THE USE OF GROWTH ANGLE OF SEMINAL ROOTS AS TRAIT TO IMPROVE THE DROUGHT TOLERANCE IN WINTER WHEAT (*Triticum aestivum* L.)

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Abstract

Root system characteristics are fundamental importance not only for the uptake of nutrients and water from soil but also for environmental stress tolerance. Root architectural traits determine the in situ space-filling properties of a root system or root architecture. The growth angle of root axes is a principal component of root system architecture that has been strongly associated with water efficiency in many crop species. The aims of this study were to examine the extent of genotypic variability for the growth angle of seminal roots in 100 Romanian haploid lines derived from a cross between two winter wheat cultivars (Triticum aestivum L.), different for osmotic adjustment and association between growth angle of seminal roots and cuticular transpiration. Also three simple sequence repeat (SSR) markers (WMC596, WMC603 and SCM9) were examined for polymorphism between Izvor and F000628 wheat genotypes and to quantify the effect of 1RS:1AL translocation (from Secale) on seminal root characteristics and association between growth angle of seminal roots and cuticular transpiration. The growth angle of seminal roots showed significant genotypic variation among the wheat genotypes with values ranging from 53 to 119°. The obtained results revealed that SSR markers studied (wmc 596 and wmc 603) and 1AL/1RS translocation (from Secale) were weakly associated with growth angle of seminal roots. The implications of genotypic variation in the seminal root characteristics are discussed with emphasis on the possible exploitation of root architectural traits in breeding for improved wheat cultivars for water-limited environments.

Key words: winter wheat, angle of seminal roots, cuticular transpiration, SSR markers, drought resistance.

INTRODUCTION

Roots play several essential roles in the plant life cycle both for the uptake of nutrients and water but also for hydric tolerance of some plants. Plants have several ways to modify their root system architecture in response to changes in the external environment (Osmont et al., 2007). Analyses of genetic factors contributing to root system architecture are still very limited, however, partly because is difficult to observe the distribution of roots in field conditions, and partly because of the complexity of the effects of environmental conditions on root system architecture.

Some study suggested that the seminal root growth angle is useful for predicting vertical root distribution. Manschadi et al. (2008) have also suggested that selection for seminal root traits might be useful in breeding to increase the drought tolerance of wheat varieties with deep root architecture. They conducted that

selection for root growth angle and number of seminal roots may help to identify genotypes with root system architecture adapted to drought tolerance. Therefore, identification of the genetic factors controlling growth angles of roots at the seedling stage is an important key to predicting root system architecture in cereals.

Drought resistance can be improved by increasing the water use efficiency, and one way to achieve this is by selecting genotypes with low cuticular transpiration. Cuticular (or residual) transpiration represents the main way of water loss during night under optimal conditions and during noon under drought conditions, when stomata are closed. It was used as selection trait in wheat breeding for drought resistance (Clarke, 1991; Balota, 1995; Petcu, 2005).

The aims of this study were to examine the genotypic variability for the growth angle of seminal roots in 100 Romanian haploid lines

derived from a cross between two winter wheat cultivars (*Triticum aestivum* L.), different for osmotic adjustment and association between growth angle of seminal roots and cuticular transpiration. This will help in establishing a strategy to improve the tolerance of winter wheat to water stress.

MATERIALS AND METHODS

The haploid lines derived from a cross between two winter wheat cultivars different for osmotic adjustment (Izvor with gene for osmotic adjustment, Or^+ and F00628 without osmotic adjustment gene, or^-) and lines with 1AL/1RS translocation (from Secale) were studied.

For determination the seminal root angle, we used 0.75L transparent pots. The transparent pots were filled with two types of soils mixture (70% turba and 30% chernozem soil). Seeds were sown at a depth of 2 cm every 2.5 cm along the pot wall. The seeds were carefully placed vertically, embryo downwards and facing the wall to facilitate root growth along the transparent wall. After sowing, the clear pots were wrapped in aluminum foil and placed in dark-colored paper bags to exclude light from the developing. The pots were watered after sowing and no additional water or nutrients were supplied thereafter.

