BACHELOR THESIS

Differences in root morphology of modern wheat and barley varieties and their wild ancestors

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BLANCA PASCHEN

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Rhine-Waal University of applied Sciences

Faculty of Life Sciences

Sustainable Agriculture

Blanca Paschen

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Abstract

Sustainable agriculture requires plants which are able to absorb water and nutrients more efficiently to avoid losses and to reduce negative effects on the environment. Root traits are vitally important to fulfill these aims. Therefore, it is important to better understand root morphology of common cereals, such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) and their wild relatives. Wild wheat and barley relatives have a superior root system and thus may provide a valuable biological resource for breeding stress tolerant cereals in the future. The objectives of this research were to characterize the variability of root traits among modern wheat and barley cultivars and their wild forms. Fifteen wheat and nine barley cultivars and wild forms were grown under optimal growth conditions without addition of nutrients in a greenhouse experiment. Cultivars and wild forms showed a high genetic variation in root morphology, as well as in shoot traits. Cultivars were divided into three domestication categories: wild forms, old cultivars and modern cultivars. A high variability while comparing cultivars at domestication level showed that it is a

weak predictor for root traits. A deep and large root system, high root length density and a high root to shoot ratio were identified as being beneficial traits for efficient nutrient and water uptake. *T. aestivum* var*. tschermakianum, Aegilops cylindrica, H. vulgare hexastichon* and *H. lagunculiforme* had highest values for these traits.

Introduction

Drought is one of the major threatening abiotic stresses towards global food supply and is predicted to increase in frequency and severity in the next years (Becker et al. 2016). In addition, declining soil fertility poses further challenges for meeting future food demand. Therefore, sustainable agriculture requires plants which make use of nutrients and water more efficiently and can adapt to water-limited and nutrient deficient environments. Plants, which use little resources while keeping up yield production.

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are global major food crops and are often grown in arid or semiarid agricultural systems and often suffer from nutrient and water deficiency (Huang et al. 2007). Hence an improvement in their water and nutrient use efficiency can play an important role in stabilizing global future food supply. Genetic diversity is a valuable resource for crop improvement (Huang et al. 2007). Crop breeding could serve as a solution to generate higher water and nutrient uptake and use efficiency. This could stabilize crop performance especially in dry climates (Nevo and Chen 2010). Until now crop breeding programs focused mainly on yield and above-ground plant traits, reducing the genetic diversity and minimizing plant tolerance to environmental stresses (Nevo and Chen 2010). Studies about drought and salt tolerance in wheat and barley found out that their wild relatives possess adaptive genetic qualities that make them generally more resistant to biotic and abiotic stresses (Akcura 2009, Akman 2017, Nakhforoosh et al. 2014, Nevo and Chen 2010, Zhang 2017). For example Huang et al. (2007) observed a significant higher water use efficiency and nutrient uptake efficiency for *Aegilops squarrosa* L., a wild wheat relative. A major focus in drought tolerance research is of course root morphology (Becker et al. 2016). According to different studies, an extensive and deep root system helps plants to take up water and nutrients more efficiently. Consequently, inputs and negative effects on the environment can be reduced as well as higher yields attained (Botwright Acuña and Wade 2012, Chloupek et al. 2010, Richards 2008). Narayanan et al. (2014) suggests that water and nutrient uptake of crops is proportional to the contact area between the root surface and soil. This indicates that a plant's uptake of resources will increase with a greater root surface area, which is generally correlated with root length and biomass. Cultivars have different strategies to maximize soil water uptake. These were identified as being a deep rooting and having high topsoil root length density, low tissue mass density and high specific root length (Nakhforoosh et al. 2014). In the durum wheat study of Motzo (1993), the cultivar with the largest root mass and highest root density in the upper soil layers showed the lowest yield reduction under drought stress. However, other studies showed that drought tolerance was not the result of a larger overall root dry weight, but rather of an increased amount of fine roots and branching in deeper soil levels. It was observed that these traits increased the ability to extract moisture from those depths (Becker et al. 2016, Reynolds et al. 2007, Wasson et al. 2012). This correlates with the findings of Akman

(2017). In his study, species with deeper root systems, such as *Triticum turgidium,* were more resistant to drought conditions. Chloupek et al. (2010) found similar results. In his study, higher grain yield was correlated with a greater root system size in barley as well as in wheat under different fertilization and water treatments. This shows that a plant with a more complex root system can absorb water and nutrients more efficiently and can provide essential resources for plant and especially grain development. Besides water extraction from deep soil layers, a more shallow root system can be beneficial to capture rain water before it percolates into deep soil layers (Ehdaie et al. 2012). Drought strategies are highly environment-dependent. For example, in a climate with erratic spring rainfall like the Mediterranean regions a shallow root density will be more efficient than a deep root density. Additionally, drought increases root growth, helping the plant to withstand stress by scavenging for water. Whereas low nutrient availability induces a smaller root system and reduced tillering (Motzo et al. 1992).

Similar to drought tolerance, for an efficient nutrient acquisition, root growth as well as long and dense root systems are important traits (Wang 2016). Wheat species differ in their nitrogen use efficiency and their performance when nitrogen is limited (Chandna et al. 2012). In the work of Kaggwa (2013) a higher nitrogen use efficiency was correlated to overall higher total root biomass and longer roots. This was also demonstrated by Bakhshandeh et al. (2016), who showed that ammonium uptake was positively related to root biomass and water uptake with high root length. In addition, Ehdaie et al. (2010) found a positive correlation between nitrogen plant content, phosphorus and potassium uptake and root biomass.

Consequently, most important plant characteristics for efficient extraction of water and nutrients were identified as being a large and deep root system (Akman 2017, Kaggwa 2013, Nakhforoosh et al. 2014), a high root length density (Chloupek et al. 2010, Nakhforoosh et al. 2014, Wang 2016), a high root to shoot ratio and a high root biomass of fine roots in deep soil (Becker et al. 2016, Wang 2016, Wasson et al. 2012) or shallow soil (Motzo et al. 1992), depending on the climate.

A targeted integration of these root traits into plant breeding programs requires reliable knowledge about the root morphological diversity of various cultivars (Nakhforoosh et al. 2014). In the past decades, scientists have carried out many studies on wheat and barley evolution and found many root morphological differences between modern cultivars, old landraces and their wild relatives. Significant differences were found in root and shoot traits, such as root length and number, average root diameter, root length density, root to shoot ratio, grain yield, tiller number and plant height (Akman 2017). However, studies of root and plant biomass across ploidy levels and domestication levels in wheat and barley are often inconsistent. Wacker (2002) found an increase of total biomass with increasing domestication level at anthesis but surprisingly no reduced investment to roots and stems in modern cultivars. Specific leaf area, leaf area ratio and number of fertile tillers were higher in wild wheat forms. Austin et al. (1982) reported that tetraploid cultivars had the lowest total plant weight, but similar to Wacker (2002) a higher specific leaf area and number of tillers was found in wild forms compared to modern cultivars. Akman (2017) investigated shoot traits of different wheat species and wild wheat relatives and found higher number of tillers and plant height for most landraces and wild wheat relatives and a significant grain yield for wild wheat forms. According to Nakhforoosh et al. (2014) and Wacker (2002) the number of fertile tillers seems to be an indirect indicator for root density and biomass. In general, modern cultivars have fewer tillers and fewer roots, whereas wild varieties develop more tillers and a higher root biomass (Nakhforoosh et al. 2014, Wacker 2002).

Root length of wild barley was up to 91 % higher than that of modern barley cultivars (Sayed 2011). Also wheat cultivars had on average slightly longer roots (217 cm) than wild wheat relatives (212 cm) (Akman 2017). Under field conditions, Nakhforoosh et al. (2014) found thinnest and thickest roots for *T. monococcum* L.*,* a diploid wheat cultivar and *T. durum* L.*,* a tetraploid wheat cultivar, respectively. Highest root length density and specific root length was observed for *T. monococcum* L.. Underutilized wheat species showed lowest values for tissue mass density. *T. monococcum* L. showed highest root to shoot ratio and *T. carthlicum* showed the lowest (Nakhforoosh et al. 2014).

The identification and integration of desirable root traits could potentially be used for breeding stress tolerant cereals in the future (Akman 2017, Nevo and Chen 2010). Selection and improvements in breeding programs for root traits has been considerable slow and lagged behind that of above-ground plant characteristics. The main reasons are time-consuming phenotyping, difficult selection for root traits and their low heritability and gene expression (Wasson et al. 2012).

Additionally, as mentioned above, root traits improving yield under drought conditions are very environment-dependent. Root differences for the same cultivar can be observed in different environmental conditions, such as soil type, water and nutrient availability and management practices (Botwright Acuña and

Wade 2012, Motzo et al. 1992, Nevo and Chen 2010, Richards 2008, Wasson et al. 2012). Root plasticity in water-limited conditions or periods can also be a highly influential factor for crop performance. Some cultivars have a better ability to react to drought, through additional root growth, than others (Ehdaie et al. 2012).

The first step for improving drought tolerance and nutrient uptake efficiency of wheat and barley cultivars in breeding programs is to better understand genotypic differences of their root morphology. Special attention has to be put on traits that have been identified to improve these desirable functions. We compared the root morphological traits as well as shoot characteristics of old and modern wheat and barley cultivars with their wild relatives to investigate the following hypotheses:

- Significant differences of root morphology and shoot traits exist between modern and old cultivars and their wild relatives
- Beneficial root characteristics for drought tolerance and nutrient uptake got lost during domestication, because a high nutrient availability through fertilization decreases the necessity of an extensive root system.
- Thus, wild wheat and barley have a more superior root system than modern cultivars. A superior root system is defined as a root system which has a high root surface, volume and length as well as a high root length density and root to shoot ratio.
- Wild forms develop more tillers than modern cultivars, however they produce fewer grains.

Plants were cultivated in a greenhouse experiment. They were well-watered and not fertilized during the cultivation period. The cultivars and wild forms were compared individually and at domestication level.

Materials and Methods

Plant Material

The root morphology of fifteen cultivars and wild forms of diploid, tetraploid and hexaploid wheat (*Triticum aestivum* L.*)* and nine diploid cultivars of barley (*Hordeum vulgare* L.*)* were investigated in a greenhouse experiment in Kleve, North Rhine-Westphalia, Germany from March to end of June 2017. The seeds used in this study were obtained from *Ludwig Watschong (Dreschflegel GbR-biologisches Saatgut, Witzenhausen, Germany).* The cultivars were grouped into three domestication levels according to the database of the *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben*: wild forms (wild), old cultivars (old) and advanced or improved cultivars (modern) (Tables 1 and 2). Wheat cultivars can further be divided according to their number of chromosomes into three classifications according to Wolfgang Frenzel (2009) and Gill et al. (1991).

1. diploid, "*Einkornreihe*", containing 14 chromosomes (2n)
2. tetraploid,"*Emmerreihe*", containing 28 chromosomes (4n)
3. hexaploid, "*Dinkelreihe*" containing 42 chromosomes (6n)

The barley cultivars used in our experiment were all diploid (Forster 2011, Wacker 2002).

Table 1: Taxonomy, ploidy and domestication level, origin, accession number and genome composition of modern and old wheat cultivars and wild wheat forms used in this study

Species	**Abbre-viations**	**Cultivar name**	**Common name**	**Origin**	**Ploidy level**	**Genome**	**Domestica-tion level**	**Accession number***
Aegilops cylindrica Host	AC	-	Jointed goat grass	Kazakhstan	diploid	BB	wild	-
T. aestivum L.	TAS	“Servus“	Winter soft wheat	Germany	hexaploid	AABBDD	modern	-
T. aestivum L.	TAN	“Naxos“	Soft wheat	Germany	hexaploid	AABBDD	modern	-
T. aestivum L.	TAW	-	Soft wheat	-	hexaploid	AABBDD	modern	-
T. aestivum L. var. *tschermakianum* Mansf.	TT	-	Soft wheat (turquoise)	-	hexaploid	AABBDD	modern	-
T. carthlicum Nevski var. *carthlicum*	TC	-	Persian wheat	Georgia	tetraploid	AABB	old	TRI 36566 + TRI 44615
T. dicoccom var*. Farrum* Schrank	TDF	-	Emmer	-	tetraploid	AABB	old	-
T. durum var*. africanum* Desf.	TDA	-	Durum wheat	-	tetraploid	AABB	old	-
T. isphahanicum Heslot.	TI	-	Isfahan spelt wheat	-	tetraploid	AABB	old	-
T. macha Dekapr. & Menabde	TM	-	Makha wheat	Georgia	hexaploid	AABBDD	old	-
T. monococcum L.	TMEG	-	Domesticated einkorn	-	diploid	AA	old	-
T. turanicum Jakubz	TTU	-	Oriental wheat	Europe	tetraploid	AABB	old	TRI 2377
T. turgidum var*. plinianum* L.	TTI	-	Domesticated emmer wheat	-	tetraploid	AABB	old	-
T. aestivum L.	TAT	“Thasos”	Summer soft wheat	Germany	hexaploid	AABBDD	modern	-
T. dicoccoides Asch. & Graebn. var*. spontaneo-villosum*	TDS	-	Wild emmer	-	tetraploid	AABB	wild	-

*Accession numbers TRI are from the gene bank in Gatersleben; Information on seeds without number and origin is not available

Table 2: Taxonomy, ploidy and domestication level, origin, accession number and genome composition of modern and old barley cultivars and wild barley forms used in this study

Species	**Abbre-viations**	**Cultivar name**	**Common name**	**Origin**	**Ploidy level**	**Genome**	**Domestica-tion level**	**Accession number***
H. agriocrithon Åberg	HA	-	Wild six-rowed barley	China	diploid	AA	wild	-
H. lagunculiforme Bacht.	HL	-	Wild barley	Turkmen-istan, Is-rael	diploid	AA	wild	-
H. vulgare L.	HVI	"Ithaka"	Spelt barley	Germany	diploid	AA	old	HOR 1620
H. vulgare distichon L.	HVD	-	Two-rowed spelt barley	Austria	diploid	AA	old	HOR 1599
H. vulgare distichon L.	HVD13	No. 1,3	Two-rowed barley	Ethiopia	diploid	AA	old	HOR14028+E 604+E312
H. vulgare hexastichon L.	HVH	No. 3,1	Six-rowed barley	Nepal	diploid	AA	old	N060
H. vulgare vulgare L.	HVV221	No. 2,2,1	Cultivated barley	Korea	diploid	AA	modern	K088
H. vulgare vulgare L.	HVV27	No. 2,7	Spelt barley	Nepal	diploid	AA	modern	N426
H. vulgare vulgare L.	HVVD	"Dante"	Cultivated barley	-	diploid	AA	modern	-

*Accession numbers HOR are from the gene bank in Gatersleben; accession numbers E, N, K are from a gene bank in Japan. Information on seeds without number and origin are not available

Experimental conditions

For germination, seeds were put in aluminum dishes filled with sand on 27th of March, 2017 and kept moist. A week later seedlings were transplanted into plastic pots containing 950 g of soil. The soil used was a silty loam soil from an arable field in Uedem, North Rhine-Westphalia (21 m a.s.l.). The soil type was a Haplic Luvisol with locally occurring stagnic properties in the subsoil. In a previous experiment the soil was used to cultivate oil and forage radish (*Raphanus sativus* L. var.*oleiformis* Pers.) and winter turnip rape (*Brassica rapa* L. var. *silvestris* [Lam.] Briggs) (Kanders et al. 2017). The soil was stored in the dark at 5°C until being used in this experiment. Each pot contained six plants of the same cultivar or wild form and was replicated five times in a randomized block design. During the entire growth period plants were watered with distilled water to keep soil moisture constant between 50 and 70 % of its water holding capacity. The average temperature in the greenhouse was 20.5 °C. Lowest temperature was 13 °C and maximum temperature was 39 °C. During the experiment the day and night cycle was 12 hours. No fertilizer was applied during the whole growth period. Once per week the growth stage was assessed according to the Zadoks scale (Zadoks et al. 1974) and the overall plant height was measured. In the middle of May (10th and 15th of May) a 1250-fold diluted fungicide *Ortiva* (Syngenta Crop Protection AG) had to be sprayed twice against an infestation with powdery mildew (*Blumeria graminis* f. sp. *tritici* (D.C.) Speer). All plants were treated in the same way.

Harvest

Plants were harvested when more than 60 % of the plants per cultivar were in growth stage (GS) of complete anthesis or early milk development (GS 69 and GS 70, according to Zadoks et al. (1974)). After anthesis, root biomass remains constant for some weeks before it declines due to root death (Hoad et al. 2001). During the first harvest on the 10th of June the following varieties were harvested: *T. aestivum* ("Naxos"), *T. aestivum* ("Servus"), *T. aestivum* ("Thasos"), *T. monococcum, T. isphahanicum, T. carthlicum* var. *carthlicum, T. macha, T. aestivum, T. turgidium* var. *plinianum, T. dicoccom* var. *farrum, H. agriocrithon, H. vulgare* ("Ithaka"), *H. vulgare vulgare* (No. 2,2,1), *H. vulgare vulgare* (No. 2,7), *H. vulgare hexastichon, H. vulgare distichon* (No. 1,3), *H. vulgare vulgare* ("Dante"). The second harvest took place on the 21st of June and the following varieties could be harvested: *T. dicoccoides* var. *spontaneo villosum, T. durum* var. *africanum, H. lagunculiforme, H. vulgare distichon*. The last harvest took place on the 30th of June. The varieties *A. cylindrica, T. aestivum* var. *tschermakianum, T. turanicum* did not develop until GS69 during the experimental period.

