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Original scientific article

SOME AGRONOMIC TRAITS AFFECTING BARLEY MYCORRHIZATION, GRAIN YIELD AND QUALITY

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Breeding barley (*Hordeum vulgare* L.) for low-input conditions may be a key factor for enhancing yields in poor environments. Arbuscular mycorrhizal (AM) symbiosis and seeding rate may also affect barley performance in alkaline, low-P soils under Mediterranean conditions. For two growing seasons, two conventionally bred and two cultivars bred under low-input conditions were tested at three seeding rates (300, 400 and 500 seeds m^{-2}) under rainfed Mediterranean conditions. Length of root colonized by AM fungi and plant height were determined at anthesis, whereas grain yield (GY), 1000-kernel weight (TKW) and protein concentration (PC) were measured at harvest. Across the growing seasons, GY was highest (2713.6 kg ha^{-1}) at the highest seeding rate. The shorter, conventionally bred cultivars yielded better compared to the low-input-bred counterparts (2872.6 vs. 2228.1 kg ha^{-1}). However, the low-input cultivars had significantly higher PC (12.63 vs. 12.04%). The six-row cultivars were more productive compared to two-row ones (2854.1 vs. 2246.6 kg ha^{-1}) with higher TKW (40.22 vs. 35.99 g). No differences between cultivars, seeding rates or breeding method were found for AM colonization of roots. Low-input breeding did not select for higher mycorrhization and did not perform better than conventionally bred barley cultivars under low-input conditions.

Keywords: *Hordeum vulgare*, low-input agriculture, mycorrhizae, phosphorus, seeding rate

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is a grain cereal with wide adaptability, which is grown mainly for animal feed. A considerable proportion (ca. 20%) of the grain produced is used for malt for brewing and a smaller amount is directed to human consumption. In general, two-row barley is used mainly for malt production, whereas six-row barley is used for feed (ARENDT and ZANNINI, 2013).

Under rainfed Mediterranean conditions, winter barley yield is unstable because it is affected heavily by water inputs before and after anthesis, which determine the number and size of seeds produced (MACHOLDT *et al*., 2020; VAHAMIDIS *et al*., 2021). In addition, barley in the Mediterranean basin often grows on alkaline soils with low P availability, a condition made worse by the low soil water availability (BARROW, 2017).

Under rainfed, low-input or organic-farming conditions, maintaining satisfactory yields may involve breeding/selecting cultivars adapted to such conditions (AHMADI *et al*., 2016; ARSHADI *et al*., 2016; VLACHOSTERGIOS *et al*., 2011). Possibly, such cultivars possess high efficiency in nutrient uptake and use (CAMPOS *et al*., 2018), altered root architecture (BROWN *et al*., 2013; HECHT *et al*., 2016, 2019) or have efficient arbuscular mycorrhiza (AM) symbiosis (ZHU *et al*., 2003; VAN DE WIEL *et al*., 2016). Mycorrhiza may have a pivotal role in plant nutrition with respect to immobile elements (e.g., P and Zn), water-stress mitigation, disease tolerance and it can result in improved soil aggregate formation (MÄDER *et al*., 2000; HOHMANN and MESSMER, 2017). These effects are considered to be more pronounced under low-input than high-input conditions (MÄDER *et al*., 2000). However, mycorrhiza's efficiency declines with rising P levels (VAN'T PADJE *et al*., 2020); the plant halts the allocation of photosynthetic carbon to the fungus once the availability of inorganic nutrients rises. This makes selection of genotypes with high mycorrhization complicated and with ambiguous results on plant nutrition and yield. BROWN *et al*. (2013) considered AM in barley-breeding programs under P deficiency. However, the cultivars developed more root hairs instead of increasing AM colonization to cope with P deficiency.

Mycorrhiza colonization has been found to relate with barley morphology, meaning that plants with higher AM colonization were taller compared to plants with lower colonization (BESLEMES *et al*., 2016; MOSHFEGHI *et al*., 2019). However, conventional agriculture has increased yields by selecting shorter cultivars and utilizing high seeding rates (HECHT *et al*., 2016). Under high plant density and low-P soils, it is believed that the depletion zones of neighbouring plants overlap, rendering the fungal contribution irrelevant (KOIDE, 1991). At high plant densities, the length of root colonized is expected to decrease (KOIDE, 1991; ZHU *et al*., 2003; SHROEDER and JANOS, 2005) as a result of competition mainly for light (FACELLI *et al*., 1999), since less irradiance results less photosynthates produced and allocated to the fungus. However, others have not found decreased root length colonization with increased plant density (DERELLE *et al*., 2015; GONG *et al*., 2022; SCHROEDER-MORENO and JANOS, 2008).

