var./C
Biscuit	Arkeos	49.8 (±0.9)	52.3 (±1.2)	+5%	53.9 (±1.0)	54.9 (±0.5)	+2%
	Bagou	51.1 (±1.2)	53.2 (±1.0)	+4%	52.5 (±1.4)	$55.4 (\pm 0.8)$	+5%
Bread	LG Ayrton	50.8 (±1.3)	$50.6 (\pm 0.6)$	=	48.8 (±1.6)	$51.4 (\pm 0.8)$	+5% (*)
	Nemo	50.1 (±2.1)	$51.0 (\pm 0.9)$	+2%	46.0 (±1.6)	$56.0(\pm 0.9)$	+22% (*)
	Solehio	$44.8 (\pm 0.9)$	$48.0(\pm 1.4)$	+7%	46.9 (±1.4)	48.7 (±1.3)	+4%
	Vivendo	45.3 (±1.4)	51.1 (±1.1)	+13% (*)	43.6 (±1.3)	$52.0(\pm 0.9)$	+19% (*)
Hard	Rebelde	$44.0 (\pm 0.7)$	49.6 (±0.6)	+13% (*)	40.9 (±1.3)	47.6 (±1.1)	+16% (*)
Old	Canove	43.6 (±1.1)	49.0 (±1.2)	+12% (*)	43.8 (±2.0)	48.0 (±1.7)	+9%
	Guá 113	$41.4 (\pm 2.0)$	48.7 (±1.3)	+18% (*)	$38.9 (\pm 0.9)$	$50.0(\pm 1.4)$	+28% (*)
	Piave	41.9 (±1.3)	49.0 (±1.3)	+17% (*)	44.1 (±3.0)	44.5 (±1.2)	+1%
Average		$46.2 (\pm 0.6)$	$50.3 (\pm 0.4)$	+9% (*)	45.9 (±0.7)	$50.8 (\pm 0.5)$	+11% (*)

For each variety and date, the percentage variation of AF treatment vs. C is reported, and asterisks indicate significant differences between treatments according to the Tukey's HSD test ( $p \le 0.05$ )

each wheat varié (p≤0.05)	ety, the percentage	e variation of AF	treatment vs. C is	s reported, and	1 asterisks indica	te significant diffe	rences between	treatments accor	ding to the Tukey	's HSD test
Varietal group	Variety name	Plant height (cn	(L		Aboveground b	iomass (g DW m <sup>-</sup>	2)	Leaf/Culm DW	ratio	
		С	AF	% var./C	С	AF	% var./C	C	AF	% var./C
Biscuit	Arkeos	70.7 (±1.9)	69.1 (± 8.6)	- 2%	1541 (±383)	814 (± 291)	-47%	0.28 (±0.02)	0.46 (±0.08)	+64%
	Bagou	62.2 (±0.8)	70.8 (±4.0)	+ 14% (*)	$1007 (\pm 172)$	1044 (±279)	+4%	$0.37 ~(\pm 0.02)$	$0.51 \ (\pm 0.02)$	+38% (*)
Bread	LG Ayrton	69.8 (±2.3)	74.8 (±2.5)	+ 7%	1717 (±263)	912 (± 197)	-47% (*)	$0.33 (\pm 0.02)$	$0.38 \ (\pm 0.02)$	+15%
	Nemo	$69.8 (\pm 1.9)$	65.0 (±2.0)	- 7%	1217 (±263)	635 (±59)	-48% (*)	$0.27 \ (\pm 0.01)$	$0.33 (\pm 0.01)$	+22% (*)
	Solehio	80.2 (±1.7)	$76.0 (\pm 10.0)$	-5%	1329 (±256)	904 (±537)	-32%	$0.30 (\pm 0.02)$	$0.43 \ (\pm 0.07)$	+43%
	Vivendo	67.4 (±3.5)	$69.3 (\pm 9.7)$	+3%	769 (±97)	878 (±370)	+ 14%	$0.36 (\pm 0.04)$	$0.38~(\pm 0.07)$	+6%
Hard	Rebelde	74.6 (±2.0)	74.6 (±2.8)	11	1124 (±204)	833 (± 142)	-26%	$0.22 \ (\pm 0.01)$	$0.29 \ (\pm 0.01)$	+32% (*)
Old	Canove	98.6 (±5.5)	142.1 (±3.4)	+44% (*)	1217 (±58)	2631 (±625)	+ 116% (*)	$0.21 \ (\pm 0.03)$	$0.19 \ (\pm 0.04)$	-10%
	Guá 113	94.8 (±5.4)	$137.6 (\pm 6.9)$	+45% (*)	1167 (±97)	$3030 (\pm 1009)$	+160%	$0.30 (\pm 0.04)$	$0.31 \ (\pm 0.02)$	+3%
	Piave	105.3 (±4.8)	123.7 (±6.1)	+17% (*)	1699 (± 228)	1709 (±293)	+1%	0.20 (±0.02)	0.24 (±0.02)	+20%

