Root:Shoot Ratio and Water Use Efficiency Differ in Cool Season Cereals Grown in Pure and Mixed Stands Under Low and High Water Levels

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ABSTRACT

This study examined the effect of low and high water levels on the root:shoot ratio (R:S) and water use efficiency (WUE) response of four cool season cereals [wheat (*Triticum aestivum*, cv. TAM III), barley (*Hordeum vulgare*, cv. P919), rye (*Secale cereale*, cv. Elbon), and oat (*Avena sativa*, cv. Walker)]. The pot experiment was conducted in the green house of Dryland Agriculture Institute, West Texas A&M University Canyon, Texas, USA during winter 2009-10. The hypothesis that crop stands and water level have no significant effect on R:S and WUE of field crops was tested in the trial. The results revealed that higher root biomass, R:S and WUE were observed for wheat under low than high water level. Wheat grown alone or mixed with barley or oat had produced higher root and shoot biomass that had positive impact on the WUE of wheat. Barley grown alone as sole crop or mixed with oat produced higher root and shoot biomass and increased its WUE. Rye grown alone as the sole crop or grown mixed with oat produced higher root and shoot biomass and with a corresponding increase in WUE. Oat grown alone as sole crop had produced higher shoot biomass and had higher WUE as compared with oat grown in mixed stands. The R:S of oat increased when grown mixed with wheat. The lower plants competition in the pure crop stands had partitioned more biomass into roots and shoots that resulted in the higher R:S and WUE. The increase in plant competitions in the mixed stands not only reduced roots and shoot biomass but also declined the R:S and WUE. The increase in R:S under low water level was observed for all four crop species due to the increase in the root biomass and the corresponding decrease in the shoot biomass.

KEYWORDS: *Triticum aestivum*; *Hordeum vulgare*; *Secale cereal*; *Avena sativa*; pure and mixed stands; water levels; R:S; water use efficiency

INTRODUCTION

The root:shoot ratio (R:S) of field crops is usually used to estimate the annual crop residue contribution to the soil from the root residues left in the soil. Research on root studies have often been ignored and the estimates of R:S for many crop species

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are not known (Bolinder et al. 2002). Determination of R:S in a mixed stands is very important to assess the overall health of the plants. The interaction between a crop species' root system and their environment influence the R:S (Amanullah and Stewart 2013), crop growth (Gregory 2006; Amanullah 2014a), and yield (Gregory 2006). R:S in crop species can be manipulated by competition among the mixed crop stands (Sadras and Calderini 2009). The competition in the mixed crop stands may occur both above and below ground portions (Rubio et al. 2001). According to Casper and Jackson (1997), below ground competition in the crop plants is more severe compared with above ground competition. According to Casper and Jackson (1997), the main factors involved in below-ground competition includes decrease in growth, survival, or fecundity of neighbors by reducing available soil resources.

Both above and below ground competitions in the crop species varies due to the differences in their genetic makeup (Dubbs 1971; Hannay et al. 1977; Amanullah and Stewart 2013); root architecture (Rubio et al. 2001); crop nutrition (Davidson 1969; Dahmane and Graham 1981; Eghball and Maranville 1993; Dunbabin et al. 2001; Amanullah et al. 2016); crop growth rates (Amanullah 2014a); competition ratios and water use efficiencies (Amanullah 2015). Sadras and Calderini (2009) reported that growing plants near to one another results in plants competing for resources. Aldrich (1984) observed that crop plants do not compete for space; on the other hand, Wilson et al. (2007) reported that competition exists in crop species for space.

Water use efficiency (WUE) can be affected by crop competition (Passioura 2006) because each individual plant interacts (Sadras and Calderini 2009) and competes with its neighbors both above and below ground (Rubio et al. 2001). Massinga et al. (2003) and Van Wychen et al. (2004) reported that the total water use by a weedy crop is similar to or much greater than the water use of a weed-free crop, but since the total dry matter accumulation is reduced, the WUE of a weedy crop will be lower than a weed-free crop. Szente et al. (1993) reported that WUE of fat hen (*Chenopodium album*) was significantly greater than that of sunflower (*Helianthus annuus*) when the two species were grown in mixed stands. Crop competition decreases the availability of growth resources (Sadras and Calderini 2009). Understanding growth attributes of different crop species in mixed stands could improve water and nutrient uptake, and adaptation to adverse conditions (Richards et al. 2007; Lynch 2007; Haling et al. 2011). Different root systems in the mixed stand enabled plants to obtain nutrients and water from different portions of the soil, and thus soil fertility and moisture content at one place is minimized (Amanullah et al. 2015). According to the USDA report (2014), growing two or more crops in close proximity to each other promotes interaction that results in improved soil and water quality while enhancing biodiversity.

