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# Morphological and Physiological Root Traits and Their Relationship with Nitrogen Uptake in Wheat Varieties Released from 1915 to 2013

**Guglielmo Puccio [1], Rosolino Ingraffia [1,2,3,*], Dario Giambalvo [1], Gaetano Amato [1] and Alfonso S. Frenda [1]**

[1] Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy; guglielmo.puccio@unipa.it (G.P.); dario.giambalvo@unipa.it (D.G.); gaetano.amato@unipa.it (G.A.); alfonso.frenda@unipa.it (A.S.F.)
[2] Plant Ecology, Institute of Biology, Freie Universität Berlin, 14195 Berlin, Germany
[3] Berlin-Brandenburg Institute of Advanced Biodiversity Research, 14195 Berlin, Germany
* Correspondence: rosolino.ingraffia@unipa.it

**Abstract:** Identifying genotypes with a greater ability to absorb nitrogen (N) may be important to reducing N loss in the environment and improving the sustainability of agricultural systems. This study extends the knowledge of variability among wheat genotypes in terms of morphological or physiological root traits, N uptake under conditions of low soil N availability, and in the amount and rapidity of the use of N supplied with fertilizer. Nine genotypes of durum wheat were chosen for their different morpho-phenological characteristics and year of their release. The isotopic tracer $^{15}N$ was used to measure the fertilizer N uptake efficiency. The results show that durum wheat breeding did not have univocal effects on the characteristics of the root system (weight, length, specific root length, etc.) or N uptake capacity. The differences in N uptake among the studied genotypes when grown in conditions of low N availability appear to be related more to differences in uptake efficiency per unit of weight and length of the root system than to differences in the morphological root traits. The differences among the genotypes in the speed and the ability to take advantage of the greater N availability, determined by N fertilization, appear to a certain extent to be related to the development of the root system and the photosynthesizing area. This study highlights some variability within the species in terms of the development, distribution, and efficiency of the root system, which suggests that there may be sufficient grounds for improving these traits with positive effects in terms of adaptability to difficult environments and resilience to climate change.

**Keywords:** wheat roots; N uptake efficiency; genotypes; N fertilizer recovery



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## 1. Introduction

The general public, sensitized to protecting the environment and health, is currently advocating for the identification of sustainable cropping systems able to guarantee adequate yields while minimizing the impact of production processes on the environment and safeguarding non-renewable resources. Particular attention has been paid to the use of nitrogen (N) fertilizers, as their misuse in mode, timing, and form can lead to the release of N into the environment, causing global warming through nitrous oxide emissions [1]; pollution of water by nitrate emissions [2]; and soil acidification, eutrophication, and loss of biodiversity of natural ecosystems when N is returned to the surface by deposition in the form of $NH_3$ [3].

In cereal systems, an often-substantial proportion of N fertilizers is not intercepted by crops [4,5]. In a study performed in Spain on durum wheat (*Triticum durum*), López-Bellido et al. [6] reported values for labelled $^{15}N$ fertilizer recovery ranging from 12.7% to 41.6% depending on the distribution method. Values in that range have also been reported for the species in other research [7–11]. This shows the need to identify solutions able to

improve the ability of crops to absorb N and reduce N loss potential; in this context, the choice of variety may be essential.

Nitrogen use efficiency (NUE) is generally defined as the grain yield produced per unit of N available from the soil and fertilizer [12]; it is the product of two physiological factors: (1) N uptake efficiency (NUpE, defined as the amount of N taken up by the crop per unit of N available to the crop) and (2) N utilization efficiency (NUtE, defined as the grain yield per unit of N taken up by the crop). A certain genetic variability for NUE has been detected in wheat [13]. Differences in NUE among genotypes do not seem clearly related to the year of release of the variety. For example, some studies have found that NUpE has increased with the introduction of improved varieties [14–17]. In contrast, other studies have found that modern wheat varieties are less efficient at recovering soil N than older varieties when no N fertilizer is applied and are more efficient only when N is applied profusely [18]. The limited progress of breeding in improving NUE under conditions of low available N in the soil may be due to the fact that plant selection is routinely carried out with sufficient to excess N, orienting selections only toward genotypes capable of responding to non-limiting N levels in the soil. NUE is a quantitative trait subject to large genotype × environment × agricultural management interactions, and currently understanding of the plant traits and mechanisms that influence and regulate it is very limited. There are still many gaps in this knowledge, in particular around the role of root traits, mainly because basic knowledge of root biology in the soil context is limited because of the difficulties of characterizing root morphology and functionality in the field [19]. The ability of a plant to use N efficiently depends on a variety of factors, including root traits (depth, length, density, speed of growth) and root N transport and metabolism [19].

Thus, the objectives of the present study were to determine whether differences exist among genotypes of durum wheat in (1) N uptake when the plants are grown in conditions of low N availability, (2) the amount and rapidity of N use as it becomes available and (3) morphological or physiological root traits. We hypothesized that (1) in conditions of low N availability, older varieties would have more efficient N uptake compared to modern ones and that this superiority would be associated with greater root length and root length density, and (2) modern varieties would be more able and quicker than older ones to intercept N when it became available after fertilization.

To this end, we studied nine genotypes chosen for their large variability in terms of plant growth habits, grain yield potential, and year of release. The isotopic tracer $^{15}N$ was used to measure the fertilizer NUpE. The information obtained is useful for identifying wheat varieties (and developing new lines) able to use N efficiently and therefore suitable for low-input systems or organic systems (i.e., those less reliant or not at all reliant on the use of chemical fertilizers).

## 2. Materials and Methods

The experiment was conducted outdoors in a wire house under a transparent plastic roof (pots were protected from the rain) with open sides at the Pietranera farm (S. Stefano Quisquina, Sicily, Italy; 37°53′ N, 13°51′ E; 162 m a.s.l.). Nine genotypes of durum wheat that varied greatly in their year of release, morpho-phenological characteristics, and productive traits were evaluated (Table 1).

Plants were grown in 4 L pots (diameter = 8 cm, height = 80 cm) filled with artificial substrate. There were 12 pots for each genotype, for a total of 108 pots. The growth substrate was composed of a mixture of 80% silica sand (Gras Calce, Trezzo sull'Adda, Italy) and 20% *w*/*w* agricultural soil; we used a high percentage of silica sand both to have a substrate poor in N and to easily extract all the roots. Both substrates were sieved through a 2 mm mesh and characterized separately. Sand total N (Kjeldahl) and available phosphorous (P; Olsen P) were 0.11 g $kg^{-1}$ and 7.44 mg $kg^{-1}$, respectively. The soil was collected from the first 30 cm of a well-structured clay soil classified as Vertic Haploxerept with the following characteristics: 267 g $kg^{-1}$ clay, 247 g $kg^{-1}$ silt, and 486 g $kg^{-1}$ sand; pH 8.0; 10.8 g $kg^{-1}$ total carbon (C; Walkley-Black); 0.86 g $kg^{-1}$ total N (Kjeldahl); 40.1 mg $kg^{-1}$ available

P (Olsen P); 598 mg $kg^{-1}$ total P; 26 cmol $kg^{-1}$ cation exchange capacity; 1.70 dS $m^{-1}$ electrical conductivity (saturated paste at 25 °C); 27.9% water content at field capacity; and 18.9% water content at the permanent wilting point. Therefore, the resulting mixture was poor in N and sufficiently supplied with P.

