

Article

Variability of Root and Shoot Traits under PEG-Induced Drought Stress at an Early Vegetative Growth Stage of Soybean

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Abstract: The performance of a soybean genotype under water deficit stress at an early vegetative stage might be related to its general tolerance to drought. To investigate the plasticity of root and shoot seedling traits in response to drought at an early vegetative stage, a set of 32 soybean genotypes adapted to southeast European growing conditions was grown under polyethylene glycol (PEG) 8000-induced drought stress and well-watered control conditions. Under drought, mean tap root length (RL), shoot length (SL), root fresh weight (RFW) and shoot fresh weight (SFW) decreased significantly by 11, 17, 38 and 34%, respectively, while root dry matter (RDM) and shoot dry matter (SDM) increased significantly by 13 and 11%, respectively. Of the four derived traits, the ratios of RL/SL, RL/RFW and SL/SFW increased significantly by 8, 45 and 28%, respectively, under drought, while the ratio of RFW/SFW decreased significantly by 4%. However, a wide variation between genotypes was observed for all 10 studied seedling traits under both control and drought conditions. Broad sense heritability ranged from 0.53 (RL) to 0.97 (SL) under control conditions and from 0.56 (RL/RFW) to 0.96 (SL) under drought conditions. The correlation coefficients between the traits were either weak or moderate, indicating that the studied traits can be modified independently by selection.

Keywords: soybean; root and shoot traits; drought stress; PEG



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1. Introduction

Soybean (*Glycine max* L. Merr.) is the largest source of vegetable protein for animal feed worldwide and an important component of the human diet in some areas of the world [1]. It has higher protein, poly- and mono-unsaturated fat, and lower carbohydrate content and digestibility than other legumes, along with high concentrations of isoflavones, phytosterols and minerals that enhance the nutritional value as a human food ingredient [2]. European soybean production increased progressively over the last years, reaching a record of almost 3 million tons produced in 2023, but this increase is not sufficient and accounts for only about 8% of actual consumption requirements within the EU [3]. Soybean yields and production are hindered by various abiotic stresses, among which the drought is considered to be a major limiting factor [4]. According to Clement et al. [5], soybean is described to be the least adapted legume species to drought. Its yield under drought can be reduced by more than 50%, causing substantial financial losses to farmers and growers [6], hence, drought has been recognized as a significant climatic risk which calls for effective mitigation strategies to sustain the supply of soybeans worldwide. In the United States, improving yield under drought stress has become a major goal of soybean breeding programs [7], but a similar, if not the same, approach has been taken by the soybean breeders in all

soybean growing regions throughout the world [8–14]. Although the soybean crop is most susceptible to drought at emergence and from flowering through pod-filling [7,13], drought stress can affect growth or lead to yield reduction at all stages of soybean growth and development [10]. Studying the effects of drought at the soybean seedling stage is also important, since the number and vigor of seedlings determines the quality of individual and population development, i.e., growth and yield at harvest of soybean [8,12].

The plasticity of the root system is critical to plant acclimation and survival under environmental stress, especially drought [15]. For example, drought could stimulate plants to develop thinner and deeper roots to improve water and nutrient acquisition [16–18], but the studied effects of drought on root traits and responses are highly contradictory [19]. Drought tolerance at the seedling or early vegetative stage has been previously studied in soybean [7,11,13,20–24]. In these studies, genetic variability for root and shoot traits during early soybean growth has been well documented. Generally, both root and shoot growth and development are affected under drought stress, but shoot length and biomass accumulation seem to be more inhibited than root length and biomass accumulation, which has been explained as the primary response to water deficit. This effect also leads to a higher root-to-shoot biomass ratio under drought stress. Boote (2011) [25] identified the characteristics of rooting traits for improved water uptake in soybean: faster rate of root depth progression, improved distribution of root length density into deeper soil layers, an increased length per unit of root mass and a greater root to shoot (R/S) biomass ratio. Soybean cultivars with such favorable root traits during early vegetative growth could be good candidates (ideotypes) for maintaining crop performance under water-stressed conditions, thereby limiting yield losses, as proposed by Dayoub et al. [13], but knowledge on how to develop such ideotypes is still limited. The same authors also noticed the scarce knowledge about the genetic variability of root traits of soybean cultivars grown in Europe.

At the soybean seedling stage, the identification of drought resistance is cheap, easy to perform and requires a shorter experimental cycle [8], and, according to Dayoub et al. [13] the phenotyping of root traits with simple, rapid and accurate methods is more feasible during early growth stage. Currently, the most commonly used substance in experiments that simulate the dehydration effects of soil dryness is polyethylene glycol (PEG) [26]. PEG is considered to be a perfect regulator of water potential, since molecules with a molecular weight of 6000 or more cannot pass through cell walls [27] and can accurately reflect the state of the plant under a specific osmotic potential [28].