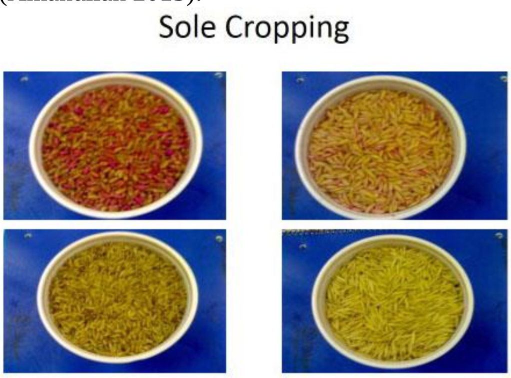
Research on crops' roots have often been ignored and the estimates of R:S and WUE for many field crop species are not known. Determination of R:S and water use efficiency in mixed stands is very important to assess the overall health of soil and crops. Up until now, competition among cool season C_3-grasses in pure and mixed stands under different water levels has not yet been investigated. The objective of this research study was to investigate the differences in R:S and WUE of cool season C_3-cereals (wheat, rye, barley, and oat) grown in pure and mixed stands under low and high water levels. The findings of this study will motivate and help the researchers and plant ecologists to study the plant growth, especially root characteristics of different crop species grown in close proximity with each other.

MATERIALS AND METHODS

Experimental site. Root to shoot ratio [*root dry weight per plant divided by shoot (leaf + stem) dry weight per plant*] and water use efficiency [*total (shoot + root) dry matter accumulation per liter water used*] response of four cool season C_3-cereal crop [wheat (*Triticum aestivum*, cv. TAM III), barley (*Hordeum vulgare*, cv. P919), rye (*Secale cereale*, cv. Elbon), and oat (*Avena sativa*, cv. Walker)] in various combinations (sole, two-, three-, and four-crop mixed stands) was investigated under low and high water level in a greenhouse experiment at Dryland Agriculture Institute, West Texas A&M University, Canyon, Texas, USA during winter 2009-2010.

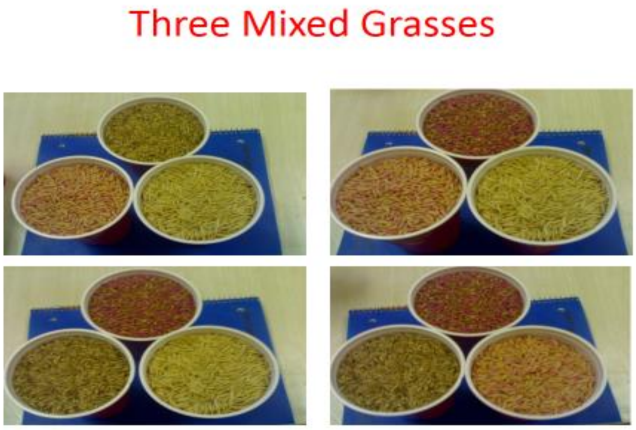
Experimental design. The experiment was arranged in a completely randomized design with three replications. A total of 30 treatments including 15 crop stands (sole crop and various mixture combinations) and two water levels (low and high water levels), i.e., 15 x 02 = 45 pots under each water level. At low water level, a known amount of water was applied to the pots (45 pots) when 75% of the available water had been used. Under high water level, a known amount of water was applied to the pots (45 pots) when 25% of the available water had been used. Under each water level, there were 45 pots (a total of 45 x 02 = 90 pots used in the whole experiment). For more detail, please see the slides at the end of the manuscript. A total of 36 plants were grown per pot. In the pure crop stands (Picture 1), 36 plants of the same crop species were grown per pot. In two-crop mixed stands (Picture 2), 18 plants of each crop species (2 species x 18 plants each crop species = 36 plants) while in three-crop mixed stands (Picture 3), 12 plants of each crop species (3 species x 12 plants each crop species = 36 plants) were grown per pot. Similarly in four crop mixed stands treatment (Picture 4), nine plants of each crop species (4 species x 9 plants each crop species = 36 plants) were grown per pot (Amanullah and Stewart 2013; Amanullah 2014a, 2014b; Amanullah 2015; Amanullah et al. 2015). The volume of each pot was about 6,283 cm^3 and contained 2,000 g of potting mix (all-purpose enriched compost) called *MIRACLE GROW*).

Miracle Grow is a formulated soil medium (compost) from 50 to 60% sphagnum peat moss, coconut husk fibers (coir pith), composted bark fines, wetting agent, and fertilizer. The nitrogen, phosphorus, and potassium sources had been coated to provide 0.10% slow-release of nitrogen (N), 0.10% slow-release of phosphate (P_2O_5), and 0.10% potash (K_2O). The ACGIH Threshold Limit Values (TLV) for nuisance (inert) dust containing less than 1% crystalline silica and no asbestos were: 10 mg m_{-3} inhalable particulates and 3 mg m^{-3} respirable particulate (Amanullah 2015). The bulk density (0.32 g cm^{-3}) and porosity (88 %) for Miracle Grow was calculated in the green house (Amanullah 2015).



Picture 1. Sole crops were grown separately in four different pots: (clockwise from upper left) wheat, barley, oat, and rye.