**Table 1.** Year of release, pedigree, plant height, and earliness of the nine genotypes of durum wheat used in the experiment.

| Genotype | Acronym | Year of Release | Pedigree | Plant Height | Heading |
|---|---|---|---|---|---|
| Cappelli | Capp | 1915 | Selection from Tunisian population | Very tall | Late |
| Capeiti 8 | Cape | 1955 | EITI 6 × Cappelli | Tall | Early |
| Trinakria | Tri | 1970 | B-14 × Capeiti-8 | Tall | Early |
| Creso | Cre | 1974 | Cpb144 × [(Yt54-N10-B)Cp2 63 Te3] | Short | Late |
| Appio | App | 1982 | Cappelli × (Gaviota × Yuma) | Medium-short | Medium-late |
| Simeto | Sim | 1988 | Capeiti-8 × Valnova | Medium-short | Medium-early |
| Svevo | Sve | 1996 | Cimmyt selection × Zenit | Medium | Early |
| Orizzonte | Ori | 2011 | Rusticano × Simeto | Short | Early |
| Antalis | Ant | 2013 | Unknown | Medium-short | Medium |

Sowing was performed on 22 January 2019. Four seeds per pot were distributed; all pots were arranged in a completely randomized design. Ten days after emergence, plants were thinned to two plants per pot. The soil water holding capacity of the substrate was determined with the gravimetric method [20]. Briefly, 10 perforated crucibles were filled with 100 g soil and placed in a basin with water up to half of the height of the crucibles. The crucibles were allowed to absorb water by capillarity until each pot was saturated. Excess water was allowed to drain, and the crucibles were weighed and oven-dried at 105 °C to a constant weight. The difference in weight between the crucibles before and after the drying process represented the soil water content at field capacity. The plants were kept in optimal conditions in terms of water supply throughout the experiment; watering was pot specific. Pots were weighed every 2 days to determine whether irrigation was needed. When the soil water content reached approximately 70% of the available water capacity threshold, a volume of water sufficient to bring the substrate back to field capacity was added. Variation in weight was attributed to evapotranspiration.

Then 90 days after emergence, 0.19 g fertilizer per pot (10% $^{15}$N-enriched ammonium sulphate) was applied.

During the experiment measurements were taken at the following three times: 90 days after emergence (just before the application of fertilizer; T1), 7 days after the application of fertilizer (T2), and 35 days after the application of fertilizer (T3). At each time, measurements were carried out on four pots per genotype (36 pots total). At each time (T1, T2, and T3), shoots from each pot were removed, separated into botanical fractions (leaves, stems, ears, dry and senescent tissue), and weighed. A fresh sample of the leaves fraction was used to determine the leaf area with an area meter (LI-3100C; LiCOR, Lincoln, NE, USA). Each fraction was oven-dried to a constant weight to determine the dry matter content. The aboveground dry matter was then reunited, finely ground using a Qiagen TissueLyser II, and analyzed for total N content by the Dumas method (flash combustion with an automatic N analyzer; DuMaster D-480; Büchi Labortechnik, Flawil, Switzerland) and for $^{15}$N content with an elemental analyzer (NA1500; Carlo Erba, Milan, Italy) paired with a mass spectrophotometer (Isoprime, Cheadle, UK).

The soil profile was sampled from top to bottom in 20 cm sections, and roots were extracted from each section by sieving and washing. Each of these sections was then oven-dried to determine the dry weight and divided into two subsamples of equal weight. One subsample was used to measure length, mean diameter, and root surface with a WinRhizoTM scanner-based system (version 2007; Regent Instruments, Quebec, QC, Canada). The other subsample of each fraction was reunited, finely ground with a TissueLyser II, and analyzed for total N content and the relative isotopic excess.

The amount of N taken up by the plants represents a valid index of their uptake efficiency (NUpE; defined as the amount of N taken up by the crop per unit of N available to the crop), given that it is reasonable to hypothesize that the N potentially available in the substrate did not vary among treatments, as the plants grew on the same substrate, the plants were managed in the same way, and no leaching was observed. The $^{15}$N concentration was used to determine the amount ($^{15}$Nrec) and percentage (%$^{15}$Nrec) of N recovered from the fertilizer, respectively, with Equations (1) and (2):

$$^{15}Nrec = N_t \times \frac{atom\%\ ^{15}Nfp\ excess}{atom\%\ ^{15}Nfert\ excess} \quad (1)$$

$$\%^{15}Nrec = \frac{^{15}Nrec}{f} \times 100 \quad (2)$$

where $N_t$ is N content (g pot$^{-1}$) in the biomass at T2 or T3, atom% $^{15}Nfp$ excess is the $^{15}$N isotopic excess (atom% $^{15}$N—0.3663) in the fertilized plant, atom% $^{15}Nfert$ is the $^{15}$N isotopic excess in the fertilizer, and $f$ is the amount of fertilizer (g pot$^{-1}$).

The specific uptake ratio of N was calculated according to the following equations:

$$SNupR1 = \frac{N_tT1}{RT1} \quad (3)$$

$$SNupR2 = \frac{(N_tT2 - N_tT1)}{RT1} \quad (4)$$

$$SNupR3 = \frac{(N_tT3 - N_tT1)}{RT1} \quad (5)$$

where $N_tT1$, $N_tT2$, and $N_tT3$ are N content (g pot$^{-1}$) in the biomass at $T1$, $T2$, and $T3$, respectively, and $RT1$ is root dry weight (g pot$^{-1}$) or root length (m pot$^{-1}$) at $T1$. $SNupR1$ represents the amount of N taken up by the plant per gram or meter of root at $T1$. $SNupR2$ and $SNupR3$ represent the amount of N taken up by the plant in the 7 and 35 days following fertilization, respectively (calculated as the difference between the amounts of N at $T2$ and $T3$ compared to $T1$, respectively), per gram or meter of root at $T1$. Therefore, $SNupR2$ and $SNRupR3$ give, respectively, an estimate of the speed and capacity of use of the N fertilizer by each variety as a function of both the weight and length of roots at the fertilization time.

The data collected at each time point were analyzed in accordance with the experimental design (a completely randomized design with four replicates). Means were compared with Fisher's least significant differences test at the 5% probability level. All analyses were performed in the R environment [21]. Furthermore, the data were analyzed in relation to the year of release of the genotype; significant results ($p < 0.05$) shown in the figures.

## 3. Results

The shoot biomass at T1 (90 days after emergence) ranged from 1.49 g pot$^{-1}$ (Creso) to 2.22 g pot$^{-1}$ (Simeto and Capeiti; Table 2); overall, the observed differences are attributable to the different phenology of the accessions, as the earlier heading ones had more shoot biomass. In the later heading genotypes (Cappelli and Creso), the percentage of leaves on the shoots (on a dry weight basis) was significantly higher (more than 50%; Figure S1). The leaf area ranged from 136 to 196 cm$^2$ (Trinakria and Cappelli, respectively; Table 2).