was instead observed in the two old varieties Canove and Guà 113, by 116% and 160% respectively vs. C (Table 3).

The leaf-to-culm DW ratio was averagely increased by 24% ( $p \le 0.05$ ; varietal average) in response to shading in AF. All the wheat varieties increased this ratio in AF, excepted for the old variety Canove which exhibited a 10% reduction vs. C, although not significant. Significant increases of the leaf-toculm ratio regarded var. Bagou, Nemo and Rebelde, with + 38%, + 22% and + 32%, respectively.

The leaf area index (LAI) of wheat varieties was on average 4.5 in controls, that was increased by 19% in response to shading in AF ( $p \ge 0.05$ ) (Fig. 2). The old wheats showed the largest response for this parameter, particularly Canove (+ 134% vs. C;  $p \le 0.05$ ) and Guà 113 (+ 225% vs. C;  $p \ge 0.05$ ). On the contrary, the modern varieties reduced LAI in AF as compared to C, with a significant decrease in Nemo (- 39%), and an increase only in Bagou (+ 30% vs. C).

The leaf-to-culm area ratio (LAI/CAI) was on average 2.3 in both the treatments (Fig. 2), although with variety-dependent variations. The old varieties increased this parameter in response to shading, particularly in Guà 113 (+55% vs. C; P $\ge$ 0.05), while different responses to AF were observed in modern varieties, i.e. with increments in the biscuit making Arkeos and Bagou (p $\ge$ 0.05), and reductions in the bread-making and hard types LG Ayrton, Nemo, Rebelde, Solehio and Vivendo, although significantly only for the latter.

#### Grain yield and quality

In the control treatment, the varietal grain yield of wheat ranged from ~ 500 g m<sup>-2</sup> to ~ 1200 g m<sup>-2</sup>, with a marked difference between the higher yielding modern vs. old varieties (979 vs. 578 g m<sup>-2</sup> respectively). In AF, the yield was significantly reduced as compared to full sun by 57% on average ( $p \le 0.05$ ), with a productivity ranging from ~ 200 g m<sup>-2</sup> to ~ 500 g m<sup>-2</sup>. The yield reduction was more marked in the modern varieties (- 60% vs. C on average), than in the old ones (- 44% vs. C), although the yield reduction of Canove was comparable to that of modern varieties (- 65% vs. C) (Table 4). Yield losses were significant in many varieties, except for Bagou, Solehio and Guà 113 (p>0.05). However, in AF the highest absolute grain yield was still recorded in the modern var.

Table 3 Morphological traits (means  $\pm$  S.E.; n = 3) of ten common wheat varieties at flowering (13 May) in agroforestry (AF) and control (C) treatments. DW: dry weight. Within



**Fig. 2** Leaf area index (LAI) (above) and ratio between LAI and culm area index (LAI/CAI) (below) at flowering (May 13) in ten common wheat varieties in agroforestry (AF) and control (C) treatments. Numbers indicate the percentage vari-

C AF

LG Ayrton

C AF

Nemo

Bread

C AF

Solehio

C AF

Vivendo

C AF

Rebelde

Hard

Solehio and LG Ayrton with ~500 g m<sup>-2</sup>, although the lowest yield reduction were measured in two old wheat varieties, i.e. Piave and Guá 113 (about -30% vs. C) followed by Solehio and LG Ayrton (-46% and -49% respectively vs. C).