Picture 2. Two mixed crops per pot were grown in six different combinations. Top row l-r: wheat + barley, wheat + rye, wheat + oat. Bottom row l-r: barley + rye, barley + oats, and rye + oat



Picture 3. Three mixed crops per pot were grown in four different combinations. Clockwise from upper left: wheat + barley + rye, wheat + barley + oat, barley + rye + oat, and wheat + rye + oat

Picture 4. Four mixed crops per pot were grown (clockwise from upper left: wheat + barley + oat + rye) in a single pot.

Data recording and handling. A total of 12 plants were uprooted at 30, 60, and 90 days after emergence (DAE) in each treatment (per pot). In the pure (sole crop) stand, 12 plants of the same crop species were uprooted at each growth stage. In two-crop mixed stand, six plants of each crop species (2 species x 6 plants each species = 12 plants) were uprooted, whereas in three-crop mixed stand, four plants of each crop species (3 species x 4 plants each species = 12 plants) were uprooted from pots at each growth stage. In four-crop mixed stands, three plants of each crop species (4 species x 3 plants each species = 12 plants) were evacuated from pots at each growth stage. However, the data were recorded on the average of three plants of each crop species in each treatment (each pot). After pulling them out, the roots of each crop species were washed separately with tap water and then divided into three portions, i.e., roots, leaves, and stems.

In paper bags, the material was dried in an oven at 80 ^{0}C for about 20-24 hours. The samples were weighed by electronic balance (*Sartorius Basic*, *BA2105*) and the average data of dry weight of root, leaf, and stem per plant was calculated. Shoot dry weight per plant was obtained by adding leaf dry weight to stem dry weight (Amanullah and Stewart 2013; Amanullah 2014b). The sum of the shoot and root dry weight was calculated as the total dry weight plant^{-1}. The R:S and WUE were calculated with the following formulae:

Root to shoot ratio (R:S) = Root dry weight plant^{-1} / Shoot dry weight plant^{-1}

Water use efficiency (WUE) = Total (shoot + root) dry matter plant^{-1} / Water used plant^{-1}

Statistical analysis. Data were subjected to analysis of variance (ANOVA) according to the methods described in Steel et al. (1997) and treatment means were compared using the least significant difference (LSD) at $P \leq 0.05$. Standard deviations (SD) were also calculated for the mean values of crop combinations and water levels of each parameter.

RESULTS

Wheat. Water levels had significant influence on root and shoot biomass, R:S, and WUE of wheat (Table 1). Wheat plants produced significantly higher shoot biomass at high (0.98 g plant^{-1}) than at low water levels (0.67 g plant^{-1}). Higher root biomass (0.43 g plant^{-1}), R:S (0.64), and WUE (2.09 g L^{-1}) were attained under low water levels. Significant consequences of crop combinations (stands) were observed on root and shoot biomass and WUE of wheat. Little differences in the R:S of wheat under different crop combinations were noticed.

In pots with wheat grown alone or mixed in two-crop stands with barley or oat, higher root and shoot biomass as well as higher WUE than other wheat combinations was recorded. The interaction between crop combinations and water levels significantly influenced the root and shoot biomass and R:S of wheat (Table 1).

Wheat root biomass increased under low water level when grown mixed in two crop stands with barley or rye, and four-crop mixed stands. Under high water level, root biomass enhanced in wheat when grown as sole crop or mixed in two-crop stands with oat. Shoot biomass of wheat was also augmented under high water levels with different crop combinations. Sole wheat crop and wheat + oat mixed stand had the statistically same R:S under low and high water levels. In all other crop combinations, R:S of wheat was higher under low than high water level and maximum increase was recorded when wheat was grown mixed with barley or rye. Although the interaction between water level and crop combinations had no significant effect on the WUE of wheat, yet WUE increased in all crop combinations under low than high water levels (Table 1).

Table 1. Root and shoot biomass (g plant^{-1}), R:S and water use efficiency (g L^{-1}) response of wheat (*Triticum aestivum* L.) as affected by crop combinations and water levels.

Crop Combinations	Roots biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root: shoot ratios	Water Use Efficiency (g L^{-1})
Wheat alone	0.44 ± 0.06	0.91 ± 0.13	0.48 ± 0.01	1.88 ± 0.52
Wheat in Barley (B)	0.48 ± 0.28	0.99 ± 0.27	0.56 ± 0.41	2.24 ± 0.97
Wheat in Rye (R)	0.49 ± 0.13	1.01 ± 0.31	0.53 ± 0.30	2.22 ± 0.81
Wheat in Oat (O)	0.34 ± 0.13	0.79 ± 0.25	0.43 ± 0.02	1.51 ± 0.11
Wheat in B+R	0.36 ± 0.04	0.92 ± 0.35	0.42 ± 0.10	1.72 ± 0.18
Wheat in B+O	0.33 ± 0.04	0.68 ± 0.18	0.51 ± 0.16	1.36 ± 0.17
Wheat in R+O	0.38 ± 0.06	0.69 ± 0.01	0.59 ± 0.25	1.49 ± 0.46
Wheat in B+R+O	0.34 ± 0.19	0.64 ± 0.09	0.54 ± 0.31	1.46 ± 0.82
$LSD_{0.05}$	**0.12**	**0.26**	**ns**	**0.45**
Water levels				
High water level	0.36 ± 0.08	0.98 ± 0.20	0.38 ± 0.07	1.38 ± 0.26
Low water level	0.43 ± 0.15	0.67 ± 0.12	0.64 ± 0.16	2.09 ± 0.53
$LSD_{0.05}$	**0.04**	**0.10**	**0.05**	**0.16**

Where: ns stands for non-significant data at $P \leq 0.05$. , and ± indicates the standard deviation between the mean values of crop combinations and water levels of each parameter.