**Table 2.** Shoot and root biomass, leaf area and root traits for the nine studied genotypes at 90 days after emergence (Time 1).

| Genotypes | Shoots | Roots | Leaf Area | RMD | Root:Shoot Ratio | RLD | SRL |
|---|---|---|---|---|---|---|---|
| | g DM pot$^{-1}$ | g DM pot$^{-1}$ | cm$^2$ pot$^{-1}$ | mm | | cm cm$^{-3}$ | m g$^{-1}$ Root |
| Cappelli | 1.83 | 1.92 | 196 | 0.23 | 1.06 | 5.01 | 105 |
| Capeiti | 2.22 | 1.57 | 172 | 0.24 | 0.71 | 3.67 | 94 |
| Trinakria | 1.78 | 1.66 | 137 | 0.26 | 0.95 | 4.07 | 98 |
| Creso | 1.49 | 1.57 | 154 | 0.23 | 1.06 | 4.24 | 108 |
| Appio | 1.61 | 1.56 | 155 | 0.24 | 0.97 | 4.08 | 105 |
| Simeto | 2.22 | 1.56 | 165 | 0.26 | 0.71 | 4.30 | 110 |
| Svevo | 1.96 | 1.57 | 162 | 0.24 | 0.82 | 4.02 | 103 |
| Orizzonte | 2.11 | 1.38 | 152 | 0.23 | 0.66 | 3.83 | 111 |
| Antalis | 1.71 | 1.58 | 146 | 0.23 | 0.96 | 4.44 | 112 |
| *p* | 0.007 | 0.011 | 0.006 | 0.156 | <0.001 | 0.006 | 0.056 |
| $LSD_{0.05}$ | 0.407 | 0.227 | 6.3 | – | 0.169 | 0.599 | 11.6 |

DM, dry matter; RMD = root mean diameter; RLD = root length density (root length per unit of soil volume); SRL = specific root length (root length per unit of biomass).

The total root biomass ranged from 1.92 g pot$^{-1}$ (Cappelli) to 1.37 g pot$^{-1}$ (Orizzonte; Table 2); the differences among genotypes were highly significant in statistical analyses. A highly significant negative relationship emerged between root biomass and the year of release of the variety ($R^2$ = 0.71; data not shown). No significant relationships between shoot and root biomass were observed. In addition, appreciable differences emerged among the genotypes in the distribution of roots along the soil profile; overall, greater uniformity in the distribution of roots along the soil profile was observed in Cappelli and Creso, whereas the less uniform varieties were Orizzonte, Capeiti, and Simeto (Figure S1).

No appreciable differences were observed among the studied genotypes in mean root diameter (Table 2), which ranged from 0.230 mm (Creso and Orizzonte) to 0.256 mm (Simeto and Trinakria). Conversely, substantial variation was observed in root length density, which ranged from 3.67 to 5.01 cm cm$^{-3}$ in Capeiti and Cappelli, respectively (Table 2).

Cappelli showed the lowest total N in the shoot tissue (27.6 mg pot$^{-1}$; Figure 1); this is attributable mainly to the low N concentration in the shoot biomass (just 1.52%; Figure S2). The same variety showed the highest N accumulated in the root biomass (11.8 mg pot$^{-1}$; Figure 1). In contrast, Orizzonte showed the highest N uptake in the shoot biomass and the lowest in the roots (40.7 and 7.8 mg pot$^{-1}$, respectively). Therefore, a large difference emerged among the genotypes in the distribution of this element between the different organs of the plant (shoot and root tissue) rather than in total N uptake. The data revealed negative relationships between root biomass and total N uptake and between root length density and total N uptake (r = −0.71 and −0.79, respectively). Overall, a weak, albeit significant, positive relationship emerged between shoot N uptake and the year of release of the variety (Figure 1a), whereas an opposite trend was observed between root N uptake and the year of release of the variety (Figure 1b).

Seven days after the application of fertilizer (T2), large and significant differences in growth emerged among the studied genotypes. Orizzonte showed the highest shoot growth (1.25 g pot$^{-1}$), whereas Simeto showed the lowest (0.57 g pot$^{-1}$; Figure 2a). Root growth ranged from 0.07 to 0.41 g pot$^{-1}$, respectively, in Trinakria and Capeiti (Figure 2b). It is interesting that Cappelli showed low shoot growth (0.67 g pot$^{-1}$, statistically not dissimilar to the worst variety) and high root growth (0.40 g pot$^{-1}$, statistically similar to the best variety); in contrast, Trinakria showed high growth of both shoots and roots (in both cases with values statistically not dissimilar to the best variety). The differences observed in shoot and root growth among the genotypes did not appear to be related to the year of their release (Figure 2a,b).

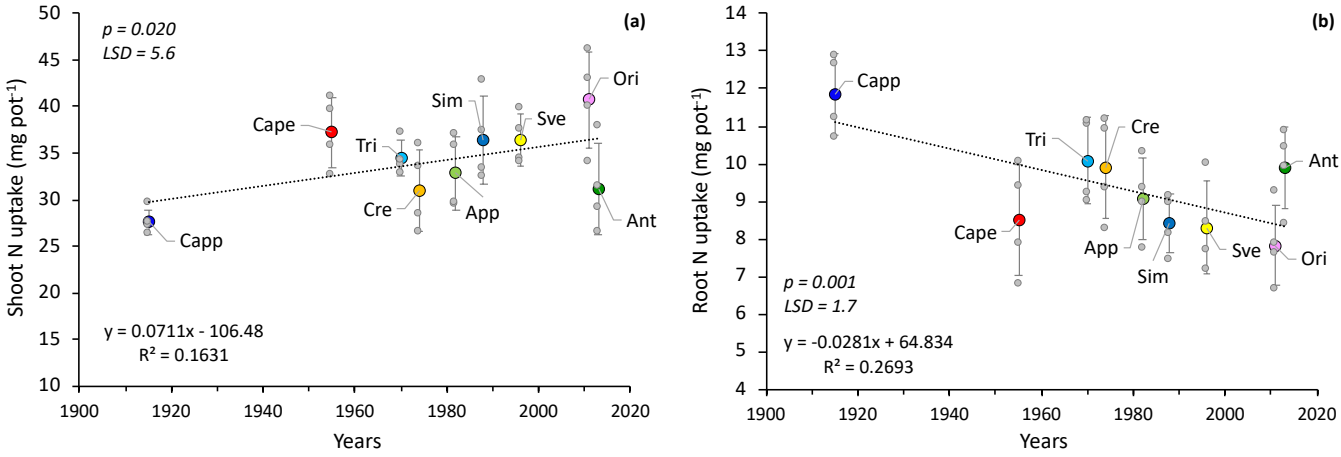


**Figure 1.** Relationships between shoot N uptake (**a**) and root N uptake (**b**) and the year of release of the nine studied genotypes at 90 days after emergence (Time 1). For each trait the *p* value and the LSD (Fisher's LSD test, $p = 0.05$) are reported. All genotype data are plotted with the mean depicted as a colored circle ± standard deviation ($n = 4$) represented by the end of the vertical black line. N, nitrogen; LSD, least significant difference.