In the full sun treatment (C), the testing weight ranged from ~73 to ~84 kg hL<sup>-1</sup> depending on the variety considered. Even though the variations between agroforestry and control treatments were small for this parameter, ranging from -4% to +1% vs. C (Table 4), some significant differences were highlighted; the testing weight was significantly reduced in AF vs. C in the var. Bagou, Piave, and Rebelde,

0

C AF

Arkeos

Biscuit

C AF

Bagou

ations of the AF treatment vs. C, and asterisks significant differences between treatments according to the Tukey's HSD test (\*= $p \le 0.05$ )

C AF

Canove

C AF

Guà 113

Old

C AF

Piave

and significantly enhanced in the var. LG Ayrton, Nemo and Solehio.

The thousand grain weight (TGW) was again reduced in response to shading in all the studied varieties (- 11% vs. C on average), with variations ranging from - 2% to - 18% as compared to the full sun (Table 4). Significant TGW reductions in AF occurred in the var. Arkeos, Bagou, Canove, and Vivendo. The minimal TGW reductions in AF were registered in the old varieties Guá 113 and Piave (<3%, p  $\geq$  0.05). Also, the Harvest Index (HI) showed a decrease under agroforestry vs. C in all the varieties (- 12% vs. C on average). The greatest worsening

HI: harvest Tukey's HS	index. For eac D test $(p \le 0.0)$	ch variety, the 5)	e percentage v	/ariation of .	AF treatment	t vs. C is repc	orted, and a	sterisks indic	ate significan	t differences	s between trea	tments accor	ling to the
Varietal	Variety	Grain yield (	(g m <sup>-2</sup> )		Testing wei	ght (Kg hL <sup>-1</sup> )		TGW (g)			H		
group	name	С	AF	% var./C	С	AF	% var./C	С	AF	% var./C	С	AF	% var./C
Biscuit	Arkeos	1215	311 (±53)	-74% (*)	73.3	73.8	+1%	35.2	29.6	- 16% (*)	0.54	0.43	-20% (*)
	Bagou	(±92) 811 (+200)	368 (土71)	-55%	$(\pm 1.3)$ 73.4 (+01)	(±0.1) 70.6 (+0.2)	-4% (*)	(±0.3) 32.9 (+0.9)	(±0.0) 29.8 (+0.2)	-9% (*)	$(\pm 0.01)$ 0.49 $(\pm 0.01)$	$(\pm 0.01)$ 0.41 $(\pm 0.02)$	-17% (*)
Bread	LG Ayrton	997 (±68)	505 (土38)	-49% (*)	$(\pm 0.1)$ $(\pm 0.1)$	81.4 (±0.3)	+1%	$(\pm 0.9)$	$(\pm 0.5)$	-7%	$(\pm 0.01)$	$(\pm 0.01)$	-8%
	Nemo	1056 (±39)	481 (± 82)	-54% (*)	79.4 (±0.1)	79.9 (±0.2)	+1% (*)	41.4 (± 0.3)	36.6 (±2.9)	-12%	0.50 (± 0.01)	0.49 (± 0.02)	-3%
	Solehio	965 (±191)	524 (±105)	-46%	80.3 (± 0.1)	81.3 (±0.2)	+1% (*)	44.8 (±1.3)	38.6 (±2.0)	- 14%	0.49 (± 0.01)	0.45 (± 0.01)	-7% (*)
	Vivendo	1064 (±87)	363 (±28)	-66% (*)	81.7 (±0.6)	80.3 (±0.1)	- 2%	36.5 (±1.1)	30.1 (±0.1)	- 18% (*)	0.49 (± 0.01)	0.44 (± 0.01)	(*) %6-
Hard	Rebelde	744 (±55)	187 (±37)	-75% (*)	84.2 (±0.5)	82.0 (±0.1)	-3% (*)	32.8 (±0.6)	27.1 (±2.2)	-17%	0.46 (± 0.01)	0.40 (± 0.03)	- 14%
PIO	Canove	613 (±69)	217 (±32)	-65% (*)	79.1 (±0.6)	77.9 (±0.2)	- 2%	41.9 (±0.9)	35.1 (±1.7)	- 16% (*)	0.33 (±0.00)	0.27 (±0.01)	-20% (*)
	Guá 113	596 (±58)	397 (±55)	- 34%	79.3 (±0.3)	78.3 (±0.4)	- 1%	36.8 (±1.1)	35.6 (±3.3)	-3%	0.40 (± 0.01)	0.35 (± 0.03)	-13%
	Piave	525 (±30)	363 (± 24)	-31% (*)	80.5 (±0.2)	77.9 (±0.5)	-3% (*)	42.5 (±0.7)	41.5 (±0.4)	-2%	0.39 (± 0.02)	0.33 (±0.01)	- 14%
Average		859 (50)	371.5 (±25)	-57% (*)	79.2 (±0.6)	78.3 (±0.6)	- 1%	37.8 (±0.8)	33.5 (±0.9)	-11% (*)	0.46 (± 0.01)	0.40 (0.01)	-12% (*)