Barley. Shoot biomass, R:S, and WUE of barley were significantly affected by water levels; however, no significant influence was observed on root biomass (Table 2). Under high water level, barley produced higher shoot biomass whereas higher R:S and WUE were recorded under low water level. Crop combinations also produced a profound influence on root and shoot biomass, R:S, and WUE (Table 2). Barley grown alone as sole crop or mixed in two crop stands with oat produced higher root and shoot biomass, WUE.

Barley R:S reached to maximum (0.58) under four-crop mixed stand. The interaction between water level and crop combinations indicated that root biomass in barley enhanced under high water level when grown as sole crop. Barley increased its R:S significantly when grown in mixture with wheat, oat, rye, or in four-crop mixed stands under low water levels. Under all other crop combinations, statistically same R:S were observed under both water levels. The WUE in barley increased under different crop stands under low than high water levels (Table 2).

Table 2. Root and shoot biomass (g plant^{-1}), R:S and water use efficiency (g L^{-1}) response of barley (*Hordeum vulgare* L.) as affected by crop combinations and water levels.

Crop Combinations	**Roots biomass (g plant^{-1})**	**Shoot Biomass (g plant^{-1})**	**Root: shoot ratios**	**Water Use Efficiency (g L^{-1})**
Barley alone	0.79 ± 0.31	1.99 ± 0.76	0.40 ± 0.01	3.67 ± 0.08
Barley in Wheat (W)	0.43 ± 0.32	1.01 ± 0.40	0.50 ± 0.45	1.62 ± 0.12
Barley in Rye (R)	0.50 ± 0.11	1.26 ± 0.52	0.41 ± 0.09	2.33 ± 0.11
Barley in Oat (O)	0.60 ± 0.07	1.28 ± 0.24	0.48 ± 0.16	2.63 ± 0.86
Barley in W+R	0.31 ± 0.15	0.96 ± 0.40	0.31 ± 0.03	1.67 ± 0.05
Barley in W+O	0.27 ± 0.05	0.88 ± 0.01	0.32 ± 0.06	1.66 ± 0.73
Barley in R+O	0.23 ± 0.04	0.62 ± 0.11	0.37 ± 0.00	1.17 ± 0.29
Barley in W+R+O	0.37 ± 0.23	0.60 ± 0.06	0.58 ± 0.30	1.47 ± 0.98
$LSD_{0.05}$	**0.16**	**0.40**	**0.11**	**0.73**
Water levels				
High water level	0.43 ± 0.28	1.29 ± 0.62	0.33 ± 0.07	1.75 ± 0.91
Low water level	0.44 ± 0.19	0.86 ± 0.29	0.51 ± 0.20	2.30 ± 0.81
$LSD_{0.05}$	**ns**	**0.14**	**0.04**	**0.27**

Where: ns stands for non-significant data at $P \leq 0.05$. , and ± indicates the standard deviation between the mean values of crop combinations and water levels of each parameter.

Rye. Data presented in Table 3 revealed that R:S and WUE of rye were significantly affected by the water levels but no significant influence was observed on root and shoot biomass. Higher R:S for rye was calculated under high versus low water levels. In contrast, higher WUE for rye was calculated under low than high water levels. Crop combinations had considerable effect on root and shoot biomass, and WUE of rye (Table 3). Rye grown alone as the sole crop or mixed in two-crop stands in oat caused higher root and shoot biomass as well as higher WUE.

The interaction between water level and crop combinations indicated that both root and shoot biomass and WUE in rye increased significantly under both levels when grown as the sole crop, followed by oat-mixed stands. Under high water level, a remarkable decrease in root biomass and shoot biomass and WUE was observed when rye was grown in a three-crop stand (rye + barley + oat) and four-crop mixed stands (rye + wheat + barley + oat).

Table 3. Root and shoot biomass (g plant^{-1}), R:S and water use efficiency (g L^{-1}) response of rye (*Secale cereale* L.) as affected by crop combinations and water levels.