Measurements

Above-ground biomass properties

Average chlorophyll content per pot was measured at GS 69 on the last fully developed green leaf with the chlorophyll meter SPAD-502 Plus (Konica Minolta Sensing, Europe). An average number of tillers, growth stage and plant height per pot was determined. The above-ground biomass was cut as low as possible above the soil surface and divided into fresh leaves, dry leaves, shoot, ear and grain (if developed). The fresh biomass was weighed for each fraction separately. Leaf area of green leaves was measured twice with LI-3100 leaf area meter (Licor, Inc., Nebr.) and the average per plant was calculated. Later, specific leaf area (SLA) was calculated by dividing the leaf area of green leaves per plant by plant dry mass.

$$\mathrm{SLA[cm^2 g^{-1}]} = \frac{\text{leaf area per plant [cm}^2\text{]}}{\text{plant dry weight [g]}} \tag{1}$$

Plant samples were dried at 70 °C and after 48 hours cooled down in a desiccator and then weighed. Remaining pots with soil were stored at 5 °C for a maximum of one week before processing.

Below-ground biomass properties

Soil was homogenized by mixing and 20 g of fresh soil was weighed into a paper bag. Soil was dried at 105 °C for 24 hours and cooled down in a desiccator before the soil dry weight for the whole pot was determined as follows:

$$\text{Total soil dry weight (SDW) [g]} = \left(\frac{\text{soil sample dry weight [g]}}{\text{soil sample fresh weight [g]}}\right) \cdot \text{total soil fresh weight [g]} \tag{2}$$

Coarse roots were collected by hand for a set time of three minutes per pot. Plant roots were then washed on a sieve with 1 mm mesh size and dried with a paper towel before fresh weight was determined. All loose roots and one shoot root (sample >7 mg) were weighed and dried at 70 °C and weighed again after 48 hours. Total coarse root content was calculated as follows:

$$\text{Dry weight of coarse roots [g]} = \left(\frac{\text{dry weight of coarse root sample [g]}}{\text{fresh weight of coarse root sample [g]}}\right) \cdot \text{total fresh weight of coarse roots [g]} \tag{3}$$

The value was then divided by the number of living plants per pot at harvest.

For fine root determination, a 400 g soil sample was washed using a sieve with 1 mm mesh size and fine roots were collected with tweezers, dried on a paper towel and then weighed. Fine roots were dried at 70 °C for 48 hours, cooled down in a desiccator and dry weight was determined. Total fine root content for each pot was calculated as follows:

$$\text{Total fine root content [g]} = \left(\frac{\text{dry weight of fine roots [g]}}{\text{soil sample [g]}}\right) \cdot \text{SDW [g]} \tag{4}$$

Values were divided by the number of living plants per pot at harvest.

Remaining intact shoot roots were put into a Falcon tube and conserved with 70 % ethanol and Neutral Red (0.35 g L^{-1}) at 5 °C. The stained roots were scanned and analyzed using WinRHIZO Pro software (Regent Instruments Inc., Québec city, Canada). Measured root parameters included root length (cm), average root diameter (mm), root surface (cm^2), root volume (cm^3) and length per volume (cm^2 m^{-3}). Values have then been divided by the number of plants.

Root tissue mass density (TMD, mg cm^{-3}), root length density (RLD, cm cm^{-3}) and specific root length (SRL, cm g^{-1}) were calculated as described by Nakhforoosh et al. (2014):

$$\text{TMD [mg cm}^{-3}\text{]} = \frac{\text{root dry mass [g]}}{\text{root volume [cm}^3\text{]}} \tag{5}$$

$$\text{RLD [cm cm}^{-3}\text{]} = \frac{\text{root length [cm]}}{\text{soil sample volume [cm}^3\text{]}} \tag{6}$$

$$\text{SRL [cm g}^{-1}\text{]} = \frac{\text{root length [cm]}}{\text{root dry mass [g]}} \tag{7}$$

Root to shoot ratio (RS, %) was calculated as follows:

$$\text{RS [\%]} = \frac{\text{Total root dry weight [g]}}{\text{Total shoot dry weight [g]}} \tag{8}$$

Statistical analysis

A one-way analysis of variance (ANOVA) was performed on all parameters with R software 3.4.0 (R Core Team, 2013). As post-hoc test TUCKEY'S HONESTLY SIGNIFICANT DIFFERENCE (HSD) was performed for balanced data sets, i.e. wild forms and cultivars using R package AGRICOLAE (Mendiburu, 2016). In the case of unbalanced datasets, i.e. domestication and ploidy levels, respectively, post-hoc analysis was done using the DUNNETT MODIFIED TUKEY-KRAMER test provided by the R package DTK (Lau, 2013). Values were transformed using the function boxcox of R package MODERN APPLIED STATISTICS WITH S (MASS) (Venables et al. 2002). Figures were plotted using R software 3.4.0 and Microsoft Excel (2010) (Version 14.0.7188.5002, Microsoft Corporation, WA), respectively. Boxplots show the median, upper and lower quartiles, outliers and minimum and maximum values excluding outliners. Letters above bars indicate significant differences between cultivars (p>0.05).

Results

Above-ground biomass properties

Already after one week, differences in plant height between cultivars and wild forms were noticed (Figure 1). After two weeks, differences in growth stage development were asserted. It is apparent that in most cases wild relatives *A. cylindrica, H. agriocrithon* and *H. lagunculiforme* had a much slower growth rate compared to the corresponding modern cultivars. Modern and old cultivars reached much higher plant heights and developed anthers, usually filled with some grain, at harvest time (except of *T. dicoccom* var*. spontaneo villosum and T. aestivum* ("Servus")). *Aegilops cylindrica, T. aestivum* var*. tschermakianum* and *T. turanicum* did not reach anthesis (GS 69) after 95 days. For the simplicity of representation, wheat and barley cultivars were selected which showed exemplary growth development for their associated domestication level (Figure 1). Wild forms and old wheat cultivars had significantly more tillers than modern ones. *Aegilops cylindrica* showed a significantly higher tiller number compared to all other wheat species, followed by *T. monococcum* and *T. turanicum* (Table 4). In barley, the wild forms *H. agriocrithon* and *H. lagunculiforme* as well as old cultivars, namely *H. vulgare distichon* and modern cultivars, *H. vulgare vulgare* (No. 2,7) had significantly higher tiller numbers compared to the other barley species (Table 6). In wheat, number of fertile tillers showed only poor correlation to root parameters (Figures 2 and 3). In barley correlation analysis was similar, figures were therefore not included. In wheat, as well as in barley, above-ground plant biomass at harvest decreased from modern to old cultivars and to wild forms. Although it must be noted that in some cases this does not imply. For example, *A. cylindrica* is not different from *T. aestivum* ("Naxos") (Table 4) and *T. isphahanicum* is considerable lower than both wild wheat forms. *T. aestivum* ("Naxos") and *T. aestivum* had the highest grain yield among wheat cultivars (Table 3).

In barley, *H. vulgare vulgare* (No. 2,2,1) had a lower plant biomass than *H. agriocrithon* (Table 6). This variation could be caused by the fact that the wild form had more leaves, due to higher tiller formation, but no anthers and grains. Whereas modern cultivars had less tillers, however they developed considerably more grains than old cultivars and wild forms (Tables 3, 4 and 9). Wild form *H. agriocrithon* developed considerable grain yield and old cultivar *H. vulgare distichon* (No. 3,1) had the highest grain yield of all cultivars. In both cereals, grain yield was not correlated to any root parameter.

Table 3: Mean values for grain yield [g] of wheat and barley cultivars and their wild forms; abbreviations see Tables 1 and 2; σ standard deviation

Wheat	**AC**	**TAN**	**TAS**	**TAT**	**TAW**	**TC**	**TDA**	**TDF**	**TDS**	**TI**	**TM**	**TMEG**	**TT**	**TTI**	**TTU**
Grain yield [g]	0	0.9	1	0.8	0.5	0	0.03	0.25	0	0.2	0.3	0.1	0	0	0
σ	0	0.2	0.4	0.2	0.3	0.1	0.06	0.2	0	0.2	0.1	0.1	0	0	0

Barley	**HA**	**HL**	**HVD**	**HVD13**	**HVH**	**HVI**	**HVV221**	**HVV27**	**HVVD**
Grain yield [g]	0.5	0.04	0	0.9	0	0.1	0.2	0.6	0.9
σ	0.4	0.1	0	0.2	0	0.2	0.2	0.2	0.3

Chlorophyll content was measured at harvest on the last fully developed green leaf. However, some plants were already dried up completely at anthesis. Hence*, T. carthlicum* and *T. durum* var*. africanum* had significantly lower chlorophyll contents compared to all other cultivars (Table 4). *Triticum macha* had the highest chlorophyll content (7.59 SPAD units). Comparing domestication levels, wild forms seem to have higher chlorophyll content than modern and old cultivars. Although it should be noted, that values do not depict a real trend. For instance, modern cultivars *T. durum* var*. africanum* and *T. aestivum* ("Thasos") are significantly different in their chlorophyll content (2.45 SPAD units, 5.07 SPAD units, respectively). In contrast to wheat, the chlorophyll measurements of barley cultivars and wild forms showed a reverse trend, decreasing from modern to old cultivars and wild forms, respectively (Table 6). The lowest chlorophyll content had *H. vulgare distichon* (No. 1,3) due to the fact that most of the plants were dry at harvest. Significantly higher chlorophyll content was measured for modern varieties (highest value for *H. vulgare vulgare* (No. 2,2,1): 6.00 SPAD units). However, wild cultivar *H. agriocrithon* is insignificantly higher than modern cultivar *H. vulgare vulgare* (No. 2,7) (4.64 and 4.32 SPAD units respectively). In wheat, both leaf area and specific leaf area (SLA) were lower in modern cultivars than in wild forms and old cultivars (Figure 4 A, Table 4). *Triticum macha* and *T. turanicum* had the highest and modern cultivars like *T. aestivum* and *T. aestivum* ("Servus") had lowest leaf area values.

In barley, *Hordeum vulgare hexastichon* and *H. vulgare vulgare* (No. 2,2,1) had the largest leaf area and SLA and *H. vulgare distichon* (No. 1,3) showed lowest values (Figure 4 B). Leaf area and specific leaf area (SLA) were higher in modern cultivars compared to wild forms. As already mentioned above, *T. carthlicum* and *T. aestivum* ("Naxos") and *H. vulgare distichon* (No. 1,3) had no green leaves, hence no leaf surface measurement was conducted for these cultivars.

Table 4: Means for shoot traits of old and modern wheat cultivars and wild forms

Cultivar	AC		TAN		TAS		TAT		TAW		TC		TDA	
Domestication level	wild		modern		modern		modern		modern		old		modern	
Days of cultivation	95		75		75		75		75		75		86	
Tiller number	0.9	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b
ABM (g)	0.6	ab	0.6	ab	0.6	a	0.7	a	0.6	abc	0.4	cd	0.4	d
Chlorophyll (SPAD units)	5	ab	4	bcd	5	abc	5	abc	5	abc	1	d	2.5	cd
LA (cm²)	14	abc	2	f	4	def	5	def	4	def	0.5	f	2	f
SLA (cm^2 g^{-1})	22	bcde	2.5	f	6	f	7	ef	7	ef	1	f	6.5	ef

Names: AC *A. cylindrica;* TAN *T. aestivum* (“Naxos”); TAS *T. aestivum* (“Servus”)*;* TAT *T. aestivum* (“Thasos”)*;* TAW *T. aestivum,* TC *T. carthlicum;* TDA *T. durum* var. *africanum*; **Measurements**: ABM Aboveground biomass (dry weight); LA Leaf area, SLA Specific leaf area; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 4 (continued): Means for shoot traits of old and modern wheat cultivars and wild forms

Cultivar	TDF		TDS		TI		TM		TMEG		TT		TTI		TTU	
Domestication level	old		wild		modern		old		old		modern		old		old	
Days of cultivation	95		86		75		75		86		75		75		95	
Tiller number	0.0	b	0.06	b	0.0	b	0.2	b	0.0	b	0.1	b	0.0	b	0.3	b
ABM (g)	0.6	abc	0.4	cd	0.4	d	0.6	a	0.5	bcd	0.5	bcd	0.4	cd	0.5	abcd
Chlorophyll (SPAD units)	4	bc	6	ab	4	bcd	8	a	5	abc	5	ab	5	ab	6	ab
LA (cm²)	13	bcd	4	ef	2	f	23	a	8	cdef	16	abc	11	cde	22	ab
SLA (cm^2 g^{-1})	24	bcd	8	def	8	ef	36	ab	15	cdef	36	ab	27	bc	43	a

Names*:* TDF *T. dicoccom* var. *farrum;* TDS *T. diccocoides* var. *spontaneo villosum;* TI *T. isphahanicum;* TM *T. macha;* TMEG *T. monococcum;* TT *T. aestivum* var. *tschermakianum;* TTU *T. turanicum;* TTI *T. turgidum* var. *plinianum*; **Measurements**: ABM Aboveground biomass (dry weight); LA Leaf area, SLA Specific leaf area; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 5: Means for shoot traits of old and modern wheat cultivars and wild forms

Cultivar	**AC**		**TAN**		**TAS**		**TAT**		**TAW**		**TC**		**TDA**	
Domestication level	wild		modern		modern		modern		modern		old		modern	
Days of cultivation	95		75		75		75		75		75		86	
Tiller number	0.9	a	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b	0.0	b
ABM (g)	0.6	ab	0.6	ab	0.6	a	0.7	a	0.6	abc	0.4	cd	0.4	d
Chlorophyll (SPAD units)	5	ab	4	bcd	5	abc	5	abc	5	abc	1	d	2.5	cd
LA (cm²)	14	abc	2	f	4	def	5	def	4	def	0.5	f	2	f
SLA (cm^2 g^{-1})	22	bcde	2.5	f	6	f	7	ef	7	ef	1	f	6.5	ef

Names: AC *A. cylindrica;* TAN *T. aestivum* (“Naxos”); TAS *T. aestivum* (“Servus”)*;* TAT *T. aestivum* (“Thasos”)*;* TAW *T. aestivum,* TC *T. carthlicum;* TDA *T. durum* var. *africanum*; **Measurements**: ABM Aboveground biomass (dry weight); LA Leaf area, SLA Specific leaf area; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 5 (continued): Means for shoot traits of old and modern wheat cultivars and wild forms

Cultivar	**TDF**		**TDS**		**TI**		**TM**		**TMEG**		**TT**		**TTI**		**TTU**	
Domestication level	old		wild		modern		old		old		modern		old		old	
Days of cultivation	95		86		75		75		86		75		75		95	
Tiller number	0.0	b	0.06	b	0.0	b	0.2	b	0.0	b	0.1	b	0.0	b	0.3	b
ABM (g)	0.6	abc	0.4	cd	0.4	d	0.6	a	0.5	bcd	0.5	bcd	0.4	cd	0.5	abcd
Chlorophyll (SPAD units)	4	bc	6	ab	4	bcd	8	a	5	abc	5	ab	5	ab	6	ab
LA (cm²)	13	bcd	4	ef	2	f	23	a	8	cdef	16	abc	11	cde	22	ab
SLA (cm^2 g^{-1})	24	bcd	8	def	8	ef	36	ab	15	cdef	36	ab	27	bc	43	a

Names*:* TDF *T. dicoccom* var. *farrum;* TDS *T. diccocoides* var. *spontaneo villosum;* TI *T. isphahanicum;* TM *T. macha;* TMEG *T. monococcum;* TT *T. aestivum* var. *tschermakianum;* TTU *T. turanicum;* TTI *T. turgidum* var. *plinianum*; **Measurements**: ABM Aboveground biomass (dry weight); LA Leaf area, SLA Specific leaf area; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 6: Means for morphological root traits of old and modern wheat cultivars and wild forms

Cultivar	AC		TAN		TAS		TAT	
CR (g)	0.3	a	0.6	c	0.6	c	0.7	c
FR (g)	0.4	a	0.02	c	0.04	c	0.08	bc
AD (mm)	0.07	ab	0.08	ab	0.09	ab	0.08	ab
LPV (m m^{-3})	14569	ab	5099	de	6406	cd	6265	cde
RLD (mm mm^{-3})	0.02	ab	0.005	de	0.006	cd	0.006	cde
RMD (mg cm^{-3})	0.7	a	0.6	a	0.5	a	0.6	a
SRL (cm g^{-1})	36	a	177	ab	104	b	71	ab
RBM (g)	0.4	a	0.1	cd	0.7	cd	0.08	cd
RS (%)	63	a	9	b	10	b	12	b
RV (cm^3)	0.6	a	0.1	de	0.1	cde	0.2	cd
RSA (cm^2)	99	ab	25	cde	31	cd	33	cd
RL (cm)	1311	ab	459	de	577	cd	564	cde

Names: AC *A. cylindrica;* TAN *T. aestivum* ("Naxos"); TAS *T. aestivum* ("Servus"); TAT *T. aestivum* ("Thasos")*;* **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; LPV Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; RV Root volume; RSA Root surface area; RL Root length; values followed by the same letter are not significantly different from each other (Tukey's HSD, $p<0.05$)

Table 6 (continued): Means for morphological root traits of old and modern wheat cultivars and wild forms

Cultivar	TAW		TC		TDA	
CR (g)	0.05	c	0.2	c	0.1	c
FR (g)	0.1	abc	0.1	abc	0.05	bc
AD (mm)	0.09	ab	0.07	ab	0.1	b
LPV (m m^{-3})	7431	bcd	4682	de	6079	de
RLD (mm mm^{-3})	0.007	bcd	0.005	de	0.006	de
RMD (mg cm^{-3})	0.5	a	0.8	a	0.8	a
SRL (cm g^{-1})	108	b	256	b	59	ab
RBM (g)	0.07	cd	0.06	cd	0.09	bcd
RS (%)	12	b	14	b	240	b
RV (cm^3)	0.2	cd	0.1	de	0.1	cde
RSA (cm^2)	36	cd	21	de	30	cde
RL (cm)	669	bcd	421	de	547	de