The roots were photographed at 7 days after sowing, then foto images of each individual seedling were transferred in PC. The angle between the two most outer seminal roots was measured with ImageJ program.

Cuticular transpiration, according to Clarke (1991) method, was measured on second leaves (five for each replicate) from plants that were stresses 14 days (without water). Following the initial weight determination, the leaves were maintained in the darkness for stomata closure under ambient room conditions, weighed again after 5 h and then dried at 90°C. Water loss was expressed in grams of water lost per gram of leaf dry matter, using the formula: [(Gi-Su)-(G5-Su)]/Su; where Gi is initial fresh weight, G5 - fresh weight after 5 h and Su is the dry weight.

A total of three simple sequence repeat (SSR) markers were analysed for polymorphism between Izvor and F000628.

DNA isolation of materials used in this study was extracted from two seeds using the SDS3 method described by Cristina et al. (2017). The molecular analysis for SSR markers WMC596 and WMC603 located on 7A chromosome were conducted according to the method described by Ciucă et al. (2010) and for SSR markers SCM9 we used the protocol by Saal and Wricke (1999).

RESULTS AND DISCUSSIONS

The anlyses of variance of the seminal root angle indicated a significant effect of genotype at the 0.1% level of significance (Table 1).

Table 1. Analyses of variance for seminal root angle

Specification	Source of variance	DF	Sum of squares	Mean square	*F value and significance*
Genotypes Or^-	Variants	24	18146	756.11	128.91***
	Error	48	281	5.86	
Genotypes Or^+	Variants	25	15311	612.46	1526***
	Error	50	20.05	0.40	
Genotypes Or^- with 1AL/1RS translocation	Variants	25	13102	524.08	1366***
	Error	50	19.6	0.38	
Genotypes Or^+ with 1AL/1RS translocation	Variants	24	15886	661.02	1056***
	Error	48	30.07	0.62	

Izvor cultivar carry gene for osmoregulation (*or*) by Banica et al. (2008), Ciucă et al. (2010). F000628 line carry 1AL: 1RS translocation by Ciucă et al. (2015). The DH lines created by crossing between Izvor and F000628 were characterized regarding the presence/absence of gene for osmotic adjustment and for presence/absence of 1AL/1RS wheat-rye translocation using molecular markers. Based on molecular markers results the lines were split into four groups, according to Table 2.

Considerable variation was observed among these cultivars concerning seminal root angle. Within each genotype group there were variations in the seminal root angle from 53 to 119°, with the average between 86 and 94°, (Table 2).

The analyzed Western Europe cultivars had seminal roots angles between 84 and 88º, the Austrian and Russian cultivars had angles of 79 and 85º, respectively and some Romanian

varieties showed a variability from 74-115º, (David, 2018).

The value of angle of seminal roots for Izvor genotype (known as drought resistant by its osmotic adjustment capacity) was 82° as compared with genotype F00642 (115°), without adjustment capacity (Figure 1).

Figure 1. The seminal root angle of Izvor (left) and F00642 (right) genotypes

Not all genotypes with gene for osmotic adjustment had small angles of seminal roots such as control genotypes (Izvor). It is noted that there are a total of eight genotypes with Or⁺ gene that have a seminal angle over 100°. Also in the group of genotypes withoth osmotic adjustment (or⁻) are many genotypes that have shallower root systems (Table 2).

Oyanagi (1994) have found that wheat varieties bred for western Japan tend to have shallower root systems than varieties bred for eastern Japan, and explained this regional difference as an adaptation to the more abundant soil moisture in western Japan. Manschadi et al. (2008) have found that drought-tolerant varieties tend to have deeper root systems compared to susceptible varieties. These results illustrate the close relationship between root system architecture and soil environmental conditions.