Crop Combinations	Roots biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root:shoot ratios	Water Use Efficiency (g L^{-1})
Rye alone	0.66 ± 0.21	2.26 ± 0.93	0.35 ± 0.18	2.54 ± 0.12
Rye in Wheat (W)	0.37 ± 0.08	0.85 ± 0.18	0.45 ± 0.01	1.90 ± 1.19
Rye in Barley (B)	0.37 ± 0.16	0.75 ± 0.21	0.47 ± 0.06	1.49 ± 0.13
Rye in Oat (O)	0.54 ± 0.27	1.21 ± 0.33	0.43 ± 0.08	2.33 ± 0.16
Rye in W+B	0.32 ± 0.14	0.73 ± 0.01	0.43 ± 0.18	1.47 ± 0.41
Rye in W+O	0.24 ± 0.03	0.70 ± 0.27	0.36 ± 0.10	1.25 ± 0.12
Rye in B+O	0.19 ± 0.11	0.47 ± 0.30	0.41 ± 0.04	1.06 ± 0.97
Rye in W+B+O	0.20 ± 0.16	0.44 ± 0.30	0.44 ± 0.10	1.05 ± 1.04
$LSD_{0.05}$	**0.20**	**0.71**	**ns**	**0.54**
Water levels				
High water level	0.40 ± 0.26	0.85 ±0.49	0.45 ± 0.09	1.27 ± 0.76
Low water level	0.32 ± 0.10	1.00 ±0.79	0.39 ± 0.09	2.00 ± 0.52
$LSD_{0.05}$	**ns**	**ns**	**0.04**	**0.20**

Where: ns stands for non-significant data at $P \leq 0.05$. , and ± indicates the standard deviation between the mean values of crop combinations and water levels of each parameter.

Oat. Data in Table 4 demonstrated the effect of water levels and crop stand on shoot and root biomass, WUE, and R:S of oat plants. Both shoot biomass and WUE were significantly affected whereas no significant differences in root biomass and R:S of oat was observed. Higher shoot biomass for oat was calculated under high than low water level. In contrast, higher WUE for oat was calculated under low than high water level. It was noticed that crop combinations produced notable influence on root and shoot biomass, R:S, and WUE of oat (Table 4).

Oats grown alone as a sole crop or mixed in a three-crop stand (wheat + barley + oat) showed higher shoot biomass than other oat combinations. The R:S of oat was significantly higher when grown mixed with wheat, while the WUE of oat was increased significantly when grown as the sole crop. The interaction between water level and crop combinations indicated that shoot biomass and WUE in oat under both levels augmented when grown as the sole crop. Under high water level, both shoot biomass and WUE of oat increased significantly when oat was grown mixed in two-crop stands with barley or mixed with rye or gown in three-crop mixed stand (wheat + barley + oat).

Table 4. Root and shoot biomass (g plant^{-1}), R:S and water use efficiency (g L^{-1}) response of oat (*Avena sativa* L.) as affected by crop combinations and water levels.

Crop Combinations	Roots biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Root:shoot ratios	Water Use Efficiency (g L^{-1})
Oat alone	0.41 ± 0.03	1.36 ± 0.19	0.30 ± 0.02	2.61 ± 1.34
Oat in Wheat (W)	0.41 ± 0.11	0.75 ± 0.07	0.53 ± 0.11	1.72 ± 0.95
Oat in Barley (B)	0.30 ± 0.08	0.78 ± 0.33	0.40 ± 0.04	1.44 ± 0.04
Oat in Rye (R)	0.35 ± 0.25	1.13 ± 0.61	0.28 ± 0.04	1.87 ± 0.36
Oat in W+B	0.13 ± 0.05	1.47 ± 1.35	0.18 ± 0.01	1.20 ± 0.11
Oat in W+R	0.20 ± 0.06	0.69 ± 0.25	0.30 ± 0.01	1.19 ± 0.08
Oat in B+R	0.14 ± 0.01	0.43 ± 0.00	0.32 ± 0.01	0.82 ± 0.35
Oat in W+B+R	0.10 ± 0.01	0.41 ± 0.10	0.27 ± 0.04	0.67 ± 0.19
$LSD_{0.05}$	**0.14**	**0.64**	**0.14**	**0.50**
Water levels				
High water level	0.28 ± 0.15	1.09 ± 0.66	0.31 ± 0.08	1.22 ± 0.53
Low water level	0.23 ± 0.15	0.67 ± 0.37	0.34 ± 0.13	1.66 ± 0.90
$LSD_{0.05}$	**ns**	**0.23**	**ns**	**0.18**

Where: ns stands for non-significant data at $P \leq 0.05$. , and ± indicates the standard deviation between the mean values of crop combinations and water levels of each parameter.

DISCUSSION

Water level. High root to shoot ratio (R:S) of wheat and barley was observed under low water levels as compared with high water level (Tables 1 and 2); however on the contrary, the R:S of rye was higher under high water level than that of under low water level (Table 3). No significant differences in the R:S of oat were observed between the two water levels (Table 4). The increase in root biomass and the corresponding decrease in shoot biomass of wheat under low water level indicated that additional carbon was invested in root biomass under low water level and so the R:S augmented in wheat. Clarkson (1985) reported that production, growth and maintenance of more roots involve more investment of photosynthetic carbon. Hamblin et al. (1990) noted that in stressful environments, the allocation of resources to below ground growth may be high which may reduce above ground growth. The increase in R:S of barley under low water level was attributed to the lower shoot biomass. According to Eghball and Maranville (1993), environmental stresses enhance the weights of roots over shoots. The increase in root biomass and the corresponding decrease in shoot biomass of rye under higher water level resulted in the higher R:S. In contrast, the decrease in root biomass and the corresponding increase in shoot biomass under low water level decreased the R:S in rye.