Names: TAW *T. aestivum,* TC *T. carthlicum;* TDA *T. durum* var. *africanum;* **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; LPV Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; RV Root volume; RSA Root surface area; RL Root length; Values followed by the same letter are not significantly different from each other (Tukey's HSD, $p<0.05$)

Table 6 (continued): Means for morphological root traits of old and modern wheat cultivars and wild forms

Cultivar	**TDF**		**TDS**		**TI**		**TM**	
CR (g)	0.08	c	0.08	c	0.03	c	0.2	b
FR (g)	0.3	abc	0.05	bc	0.05	bc	0.1	abc
AD (mm)	0.06	ab	0.08	ab	0.11	ab	0.07	ab
LPV (m m^{-3})	9020	bcd	6962	cd	2654	e	12861	abc
RLD (mm mm^{-3})	0.009	bcd	0.007	cd	0.003	e	0.01	abc
RMD (mg cm^{-3})	0.5	a	0.7	a	0.7	a	0.4	a
SRL (cm g^{-1})	74	ab	67	ab	149	ab	68	ab
RBM (g)	0.1	bc	0.1	bcd	0.03	d	0.2	b
RS (%)	22	b	21	b	9	b	28	b
RV (cm^3)	0.3	bc	0.14	cde	0.06	e	0.5	ab
RSA (cm^2)	51	bc	33	cd	13	e	85	ab
RL (cm)	812	bcd	627	cd	239	e	1158	abc

Names: TDF *T. dicoccom* var. *farrum;* TDS *T. diccocoides* var. *spontaneo villosum;* TI *T. isphahanicum;* TM *T. macha;* **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; LPV Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; *RV* Root volume; RSA Root surface area; RL Root length; values followed by the same letter are not significantly different from each other (Tukey's HSD, $p<0.05$)

Table 6 (continued): Means for morphological root traits of old and modern wheat cultivars and wild forms

Cultivar	**TMEG**		**TT**		**TTI**		**TTU**	
CR (g)	0.1	c	0.3	a	0.07	c	0.3	a
FR (g)	0.06	bc	0.3	abc	0.05	bc	0.4	ab
AD (mm)	0.04	a	0.08	ab	0.06	ab	0.07	ab
LPV (m m^{-3})	8439	bcd	19572	a	6684	cd	17908	a
RLD (mm mm^{-3})	0.008	bcd	0.02	a	0.007	cd	0.02	a
RMD (mg cm^{-3})	0.5	a	0.5	a	0.5	a	0.6	a
SRL (cm g^{-1})	95	b	59	ab	84	ab	46	ab
RBM (g)	0.08	cd	0.3	a	0.07	cd	0.4	a
RS (%)	19	b	69	a	17	b	72	a
RV (cm^3)	0.2	cd	0.8	a	0.2	cd	0.6	a
RSA (cm^2)	40	cd	129	a	36	cd	113	a
RL (cm)	160	bcd	1762	a	602	cd	1612	a

Names: TMEG *T. monococcum;* TT *T. aestivum* var. *tschermakianum;* TTU *T. turanicum;* TTI *T. turgidum* var. *plinianum*; **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; LPV Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; *RV* Root volume; RSA Root surface area; RL Root length; values followed by the same letter are not significantly different from each other (Tukey's HSD, $p<0.05$)

Table 7: Means for shoot traits of old and modern barley cultivars and wild forms

Cultivar	HA		HL		HVD		HVD13		HVH		HVI		HVV221		HVV27		HVVD	
Domestication level	wild		wild		Old		old		old		old		modern		modern		modern	
Days of cultivation	75		86		75		86		75		75		75		75		75	
Tiller number	0.03	ab	0.14	a	0.06	ab	0.1	ab	0.03	ab	0.0	b	0.0	b	0.09	ab	0.0	b
ABM (g)	0.6	abc	0.5	a	0.6	abc	0.7	bc	0.6	ab	0.7	c	0.5	a	0.7	bc	0.7	c
Chlorophyll (SPAD units)	5	ab	3	b	5	ab	1	c	5	ab	6	a	6	a	4	ab	6	a
LA (cm^2)	8	bcd	9	bcd	6	cd	0.8	d	32	a	16	b	29	a	12	bc	12	bc
SLA ($cm^2\ g^{-1}$)	14	bc	18	bc	9	bc	0.9	c	59	a	23	b	55	a	18	bc	15	bc

Names*:* HA *H. agriocrithon;* HL *H. lagunculiforme;* HVD *H. vulgare distichon;* HVD13 *H. vulgare distichon* (No. 1,3); HVH *H. vulgare hexastichon;* HVI *H. vulgare* ("Ithaka"); HVV221 *H. vulgare vulgare* (No. 2,2,1)*;* HVV27 *H. vulgare vulgare* (No. 2,7); HVVD *H. vulgare vulgare* ("Dante") **Measurements**: ABM Aboveground biomass (dry weight); LA Leaf area, SLA Specific leaf area; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 8*:* Means for morphological root traits of old and modern barley cultivars and wild forms

Cultivar	HA		HL		HVD		HVD13		HVH	
CR (g)	0.09	bc	0.26	a	0.14	b	0.08	cd	0.29	a
FR (g)	0.03	bc	0.01	c	0.04	b	0.3	b	0.1	a
AD (mm)	0.06	abc	0.1	c	0.06	abc	0.1	bc	0.07	abc
LPV ($m\ m^{-3}$)	6597	c	14989	a	7936	c	7283	c	13174	ab
RLD ($mm\ mm^{-3}$)	0.007	c	0.02	a	0.008	c	0.007	c	0.013	ab
RMD ($mg\ cm^{-3}$)	0.5	ab	0.4	a	0.7	b	0.7	ab	0.7	b
SRL ($cm\ g^{-1}$)	57	abc	51	abc	42	abc	69	ab	30	c
RBM (g)	0.1	def	0.3	b	0.2	cd	0.1	ef	0.4	a
RS (%)	19	a	52	a	29	a	16	a	72	a
RV (cm^3)	0.2	de	0.8	a	0.3	de	0.2	e	0.6	ab
RSA (cm^2)	42	c	117	a	49	bc	38	c	97	a
RL (cm)	594	c	1349	a	714	c	656	c	1186	ab

Names*:* HA *H. agriocrithon*; HL *H. lagunculiforme*; HVD *H. vulgare distichon*; HVD13 *H. vulgare distichon* (No. 1,3)*;* HVH *H. vulgare hexastichon*; **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; *LPV* Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; RV Root volume; RSA Root surface area; RL Root length; values followed by the same letter are not significantly different from each other (Tukey's HSD, p<0.05)

Table 8 (continued): Means for morphological root traits of old and modern barley cultivars and wild forms

Cultivar	HVI		HVV221		HVV27		HVVD	
CR (g)	0.05	d	0.21	a	0.11	bc	0.10	bc
FR (g)	0.04	b	0.04	b	0.03	bc	0.03	b
AD (mm)	0.05	a	0.07	abc	0.06	ab	0.06	abc
LPV ($m\ m^{-3}$)	6712	c	9557	abc	8168	bc	6650	c
RLD ($mm\ mm^{-3}$)	0.007	c	0.01	abc	0.008	bc	0.007	c
RMD ($mg\ cm^{-3}$)	0.4	a	0.6	ab	0.5	ab	0.7	ab
SRL ($cm\ g^{-1}$)	75	a	38	bc	56	abc	53	abc
RBM (g)	0.08	f	0.3	bc	0.1	de	0.1	def
RS (%)	12	a	48	a	20	a	18	a
RV (cm^3)	0.3	de	0.5	bc	0.3	cd	0.2	de
RSA (cm^2)	44	bc	70	ab	53	bc	41	c
RL (cm)	604	c	860	abc	735	bc	599	c

Names: HVI *H. vulgare* (“Ithaka”); HVV221 *H. vulgare vulgare* (No. 2,2,1); HVV27 *H. vulgare vulgare* (No. 2,7); HVVD *H. vulgare vulgare* (“Dante”); **Measurements**: CR Dry weight of coarse roots; FR Dry weight of fine roots; AD Average root diameter; *LPV* Length per volume; RLD Root length density; RMD Root tissue mass density; SRL Specific root length; RBM Root biomass (dry weight); RS Root to shoot ratio; RV Root volume; RSA Root surface area; RL Root length; values followed by the same letter are not significantly different from each other (Tukey's HSD, $p<0.05$)

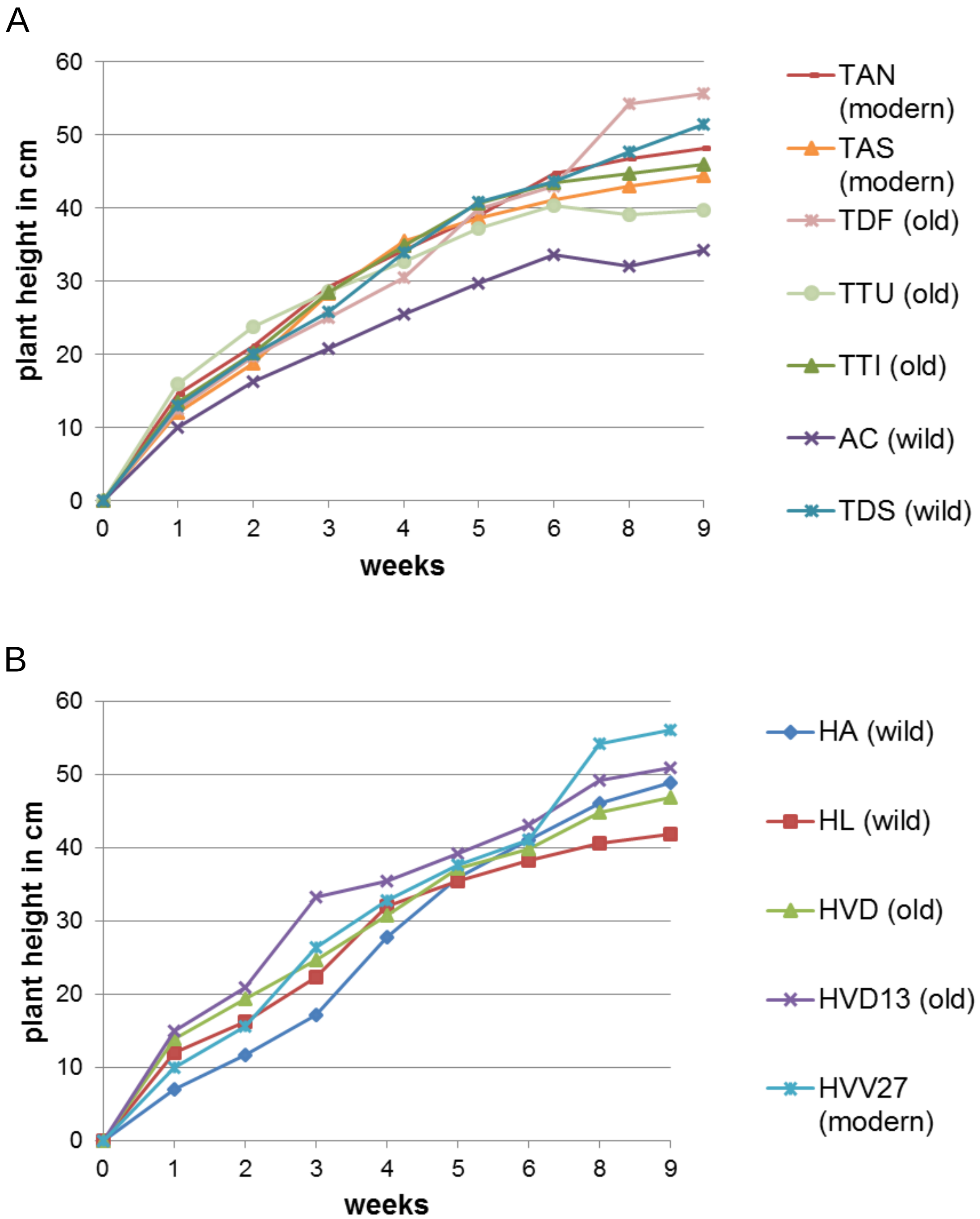


Figure 1: Plant growth of selected wheat (A) and barley (B) cultivars and wild forms; abbreviations see Tables 1 and 2

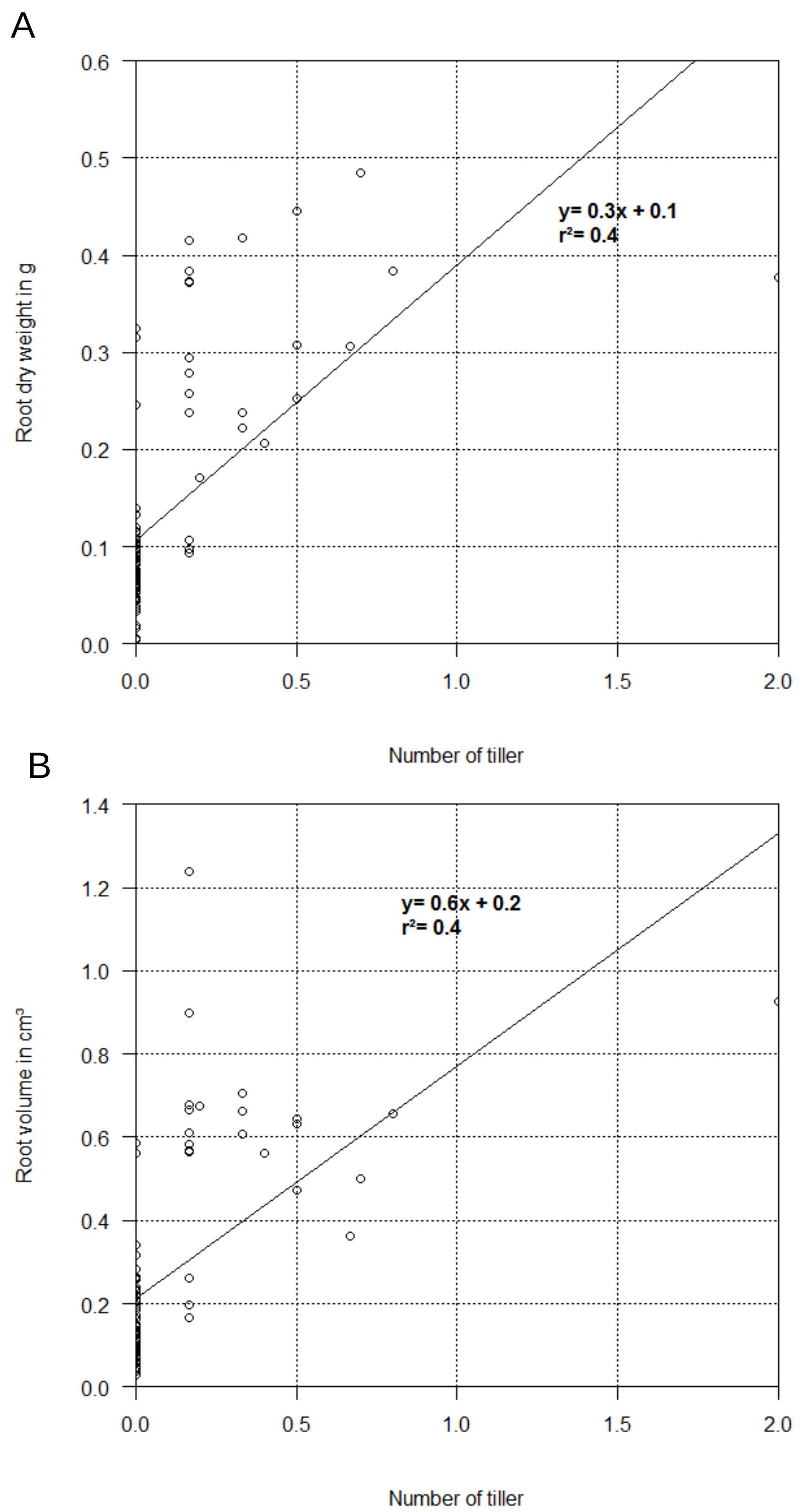


Figure 2: Correlation of root dry weight (A), root volume (B), root length (C) and RLD (D) and number of fertile tiller of wheat cultivars and wild forms