There is a severe scarcity of field works on how seeding rate actually affects AM fungal root colonization in grain cereals, as most data have derived from pot experiments. SASVÁRI and POSTA (2010) found no colonization difference in maize (*Zea mays* L.) between two plant densities. However, plant growth response to AM generally decreases with increasing plant density, even if colonization is unaffected (FACELLI *et al*., 1999).

In Greece, the Hellenic Agricultural Organization “Demeter” had run a barley breeding program focusing on best-performing cultivars under low-input conditions, without considering their mycorrhizal dependency (BLADENOPOULOS and KORPETIS, 2012). Breeding under high-input systems led to genotypes with low AM colonization (HETRICK *et al*., 1993). On the other hand, under low-P supply, P-efficient cultivars are not expected to benefit from AM compared to P-inefficient genotypes (BAON *et al*., 1993a). It is not evident that cultivars bred under low-input conditions are more responsive to AM or have high colonization compared to conventionally bred ones. However, cv. ‘Tritptolemos’, bred for low-input cultivation, showed a positive response to field inoculation with AM with or without organic or inorganic P fertilization (BESLEMES *et al*., 2016).

A field study was conducted aiming to 1) compare the agronomic performance of conventional and low-input cultivars and 2) identify possible associations with AM colonization under different plant densities. Thus, two- and six-row barley cultivars (a total of four cultivars) were grown under three seeding rates (300, 400 and 500 seeds m^{-2}) for two growing seasons. The hypothesis was that under rainfed, low-P, calcareous conditions the low input cultivars shall perform better and have higher AM colonization.

MATERIALS AND METHODS

Experimental Design and Conditions

Field experiments were conducted for two growing seasons [2013-2014 (hereafter 2014) and 2014-2015 (hereafter 2015)], in adjacent sites, in the Hellenic Agricultural Organization-«Demeter», Institute of Plant Breeding and Genetic Resources, Thermi, Greece (40^o 32’ 22 N, 23^o 00’ 32 E, 21 m asl). The soil was a clay loam in 2014 and a loam in 2015, with low P concentrations (Table 1). The climate is typical Mediterranean with mild and rainy winters (Fig. 1).

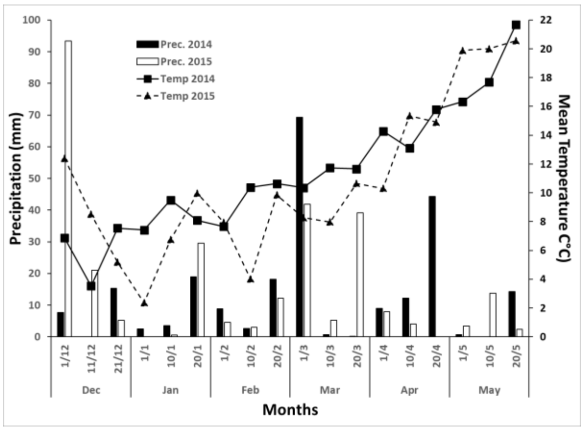

Fig. 1. Ten-day mean air temperature (Temp.) and precipitation (Prec.) during the two growth seasons (December to May).

Table 1. Some soil properties before the establishment of the experiments.

	2014	**2015**
Sand (%)	40.9	40.7
Silt (%)	30.5	37.0
Clay (%)	28.6	22.3
Texture	CL[†]	L[‡]
pH (1:2.5)	8.3	8.4
Organic matter (%)	2.15	1.70
$CaCO_3$ (%)	1.68	0.90
NO_3-N (mg kg^{-1})	7.71	23.66
Olsen-P (mg kg^{-1})	4.75	10.40
K (mg kg^{-1})[*]	117.6	221.8
Na (mg kg^{-1})[*]	20.7	49.3
Ca (mg kg^{-1})[*]	3878	3755
Mg (mg kg^{-1})[*]	684.8	310.3
Cu (mg kg^{-1})[1]	2.37	1.76
Zn (mg kg^{-1})[1]	0.95	0.57
Fe (mg kg^{-1})[1]	7.60	6.35
Mn (mg kg^{-1})[1]	13.44	12.9
EC[§] (mS/cm)	0.19	0.30

[†]CL: clay loam. [‡]L: loam. [§]EC: electrical conductivity. *Extraction with 1M CH_3COONH_4 method [1]DTPA extraction