The higher WUE of all four crop species under study (wheat, barley, rye, and oat) under low water level indicated that the less available water enabled the crop to efficiently utilize the water. On the other hand, the lower WUE of crop species under high water level may be due to the higher transpiration rate because of the higher canopy development (higher shoot biomass) under high than low water level. The shoot biomass of rye under low and high water levels were statistically the same and therefore no significant influence of water levels was observed on the WUE of rye.

Crop combination. Impact of crop combinations significantly influenced the R:S of barley (Table 2) and oat (Table 4) but no momentous consequence on the R:S of wheat (Table 1) and rye (Table 3) at $P \leq 0.05$. Barley R:S attained maximum level in the four-crop mixed stand, followed by barley grown mixed with wheat. The increase in R:S of barley in the wheat mixed stand and four-crop mixed stand was attributed to the significant increase in the root biomass of barley indicating lower underground competition of barley roots. The R:S of barley decreased to minimum when grown in mixture with three-crop stands (wheat + barley + rye or wheat + barley + oat). The decrease in the R:S of barley in three-crop mixed stands was due to the significant decrease in root biomass of barley. This indicated that the existence of higher below ground plants competition of barley with other crop that decreased root biomass as well as its R:S.

Oat grown alone as the sole crop or mixed in three-crop stand (wheat + barley + oat) produced higher shoot biomass. The decrease in shoot biomass of oat in all the crop combinations indicated negative impacts on the shoot development of oat (Sadras and Calderini 2009). The R:S of oat was significantly higher when grown mixed with wheat. The increase in R:S of oat in wheat mixed stand was attributed to more root biomass but the corresponding decrease in shoot biomass of oat. This indicated that oat has strong below-ground competition with wheat that resulted in higher R:S in oat (Sadras and Calderini 2009).

Crop combination had significantly ($P \leq 0.05$) influenced the WUE in all four crop species (Tables 1, 2, 3, and 4). In all the four crop species in this study, the WUE was higher when grown in pure (sole crop) stands. Interestingly, our similar study on C_4 summer crop mixed stands indicated that the higher intra-plants competition among pure corn plants significantly decreased shoots and roots dry biomass (Amanullah and Stewart 2013), and as a result, the WUE of pure corn plants was declined. There was an increase in the WUE of corn plants when it was grown in mixed stand with sorghum and millets (corn + sorghum + millets) because of the reduction in the crop growth rate (Amanullah 2014a) and total dry matter accumulation of both millets and sorghum (Amanullah and Stewart 2013).

In the current study, wheat grown alone as a sole crop or grown mixed with another crop of barley or oat produced higher root and shoot biomass which enhanced its WUE significantly ($P \leq 0.05$). The increase in root and shoot biomass of sole wheat crop was probably due to less intra-plants competition in pure wheat crop. However, Dubbs (1971) found more competition among the sole alfalfa crop and found less competition of alfalfa plants in the mixed stands. The lower above-ground competition of wheat with barley and rye increased wheat shoot biomass and so the WUE was also increased.

According to Bazzaz (1998), plant canopy architecture is very important in plants competition. In the wheat and oats mixed stands, wheat reduced its root biomass especially under low water level, which decreased the WUE in wheat. Barley grown alone as the sole crop or mixed in two-crop stands with oat had higher root and shoot biomass as well as higher WUE. The increase in the root and shoot biomass of barley in the pure stands showed lower plant competition above and below the ground among the pure barley plants.

Competition among roots for water and nutrients is stronger than competition among shoots for light interception (Casper and Jackson 1997). The lower competitions below and above ground in the pure barley plants accumulated more total dry matter (root + shoot biomass) and the WUE was increased significantly (3.67 g L^{-1}). However, Dubbs (1971) observed stronger plants competition in the same alfalfa plants than competition of alfalfa with crop species. The increase in WUE of barley in two-crop mixed stands with rye or oat was due to the significant increase in shoot biomass (better canopy architecture) of barley and so the WUE significantly increased. Therefore, the canopy architecture may be considered important criteria in plants competition (Sorrenson et al. 1993).

Rye grown alone as the sole crop or mixed in two-crop stands in oat resulted in higher WUE. The increase in WUE in pure rye stand and oat mixed stand was due to the higher total biomass accumulation (root + shoot biomass) of rye crop. The decrease in WUE of rye in three- and four-crop mixed stands was attributed to the decline in both root and shoot biomass of rye. This indicated that rye had the least above and below ground competitions with other species while increasing the number of species in a mixture.