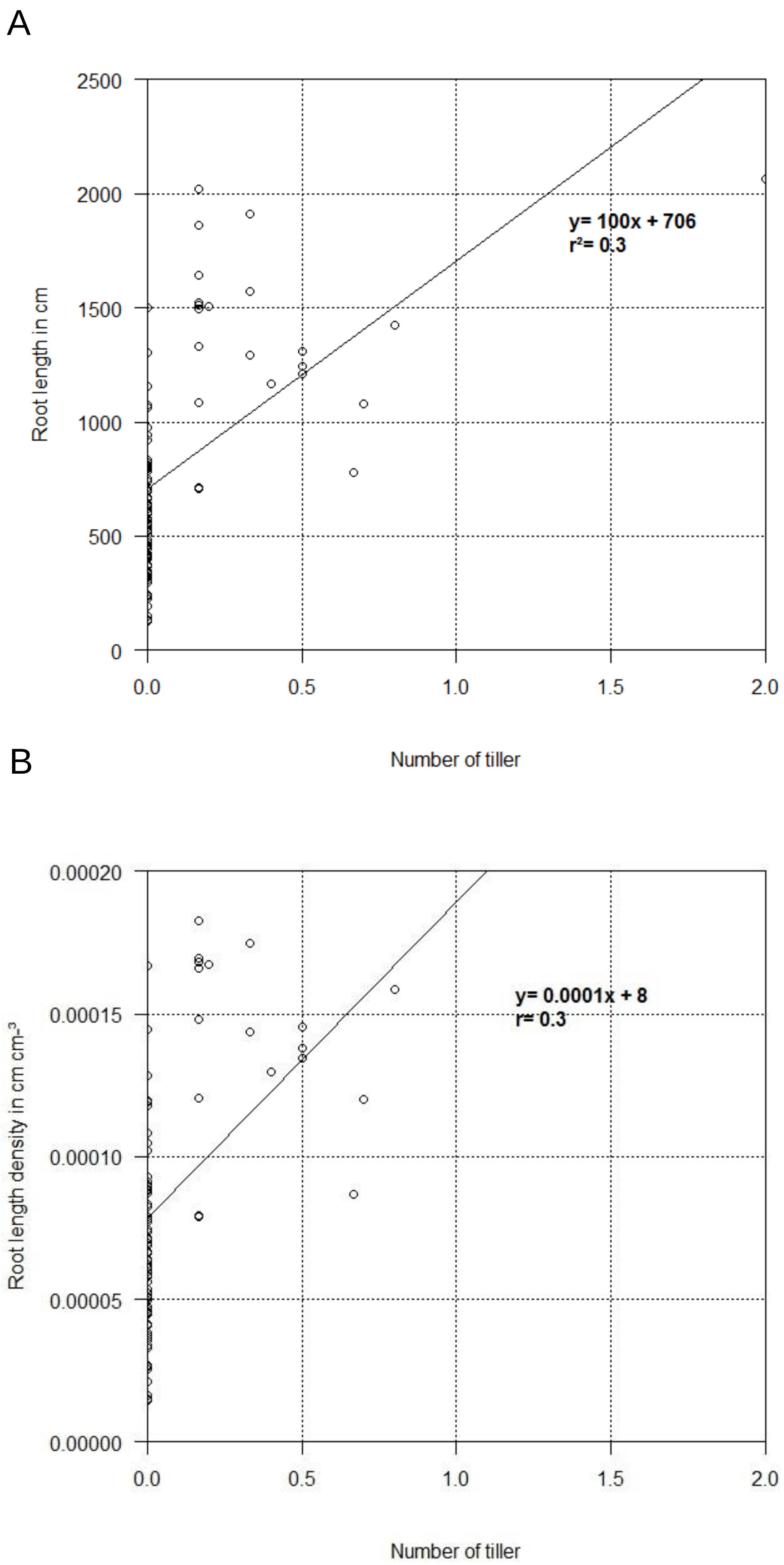


Figure 3: Correlation of root length (A) and RLD (B) and number of fertile tiller of wheat cultivars and wild forms

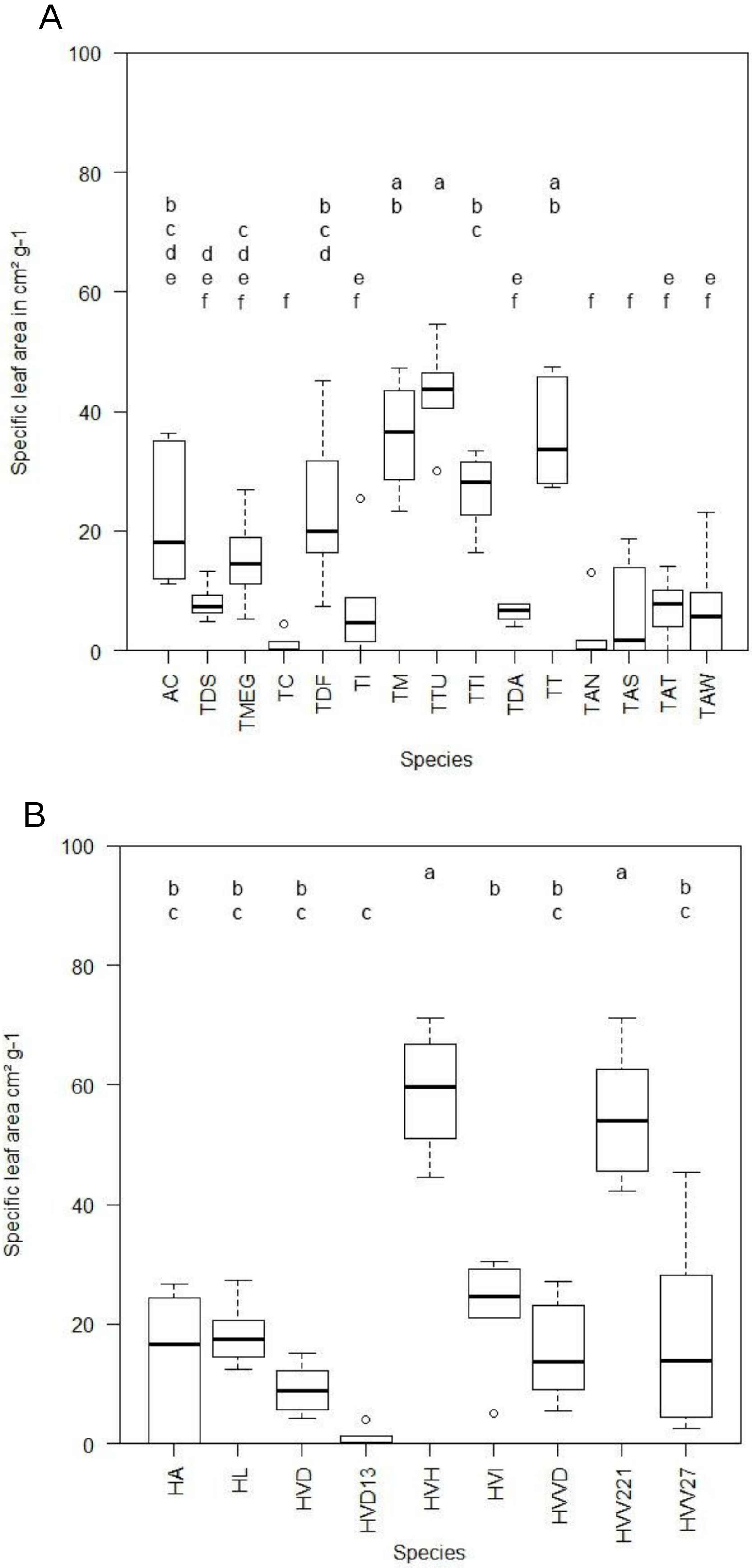


Figure 4: Specific leaf area of wheat (A) and barley (B) cultivars and wild forms; abbreviations see Tables 1 and 2

Total root biomass

Besides *A. cylindrica* and *T. turanicum* a high total root biomass was also observed for *T. aestivum* var. *tschermakianum* (Figure 5 A). Lowest root biomass had *T. carthlicum* and *T. aestivum* ("Naxos"). A significant higher amount of root dry weight was contributed by the amount of coarse roots, except for the modern varieties *T. isphahanicum, T. aestivum* var. *tschermakianum* and *T. aestivum*. In general, wild forms had a higher root dry mass than modern cultivars (Figure 5 B). Root biomass of old cultivars was close to that of modern cultivars. In barley, *H lagunculiforme* and *H. vulgare hexastichon* had the highest root dry mass (Figure 6 A) Modern barley cultivar *H. vulgare* ("Ithaka") had the lowest total root mass. Similar to wheat, though not significant, the wild-forms of barley had on average a higher root biomass than modern cultivars, whereas old cultivars were more similar to wild forms (Figure 6 B). Interestingly, a higher amount of coarse roots does not mean a higher amount of fine roots (Figure 7). As unexpected, a higher root biomass did not lead to a higher grain yield. For instance, *A. cylindrica* with the highest root biomass did not develop any grain or anthers until harvest. Correlation analysis further revealed that a higher root dry weight is caused by longer roots rather than thicker roots and by dry weights of coarse roots (Figure 8).

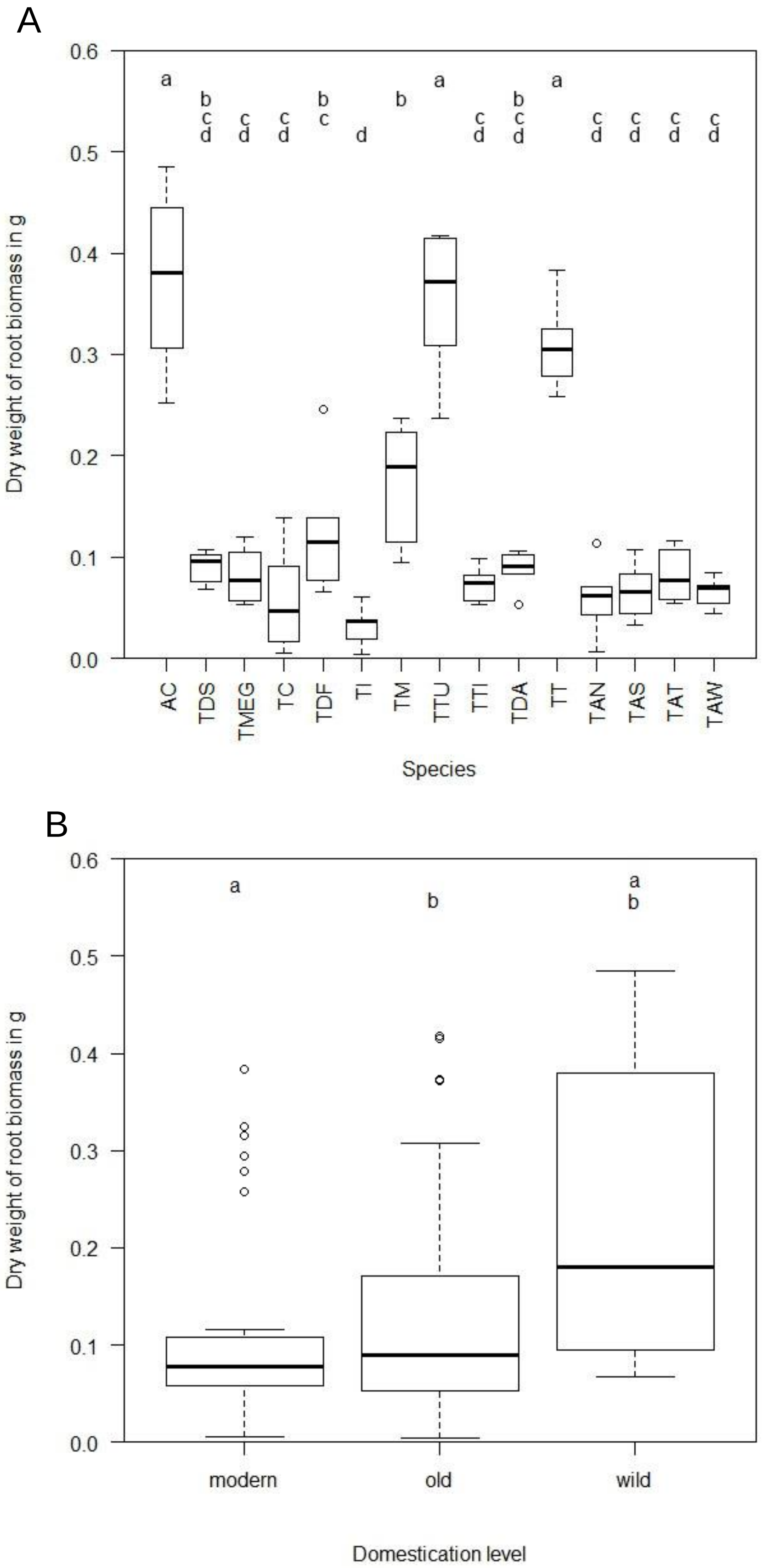
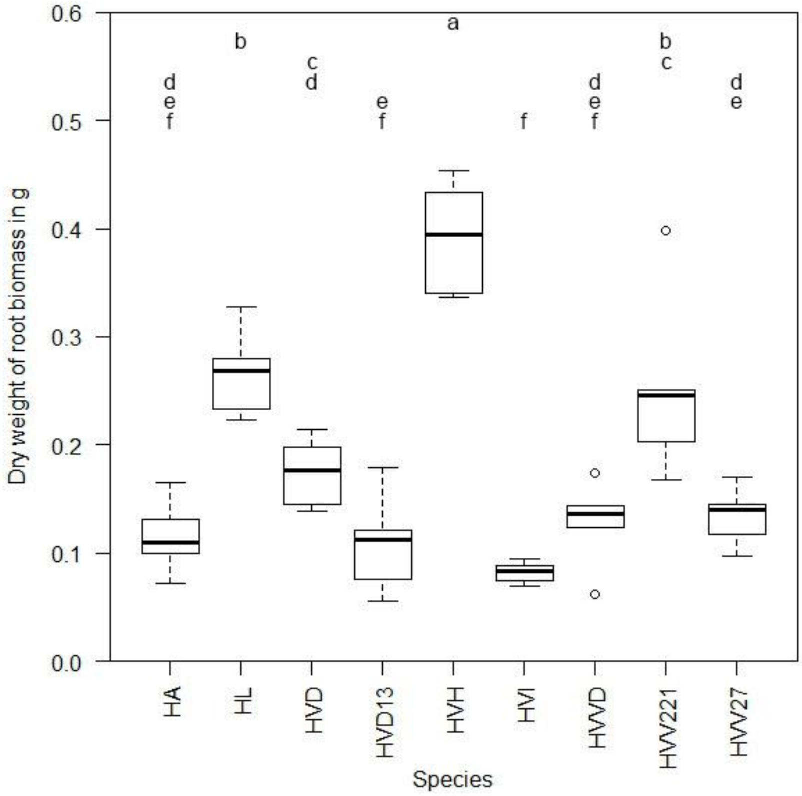


Figure 5*:* Dry weight of root biomass of wheat cultivars and wild wheat forms; abbreviations see Table 1

A



B

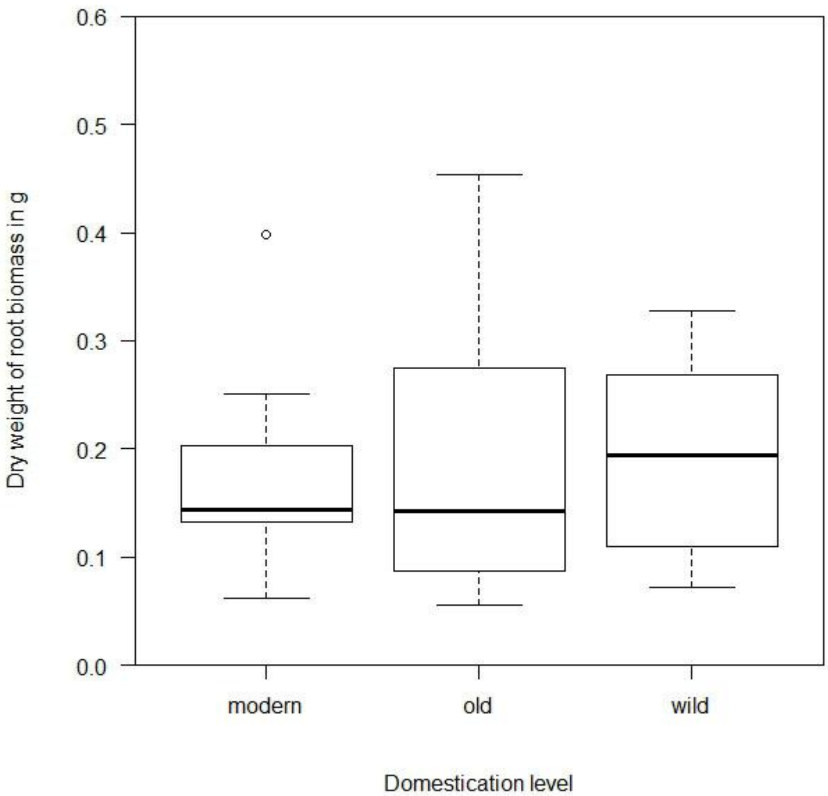


Figure 6*:* Dry weight of root biomass of barley cultivars and wild forms (A) and grouped according to domestication levels (B); there were no significant differences between domestication levels; abbreviations see Table 2

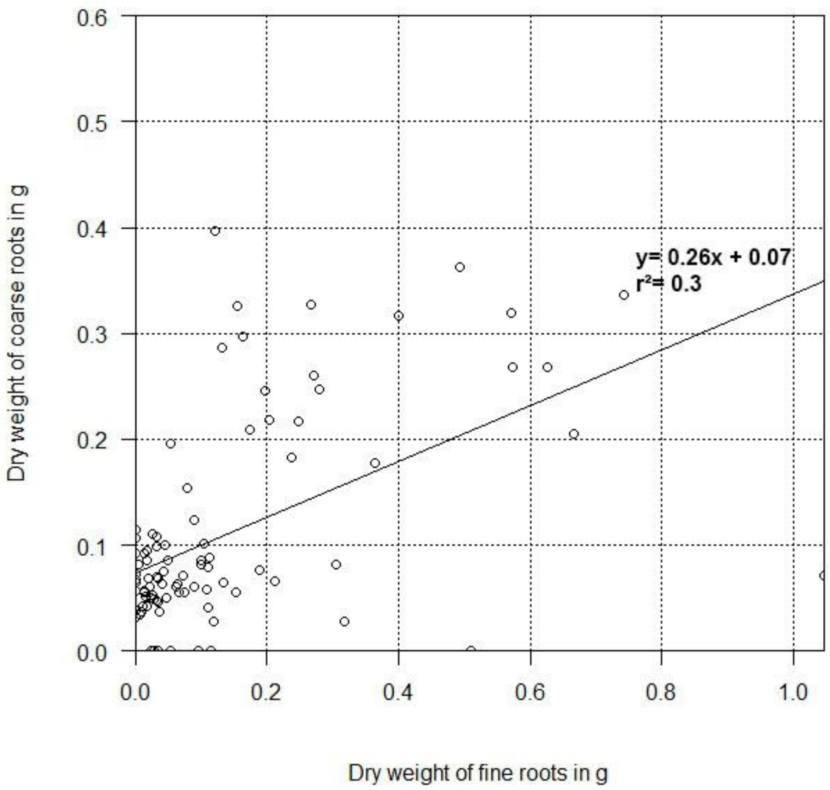


Figure 7: Correlation of dry weight of coarse roots to dry weight of fine roots of wheat cultivars and wild forms