Four barley cultivars, the conventionally bred cvs. ‘Chill’ (two-row) and ‘Mucho’ (six-row), both imported commercial cultivars, and cvs. ‘Makedonia’ (two-row) and ‘Triptolemos’ (six-row), bred under low-input conditions (registered by the Hellenic Agricultural Organization-«Demeter», Institute of Plant Breeding and Genetic Resources, Thermi, Greece) were seeded by hand on 30 November 2013 and 25 November 2014. The experimental design was a split-plot design with four replications; three seeding rates (300, 400 and 500 seeds m^{-2}) were in the main plots and the four cultivars were in the subplots. Breeders' seeds with emergence percentage higher than 93% were used for seeding. Each subplot consisted of seven, 5 m-long rows at 0.25 m spacing. Nitrogen in the form of NH_4NO_3 was applied at a rate of 40 kg ha^{-1} as top-dressing when two nodes were detectable (BBCH 32, LANCASHIRE *et al.*, 1991). No irrigation was supplied at any growth stage. Weeds were controlled by a combination of hand-weeding and hoeing later in the season.

Plant Harvest and Analysis

At anthesis (BBCH 65), 10 plants per subplot were uprooted and the height of the main stem up to the end of spike (PH) was measured using a common ruler. Then, the roots were cut off, washed on a sieve and approximately 0.5 g of roots was stained with trypan blue for estimation of the length of root colonized by the arbuscular mycorrhizal fungi (AMF) (SYLVIA, 1994).

At harvest maturity (BBCH 89), the five internal rows per subplot were mechanically harvested using a plot combine harvester (Type INM, F. Walter-H. Wintersteiger KG, Ried, Austria). After further hand-threshing, grain weight per subplot was measured, adjusted to 10% seed moisture and grain yield (GY) was calculated as kg ha^{-1}. After harvest, thousand-kernel weight (TKW) was assessed on 100 grains randomly selected per subplot and protein concentration (PC) was estimated on a near-infrared spectrometer (PerCon Inframatic 8620, Perten Instruments GmbH, Hamburg, Germany) after adjusting at 10% seed moisture concentration.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the statistical software JMP 8 (SAS INSTITUTE, 2009). The same analysis was run when data were grouped according to breeding method (conventional and low-input) and barley type (two- and six-row cultivars). The *F*-ratios were used to test the effects for the randomized complete block experiment combined across years considering treatments as fixed and years as random (MCINTOSH, 1983). The least significant difference (LSD) test at $P \leq 0.05$ was applied to compare means.

RESULTS AND DISCUSSION

The two growing seasons differed in climatic parameters, with 2014 being more wet and with higher temperatures (Fig. 1). Therefore, growing seasons affected significantly all the traits measured (Table 2, but also see Supplemental Tables S1-3). Distribution of rain in the season, combined with sowing time may affect grain yield (MIROSAVLJEVIC *et al*., 2015). Grain yield was less than half in 2015 (1637.8 kg ha^{-1}) compared to 2014 (3462.9 kg ha^{-1}). This was the result of the higher and earlier infection by leaf diseases [blotch caused by *Pyrenophora teres* Drechs. (1923) and rhynchosporium caused by *Rhynchosporium secalis* (Oudem.) Davis (1922)], which were favored by the wetter winter (December to March) in 2015 growing season. Yield reduction was accompanied by reductions in grain protein concentration (11.02% vs. 13.64%) and grain size (39.72 g vs. 36.50 g).

AM colonization was almost four-times higher in 2015 (32.5%) compared to 2014 growing season (8.5%), and similar for all cultivars. Such low AM colonization (8.5%) has already been reported in barley fields in Tunisia, where AM colonization was negatively correlated with available soil P and annual rainfall, but it was positively correlated with temperature (JERBI *et al*., 2020). However, in our study, the higher AM colonization was recorded in the wetter and cooler growing season, with the higher soil P availability. This response could be ascribed to the fact that similar, but different fields were used each year and likely they had different inoculum potential.

Interestingly, the cultivars differed significantly in all the traits but AM colonization. Significant growing season × cultivar interactions were evident for PH and GY, but in both growing seasons, the shortest (82.0 and 56.7 cm) cv. ‘Mucho’ was the highest yielding (4830.9 in 2014 vs. 2031.9 kg ha^{-1} in 2015) cultivar (Table 2). These differences were also evident between breeding methods and barley types (Table 2). Specifically, the conventional cultivars were overall shorter (73.6 cm vs. 95.9 cm) and more productive (2872.6 kg ha^{-1} vs. 2228.1 kg ha^{-1}) because of the six-row, very short (69.3 cm) and large-seeded (41.83 g) cv. ‘Mucho’, which was by far the highest-yielding genotype (3431.4 kg ha^{-1}). In contrast, the conventional, two-row, malt-producing cv. ‘Chill’ was also short (77.9 cm), but low-yielding (2313.7 kg ha^{-1}) and with the lowest TKW (33.57 g). Both conventional varieties were shorter, but it was the six-row conventional variety that was actually more productive than the low-input bred varieties.