Table 4 Grain yield parameters (means ± S.E.; n = 3) in ten common wheat varieties at harvest in agroforestry (AF) and control (C) treatments. TKW: thousand kernel weight;

were associated with high leaf expansion (LAI/CAI increase) in modern var. Arkeos and Bagou and in the three old varieties (Fig. 2). HI was on average 0.49 in C and 0.42 in AF, close to the expected value of 0.5 of modern wheats. HI reductions in AF were significant in var Arkeos, Bagou, Canove, Solehio, and Vivendo, ranging from -7% to -20%. The minimal reductions in AF occurred in the variety Nemo (-3% vs. C).

The average protein content of wheat grains under full sun C was 12% DW, significantly lower than 16.5% of the AF treatment as average of all the varieties (Table 5), although associated with lower yield. In AF the highest grain protein content was achieved by the old varieties Canove and Piave (20.6 and 17.9%, respectively) and the hard var. Rebelde (18.8%) (Table 5). The greatest increases in protein content between AF and C was found in the old var. Guà 113 (+5.4% vs. C; absolute percentage variation) and in the modern var. Rebelde and Vivendo (+6.8% and +5.3%, respectively).

AF also led to significantly increases of the grain gluten content, 36.7% DW vs. 18.2% of C (+17.4% as absolute percentage increase) (Table 5). As for proteins, the highest gluten values under agroforestry were measured in old var. Canove (43.8%) and modern var. Rebelde (43.1%), but in general in the old varieties, also including Guà 113 and Piave (38.8 and 40.3%, respectively). The greatest improvements in AF vs. C were observed in the modern var. Nemo,

**Table 5** Grain qualitative parameters (mean  $\pm$  S.E.; n=3) of ten common wheat varieties at harvest in agroforestry (AF) and control (C) treatments. DW: Dry Weight. For each variety,

Rebelde and Vivendo (+14.8, +19.3 and +17.4, respectively, vs. C).

#### PCA and MDA

#### Control (C) treatment

Principal Component Analysis (PCA) applied on data from the control treatment (C) identified two synthetic variables, F1 and F2, which explained 85.52% and 8.33% variability respectively, and 93.85% together (Fig. 3). The most significant variables (loadings>l0.5l) in F1 were grain protein content, HI, grain yield, and additionally plant height and the leaf-to-culm area ratio (LAI/CAI). According with the vector directions of each variable in PCA, there is strict positive correlation between yield, Leaf/Culm DW ratio, LAI/CAI and HI, and negative correlation between yield and grain protein content. A positive correlation was also revealed between soil moisture in April and the vegetational indexes NDVI and SPAD.

According to the centroids position and cluster separation in MDA, a different behavior was revealed among varietal groups, according to the choice of modern vs. old varieties, and the bakery use as defined by the Synthetic Index of Grain Quality (Table S1) within modern varieties. Under full sun conditions the old varieties had the highest protein content, plant height and aboveground biomass, and TGW. For the modern varieties, those biscuit making

the percentage variation of AF treatment vs. C is reported, and asterisks indicate significant differences between treatments according to the Tukey's HSD test ( $p \le 0.05$ )