Previous studies on seminal root angle reported lower values, which could be due to their measurement of the angle between primary seminal root and one of the first pair of seminal roots and also using entirely different genotypes than the ones used in your study (Richard et al., 2015). Manschadi et al. (2006) reported that a narrower seminal root angle was associated with higher grain yield in moisture limiting condition.

The rye translocation increased biomass of root and shoot in some cases, reduced plant height, and delayed maturity in some cases. The 1RS.1BL translocation produced the highest grain yield associated with the lowest root and shoot biomass under both well watered and water stressed conditions.

In our study the 1AL/1RS translocation increased a little bit the seminal root angle, the average of the two groups of genotypes possessing the rye translocation was 93 and 94°, respectively, compared to 86 and 91°, the values of the two groups without 1AL/1RS translocation (Table 2).

Table 2. The seminal root angle of studied genotypes

Genotypes without osmotic adjustment (or⁻)		Genotypes with osmotic adjustment (Or⁺)		Genotypes with 1AL/1RS translocation and or⁻		Genotypes with 1AL/1RS translocation and Or⁺	
AiII 65	53	BiII 18	54	AiII 55	60	AiI 1	89
BiII 13	61	BiII 71	68	Bi II 3	76	BiII-2	102
AiII232	62	BiII 107	74	AiII 271	76	BiI - 8	85
BiII 81	64	Bi II 87	78	AiI- 33	79	AiII -19	83
AiII 118	68	Bi II 141	80	Ai I - 76	81	BiII- 24	62
BiII 140	71	Ai I - 44	82	Ai I - 73	85	Ai I 24	99
BiII 96	73	**Izvor**	82	Ai II 115	88	Bi I 32	106
BiII 114	79	Bi II 78	84	Ai I - 65	88	Bi I 44	86
BiII 79	84	AiII- 130	85	AiII 141	89	Ai I 45	71
BiII 110	86	Ai II 253	87	Ai II 51	89	Ai II 66	106
BiII 86	86	AiII- 215	89	Bi II 147	91	Ai II 104	73
AiII 90	88	AiII- 238	89	Bi I 36	93	Bi II 109	110
AiII 230	88	Bi II 10	89	BiII 100	94	Bi II 129	82
AiII - 82	89	Ai II 251	90	Bi II 1	95	Ai II 137	80
AiII 204	89	Bi II 16	91	Ai I 248	96	Ai II 159	69
BiII 143	93	AiII- 233	93	Ai II 34	97	Ai II 163	106
AiII 241	94	Bi II 85	96	Ai II 225	99	AiII 172	101
AiII - 89	94	Bi I 23	97	Bi II 142	99	Ai II 175	100
AiII - 83	96	Bi II 25	102	AiI - 20	100	AI II 183	118
AiII 167	97	Bi II 95	103	BiII 72	101	AiII 184	111
BiII 137	100	Bi II 29	104	Ai II 14	101	Ai II 202	95
AiI - 62	105	Bi II 122	104	Ai II 20	104	Ai II 231	93
BiII 75	105	BiII 144	106	Ai II 86	108	Ai II 237	111
AiII 126	106	BiII 104	112	AiII 47	113	AiII 239	96
AiII - 54	114	Bi II 134	114	**F00628**	115	AiII 259	97
		Ai II 240	114	Ai II 45	119		
Average	*86*		*91*		*94*		*93*
LD 5%	*3.9*		*1.03*		*1.36*		*1.8*
LD 1%	*5.3*		*1.38*		*1.81*		*2.4*