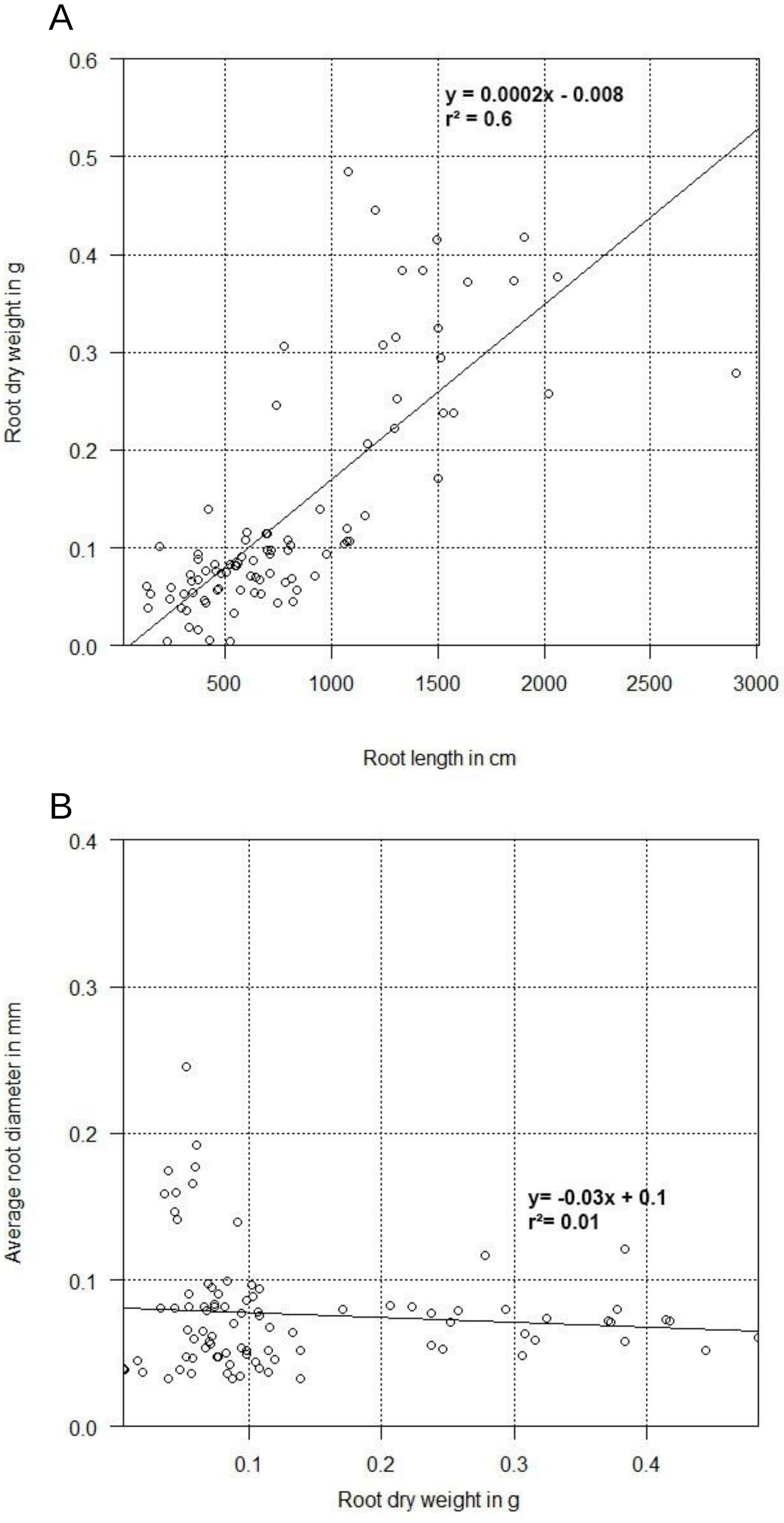


Figure 8: Correlation of total root dry weight and root length (A) and average diameter (B) of wheat cultivars and wild forms

C

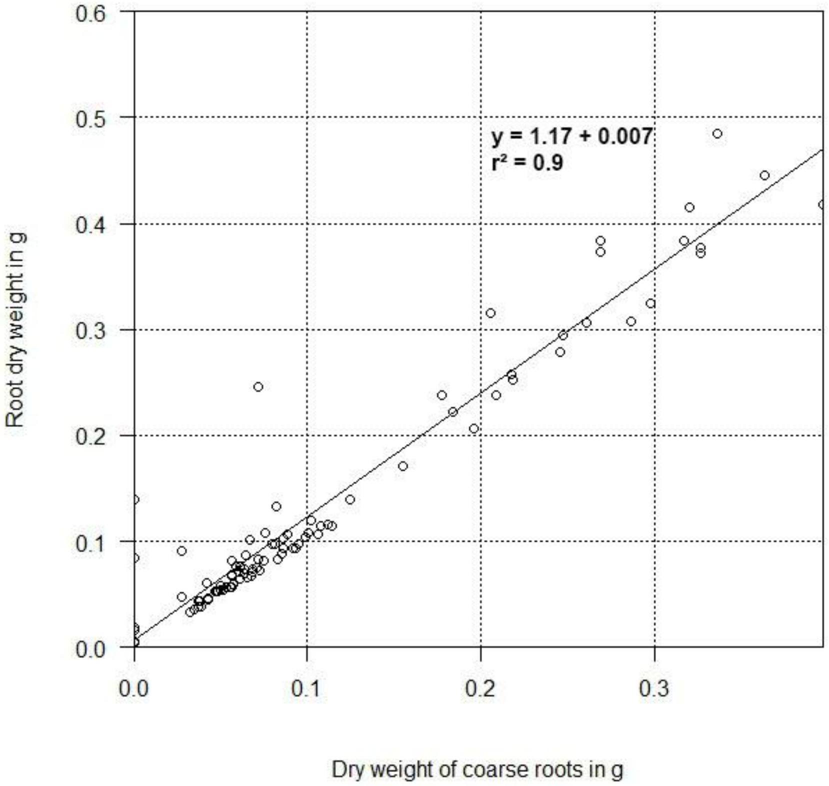
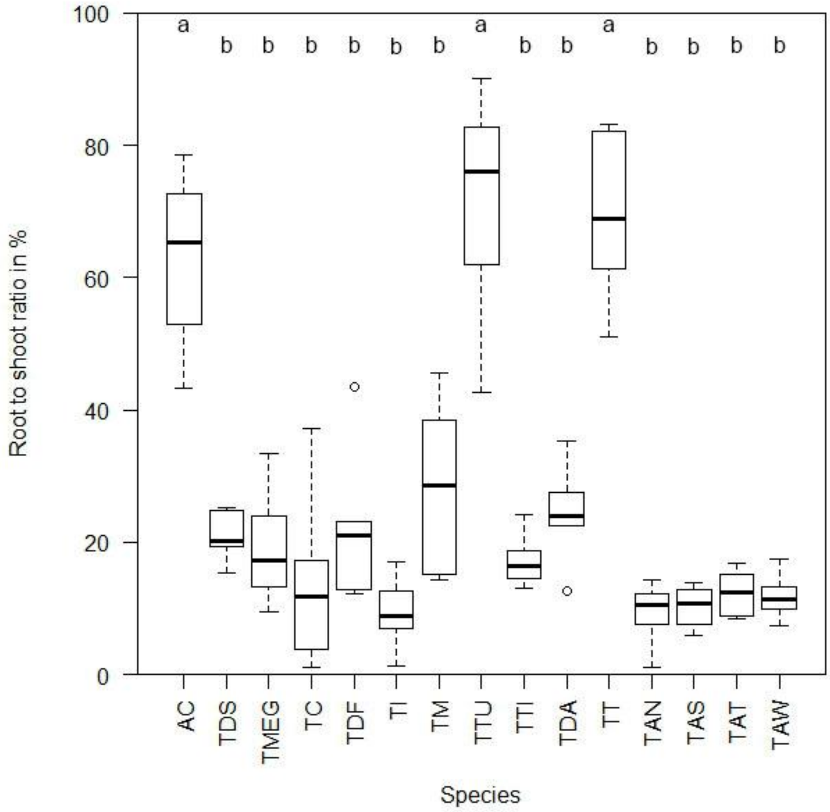


Figure 8: Correlation of total root dry weight and coarse roots (C) of wheat cultivars and wild forms

Root to shoot ratio

A much higher root to shoot ratio (RS) was exhibited for the cultivars *A. cylindrica, T. turanicum* and *T. aestivum* var*. tschermakianum* compared to the other cultivars (Figure 9 A). When looking at differences between domestication levels, a high variability can be observed (Figure 9 B). It shows that root to shoot ratio is depending stronger on genotype than on domestication level. This is due to the fact that *T. turanicum* belongs to the old cultivars, and *T. aestivum* var*. tschermakianum* is a modern cultivar. When comparing ploidy levels (Figure 9 C), diploid genotypes have the highest RS. High variability can be observed in hexaploid and tetraploid genotypes. In barley, *H. lagunculiforme* and *H. vulgare hexastichon* had the highest root to shoot ratio (Figure 10 A), nevertheless, no significant differences were observed between domestication levels (Figure 10 B). In wheat, correlation analysis revealed that a high root to shoot ratio is mainly explained by longer roots rather than thicker roots (Figure 11).

A



B

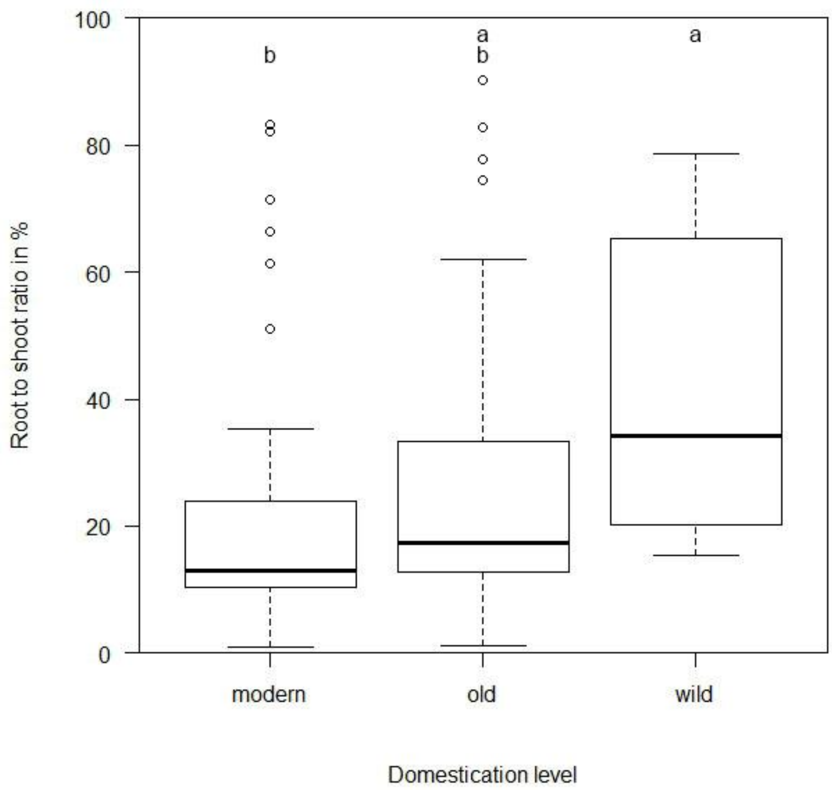


Figure 9: Root to shoot ratio of wheat cultivars and wild forms (A) and compared at domestication level (B); abbreviations see Table 2

C

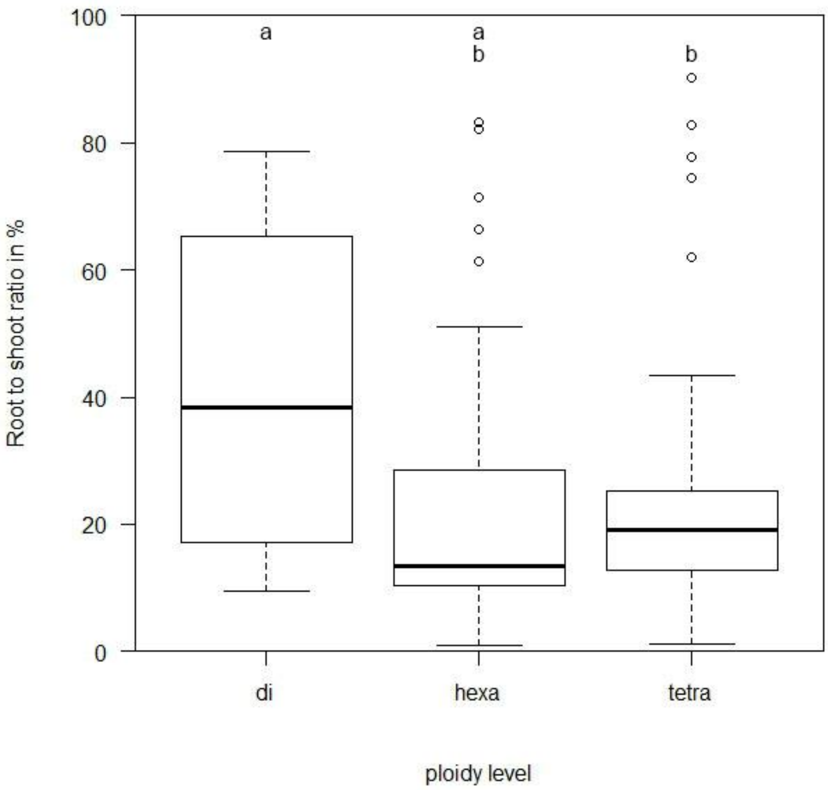


Figure 9: Root to shoot ratio of wheat cultivars and wild forms compared at ploidy level (C); abbreviations see Table 2

A

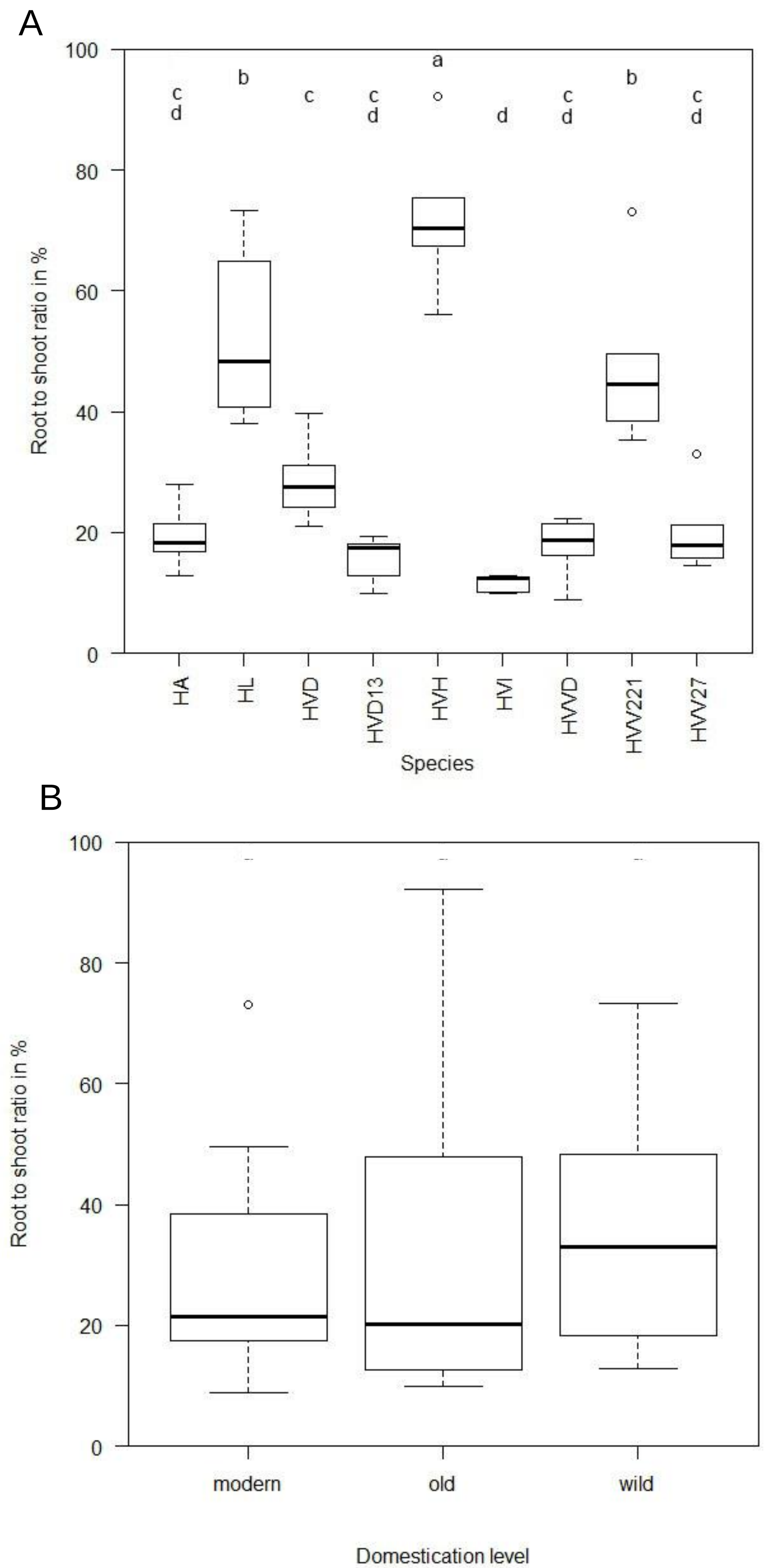


Figure 10: Root to shoot ratio of barley cultivars and wild forms (A) and grouped according to domestication level (B); there were no significant differences between domestication levels; abbreviations see Table 2