Varietal group	Variety name	Protein conten	t (% DW)		Wet gluten (% DW)		
		С	AF	var./C	С	AF	var./C
Biscuit	Arkeos	11.5 (±0.1)	16.1 (±0.1)	+4.6 (*)	22.4 (±0.1)	36.9 (±0.3)	+ 14.5 (*)
	Bagou	$11.6(\pm 0.1)$	$15.4 (\pm 0.1)$	+3.8 (*)	22.7 (±0.1)	$33.4(\pm 0.1)$	+10.8 (*)
Bread	LG Ayrton	$11.3 (\pm 0.1)$	$14.9(\pm 0.1)$	+3.6 (*)	21.5 (±0.3)	32.9 (±0.2)	+11.4 (*)
	Nemo	$10.1 (\pm 0.1)$	$14.2 (\pm 0.1)$	+4.1 (*)	16.7 (±0.3)	31.5 (±0.1)	+14.8 (*)
	Solehio	$11.0(\pm 0.1)$	$14.3 (\pm 0.1)$	+3.3 (*)	21.5 (±0.3)	30.7 (±0.2)	+9.2 (*)
	Vivendo	$10.3 (\pm 0.1)$	$15.6(\pm 0.1)$	+5.3 (*)	$18.2 (\pm 0.1)$	35.6 (±0.1)	+17.4 (*)
Hard	Rebelde	$11.9(\pm 0.1)$	18.8 (±0.1)	+6.8 (*)	$23.3 (\pm 0.2)$	43.1 (±0.1)	+19.3 (*)
Old	Canove	16.3 (±0.1)	20.6 (±0.1)	+4.3 (*)	33.6 (±0.2)	43.8 (±0.1)	+10.2 (*)
	Guá 113	11.9 (±0.1)	17.3 (±0.1)	+5.4 (*)	$24.0(\pm 0.1)$	38.8 (±0.1)	+14.8 (*)
	Piave	$13.0(\pm 0.1)$	17.9 (±0.1)	+4.9 (*)	$28.9(\pm 0.2)$	$40.3 (\pm 0.2)$	+11.4 (*)
Average		$11.9 (\pm 0.3)$	$16.5 (\pm 0.4)$	+4.6 (*)	$23.3 (\pm 0.9)$	36.7 (±0.8)	+13.4(*)



# Control (C) treatment

Variables	F1	F2
Yield	0.530	0.390
TGW	-0.281	-0.685
HI	0.818	0.459
Testing weight	0.100	-0.525
Protein content	-0.989	-0.018
NDVI	0.504	-0.056
SPAD	0.164	-0.038
Plant height	-0.674	-0.593
Aboveground DW	-0.098	-0.009
Leaf/Culm DW ratio	0.509	0.221
LAI	0.112	0.210
LAI/CAI	0.551	0.469
Soil moisture 28-April	0.513	-0.247
Soil moisture 31-May	0.022	-0.248

Fig. 3 Multigroup discriminant analysis (MDA; left) and principal component analysis (PCA; right) for morpho-physiological parameters at flowering, yield and quality parameters at harvest, and soil moisture at the beginning of heading (April 28) and grain filling (May 31), of ten common wheat varieties in control (C) full sun treatment. TKW: thousand kernel weight; HI: harvest index. LAI: leaf area index. CAI: culm area index. DW: dry weight. Abv: Aboveground. The isodensity confidence circles contain 75% of variability. In the table (bottom) the highly informative variables (loadings >|0.5|) are highlighted in bold, within synthetic variables F1 and F2. Different groups of varieties, i.e. bread-making, biscuit-making and hard within modern varieties, and old varieties, are highlighted with different colors in MDA were mostly linked to morphological traits, such as LAI, LAI/CAI and HI, and productivity, while those bread making to physiological traits, i.e. the vegetational indexes SPAD and NDVI, and the Leaf/Culm DW ratio. Soil moisture appears to be little informative with regard to the measurement of both 28 April and 31 May.

#### Agroforestry (AF) treatment

The two synthetic variables identified with PCA on data from the agroforestry treatment explained an overall variability of 97.69%, F1 being 64.37% and F2 33.32% (Fig. 4). The most significant variables (load-ings >|0.5|) in the relevant F1 were grain protein content, HI, grain yield, and additionally plant height, the aboveground biomass of wheat and the leaf/culm DW ratio. The vector directions of each variable in the PCA indicate that under shading there is strict positive correlation between yield, and HI, soil moisture at end April (at wheat heading), and NDVI. As regard grain quality, positive is the correlation between protein content, and plant height, biomass and LAI.