Most of the wheat lines with osmotic adjustment capacity and 1AL/1RS translocation from rye exhibited a cuticular transpiration of between 0.6 and 0.9 g/g while those without osmotic adjustment had a cuticular transpiration over 1 g/g, with some exceptions (Figure 1).
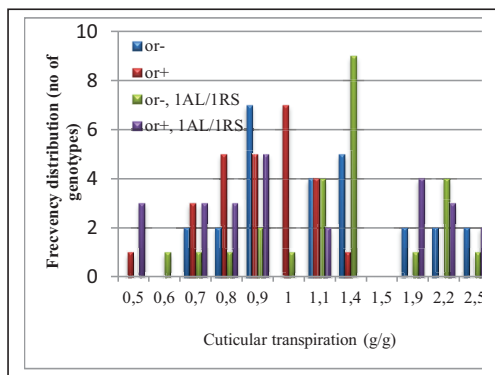
Figure 1. The frecvency distribution for cuticular transpiration

Correlation studies between characters are very important in plant breeding for indirect selection and have also been of great value in the determination of the most effective breeding procedures. In our study, regression analysis shows the existence of the correlation between seminal root angle and cuticular transpiration at the 0.1% level of significance for the genotypes without osmotic adjustment capacity and 1AL/1RS translocation and witout significance for the genotypes with Or^+ gene. (Table 3). This results suggest that seminal root angle can be selected for wheat improvement as it showed association with cuticular transpiration.

Table 3. Coefficients of correlation between seminal root angle and cuticular transpiration respectively WTS of studied winter wheat genotypes

	Genotypes or^-	Genotypes Or^+	Genotypes or^- and 1AL/1RS	Genotypes Or^+ and 1AL/1RS
Cuticular transpiration				
Seminal root angle	r = 0.60**	r = 0.36	r = 0.79***	r = 0.82***
The weight of thousand seeds				
Seminal root angle	r = 0.02	r = -0.15	r = 0.07	r = 0.12
Cuticular transpiration	r = - 0.39	r = -0.18	r = 0.2	r = 0.008

We conducted correlation analysis of seminal root angle with MMB and also between WTS and culticular transpiration and did not find significant correlation except the correlation between cuticular transpiration and WTS at the 0.5% level of significance for the genotypes without osmotic adjustment capacity (Table 3).

This results are in concordance with many studies but other reported a weak correlation of yield with the seminal root number or evidentiate that root growth angle (RGA) was negatively correlated with grain yield, positively with canopy temperature depression, negative correlations with SPAD in the managed drought and irrigated experiments Madhav Pandey et al. (2015).

Molecular analyzes have shown that molecular markers responsible for osmotic adjustment (WMC603 and WMC596) can explain part of the phenotypic variation of the seminal root angle because only 45.5% of the DH lines with Or+ gene had an electrophoretic profile similar to that of the variety Izvor. Xie et al. (2017) found a QTL associated with emergence angle of seminal root in wheat and this is located on 7D chromosome. In this respect, molecular analyses will be focused on chromosome 7D.

Similar studies of wheat, which compared the architecture of drought-adapted genotypes to that of standard genotypes, have the relevance that drought-adapted genotypes have a compact root system (the roots are laterally dispersed at a maximum of 45 cm from the main stem), the roots occupy a volume of soil uniformly and grow more in the depths of soil (3.8 times more than standard) (Manschadi et al., 2006). This type of root system allows the plant to access moisture in deeper layers. At the same time, large seminal root angle should be considered as they re-use water from superficial soil layers. This could be beneficial in capitalizing small amounts of precipitation during periods of drought. It will be necessary also to evaluate evolutions of rainfall from our zone in order to established what kind of seminal root angle is better to improve the drought resistance of wheat for the future.

CONCLUSIONS

Our results have confirmed the presence of significant genotypic differences in seminal root angle among our set of 100 genotypes. The genotypes with the narrow seminal root angle grow more in the depths of soil and this type of root system will allow the plant to access moisture in deeper layers. At the same time many varieties have the shallow seminal root angle, this might be an adaptation to the high

moisture content of the soil and could be beneficial in capitalizing small amounts of precipitation during periods of drought/vegetation. The existence of positive correlations between the seminal angle and the cuticular transpiration, opens up new possibilities of amelioration of winter wheat.

ACKNOWLEDGEMENTS

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