Table 2. Mean comparisons of plant height at anthesis (PH), arbuscular mycorrhiza percentage of colonization (Myc), grain yield (GY), seed protein concentration (PC), 1000-kernel weight (TKW).

	PH cm	Myc %	GY kg ha^{-1}	PC %	TKW g
Growing season (Gs)					
2014	101.2±16.3a	8.5±2.7b	3462.9±939.5a	13.64±0.8a	39.72±4.7a
2015	68.2±13.2b	32.5±16.7a	1637.8±412.9b	11.02±1.5b	36.50±4.0b
Cultivar (C)					
Triptolemos (Tri)	88.8±22.2b	21.5±22.0a	2276.8±1047.4b	12.55±1.7a	38.63±4.7b
Chill (Chi)	77.9±14.1c	20.0±13.9a	2313.7±715.1b	11.77±2.0b	33.57±3.7c
Makedonia (Mak)	102.9±22.0a	21.4±14.9a	2179.4±810.3b	12.70±1.7a	38.41±2.7b
Mucho (Muc)	69.3±14.2d	19.1±16.8a	3431.4±1507.5a	12.31±1.6ab	41.83±3.0a
Gs × C					
2014					
Tri	110.2±3.8b		3235.8±499.1b		
Chi	90.7±4.2c		2917.8±424.8bc		
Mak	122.1±3.5a		2867.0±365.6c		
Muc	82.0±3.5d		4830.9±631.5a		
2015					
Tri	83.8±5.0d		1317.8±195.7f		
Chi	67.5±6.0e		1709.6±304.2de		
Mak	65.0±14.2e		1491.8±455.9ef		
Muc	56.7±7.8f		2031.9±282.5d		
Breeding method					
Conventional	73.6±14.6 b	19.6±15.2a	2872.6±1296.7a	12.04±1.8 b	37.70a±5.4
Low-input	95.9±23 a	21.4±18.6a	2228.1±927.7b	12.63±1.7 a	38.52a±3.8
Barley type					
2-row	90.4±19.9a	20.3±14.2a	2246.6±752.3b	12.24±1.9a	35.99±4.1b
6-row	79.1±20.9b	20.7±19.4a	2854.1±1410.5a	12.43±1.6a	40.23±4.2a
Seeding rate					
300	84.6±22.7a	21.8±18.1a	2461.6±1064.3b	12.36±1.8a	38.05±4.5a
400	84.8±22.4a	19.2±16.0a	2475.8±1153.7b	12.36±1.8a	37.79±4.0a
500	84.8±22.3a	20.6±17.0a	2713.6±1292.4a	12.27±1.8a	38.48±5.4a

Within a column, for each factor (cultivar, breeding method, barley type, seeding rate and growing season), means followed by the same letter did not differ significantly according to LSD at $P \leq 0.05$.

Low-input cultivars showed a small, not significant increase in AM colonization compared to conventional ones (21.4% vs. 19.6%, Table 2), and therefore any effect on PH, GY and quality traits was not due to AM colonization, in contrast to previous works (e.g., CRIADO *et al*., 2015; BESLEMES *et al*., 2016). At low P supply, root hairs of barley may be more critical for P acquisition than AM colonization (BROWN *et al*., 2013). In the case of low-input cvs. ‘Triptolemos’ and ‘Makedonia’, there was no indication that breeding for low-input conditions led to selection for higher root length colonization and therefore indirectly for higher mycorrhizal response (Table 2, S5). Previously, no differences in AM colonization percentage have been found between P-efficient and P-inefficient barley cultivars (BAON *et al*., 1993b). On the other hand, modern varieties in other crops were as responsive to AMF (WANG *et al*., 2020) or even more colonized by AMF than older varieties (WANG *et al*., 2021).

Regarding barley type, six-row cultivars were shorter (79.1 cm vs. 90.4 cm), more productive (2854.1 kg ha^{-1} vs. 2246.6 kg ha^{-1}) and with higher TKW (40.23 g vs. 35.99 g) compared to two-row cultivars, actually expressing the respective traits of cv. 'Mucho'. It is noteworthy that in the low-yielding season (2015), there was no significant difference in GY between the two types (Table 3). A significant growing season × breeding method interaction was evident for PH, with all the cultivars being shorter in 2015 than in 2014 (Table 3) and the conventional cultivars always being shorter than the low-input ones. On the other hand, it is always possible that the impact of weather effects (rain patterns, low winter temperatures) on the yield of the two breeding types may have exceeded the soil fertility impact, nullifying any potential advantage of the low input cultivars at low fertility, if there was any.