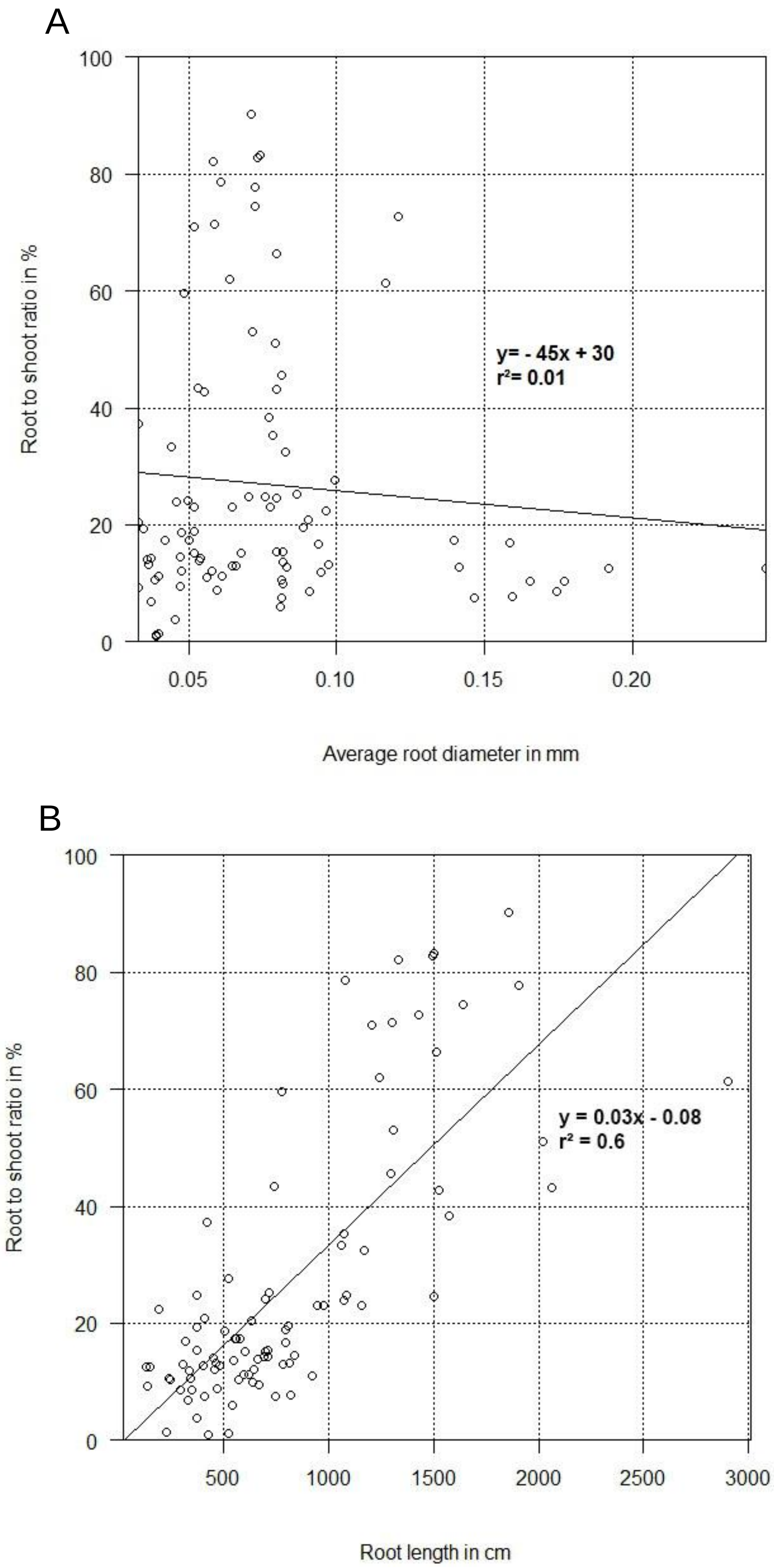
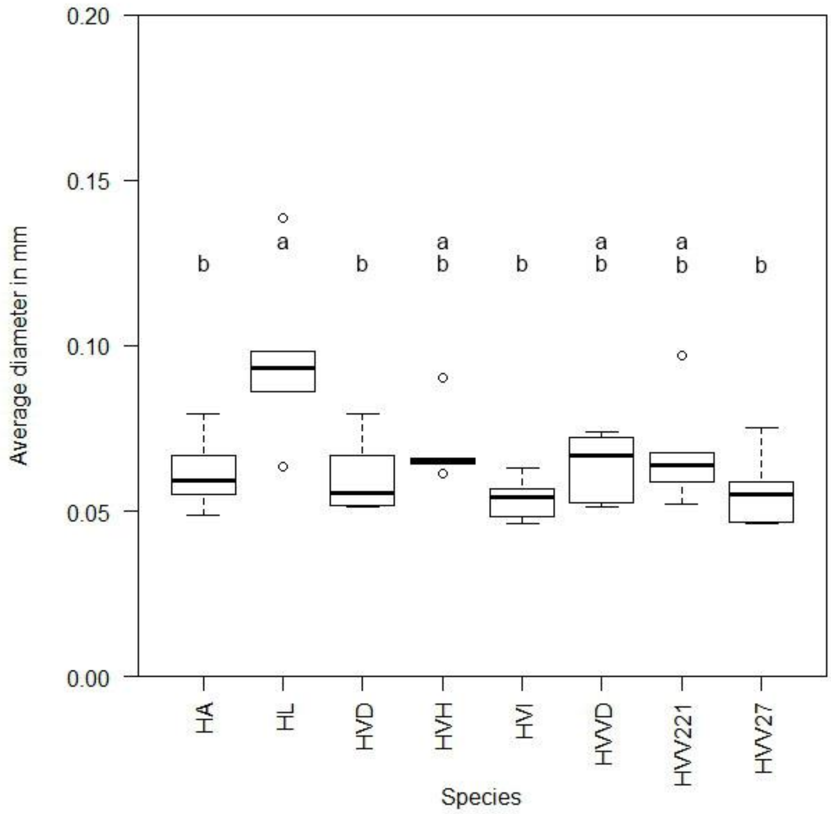


Figure 11: Correlation of RS and average root diameter (A) and root length (B) of wheat cultivars and wild forms

Root diameter

Average root diameter showed no significant differences between wheat cultivars and wild forms (p =0.2, F=1.2). In barley wild form *H. lagunculiforme* and old cultivar *H. vulgare distichon* (No. 1,3) had thicker roots compared to the others (Figure 12A). Furthermore average root diameter decreases with increasing domestication level (Figure 12 B). Due to high data variability in cultivar *H. vulgare distichon* (No. 1,3) (min: 0.05 mm, max: 0.2 mm), it was left out of the figure.

A



B

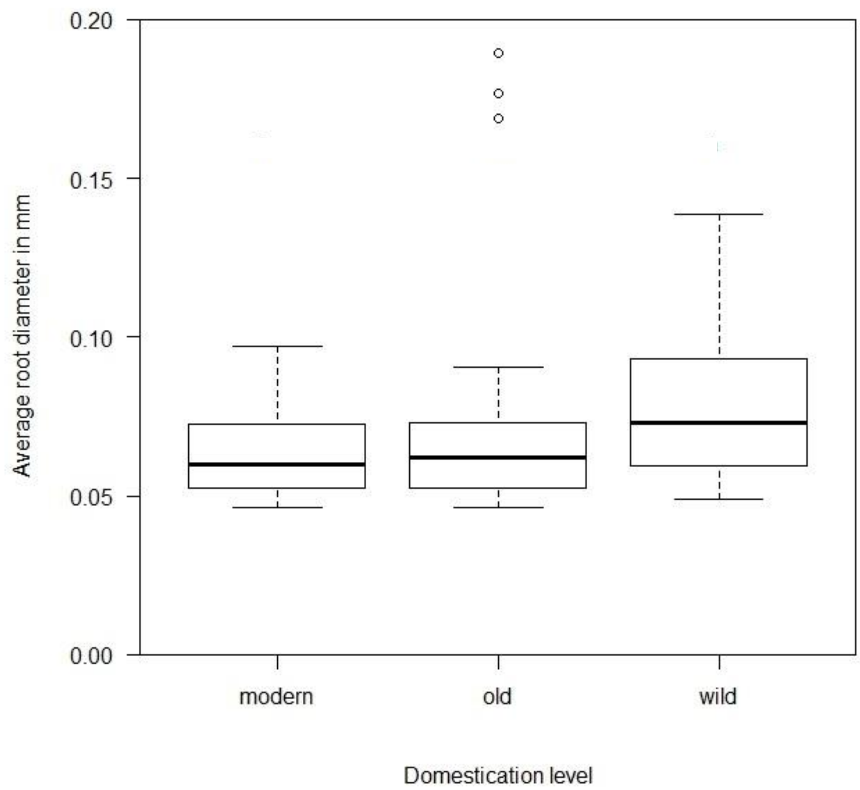


Figure 12: Average root diameter of barley cultivars and wild forms (A) and grouped according to domestication level (B); there were no significant differences between domestication levels; abbreviations see Table 2

Length per volume

Varieties *T. turanicum* and *T. aestivum* var*. tschermakianum* had the highest root length per volume (LPV), respectively. The lowest LPV was observed for *T. isphahanicum* (Table 5)*.* In general, wild wheat forms had insignificantly higher values than old and modern cultivars. In barley*, H. lagunculiforme* and *H. vulgare hexastichon* had the highest LPV, respectively (Table 7). No significant differences could be observed between domestication levels.

Root volume

For the cultivars *T. aestivum* var*. tschermakianum, T. turanicum* and *A. cylindrica* a much higher root volume could be observed compared to the other cultivars (Table 5). The smallest root volume had *T. isphahanicum*. On average the wild forms showed a higher root volume compared to old and modern cultivars. The same accounts for barley. *Hordeum lagunculiforme* had the overall highest root volume followed by *H. vulgare hexastichon* (Table 7). Root volume is positively correlated with root length (Figure 13 A), however not necessarily with average root diameter (Figure 13 B). This means, that a higher root volume is mainly triggered by longer roots rather than thicker roots.

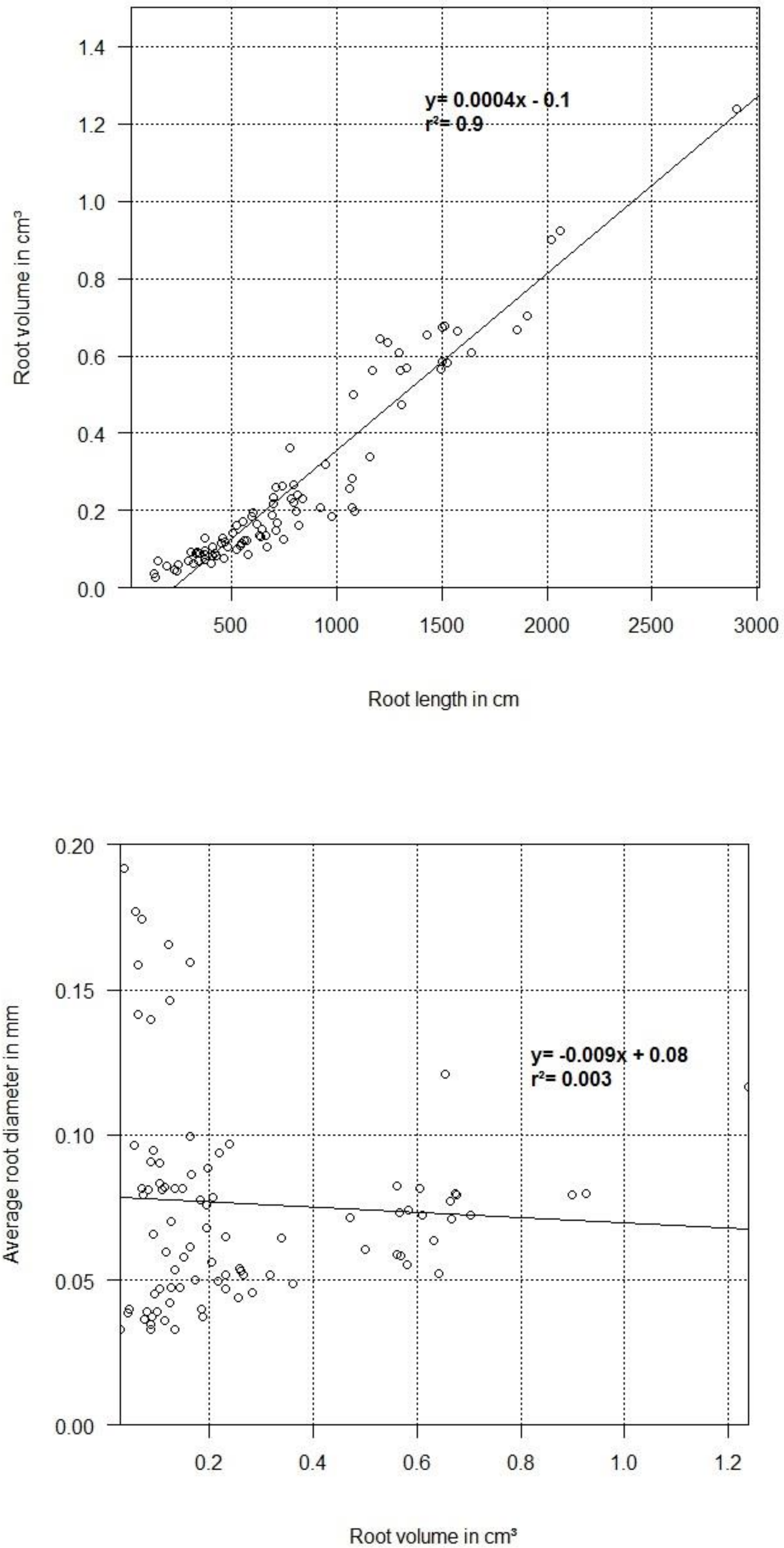


Figure 13: Correlation of root volume and root length (A) and average root diameter (B) of wheat cultivars and wild forms

Root length and surface area

Root length and surface area can be controlling variables for nutrient and water uptake in plants. Significant differences could be observed between genotypes concerning root length in both cereals. *Triticum isphahanicum* showed the lowest root length in wheat, whereas *T. aestivum* var*. tschermakianum* had the longest roots (Figure 14 A). In barley, *H. lagunculiforme and H. vulgare hexastichon* had the longest roots and *H. agriocrithon* had the shortest roots (Figure 14 B). Wild forms had not significantly longer roots than old and modern barley and wheat cultivars (Figure 15). In wheat as well as in barley, root surface area was significantly higher for wild forms than for modern and old cultivars (Figure 16).