Multigroup Discriminant Analysis (MDA) revealed a different behavior by wheat varieties (Fig. 4). Within modern varieties, the effects of shading on those biscuit-making were mostly associated with variations in LAI/CAI, leaf/culm DW ratio and SPAD, while those bread-making with yield, NDVI and HI. On the contrary, old varieties were mostly linked to protein content, plant height, leaf area and aboveground biomass.

#### Discussion

This study provides results on a varietal screening regarding 10 wheat genotypes intercropped with 4-year old poplar trees in the temperate climate of NE Italy for assessing their suitability to agroforestry farming systems. The main originality of this investigation concerns the choice to carrying out the varietal comparison in open field conditions within a specialized poplar plantation, which created natural shading, instead of artificial shading and/or in pot cultivation. The inter-row of the poplar plantation was extremely narrow as compared to usual silvoarable alley cropping designs in temperate climates, where the inter-row ranges from 15 to 40 m (Beuschel et al. 2020; Inurreta-Aguirre et al. 2018; Piotto et al. 2023).

Although very challenging for wheat, the extreme shading conditions of our trial were functional to: (i) set small wheat plots to be placed at the center of poplar alleys, and test a relatively large number of varieties, (ii) maintain belowground interactions and competition for water between wheat and poplar roots, and (iii) highlight the impact of intense shading on wheat varieties. The average seasonal amount of available PAR for the wheat crop was indeed strongly reduced (-42%) as compared to the full sun control, although mainly impacting of the last part of the wheat cycle after tree foliation. Under these conditions all the wheat varieties exhibited a longer maintenance of leaf greenness at end growing cycle, as highlighted by higher NDVI and chlorophyll content (SPAD) of the flag leaf as compared to controls. Increased stay green mainly involved the youngest uppermost leaves and is demonstrated to be crucial under lowirradiance environments (Brouwer et al. 2012; Shimoda and Sugikawa 2020). An increase of the flag leaf chlorophyll content is also an adaptive physiological trait to increased shade level, as previously documented by several authors (Li et al. 2010; Arenas-Corraliza et al. 2021; Wang et al. 2014; Xu et al. 2013; Zheng et al. 2011; Lakshmanakumar et al. 2013), thus allowing wheat to improve both radiation harvesting and use efficiency (Shaver et al. 2008; Liu et al. 2007). Indeed, increasing the content of photosynthetic pigments in the flag leaf can positively affects wheat grain yield (Luo et al. 2006; Marinaccio et al. 2015), the kernel size and its quality (Entz and Fowler 1990; Chen et al. 2010), and exert a key role in driving shading acclimation (Xu et al. 2013; Lakshmanakumar et al. 2013; Li et al. 2010; Arenas-Corraliza et al. 2019). However, sparse literature clearly documented a positive contribution of delayed canopy senescence to grain filling and wheat productivity, specifically when light reduction was moderate (-12% PAR) and applied after anthesis (Xu et al. 2016). In our trial, these physiological acclimations did not compensate for the impact of intense tree shading, which resulted in decreased grain yield. The average - 42% PAR recorded here was associated with proportionally higher yield impairment (i.e., - 57%), corroborating results from



#### Agroforestry (AF) treatment

Variables	F1	F2
Yield	-0.670	-0.083
TGW	-0.022	0.191
HI	0.868	0.101
Testing weight	0.118	0.985
Protein content	-0.992	0.106
NDVI	0.278	-0.030
SPAD	0.199	-0.442
Plant height	-0.712	0.068
Aboveground DW	-0.519	-0.024
Leaf/Culm DW ratio	0.611	-0.473
LAI	-0.398	-0.176
LAI/CAI	0.464	-0.643
Soil moisture 28-April	0.379	0.019
Soil moisture 31-May	-0.453	-0.370

Fig. 4 Multigroup discriminant analysis (MDA; left) and principal component analysis (PCA; right) for morpho-physiological parameters at flowering, yield and quality parameters at harvest, and soil moisture at the beginning of heading (April 28) and grain filling (May 31), of ten common wheat varieties in the agroforestry (AF) treatment. TKW: thousand kernel weight; HI: harvest index. LAI: leaf area index. CAI: culm

deciduous tree species-wheat alley-cropping systems in temperate regions on the exponential relationship between PAR and crop yield reductions. area index. DW: dry weight. Abv: aboveground. The isodensity confidence circles contain 75% of variability. In the table (bottom), the highly informative variables (loadings > 0.51) are highlighted in bold, within synthetic variables F1 and F2. Different groups of varieties, i.e. bread-making, biscuit-making and hard within modern varieties, and old varieties, are highlighted with different colors in MDA

Indeed, under conditions similar to ours, PAR reductions being between -40% and -60%, wheat yield losses ranged from -25% to -80% vs. full

sun conditions (Artru et al. 2017; Xu et al. 2016; Lakshmanakumar et al. 2013).