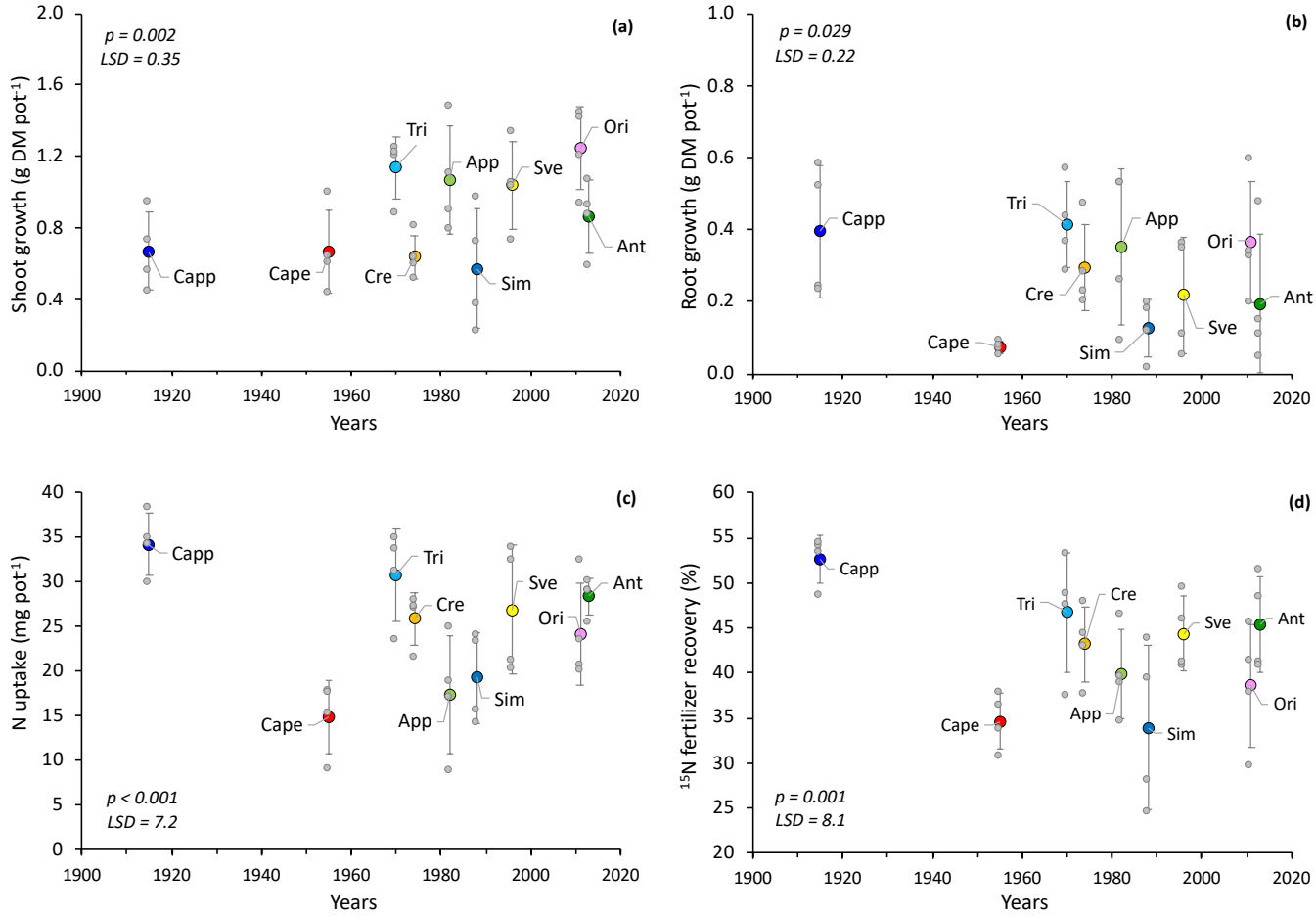


**Figure 2.** Relationships between shoot growth (**a**), root growth (**b**), N uptake (**c**) and $^{15}$N fertilizer recovery (**d**) 7 days after the application of N fertilizer (Time 2) and the year of release of the nine studied genotypes. For each trait the *p* value and LSD (Fisher's LSD test, $p = 0.05$) are reported. All genotype data are plotted with the mean depicted as a colored circle ± standard deviation ($n = 4$) represented by the end of the vertical black line. N, nitrogen; LSD, least significant difference; DM, dry matter.

Large differences were observed among the genotypes in the quantity of total N taken up in the 7 days following fertilizer distribution (from 14.92 to 34.26 mg pot$^{-1}$, respectively, for Capeiti and Cappelli; Figure 2c). No relationship emerged between growth rate and N uptake 7 days after fertilizer distribution (r = 0.319). Seven days after the application of fertilizer, Cappelli had already intercepted more than 50% of the N applied (calculated using the $^{15}$N isotope as a tracer); whereas Capeiti and Simeto intercepted N most slowly (34% and 35%, respectively; Figure 2d). No significant relationships emerged between N uptake and year of release or between $^{15}$N fertilizer recovery and year of release (Figure 2c,d).

The growth of both shoots and roots in the 35 days following the application of fertilizer varied by genotype; shoot growth ranged from 5.7 g pot$^{-1}$ (Capeiti) to 7.4 g pot$^{-1}$ (Cappelli; Figure 3a), whereas root growth ranged from 0.77 g pot$^{-1}$ (Orizzonte) to 1.65 g pot$^{-1}$ (Creso; Figure 3b). The amount of N accumulated in the phytomass, both shoot and root, during the same interval was significantly higher in Cappelli (77 mg pot$^{-1}$) than in any other genotype (from 43 to 57 mg pot$^{-1}$, respectively, in Capeiti and Creso; Figure 3c). Furthermore, as regards N fertilizer recovery, the highest value was observed in Cappelli (about 95%), whereas in the other genotype values ranged from 69% to 76% (Trinakria and Appio, respectively; Figure 3d). The differences observed among the genotypes in N uptake and $^{15}$N fertilizer recovery appeared, to some extent, negatively related to their year of release.