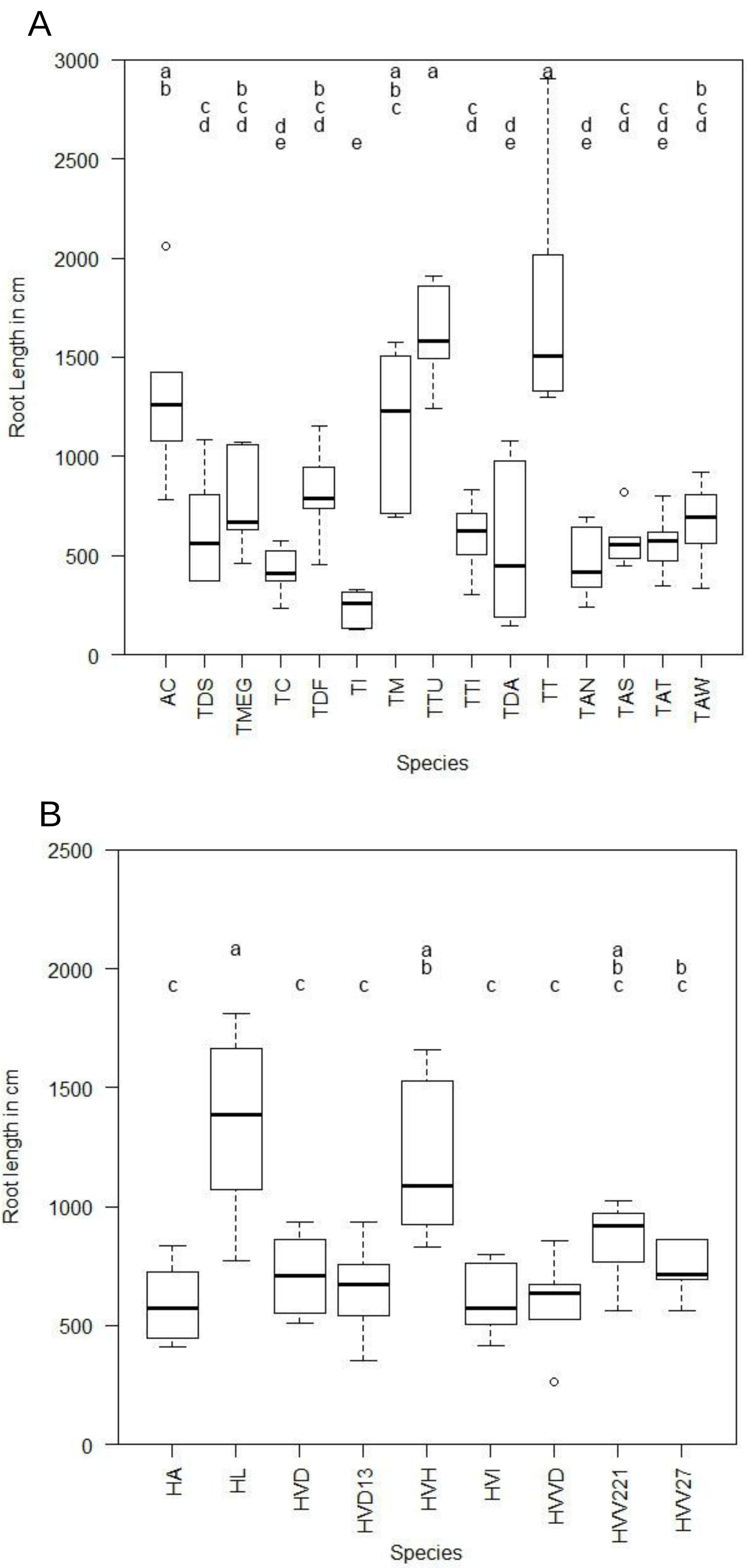


Figure 14: Root length of wheat (A) and barley (B) cultivars and wild forms; abbreviations see Tables 1 and 2

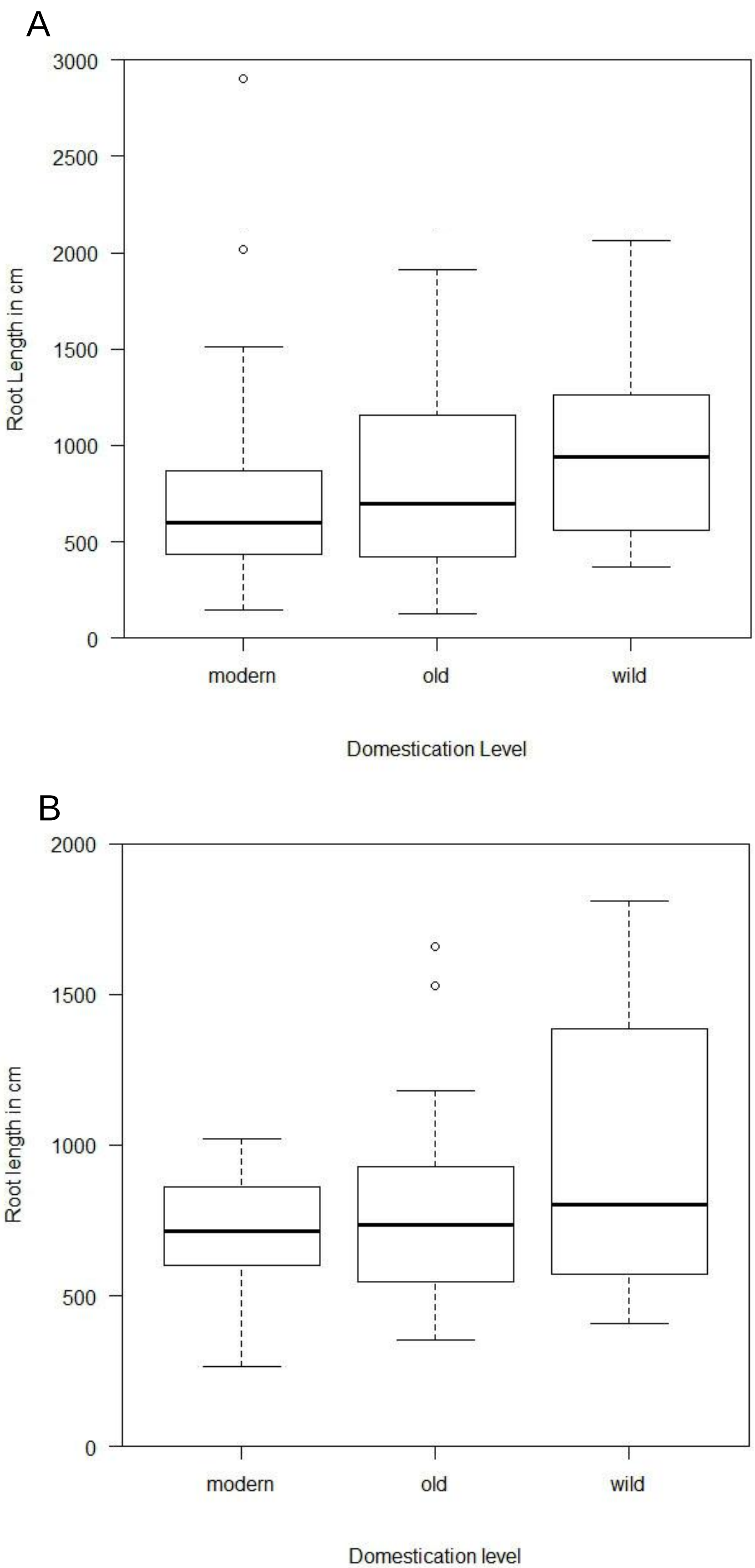


Figure 15: Root length of wheat (A) and barley (B) cultivars and wild forms grouped according to domestication level; there were no significant differences between domestication levels

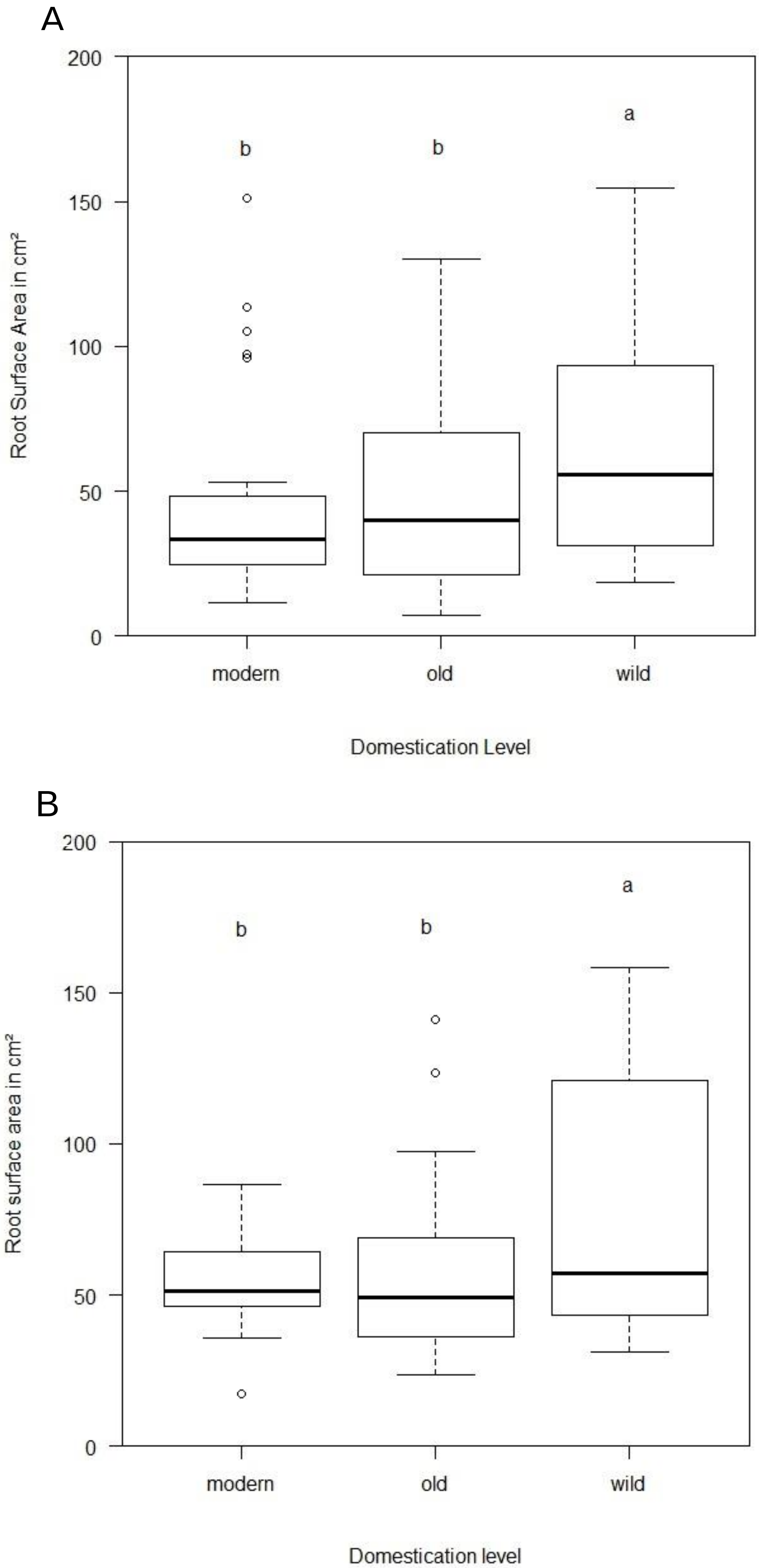


Figure 16: Root surface area of wheat (A) and barley (B) cultivars and wild forms grouped according to domestication level

Root length density

Root length density (RLD), is the length of roots per unit volume of soil. It is an important parameter required to understand root morphology and plant performance under drought conditions. Perceptibly, *T. aestivum* var*. tschermakianum* and *T. turanicum* have had highest values for RLD. Lowest RLD was measured for *T. isphahanicum* (Table 5).

In barley *H. langunculiforme* and *H. vulgare hexastichon* had highest RLD values and *H. agriocrithon* lowest (Table 7).

Specific root length

Specific root length (SRL) is the ratio of root length to root dry mass. More generally, it is a ratio of attainment of root length to resource investment of dry root mass. Modern varieties had considerable higher SRL mean values than old and wild cultivars, for instance *T. aestivum* ("Naxos") and *A. cylindrica* (Table 5). With a high variability, highest SRL values were found for *T. carthlicum*. At domestication level, wild wheat forms had a significantly lower SRL than old and modern cultivars (Figure 17 A).

In barley *H. vulgare hexastichon* had the lowest SRL and *H. vulgare* ("Ithaka") the absolute highest value (Table 7). No significant differences between domestication levels could be observed (Figure 17 B).

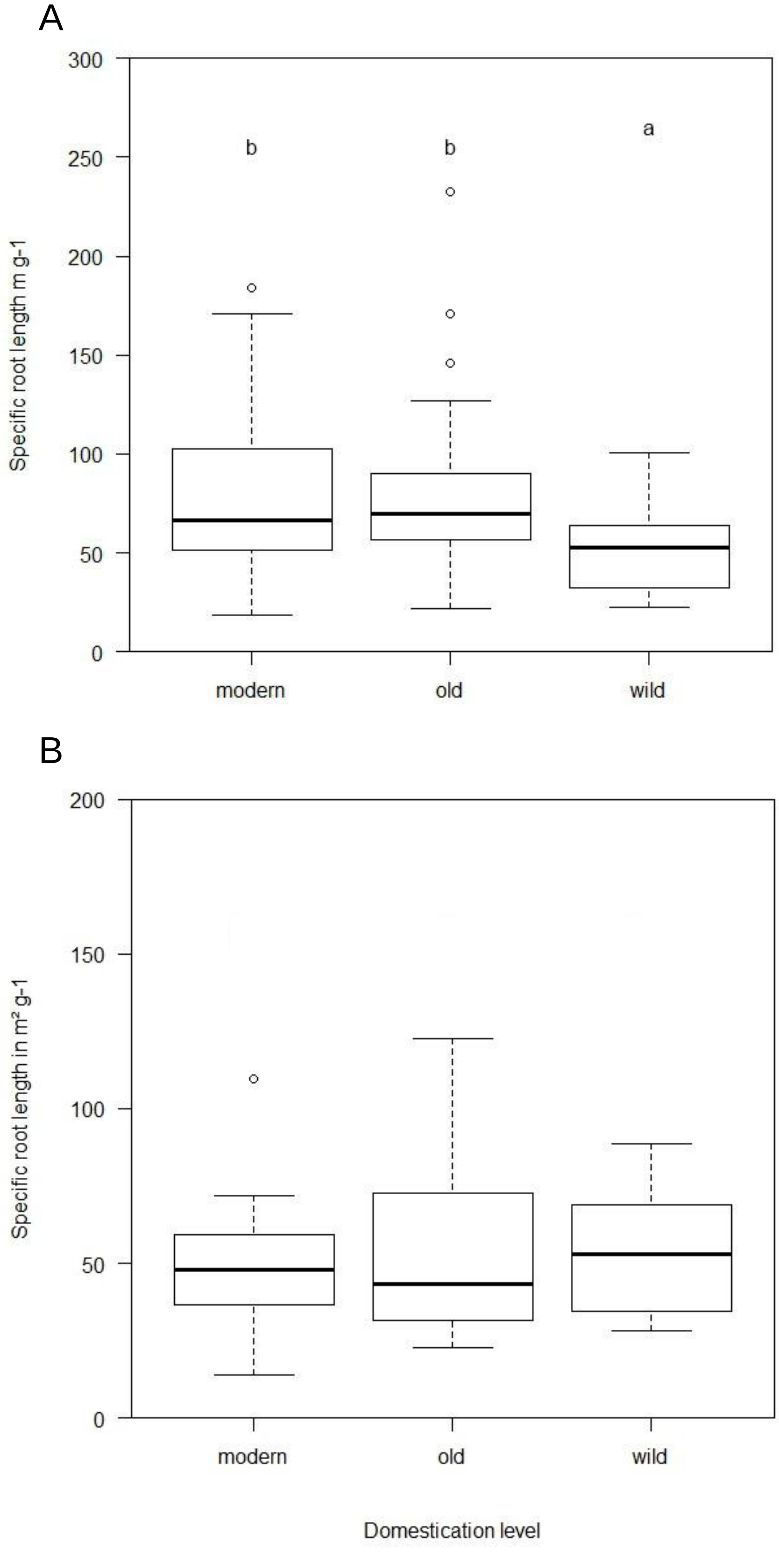


Figure 17: Specific root length wheat (A) and barley (B) cultivars and wild forms compared at domestication level; there were no significant differences between domestication levels in barley

Root mass density

A high amount of root dry mass to low root volume yields a high RMD. In wheat, highest RMD was observed for *T. carthlicum* and lowest for *T. macha* (Figure 18).

In barley, significant differences between cultivars could be observed (p= 13E-06, F=8.5). *Hordeum lagunculiforme* showed the lowest root mass density (RMD) followed by *H. vulgare* (“Ithaka”), while *H. vulgare distichon* and *H. vulgare vulgare* (“Dante”) exhibited the highest RMD (Figure 19). There were no significant differences between domestication levels.

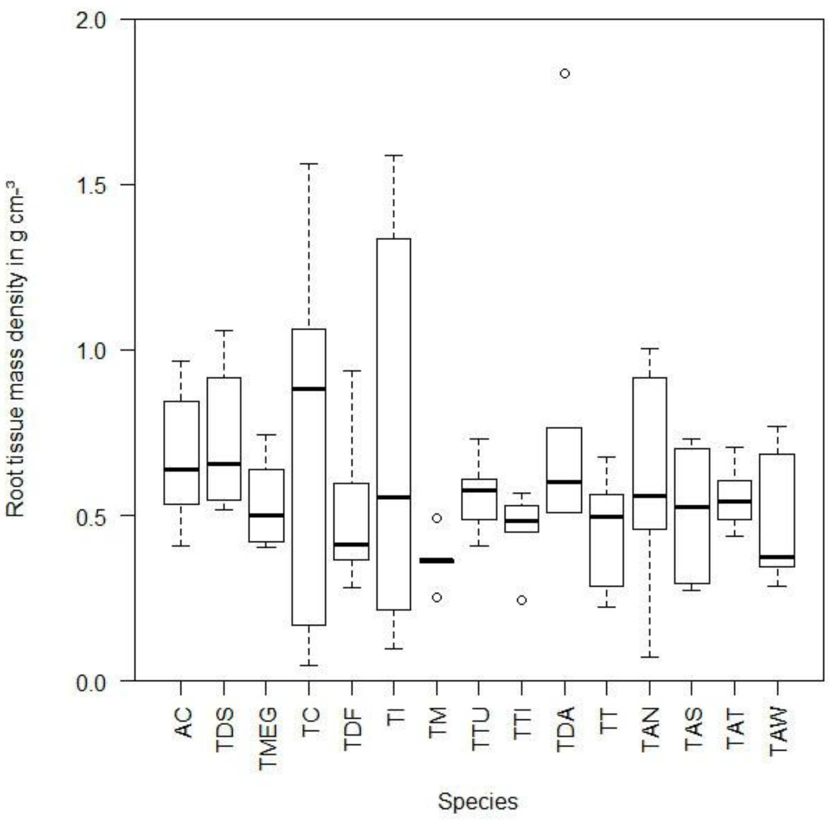


Figure 18: Root tissue mass density of wheat cultivars and wild forms; abbreviations see Table 1; there were no significant differences between cultivars