The WUE of oat was increased significantly when grown as a sole crop and decreased significantly when oat was grown in three- and four-crop mixed stands. The increase in WUE in pure oat stand was attributed to the significant increase in the shoot biomass of oat. This indicted that the above-ground competition of oat in mixed stands decreased the shoot biomass and so the WUE was decreased significantly.

Therefore, understanding canopy architecture is very important in plants competition (Sorrenson et al. 1993). Amanullah (2015) reported that very well developed canopy and root architecture of corn had negative impacts on the growth and total dry matter accumulation of millets and sorghum plants that adversely reduced the WUE of both sorghum and millets in the corn mixed stands. Likewise, Bazzaz (1998) reported that plant parts in space and their mode of display (plant architecture) are very important in plant–plant interactions.

Interactions. The interaction between crop combinations x water levels significantly affected R:S of wheat, barley and rye (Tables 1, 2, and 3, respectively) but no significant effect was observed on the R:S of oat (Table 4). Similar statistically the same R:S was observed for the sole wheat crop and wheat + oat mixed stands under both water levels. In all other crop combinations, the R:S of wheat was higher under low than high water level and maximum increase was recorded in wheat mixed stands with barley or rye. Barley increased its R:S significantly when grown in a mixture with wheat, oat, rye, or in four-crop mixed stands under low water level. Under all other crop combinations, barley had statistically the same R:S under both water levels. The WUE in barley increased under different crop stands at low than high water level. Except in the wheat + rye + oat mixed stand where the R:S of rye was higher under low than high water level, in all other rye combinations, the R:S of rye was higher under high than low water level. The increase in root biomass and corresponding decrease in shoot biomass of rye under high water level increased the R:S of rye crop.

The interaction between crop combinations into water levels had no significant effects on WUE of wheat and barley (Tables 1 and 2); however, significant effect on the WUE of rye and oat was observed (Tables 3 and 4). The interaction between water level and crop combinations (Table 3) indicated that both root and shoot biomass and WUE in rye increased significantly under both levels when grown as a sole crop, followed by rye and oat mixed stand. Under high water level, a remarkable decrease in root and shoot biomass and WUE was observed when rye was gown mixed in the three- (rye + barley + oat) and four-crop stands (wheat + rye + barley + oat). Plants with contrasting root architecture may reduce the extent of competition (Rubio et al. 2001).

The interaction between water level and crop combinations (Table 4) indicated that shoot biomass and WUE in oat under both levels increased when grown as sole crop. Under high water level, both shoot and root biomass and WUE of oat increased significantly when oat was grown mixed in two-crop stands with barley or rye or gown in three-crop mixed stand (wheat + barley + oat). Massinga et al. (2003) and Van Wychen et al. (2004) reported that the WUE of a weedy crop (mixed stand) will be lower than a weed-free crop (pure stand). Amanullah (2015) reported that the inclusion of corn in mixtures with millets (corn + millets or corn + sorghum + millets) produced negative impacts on the shoot and root development of millets and decreased the total plant dry weight of millets, and so the WUE was reduced at different water levels. The interaction between crop root systems and their environment limits growth and yield (Gregory 2006). According to Kroon and Visser (2003), the successful competitor has a root system that is able to rapidly proliferate in resource-rich volumes of soil, depleting the resources before competing with other plants, and have a high crop growth rate, thus a high demand for mineral nutrients and water.

CONCLUSIONS

Considerable variations in root biomass, shoot biomass, root to shoot ratios, and water use efficiencies of cool season cereal crop (wheat, rye, barley, and oat) was observed under different crop combinations and water levels. Due to the lower intra-plants competitions in the pure crop stands (sole crop), more biomass was partitioned into roots and shoots that resulted in the higher water use efficiency. Higher inter-plant competitions in the mixed stands reduced roots and shoot biomass and so declined the water use efficiency. The root to shoot ratios increased under low water level due to the increase in the root biomass and the corresponding decrease in shoot biomass. The increase in water use efficiencies of crop species under low water level was attributed to the most efficient use of water.

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REFERENCES

Aldrich RJ. 1984. Nature of weed competition. In: Weed-Crop Ecology. Principles in Weed Management. Breton Publishers. Belton, CA, pp. 189-213.

Amanullah. 2014a. Crop growth rate differs in warm season C_4-grasses grown in pure and mixed stands. African J. Biotech. 13(30):3036-3051.

Amanullah. 2014b. Wheat and rye differ in dry matter partitioning, shoot-root ratio and water use efficiency under organic and inorganic soils. J. Plant Nutr. 37:1885-1897.

Amanullah. 2015. Competition among warm season C_4-cereals influence water use efficiency and competition ratios. Cogent Food & Agri. 1:1011466, 1-14 doi:10.1080/23311932.2015.1011466

Amanullah, Khan F, Muhammad H, Jan AU, Ali G. 2016. Land equivalent ratio, growth, yield and yield components response of mono-cropped vs. inter-cropped common bean and maize with and without compost application. Agric. Biol. J. N. Am. 7(2):40-49.