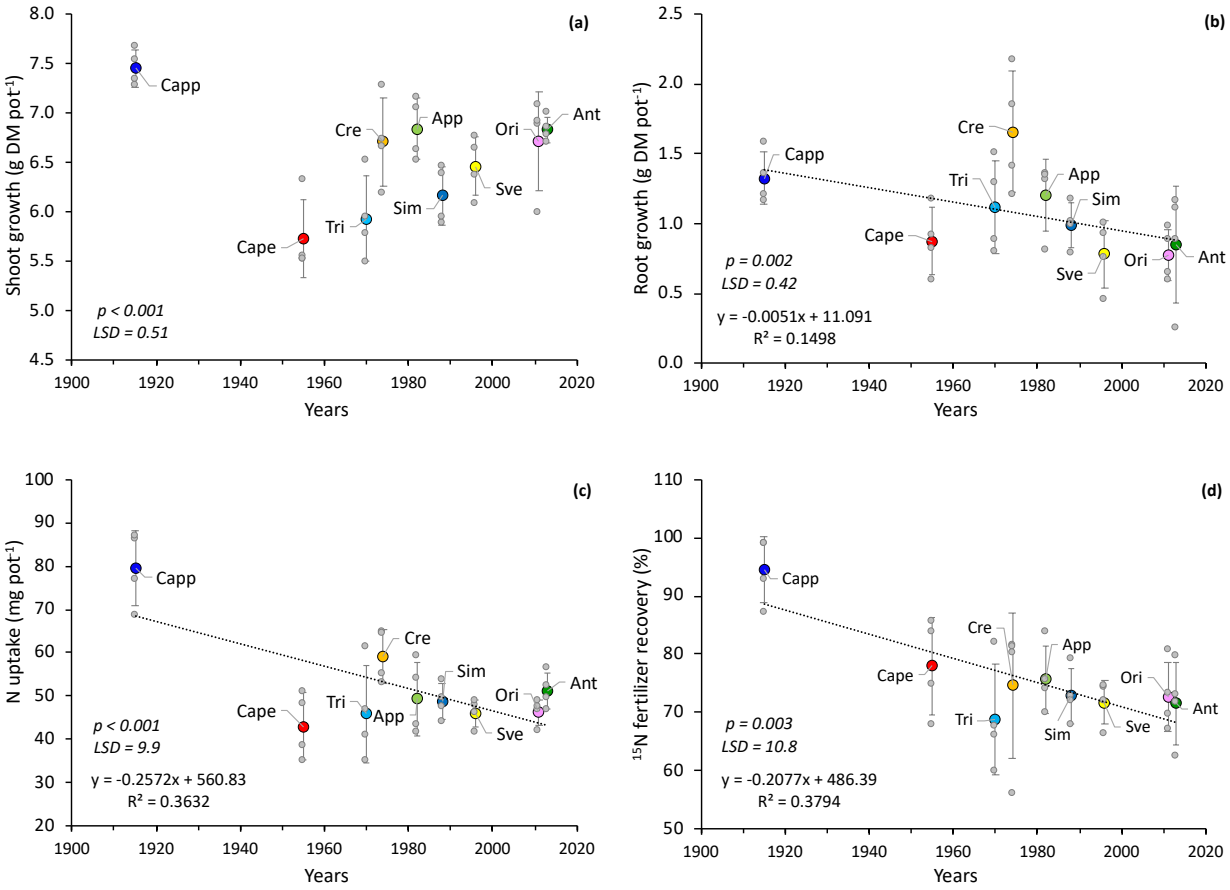


**Figure 3.** Relationships between shoot growth (**a**), root growth (**b**), N uptake (**c**), and $^{15}$N fertilizer recovery (**d**) 35 days after the application of N fertilizer (Time 3) and the year of release of the nine studied genotypes. For each trait the *p* value and LSD (Fisher's LSD test, $p = 0.05$) are reported. All genotype data are plotted with the mean depicted as a colored circle ± standard deviation ($n = 4$) represented by the end of the vertical black line. N, nitrogen; LSD, least significant difference; DM, dry matter.

The *SNupR*1 index, which represents the amount of N taken up by the plants per gram or per meter of root at T1, varied widely among the genotypes, being highest in Orizzonte (35.0 mg N $g^{-1}$ root and 0.32 mg N $m^{-1}$ root) and lowest in Cappelli (20.6 mg N $g^{-1}$ root and 0.20 mg N $m^{-1}$ root; Table 3). Large differences were also observed among the genotypes in the efficiency of recovery of the N applied with the fertilizer (both as gram or meter of root); the *SNupR*2 index (calculated 7 days after N fertilization) was between 9.5 (Capeiti) and 18.5 (Trinakria) mg N per gram of root, whereas the *SNupR*3 index (calculated 35 days after N fertilization) was between 27.4 (Capeiti) and 41.5 (Cappelli) mg N per gram of root.

**Table 3.** Specific uptake ratios of N for the nine studied genotypes.

| Genotypes | *SNupR*1 | | *SNupR*2 | | *SNupR*3 | |
|---|---|---|---|---|---|---|
| | mg N $g^{-1}$ Root | mg N $m^{-1}$ Root | mg N $g^{-1}$ Root | mg N $m^{-1}$ Root | mg N $g^{-1}$ Root | mg N $m^{-1}$ Root |
| Cappelli | 20.6 | 0.20 | 17.9 | 0.17 | 41.5 | 0.40 |
| Capeiti | 29.2 | 0.31 | 9.5 | 0.10 | 27.4 | 0.29 |
| Trinakria | 27.0 | 0.27 | 18.5 | 0.19 | 27.6 | 0.28 |
| Creso | 26.0 | 0.24 | 16.5 | 0.15 | 37.6 | 0.35 |
| Appio | 27.1 | 0.26 | 11.2 | 0.11 | 31.6 | 0.30 |
| Simeto | 28.7 | 0.26 | 12.4 | 0.11 | 31.1 | 0.28 |
| Svevo | 28.8 | 0.28 | 17.2 | 0.17 | 29.3 | 0.29 |
| Orizzonte | 35.0 | 0.32 | 17.5 | 0.16 | 33.4 | 0.30 |
| Antalis | 26.1 | 0.23 | 18.0 | 0.16 | 32.3 | 0.29 |
| *p* | <0.001 | <0.001 | 0.006 | 0.007 | 0.008 | 0.041 |
| $LSD_{0.05}$ | 3.94 | 0.042 | 5.19 | 0.049 | 7.32 | 0.072 |

*SNupR*1 represents the amount of N taken up by the plants per gram or meter of root at Time 1; *SNupR*2 and *SNupR*3 represent the amount of N taken up by plants in the 7 and 35 days following fertilization, respectively (calculated as the difference between the amounts of N at Time 2 and Time 3 compared to Time 1, respectively), per gram or meter of root at Time 1.

The differences observed among the genotypes in rapidity and ability to intercept the N applied with fertilizer were, to an appreciable extent, correlated with both root length and leaf area, as is clearly shown by the relationships reported in Figure 4.

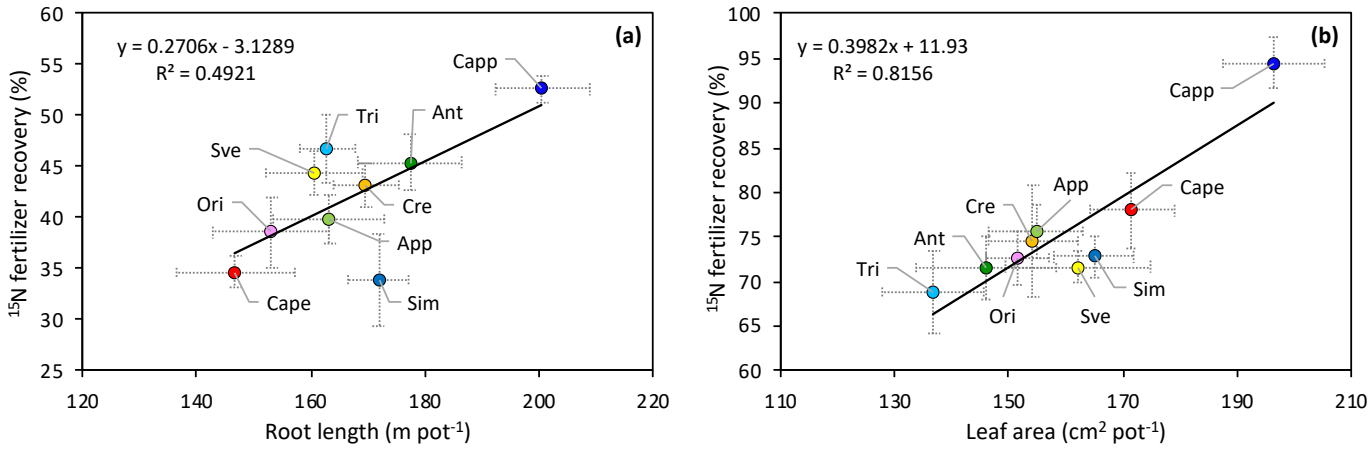


**Figure 4.** Relationships between root length (measured 90 days after emergence; Time 1) and the percentage of $^{15}N$ recovered from the fertilizer (%$^{15}$Nrec) 7 days after fertilization (**a**) and leaf area (measured at 90 days after emergence; Time 1) and the percentage of $^{15}N$ recovered from the fertilizer (%$^{15}$Nrec) 35 days after fertilization (**b**).