While this suggests avoiding excessive shading severity for preserving acceptable crop productivity, most importantly our study highlights marked genetic variability among wheat varieties in agreement with recent investigations (Gill et al. 2009; Panozzo et al. 2020). Here it was explored the variability among different varietal groups, namely between old and modern varieties, and among bread-making, biscuitmaking and hard types of modern varieties. Under shading, yield reduction was minimal in the old var. Piave (-31%) and maximum in the modern hard var. Rebelde (-75%). The average yield of old varieties under full sun conditions was largely lower than modern ones (-41%) likely due to poor harvest index, which generally worsened further under shading, with maximum reductions in some modern genotypes. This would explain why yield impairments were much larger in modern varieties with respect to the old ones (-60% vs. - 44%) when moving from full sun to shading conditions. In particular, the old var. Piave and Guà 113 seemed the best tolerant in AF, by recording 32% of yield reduction only. These results apparently suggest a less tolerant response to shading in ameliorated high-yielding varieties, but it was not a rule. Indeed, the highest yield performance (absolute value) under shading were recorded in the breadmaking varieties Solehio, LG Ayrton and Nemo, with ~ 500 g/m<sup>2</sup> of grains. According with PCA, what characterized these best performing varieties when moving from full sun to shading conditions was small and stable plant height, similar or superior NDVI and SPAD dynamics, and particularly a marked increase of the leaf-to-shoot biomass ratio, that ensured elevated light interception and favorable biomass partitioning among sink and sources (Trapani et al. 1992; Valladares et al. 2003; Li et al. 2010).

Identifying the most suitable wheat varieties able to maintain high yield levels under agroforestry is recognized to be the key point to implement highproductive silvoarable systems. Agroforestry-oriented screening and breeding programs will depend on the identification of the key traits for adaptability to intercropping with trees, and for this purpose the analysis of yield components and morpho-physiological changes here resulted extremely useful. In recent literature, the decrease of wheat yield in the interaction zone with trees is reported to be mainly related to reductions in both the number of grains per surface area, the weight of grains, and harvest index, the latter also observed in our study, with the majority of the authors documenting the highest detrimental impact on the numbers of grains (Inurreta-Aguirre et al. 2018; Acreche and Slafer 2009; Artru et al. 2017; Dufour et al. 2013; Li et al. 2008). Concerning the thousand kernels weight (TKW), contrasting results are reported in the literature, with some authors observing a negative effect of pre-anthesis (Dufour et al. 2013) and post-anthesis shading (Chen et al. 2013), while others reporting increased TKW under shading conditions (Xu et al. 2016; Li et al. 2010). In our study a general decrease of both HI and grain weight was revealed under shading, but with large variability among varieties. Among yield components, the testing weight showed the smallest variations between treatments, suggesting that soil water availability allowed for adequate grain filling in both systems.

The most relevant variations in response to shading were linked to morpho-physiological traits, and the idea to classify wheat varieties into groups was intended to identify a possible common behavior. Indeed, for optimizing light interception under agroforestry, old varieties showed the highest plasticity, as revealed by much longer maintenance of leaf greenness, higher contents of leaf pigments, and exceptional increases of both plant height and leaf area, particularly in the var. Guà 113 and Canove, as compared to modern varieties. An increase of plant height is commonly observable in shaded environments, mainly through the elongation of the last internode (Li et al. 2010; Wang et al. 2015; Arenas-Corraliza et al. 2021). Shading tolerance was also found associated to improved leaf area by some authors (Dufour et al. 2013; Li et al. 2010; Arenas-Corraliza et al. 2019, 2021), although unbalanced biomass partitioning, and HI were the major reasons of yield failure of our old varieties. This was observed particularly in var. Canove, originally selected in the Italian pre-Alps, which extreme morphological plasticity brought to the most relevant yield and TGW reductions in AF vs. C, as compared to the other two old varieties. When the canopy biomass is maintained above an adequate threshold, improvements of the leaf-to-culm biomass ratio rather than the leaf-to-culm area ratio seems one of the most important adaptive traits for ensuring sustainable wheat grain yield in agroforestry.