Amanullah, Stewart BA. 2013. Shoot: root differs in warm season C_4-cereals when grown alone in pure and mixed stands under low and high water levels. Pak. J. Botany 45(Special Issue):83-90.

Amanullah, Stewart BA, Hidayatullah. 2015. Cool season C_3-grasses (Wheat, Rye, Barley, and Oat) differ in shoot: root ratio when applied with different NPK sources. J. Plant Nutr. 38:189-201.

Bazzaz FA. 1998. Plants in changing environments. Cambridge University Press, UK.

Bolinder MA, Angers DA, Bélanger G, Michaud R, Laverdière MR. 2002. Root biomass and R:S of perennial forage crop in eastern Canada. Can. J. Plant Sci. 82:731-737.

Casper BD, Jackson RB. 1997. Plant competition underground. Annual Review of Ecology and Systematics. 28:545-570.

Clarkson DT. 1985. Factors affecting mineral acquisition by plants. Annu. Rev. Plant Physiol. 36:77-115.

Dahmane ABK, Graham RD. 1981. Effect of phosphate supply and competition from grasses on growth and nitrogen fixation of *Medicago truncula*ta. Australian J. Agri. Res. 32:761-772.

Davidson RL. 1969. Effects of soil nutrients and moisture on root/shoot ratios in *Lolium perenne* L., and *Trifolium repens* L. Ann. Botany. 33:571-577.

Dubbs AL. 1971. Competition between Grass and Legume Speciess on Dryland. Agron. J. 63:359-362.

Dunbabin V, Rengel Z, Diggle A. 2001. The root growth response to heterogeneous nitrate supply differs for *Lupinus angustifolius* and *Lupinus pilosus*. Australian J. Agri. Res. 52:495-503.

Eghball B, Maranville JW. 1993. Root development and nitrogen influx of corn genotypes grown under combined drought and nitrogen stresses. Agron. J. 85:147-152.

Gregory PJ. 2006. Plant Roots: Their growth, activity and interaction with soils. Blackwell Scientific Publications, Oxford, UK.

Haling RE, Simpson RJ, McKay AC, Hartley D, Lambers H, Keller KO, Wiebkin S, Herdina, Riley IT, Richardson AE. 2011. Direct measurement of roots in soil for single and mixed species using a quantitative DNA-based method. Plant Soil. 348:123-137

Hamblin AP, Tennant D, Perry MW. 1990. The cost of stress: Dry matter partitioning changes with seasonal supply of water and nitrogen to Dryland wheat. Plant Soil. 122:47-58.

Hannay MR, Kozub GC, Smoliak S. 1977. Forage production of sainfoin and alfalfa on dryland in mixed- and alternate-row seedlings with three grasses. Canadian J. Plant Sci. 57:61-70.

Kroon H, Visser EJW. 2003. Ecological Studies 168: Root Ecology. Springer, Heidelberg, Germany.

Lynch JP. 2007. Roots of the second green revolution. Aust. J. Bot. 55:493-512

Massinga RA, Currie RS, Trooien TP. 2003. Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci. 51:523-531.

Passioura J. 2006. Increasing crop productivity when water is scarce—From breeding to field management. Agricultural Water Management. Special Issue on Water Scarcity: Challenges and opportunities for crop science, 80, 176-196.

Richards RA, Watt M, Rebetzke GJ. 2007. Physiological traits and cereal germplasm for sustainable agricultural systems. Euphytica. 154:409-425

Rubio G, Walk T, Ge Z, Yank X, Liaok H, Lynch JP. 2001. Root gravitropism and below-ground competition among neighboring plants: A modeling approach. Ann. Botany. 88:929-940.

Sadras VO, Calderini DF. 2009. Crop Physiology: Applications for Genetic Improvement and Agronomy. Elsevier and Academic Press. New York, USA.

Sorrenson CKA, Ford ED, Sprugel DG. 1993. A model of competition incorporating plasticity through modular foliage and crown development. Ecol. Monograph. 63:277-304.

Steel RGD, Torrie JH, Dicky DA. 1997. Principles and procedures of statistics, a biometrical approach. 3rd Ed. McGraw Hill, Inc. Book Co. NY, United States.

Szente K, Tuba Z, Nagy Z, Csintalan Z. 1993. Competition between Chenopodium album and Helianthus annuus as reflected in photosynthesis and respiration. Photosynthetica, 28, 465-472.

United States Department of Agriculture (USDA). 2014. Intercropping to improve soil quality and increase biodiversity. Accessed on 01-01-2017 available at https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid... ext=pdf

Van Wychen LR, Maxwell BD, Bussan AJ, Miller PR, Luschei EC. 2004. Wild oat (*Avena fatua*) habitat and water use in cereal grain cropping systems. Weed Science. 52:352-358.

Wilson JB, Steel JB, Steel SLK. 2007. Do plants ever compete for space? Folia Geobotanica. 42:431-436.