## 4. Discussion

This research highlights great variability among the nine studied genotypes in terms of root traits and N uptake when grown in conditions of low N availability and of rapidity and

type of response when N has been made available. However, in contrast to our hypothesis, the results did not show a clear relationship between the year of release of the variety and N uptake efficiency when plants were grown under conditions of low N availability. The new varieties, in contrast to what we had hypothesized, were less able than the old ones to intercept N when it became available after fertilization. In fact, after fertilization, and with more available N, the N uptake and $^{15}$N fertilizer recovery of the varieties appeared to be (albeit weakly) negatively related to their year of release. Therefore, it seems that breeding, which has led to a progressive increase in yield and grain quality [22], has not had univocal effects on root traits and on the efficiency of the root system.

We observed large differences among the studied genotypes in root weight and length (greater in the older variety Cappelli) as well as the distribution of roots along the soil profile. Overall, the later heading genotypes (Cappelli and Creso, which reach heading on average 2–3 weeks after the earlier heading genotypes) had a more uniform distribution of the root system compared to the earlier heading genotypes (Orizzonte, Capeiti, Simeto), which had more of the root system in the top layers. These results, even if obtained from a pot experiment, are of interest, as information about variability in root development within the species is limited. In this study, when the genotypes were grown in a shortage of N, a negative relationship emerged between the year of release of the genotype and root biomass but not shoot biomass. This is in line with Zhang et al. [23], who observed in *Triticum aestivum* that total root length decreased slightly from earlier cultivars to recently released ones; the authors stated that wheat breeding to reduce plant height also reduced root size (in particular in the upper soil layers), resulting in a smaller root-to-shoot ratio. In this experiment a significant negative relationship was observed between year of release and root-to-shoot ratio (values ranging from $-0.417$ to $-0.633$ depending on the time of measurement; data not shown), which highlights how breeding has influenced the belowground plant organs more than the aboveground ones.

Large differences among the genotypes were also seen in N uptake values in the first 90 days of growth in which the plants were grown in conditions of marked N deficiency. As mentioned, weak relationships, with opposite trends, emerged between both shoot and root N uptake and year of release of the variety; so altogether, no relationship between total N uptake and year of release emerged, which suggests that breeding has had little influence on this important trait. Indeed, Foulkes et al. [24] found that when N fertilizer was applied at optimal rates, modern varieties were more efficient than older ones, whereas the opposite was true when N was a limiting factor. Other authors [5,15,25] have found that N uptake is greater among newly released varieties compared to older ones, which indicates that N uptake ability has increased through breeding, regardless of the amount of N fertilizer applied. Moreover, other authors have found no relationship between N uptake and the year of release of the variety [26–29]. They have ascribed the general increase in NUE resulting from wheat breeding mainly to the improved ability of new genotypes to use the assimilated N to increase grain yield rather than to any improvement in their capacity to extract N from the soil. However, it should be highlighted how the discrepancy in results may be partly due to different methodologies to assess NUE and its components.

In this research, negative relationships unexpectedly emerged between some characteristics of the root system (root biomass and root length density) and the amount of N taken up when the genotypes were grown in conditions of N deficiency (in the first 90 days). Therefore, the differences in N uptake capacity among the genotypes seem to be attributable to different levels of efficiency in N uptake capacity per unit of root length and root weight rather than to morphological differences in the root system. Aziz et al. [30] showed that the selection for wheat productivity carried out in Australia between 1958 and 2007 reduced the total root length and at the same time increased N uptake by increasing the efficiency of the root system for capturing N; this is partially in agreement with the results of the present study. Liu et al. [31] compared two wheat lines and found that the line with the lower root biomass absorbed more N than the other. This was associated with differential expression of nitrate and ammonium transporter genes. Therefore, it

would seem that to improve the N uptake efficiency of wheat it would be more advantageous to select for high $NO_3^-$ and $NH_4^+$ affinity rather than for a vigorous root system. However, other research has associated a more developed root system with improved N uptake [32–35]. Liao et al. [36] also highlighted how N harvesting efficiency appears to be strongly correlated with early vigorous root and shoot growth, underlining how these characteristics should be considered in wheat breeding programs to improve N efficiency. According to Foulkes et al. [24], the selection for deep rooting could represent an unexploited approach to increasing NUE given that through a deeper root system plants can improve N uptake from the subsoil, with positive effects on production, cropping system management, and the environment. In fact, many researchers have highlighted how subsoil can contribute in an important way to plant nutrition, especially when topsoil is dry or depleted of nutrients [37,38]. Severini et al. [39] found a stronger correlation between root depth and yield than between root depth and shoot biomass, which suggests that a deeper root system may offer advantages during the late phase of the crop cycle, providing nutrients and water to support transpiration during grain filling. Others have shown that rooting depth is an important factor in N uptake from the deep layers of the soil [40–43]; this certainly has important environmental implications, as more deeply rooted plants will be able to utilize some N that would otherwise be lost into the environment through leaching, causing surface water and groundwater pollution. The discrepancies in these results could be explained by differences in genotypes, experimental conditions (pot or field), crop management (e.g., the timing and method of N fertilizer application), or climate and soil conditions (i.e., available water, soil fertility, and available N), as well as differences in the timing of measurement, as varieties may have different N uptake patterns in different phenological stages.

After fertilization, with more available N, the N uptake and $^{15}N$ fertilizer recovery of the varieties appeared (albeit weakly) negatively related to their year of release. These relationships are partly attributable to the Cappelli variety, selected by the geneticist Strampelli in 1915, which differed greatly from the other accessions not only in morphological characteristics of both above- and belowground organs but also in its greater ability and rapidity of intercepting N when it became available. We hypothesize that this capacity of Cappelli (a much later heading variety than the others) is partly due to the perfect synchrony between the increase in available N induced by the application of fertilizer and the demand for N by the crop (with the maximum coinciding with the stem elongation stage). Moreover, in this research, the differences observed among the studied genotypes in terms of capacity and rapidity to intercept the N supplied were related to both the length of the root system and the photosynthesizing area. It would therefore seem that differences in N fertilizer recovery are attributable in part to the development of the root system (in particular in the deeper layers), which certainly increases the possibility of intercepting N supplied with the fertilizer, and in part to the increased demand for N by genotypes. In fact, it is easy to argue that greater leaf expansion favors growth and consequently increases the need for N to support the growth itself. This last result would seem to confirm the findings of other experiments on wheat and other species that have shown close relationships between the overall N accumulated in the canopy and the leaf area [44,45]. Furthermore, other studies have highlighted significant relationships between root traits (length, distribution, and density in the different soil layers), grain yield, nutrient uptake capacity, and water use efficiency [23,46–50].