As regards grain quality, a marked increase of protein content is expected in wheat under shading, in our trial being 16.5% vs. 12% DW of the full sun control, but an even higher gluten content improvement. Further investigations are necessary to elucidate the effects of shading on protein gluten patterns of flours, as gliadins and glutenins abundance, but it seems that shading has a positive impact on quality for high-leavened products. Higher protein concentration in wheat grains is reported in the literature under both artificial shading (Arenas Corraliza et al. 2021; Artru et al. 2017; Dufour et al. 2013) and silvoarable systems in the neighboring of tree rows (Pardon et al. 2018; Panozzo et al. 2020; Arenas-Corraliza et al. 2018). These improvements can be due to better N remobilization from green tissues at ripening, and also to the wellknown negative correlation between grain yield and protein content. The potential to enhance the grain quality and commercial value of wheat grains in agroforestry systems deserves attention and should be further investigated for implementing market strategies.

This preliminary study on varietal screening highlights that the largest morpho-physiological changes to maximize light capture occur in old wheat genotypes, but at the expense of yield. Despite halved yields, modern bread-making varieties can instead represent a sustainable choice, potentially showing even better performances under larger tree inter-rows as compared to our poplar plantation, thereby reducing the yield gap between full sun and shaded conditions. Similarly to the old ones, the biscuit-making varieties, namely Bagou and Arkeos, provided poor productivity in agroforestry, although showing high LAI-to-CAI ratio, SPAD and NDVI; as they are appreciated for the low protein content and low leavening commonly achieved under full sun, a reclassification of use will be likely necessary when cultivated in agroforestry because of their increased protein content. Under shading, wheat yield was also correlated with the soil water content in late April, beside to HI and NDVI. At that time, both crop and trees are highly water-demanding, wheat being at headingflowering stage and poplars at half of the complete leaf development (Ray and Savage 2019). Thus, a competition for water absorption was likely to occur in our AF system, as documented by low soil moisture measurement at that time, especially in the highsized old varieties, which likely reduced the yield potential of the whole set of wheat varieties.

For a complete validation of the varietal comparison, these varieties should be also compared within common alley-cropping systems with larger interrows, and at various distances from the trees along transects, in order to confirm the key morphological and physiological traits associated with shadetolerance and the varietal yield ranking. A thorough investigation on root growth and its plasticity will be also necessary to fully understand belowground croptree interactions, and the effects of shading and water

scarcity on yield losses according to variety choice.

# Conclusions

Screening of wheat varieties within the narrow alleys of a specialized poplar plantation at about its half lifespan is a novel approach that demonstrates to be suitable for highlighting key traits for shade-tolerance and contrasting responses within a set of varieties. Although from one year only, this trial provided useful preliminary information on shade tolerance of wheat varieties, to be confirmed in other years and locations. Here, the old high-sized wheat varieties were the most responsive ones to extreme shading, by increasing their aboveground biomass further (already elevated) and stay green, thereby appearing promising under agroforestry for improving light capture and use efficiency. The modern wheat varieties studied here, despite lower morpho-physiological plasticity and greater relative yield losses, still guaranteed the highest productivity under shading, particularly in the bread-making rather than the biscuit-making type. Old varieties collected from local germoplasm banks remain, however, important genetic resources to be exploited under severe shading of challenging agroforestry designs, and lowinput or organic farming systems. On the opposite, modern varieties would reasonably provide more sustainable output yield, with the expectation to improve their efficiency under alley-cropping systems with larger tree inter-rows, taking better advantage of more abundant resources availability. Here, some key traits of adaptability to agroforestry can be proposed, such as elevated leaf-to-culm biomass ratio, leaf-to-culm area ratio, harvest index, and canopy stay green, the latter being essential under heat waves during seed filling. A thorough investigation on root morphology of wheat and belowground competition with trees would be necessary to fully characterize wheat varieties particularly under low soil water and nutrients availability.

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#### Declarations

**Conflict of interest** The authors declare no conflict of interest.

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