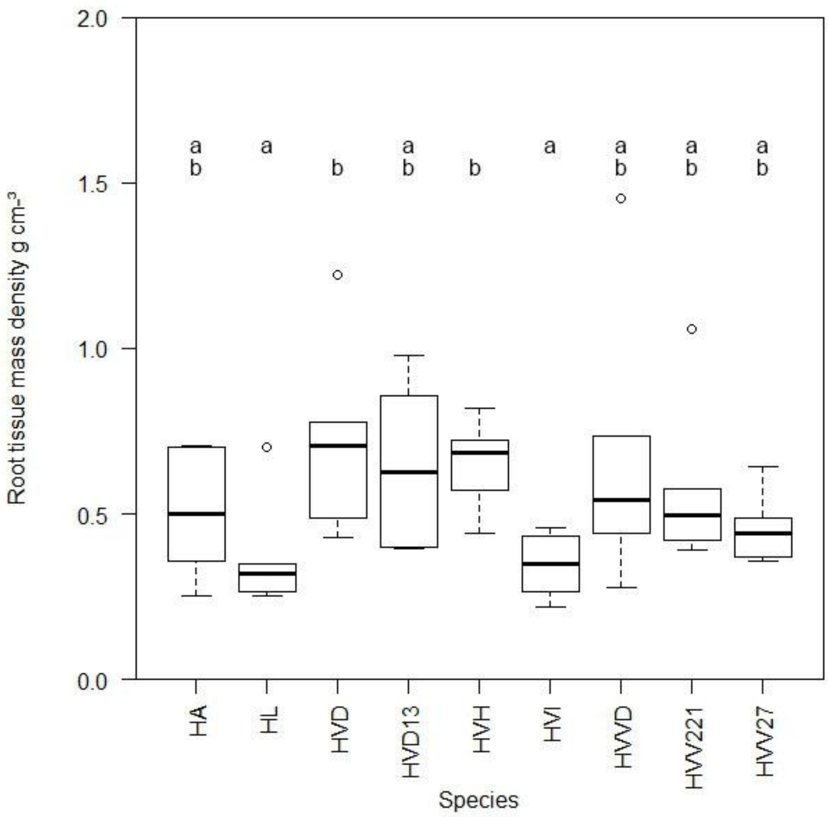
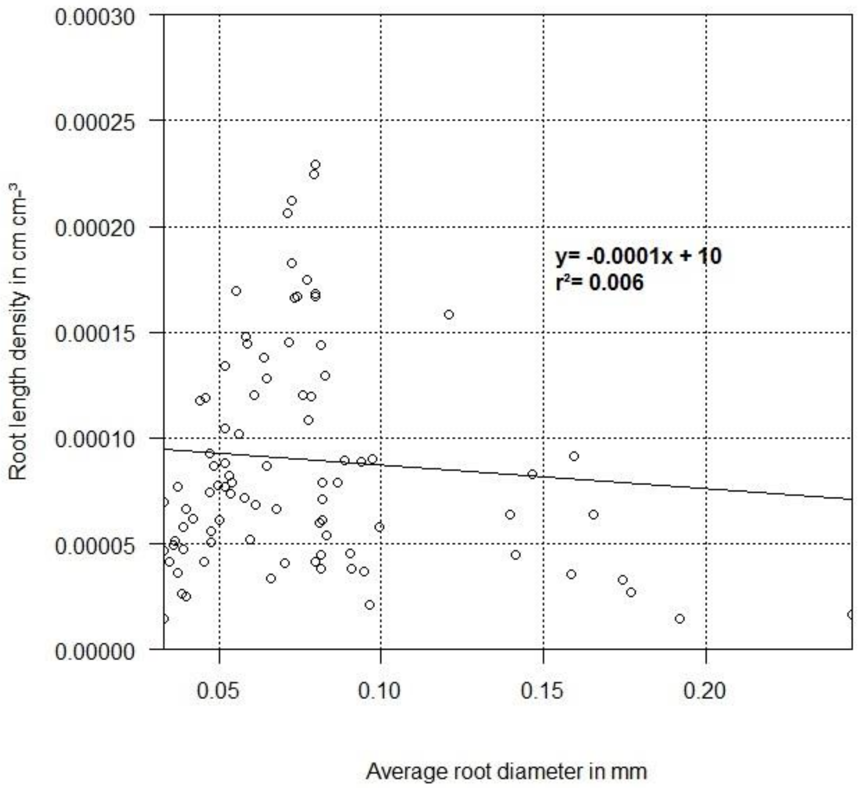


Figure 19: Root tissue mass density of barley cultivars and wild forms; abbreviations see Table 2

Root length density

In following order, *T. aestivum* var. *tschermakianum, T. turanicum* and *A. cylindrica* had the highest root length density (RLD) amongst wheat cultivars (Table 5). In barley the highest RLD could be observed for the cultivars *H. lagunculiforme* and *H. vulgare hexastichon* (Table 7). Domestication levels were not significantly different from each other; nevertheless wild forms exhibited a slightly higher RLD in wheat as well as in barley. Correlation analysis revealed that RLD can rather be explained by a higher root volume than by thicker roots (Figure 20).

A



B

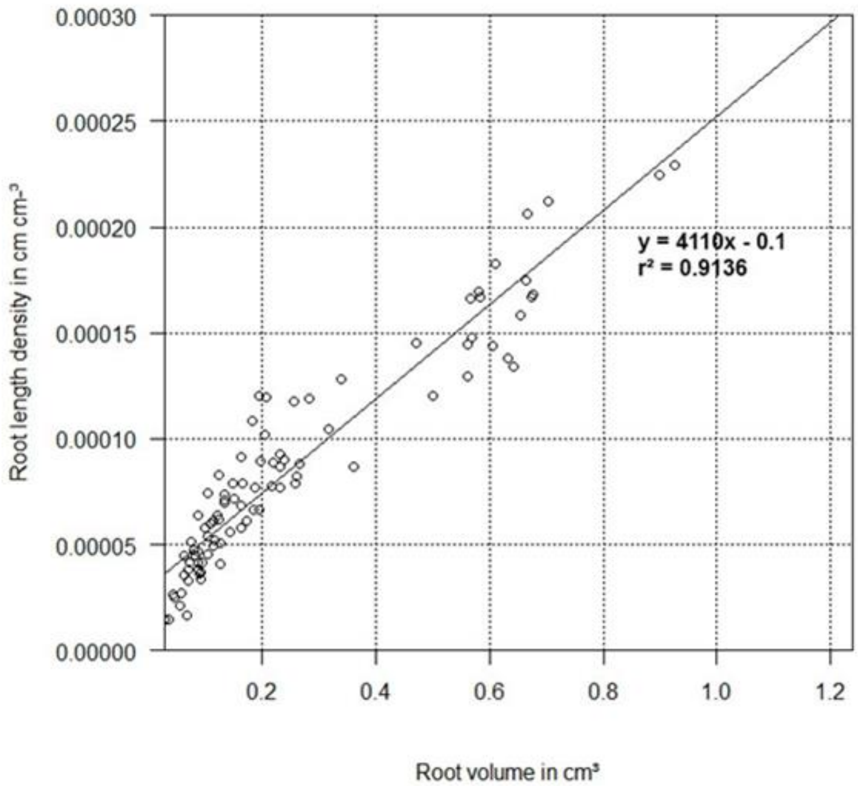


Figure 20: Correlation of root length density and average diameter (A) and root volume (B) of wheat cultivars and wild forms

Discussion

In both cereals, total plant biomass per individual increased with domestication level. Modern cultivars had higher dry matter yield compared to wild forms, thus confirming the results of Austin et al. (1986) and Wacker (2002). In their studies, a lower dry matter yield was correlated to a lower seed weight in wild forms. In our study this could be true for most wheat cultivars but not for all. For instance *A. cylindrica* had no grains, but similar plant biomass than all modern cultivars. In barley, plant biomass was similar for all cultivars and wild forms whereas grain yield was higher in all modern cultivars and *H. agriocrithon*. Wild forms and old cultivars *A. cylindrica, T. aestivum* var. *tschermakianum* and *T. turanicum* did not reach full maturity and did not develop any ears. Austin et al. (1986) reported that wild forms and old cultivars reached anthesis one to two weeks later than modern cultivars. In our study the cultivars mentioned above did not even show ear development after ten days of additional cultivation compared to other cultivars. Nonetheless, a similar above-ground biomass was observed for *A. cylindrica* and *T. aestivum* (“Naxos”) (0.60g and 0.61g, respectively). A reason for this is a higher tiller and leaf formation. Modern cultivars generally developed only one ear bearing shoot in a shorter time period than wild forms. Consequently, they had a longer grain filling period but less leaves. Whereas wild forms developed early a lot of small but mostly sterile shoots and only in some cases one ear bearing shoot which had only poorly filled grain. This effect equals out plant biomass yield between cultivars of different domestication levels. Lower grain yield and higher tiller number for old cultivars and wild forms was also noticed by Akman (2017). However, different to our study, a higher plant height was observed for wild wheat relatives. In our study, plant observations showed that wild forms were shorter, more “leafy” and “bushy” whereas modern cultivars had longer stems with fewer leaves and only one ear. This proves our hypotheses that wild wheat and barley relatives develop more tillers and have a bigger plant height than modern cultivars and produce less grain. Truly, it is difficult and not realistic to describe and to draw conclusions about plant characteristics from plants grown in a greenhouse experiment. Nonetheless, the trend of a higher tillering capacity for wild relatives which was noticed throughout the experiment is conform with other studies and comparison of in field cultivation of modern cultivars and wild forms of wheat and barley . A higher tiller number seems to positively affect RLD. However it does not mean a higher grain yield. These findings are in agreement with the reports by Manske et al. (2000) and Nakhforoosh et al. (2014) which are indicating a positive correlation of RLD and number of tiller. Nakhforoosh et al. (2014) states, that a high tiller number is therefore an important trait, which can be an advantage for cereals in the competition for nutrients and water. This might be misleading; certainly the determining trait for drought tolerance is not a high amount of tillers but a high density of roots. SLA of wheat cultivars and wild forms was similar to the results of Wacker (2002). Namely, wild wheat relatives had a higher specific leaf area compared to modern cultivars. This difference is most probably caused by a higher number of tillers and leaves in wild forms. In contrast, modern barley cultivars had a higher leaf area and SLA than old cultivars and wild barley forms. A lower SLA shows that plants produced less leaf area per unit leaf dry mass.

The study from Akman (2017) in which wheat was grown in long tubes in the field, showed that most important traits in drought tolerance are root length and root to shoot ratio. Deep-rooted cultivars can absorb water and nutrients from deep soil when soil water of shallow soil levels gets depleted during cultivation period. According to Akman (2017), wild wheat relatives with long roots and high grain yield can be considered for improving modern cultivars. In his study *T. turgidum* had the longest roots and highest grain yield amongst wheat cultivars. Confirming the findings of Sayed (2011), our results show that, wild barley form *H. lagunculiforme* had the longest roots. In wheat, old landrace *T. aestivum* var*. tschermakianum* had highest root length. Both old cultivar and wild form had as well highest root volume, surface and as well highest average root diameter and root to shoot ratio. In essence, longer roots did not necessarily infer a higher average root diameter, but certainly a higher root surface and volume. Consequently, root length rather than the thickness of roots determines root surface area and volume. Therefore *H. lagunculiforme, H. vulgare hexastichon, T. aestivum* var*. tschermakianum* and *T. turanicum* have had also the largest root surface area compared to other cultivars. Root length density was highest for these cultivars as well. *H. agriocrithon* and *T. isphahanicum* had lowest RLD values but belong to the same domestication level as *H. lagunculiforme* and *T. aestivum* var *tschermakianum,* respectively. This shows that general statements about wild forms and old and modern cultivars are difficult to make in our study and cultivars vary within the same domestication level.

Another investigated root characteristic was SRL. Plants with high SRL attain a higher root length for a given root biomass investment. As a result, these plants could possibly take up water and nutrients in higher rates per unit of dry mass (Pérez-Harguindeguy et al. 2013). Unexpected, the wild wheat variety

A. cylindrica had a much lower SRL compared to all other genotypes. The amount of fine roots in deep soil layers can further help to take up water and nutrients. Because the plants were cultivated in a pot experiment, only total fine root dry mass was determined. *Hordeum vulgare hexastichon* and *A. cylindrica* had the highest amount of fine roots - followed by *T. turanicum* and *T. aestivum* var*. tschermakianum.* Lowest average root diameter was measured for *H. vulgare* (“Ithaka”)*,* a modern cultivar and *T. monococcum*, an old cultivar. This confirms values of Nakhforoosh et al. (2014). Wild forms *A. cylindrica, T. dicoccoides* var*. spontaneo villosum* and *H. lagunculiforme* had relatively thick roots compared to old and modern cultivars. One effect of drought in cereals is the increase of their root to shoot ratio (RS). In Motzo et al. (1992) RS increased by 37% on average at tillering and 14% at early dough development under drought conditions. RS is a very flexible and environment-dependent root characteristic. Nonetheless a high genotypic root to shoot ratio could be a reason for certain cereals to be more tolerant against drought. Furthermore the flexibility of a crop’s root development under certain conditions like drought stress, in terms of adaptability, can also be considered a valuable trait.

There are two main strategies for water extraction in dryland systems: Deep and shallow water extraction. In systems with monsoonal rain events, crops rely on moisture stored in the soil from the previous rainy season. The need of deeper soil water extraction increases with growth development and is usually highest at grain development. At this time crops are especially vulnerable to drought and water use for grain growth becomes highly valuable. Therefore, in systems with frequent but little rainfall, a shallow root system can help to capture water, as soil moisture may dry up before new roots become efficient

(Wasson et al. 2012). Thus certain root traits can be beneficial in one climate whereas in another they are not. Root systems are very environment-dependent. Roots adapt depending on climatic and soil conditions as well as on their resource demand.

In our study significant differences of root morphology as well as shoot traits were observed between wheat and barley cultivars and their wild relatives. However, a strict classification according to domestication level is not really applicable. Nonetheless, one can say, that overall old cultivars and wild forms develop a superior root system than modern cultivars. Previous research showed that plant breeding focused mainly on shoot traits instead of root traits and the need for a larger root system decreased with increasing agricultural inputs. Wild barley and wheat relatives, in contrast to modern cultivars, appear to have more tillers but rarely develop grains under nutrient-deficient conditions. Knowledge about differences in root morphology of wheat and barley cultivars can help breeders in the future to improve modern cultivars with beneficial root traits, which are able to take up water and nutrients more efficiently. From this, agriculture could benefit economically, because less input would be needed and consequently negative impacts on the environment will be reduced. Hence, this study aims to help to approach a more sustainable agriculture in the future.

Limitation of methods and alternatives

Root washing was done on 1mm sieves and roots were then collected with the help of tweezers. This method is really labour and time intensive. Furthermore, small amounts of roots can be lost through the mesh and debris and roots are often linked almost inseparably. Therefore, the accuracy of this technique is questionable. Unfortunately until now, methods for examining root traits are limited. This implies even more for field experiments. An interesting method for root screening in the field was used by Akman (2017). He cultivated different wheat and barley genotypes in two meter long cylindrical PVC tubes. In his study, the most significant root trait was root length. Another possibility for root screening is root electrical capacitance. This method was used by Chloupek et al. (2010). He measured the root system size of barley varieties by creating an electrical field and measuring its electrical capacitance. The value depends on the materials, for example a high value indicates high amount of water and a very low value indicates high amount of air. The results of Nakhforoosh et al. (2014) showed that root electrical capacitance depends strongly on soil moisture, thus it is only possible to use in homogenous conditions and not on different days. This method is non-destructive and can therefore be used in the field, at different growth stages. However it still lacks sufficient knowledge about the complex electrical circuit used in this system (Chloupek et al. 2010, Nakhforoosh et al. 2014). Overall, fast methods for root phenotyping in the field are not available yet (Wasson et al. 2012). Furthermore, the transferability of results from greenhouse experiments to field conditions has to be evaluated carefully. Root characteristics are highly influenced by soil type, climatic conditions and water and nutrient availability, this poses the risk that traits selected in the greenhouse cannot be transferred to mature root systems in the field.

Next steps

Further research with wheat and barley evolution material should be conducted to determine nitrogen use efficiency of cultivars and wild forms in relation to root morphology using ^{15}N labelling approach. In addition, further analysis of soil microbial properties of the rhizosphere of wild, old and modern cultivars should be performed, because besides root morphology, soil microbial interactions may contribute to stress tolerance and more efficient nutrient use of plants. An experiment to investigate drought tolerance of cultivars with superior root characteristics like *T. aestivum* var. *tschermakianum, A. cylindrica, T. turanicum, H. lagunculiforme* and *H. vulgare hexastichon* and cultivars with small root systems and shorter roots like *T. isphahanicum* and *H. vulgare* ("Ithaka") could give information whether these cultivars can cope better with water-limited conditions or not. Another root trait of interest could be root growth flexibility under drought conditions.

Conclusions

The evaluated wheat and barley cultivars and their wild relatives showed wide variation in their root morphology and shoot traits. A high variability of results within domestication levels for root traits was noticed. This showed that the domestication level is actually a weak predictor for root characteristics and general statements about root morphology of modern cultivars and wild forms are difficult to make. In plant growth development and shoot traits clear trends for wild, old and modern cultivars could be seen. The study showed that modern cultivars had less tillers but much higher grain yield than old landraces or wild forms, because most wild forms did not reach full maturity during the experimental period. *Triticum macha, T. dicoccom* var. *farrum* and *H. agriocrithon* had the highest grain yield of the ancient wheat and barley forms. However, wild ancestors are known to be more stress tolerant. *Triticum aestivum* var. *tschermakianum, T. turanicum, A. cylindrica, H. lagunculiforme,* *H. vulgare hexastichon* had the biggest root dry weight, longest roots, highest root to shoot ratio, highest root volume, highest RLD and LPV, biggest root surface and a relative high amount of fine roots (except of *H. lagunculiforme)* of all wheat and barley cultivars and wild forms. These cultivars belong to the old cultivars and wild forms. Their beneficial root characteristics could potentially serve as biological resource to improve modern cultivars to withstand drought and nutrient-deficient conditions and consequently increasing crop productivity and stability in the future. However further experiments on differences of these cultivars' drought tolerances, nitrogen uptake efficiencies and soil microbial properties have to be conducted to show their potential more concretely.

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