In conclusion, this research shows that breeding activity in durum wheat has not had univocal effects on the characteristics of the root system (weight, length, specific root length, etc.) or N uptake capacity. The differences in N uptake observed among the studied genotypes varied with N availability. When plants were grown in conditions of low N availability, plant N uptake appeared to be related more to differences in uptake efficiency per unit of weight and length of the root system than to differences in morphological root traits. Conversely, the speed and ability to exploit the increase in N availability due to fertilization appeared, to a certain extent, to be related to root length and leaf

area. Therefore, breeding to improve N utilization efficiency must aim to select for both a vigorous root system and high efficiency of N capture per unit of root length and root weight.

Furthermore, identifying genotypes with a root system able to better explore the substrate (i.e., with a greater length per unit of soil volume and a greater depth) would increase opportunities not only to intercept resources available in the substrate (water, nutrients) but also to activate symbiotic and associative relationships with soil microorganisms (mycorrhiza, plant growth-promoting rhizobacteria, etc.), with positive effects in terms of adaptability to difficult environments and resilience to climate change. To do this, experts must identify new solutions and techniques that allow for evaluations of the conformation and functionality of root systems in open field conditions. Finally, the present research, although performed among a limited number of genotypes, highlights certain variability in the development and distribution of the root system of this species, which suggests that there may be sufficient room for improving this trait within the species.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061149/s1, Figure S1: Shoot dry matter by botanic fraction (in percent of total shoot dry matter; left) and root dry matter by soil layer (in percent of total root dry matter; right) for the nine studied genotypes at 90 days after emergence (T1). Figure S2: Shoot (left) and root (right) N concentration for the nine studied genotypes at 90 days after emergence (T1).

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## References

1. Bouwman, A.F.; Boumans, L.J.M.; Batjes, N.H. Emissions of $N_2O$ and NO from fertilized fields: Summary of available measurement data. *Glob. Biogeochem. Cycles* **2002**, *16*, 6-1–6-13. [CrossRef]
2. Wang, Y.; Ying, H.; Yin, Y.; Zheng, H.; Cui, Z. Estimating soil nitrate leaching of nitrogen fertilizer from global me-ta-analysis. *Sci. Total Environ.* **2019**, *657*, 96–102. [CrossRef] [PubMed]
3. Beusen, A.; Bouwman, A.; Heuberger, P.; Van Drecht, G.; Van Der Hoek, K. Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. *Atmospheric Environ.* **2008**, *42*, 6067–6077. [CrossRef]
4. Raun, W.; Johnson, G.V. Improving Nitrogen Use Efficiency for Cereal Production. *Agron. J.* **1999**, *91*, 357–363. [CrossRef]
5. Sylvester-Bradley, R.; Kindred, D.R. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *J. Exp. Bot.* **2009**, *60*, 1939–1951. [CrossRef]
6. López-Bellido, L.; López-Bellido, R.J.; López-Bellido, F.J. Fertilizer nitrogen efficiency in durum wheat under rainfed Mediterranean conditions: Effect of split application. *Agron. J.* **2006**, *98*, 55–62. [CrossRef]
7. Sanaa, M.; Van Cleemput, O.; Baert, L.; Mhiri, A. Field study of the fate of labelled fertilizer nitrogen applied to wheat on calcareous Tunisian soils. *Pedologie* **1992**, *42*, 245–255.

8. Pilbeam, C.J.; McNeill, A.; Harris, H.C.; Swift, R.S. Effect of fertilizer rate and form on the recovery of 15N-labelled fertilizer applied to wheat in Syria. *J. Agric. Sci.* **1997**, *128*, 415–424. [CrossRef]
9. Giambalvo, D.; Ruisi, P.; Di Miceli, G.; Frenda, A.S.; Amato, G. Nitrogen Use Efficiency and Nitrogen Fertilizer Recovery of Durum Wheat Genotypes as Affected by Interspecific Competition. *Agron. J.* **2010**, *102*, 707–715. [CrossRef]
10. Ruisi, P.; Giambalvo, D.; Saia, S.; Frenda, A.S.; Plaia, A.; Amato, G.; Di Miceli, G. Conservation tillage in a semiarid Mediterranean environment: Results of 20 years of research. *Ital. J. Agron.* **2014**, *9*, 1–7. [CrossRef]
11. Ruisi, P.; Saia, S.; Badagliacca, G.; Amato, G.; Frenda, A.S.; Giambalvo, D.; Di Miceli, G. Long-term effects of no tillage treatment on soil N availability, N uptake, and 15 N-fertilizer recovery of durum wheat differ in relation to crop sequence. *Field Crop. Res.* **2016**, *189*, 51–58. [CrossRef]
12. Moll, R.H.; Kamprath, E.J.; Jackson, W.A. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron. J.* **1982**, *74*, 562–564. [CrossRef]
13. Fageria, N.K.; Baligar, V.C.; Li, Y.C. The Role of Nutrient Efficient Plants in Improving Crop Yields in the Twenty First Century. *J. Plant. Nutr.* **2008**, *31*, 1121–1157. [CrossRef]
14. Le Gouis, J.; Béghin, D.; Heumez, E.; Pluchard, P. Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. *Eur. J. Agron.* **2000**, *12*, 163–173. [CrossRef]
15. Brancourt-Hulmel, M.; Doussinault, G.; Lecomte, C.; Berard, P.; Le Buanec, B.; Trottet, M. Genetic improvement of agro-nomic traits of winter wheat cultivars released in France from 1946 to 1992. *Crop Sci.* **2003**, *43*, 37–45. [CrossRef]
16. Guarda, G.; Padovan, S.; Delogu, G. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *Eur. J. Agron.* **2004**, *21*, 181–192. [CrossRef]
17. Guttieri, M.J.; Frels, K.; Regassa, T.; Waters, B.M.; Baenziger, P.S. Variation for nitrogen use efficiency traits in current and historical great plains hard winter wheat. *Euphytica* **2017**, *213*, 87. [CrossRef]
18. Foulkes, M.J.; Sylvester-Bradley, R.; Scott, R.K. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. *J. Agric. Sci.* **1998**, *130*, 29–44. [CrossRef]
19. Garnett, T.; Conn, V.; Kaiser, B.N. Root based approaches to improving nitrogen use efficiency in plants. *Plant. Cell Environ.* **2009**, *32*, 1272–1283. [CrossRef]
20. Dobriyal, P.; Qureshi, A.; Badola, R.; Hussain, S.A. A review of the methods available for estimating soil moisture and its implications for water resource management. *J. Hydrol.* **2012**, *458–459*, 110–117. [CrossRef]
21. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
22. De Vita, P.; Nicosia, O.L.D.; Nigro, F.; Platani, C.; Riefolo, C.; Di Fonzo, N.; Cattivelli, L. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. *Eur. J. Agron.* **2007**, *26*, 39–53. [CrossRef]
23. Zhang, X.; Chen, S.; Sun, H.; Wang, Y.; Shao, L. Root size, distribution and soil water depletion as affected by cultivars and environmental factors. *Field Crop. Res.* **2009**, *114*, 75–83. [CrossRef]
24. Foulkes, M.; Hawkesford, M.; Barraclough, P.; Holdsworth, M.; Kerr, S.; Kightley, S.; Shewry, P. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crop. Res.* **2009**, *114*, 329–342. [CrossRef]
25. Ortiz-MonasterioR, J.I.; Sayre, K.D.; Rajaram, S.; McMahon, M. Genetic Progress in Wheat Yield and Nitrogen Use Efficiency under Four Nitrogen Rates. *Crop. Sci.* **1997**, *37*, 898–904. [CrossRef]
26. Slafer, G.A.; Andrade, F.H.; Feingold, S.E. Genetic improvement of bread wheat (*Triticum aestivum* L.) in Argentina: Relationships between nitrogen and dry matter. *Euphytica* **1990**, *50*, 63–71. [CrossRef]
27. Calderini, D.F.; Torres-León, S.; Slafer, G.A. Consequences of wheat breeding on nitrogen and phosphorus yield, grain nitrogen and phosphorus concentration and associated traits. *Ann. Bot.* **1995**, *76*, 315–322. [CrossRef]
28. Motzo, R.; Fois, S.; Giunta, F. Relationship between grain yield and quality of durum wheats from different eras of breeding. *Euphytica* **2004**, *140*, 147–154. [CrossRef]
29. Ruisi, P.; Frangipane, B.; Amato, G.; Frenda, A.S.; Plaia, A.; Giambalvo, D.; Saia, S. Nitrogen uptake and nitrogen fertilizer recovery in old and modern wheat genotypes grown in the presence or absence of interspecific competition. *Front. Plant. Sci.* **2015**, *6*, 185. [CrossRef]
30. Aziz, M.M.; Palta, J.A.; Siddique, K.; Sadras, V.O. Five decades of selection for yield reduced root length density and increased nitrogen uptake per unit root length in Australian wheat varieties. *Plant. Soil* **2017**, *413*, 181–192. [CrossRef]
31. Liu, J.; Fu, J.; Tian, H.; Gao, Y. In-season expression of nitrate and ammonium transporter genes in roots of winter wheat (*Triticum aestivum* L.) genotypes with different nitrogen-uptake efficiencies. *Crop. Pasture Sci.* **2015**, *66*, 671–678. [CrossRef]
32. Palta, J.A.; Chen, X.; Milroy, S.; Rebetzke, G.; Dreccer, M.F.; Watt, M. Large root systems: Are they useful in adapting wheat to dry environments? *Funct. Plant. Biol.* **2011**, *38*, 347–354. [CrossRef]
33. Palta, J.A.; Yang, J. Crop root system behaviour and yield. *Field Crop. Res.* **2014**, *165*, 1–4. [CrossRef]
34. Pang, J.; Palta, J.A.; Rebetzke, G.J.; Milroy, S.P. Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. *Funct. Plant. Biol.* **2014**, *41*, 215–222. [CrossRef]
35. Pang, J.; Milroy, S.P.; Rebetzke, G.J.; Palta, J.A. The influence of shoot and root size on nitrogen uptake in wheat is affected by nitrate affinity in the roots during early growth. *Funct. Plant. Biol.* **2015**, *42*, 1179. [CrossRef]