Table 3. Mean comparisons: for plant height at anthesis (PH) of the significant growing season × breeding method interaction, for grain yield (GY) of the significant growing season × barley type interaction.

	PH cm		GY kg ha^{-1}
		2014	
Conventional	86.4±5.8b	2-row	2892.4±388.4b
Low-input	116.1±7.1a	6-row	4033.4±986.7a
		2015	
Conventional	60.8±8.0d	2-row	1600.7±395.0c
Low-input	75.7±13.3c	6-row	1674.9±435.4c

Means followed by the same letter did not differ significantly according to LSD at $P \leq 0.05$

Under the rainfed, low-input and P-poor conditions of the present study, seeding rate had a significant effect only on GY, with the higher rate (500 seeds m^{-2}) yielding better (2713.6 kg ha^{-1}) (Table 2). This was in contrast to HECHT *et al*. (2016), who in a highly productive environment in Germany, obtained the highest yield (~7.5 t ha^{-1}) at a rate of 230 seeds m^{-2}. Possibly, the less tillering of the barley plants at higher seeding rates is advantageous in an environment of limited resources, since less energy is invested in tillers, which cannot be supported by the limited photosynthetic products. Regarding AM colonization, there was an absence of response to seeding rates, which is contrary to the lower colonization with increasing density found in pot studies, but not confirmed in field studies (KOIDE, 1991; SASVÁRI and POSTA, 2010). On the other hand, if a stress factor like drought is present (DUAN *et al*., 2021) or there is competition with other plant species, like in intercrops (DE FREITAS *et al*., 2018; DERELLE *et al*., 2015), this decrease is not observed. Possibly, the rainfed, low P, calcareous conditions were rather stressful, and the plants were still benefited by AMF, even at high densities.

The initial hypothesis, that under semiarid conditions in an alkaline, P-poor soil, cultivars developed under low-input conditions would perform better and have a better interaction with AMF is not supported by the results. Low-input breeding did not select for increased mycorrhization and did not perform better than conventionally bred barley cultivars, under low-input conditions. This may also be stated reversely, that conventionally bred barley cultivars did not lose their ability to form an efficient symbiosis. In addition, all the cultivars-maintained AMF colonization at the same levels at all densities, indicating that the symbiosis is still functionally significant at higher densities, at the given conditions. Barley breeding strategies exploiting the AM symbiosis should focus on identifying efficient cultivar-fungal community combinations, although this would be also challenging in field conditions.

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NEKE AGRONOMSKE OSOBINE KOJE UTIČU NA MIKORIZACIJU, PRINOS ZRNA I KVALITET JEČMA

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Izvod

Oplemenjivanje ječma (*Hordeum vulgare* L.) za uslove sa niskim inputom može biti ključni faktor za povećanje prinosa u manje povoljnim spoljašnjim sredinama. Arbuskularna mikorizna (AM) simbioza i setvena norma takođe mogu uticati na performanse ječma u alkalnim zemljištima sa niskim P u uslovima Mediterana. Tokom dve vegetacione sezone, dve konvencionalno stvorene i dve sorte stvorene u uslovima niskog inputa testirane su pri tri setvene norme (300, 400 i 500 semena m-2) u mediteranskim uslovima bez navodnjavanja. Dužina korena kolonizovanog AM gljivama i visina biljke određivani su pri cvetanju, dok su prinos zrna (GY), masa 1000 zrna (TKW) i koncentracija proteina (PC) mereni pri žetvi. Tokom vegetacije, GY je bio najveći (2713,6 kg ha-1) pri najvišoj setvenoj normi. Niže, konvencionalno stvarane sorte dale su bolji prinos u poređenju sa sortama koje se stvaraju na niski input (2872,6 prema 2228,1 kg ha-1). Međutim, sorte sa niskim unosom imale su značajno veći PC (12,63 prema 12,04%). Šestoredne sorte su bile produktivnije u odnosu na dvoredne (2854,1 prema 2246,6 kg ha-1) sa većom TKW (40,22 prema 35,99 g). Nisu nađene razlike između sorti, setvene norme ili načina oplemenjivanja za AM kolonizaciju korena. Oplemenjivanje sa niskim inputom nije izvršilo selekciju za veću mikorizaciju i nije imala bolje rezultate od konvencionalno stvorenih sorti ječma u uslovima niskog inputa.

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