36. Liao, M.; Fillery, I.R.P.; Palta, J.A. Early vigorous growth is a major factor influencing nitrogen uptake in wheat. *Funct. Plant. Biol.* **2004**, *31*, 121–129. [CrossRef]
37. Kirkegaard, J.A.; Lilley, J.M.; Howe, G.N.; Graham, J.M. Impact of subsoil water use on wheat yield. *Aust. J. Agric. Res.* **2007**, *58*, 303–315. [CrossRef]
38. Kautz, T.; Amelung, W.; Ewert, F.; Gaiser, T.; Horn, R.; Jahn, R.; Javaux, M.; Kemna, A.; Kuzyakov, Y.; Munch, J.-C.; et al. Nutrient acquisition from arable subsoils in temperate climates: A review. *Soil Biol. Biochem.* **2013**, *57*, 1003–1022. [CrossRef]
39. Severini, A.D.; Wasson, A.P.; Evans, J.R.; Richards, R.A.; Watt, M. Root phenotypes at maturity in diverse wheat and triticale genotypes grown in three field experiments: Relationships to shoot selection, biomass, grain yield, flowering time, and environment. *Field Crop. Res.* **2020**, *255*, 107870. [CrossRef]
40. Kristensen, H.L.; Thorup-Kristensen, K. Root growth and nitrate uptake of three different catch crops in deep soil layers. *Soil Sci. Soc. Am. J.* **2004**, *68*, 529–537. [CrossRef]
41. Kristensen, H.L.; Thorup-Kristensen, K. Uptake of15N labeled nitrate by root systems of sweet corn, carrot and white cabbage from 0.2–2.5 meters depth. *Plant. Soil* **2004**, *265*, 93–100. [CrossRef]
42. Thorup-Kristensen, K.; Cortasa, M.S.; Loges, R. Winter wheat roots grow twice as deep as spring wheat roots, is this important for N uptake and N leaching losses? *Plant. Soil* **2009**, *322*, 101–114. [CrossRef]
43. Rasmussen, I.S.; Dresbøll, D.B.; Thorup-Kristensen, K. Winter wheat cultivars and nitrogen (N) fertilization—Effects on root growth, N uptake efficiency and N use efficiency. *Eur. J. Agron.* **2015**, *68*, 38–49. [CrossRef]
44. Grindlay, D.J.C. REVIEW Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *J. Agric. Sci.* **1997**, *128*, 377–396. [CrossRef]
45. Gastal, F.; Lemaire, G. N uptake and distribution in crops: An agronomical and ecophysiological perspective. *J. Exp. Bot.* **2002**, *53*, 789–799. [CrossRef]
46. Chen, X.Y.; Liu, X.Y.; Luo, Y.P. Effects of soil moisture on dynamic distribution of dry matter between winter wheat root and shoot. *Agric. Sci.* **2003**, *10*, 1144–1150.
47. Wang, C.; Liu, W.; Li, Q.; Ma, D.; Lu, H.; Feng, W.; Xie, Y.; Zhu, Y.; Guo, T. Effects of different irrigation and nitrogen regimes on root growth and its correlation with above-ground plant parts in high-yielding wheat under field conditions. *Field Crop. Res.* **2014**, *165*, 138–149. [CrossRef]
48. Kamiji, Y.; Pang, J.; Milroy, S.; Palta, J.A. Shoot biomass in wheat is the driver for nitrogen uptake under low nitrogen supply, but not under high nitrogen supply. *Field Crop. Res.* **2014**, *165*, 92–98. [CrossRef]
49. Guo, B.-B.; Liu, B.-C.; He, L.; Wang, Y.-Y.; Feng, W.; Zhu, Y.-J.; Jiao, N.-Y.; Wang, C.-Y.; Guo, T.-C. Root and nitrate-N distribution and optimization of N input in winter wheat. *Sci. Rep.* **2019**, *9*, 1–12. [CrossRef]
50. Lamichhane, S.; Murata, C.; Griffey, C.; Thomason, W.; Fukao, T. Physiological and Molecular Traits Associated with Nitrogen Uptake under Limited Nitrogen in Soft Red Winter Wheat. *Plants* **2021**, *10*, 165. [CrossRef]