In the present study, the root and shoot traits of 32 market-leading soybean cultivars and most promising breeding lines adapted to southeast European conditions were investigated under control conditions (no water stress) and under simulated drought stress conditions achieved by using PEG to gain insight into the genetic variation of soybean root and shoot traits at the seedling stage. The performance of a genotype under the water deficit stress at an early vegetative stage might be related to its general tolerance to drought.

2. Materials and Methods

2.1. Plant Material and Experimental Design

A growth chamber experiment was conducted in 2018 at the Department of Plant Breeding, Genetics and Biometrics at the Faculty of Agriculture, of the University of Zagreb, Croatia. The experimental material consisted of 32 soybean genotypes adapted to southeast European conditions, including 24 cultivars widely used in Croatia for commercial cultivation, as well as eight breeding lines of maturity groups (MG) ranging from 00–0 to I. Genotypes were developed by five breeding institutions from four countries (Table 1).

The seeds of 32 genotypes were germinated in moist quartz sand at 22 °C and on the 8th day after sowing, the seedlings were transplanted into plastic tubes 40 cm long and 5 cm in diameter filled with fine vermiculite (granules 2–3 mm) (Figure 1).

Table 1. List of soybean genotypes used in the growth chamber experiment.

Code	Genotype	MG	Country of Origin	Breeding Company	Code	Genotype	MG	Genotype Status	Breeding Company
1	Zlata	0–I	Croatia	UniZg	17	Buga	0	Croatia	UniZg
2	Ružica	0–I	Croatia	UniZg	18	Gabriela	0	Croatia	UniZg
3	Zagrebčanka	I	Croatia	UniZg	19	Sanda	0	Croatia	AIO
4	Pedro	0–I	Italy	ERSA	20	Sonja	0	Croatia	AIO
5	Bahia	0–I	Italy	ERSA	21	Toma	0	Croatia	AIO
6	Ascasubi	I	Italy	ERSA	22	Ema	00–0	Croatia	AIO
7	Ika	0–I	Croatia	AIO	23	Korana	00–0	Croatia	AIO
8	OS Zora	0–I	Croatia	AIO	24	Lucija	0	Croatia	AIO
9	Tena	0–I	Croatia	AIO	25	OS-1	0	Croatia	AIO
10	Sara	0–I	Croatia	AIO	26	OS-2	00–0	Croatia	AIO
11	Seka	I	Croatia	AIO	27	OS-4	00–0	Croatia	AIO
12	Tisa	I	Croatia	AIO	28	OS-5	0	Croatia	AIO
13	OS-3	0–I	Croatia	AIO	29	OS-6	0	Croatia	AIO
14	OS-8	0–I	Croatia	AIO	30	OS-7	00–0	Croatia	AIO
15	DH 5170	I	Canada	UniG	31	Merkur	00	Serbia	IFVC
16	Galina	0	Serbia	IFVC	32	Xonia	00	Italy	ERSA

UniZg—University of Zagreb Faculty of Agriculture; AIO—Agricultural Institute Osijek; IFVC—Institute for Field and Vegetable Crops Novi Sad; ERSa—Regional Agency for Rural Development of Friuli Venezia Giulia; UniG—University of Guelph Ridgetown College of Agricultural Technology.



Figure 1. Soybean seedlings in vermiculite-filled tubes.

The vermiculite was prevented from scattering out of the tube by a white water-permeable, polypropylene nonwoven agrotexile with a weight of 30 g m² (BR Garden, Nottingham, UK), which was attached to the bottom of the tube with a rubber band. Before planting the plants, 60 mL of water was added to each tube, another 10 mL immediately after planting and 10 mL the next day. The tubes were placed in containers filled with 1/2-strength Hoagland modified basal salt mixture solution (Phyto Technology Laboratories, Shawnee Mission, St, Lenexa, KS, USA) or the same solution with 6% diluted polyethylene glycol (PEG) 8000 (Acros Organics, Geel, Belgium) (drought). The osmotic pressure of the PEG solution prepared in this way was −0.64 MPa (at 22 °C), calculated according to the formula of Michel [29]. For each tube, 300 mL of the solution was added to the container (which had proved to be sufficient in preliminary tests) and thereafter no more was supplemented. Gradually, the capillary rise of the solution reached the top of the tube.

The plants were grown in a growth chamber at a photoperiod of 16/8 day/night of LED tubes (Valoya L35 NS12) and a light intensity of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at a temperature of 22°C for 26 days.

2.2. Trait Measurements

Twenty-six days after transplanting, the whole plants were removed from the tubes and the roots were carefully separated from the vermiculite on a wire mesh. The shoot length (SL) and the taproot length (RL) of each plant were measured. The roots were then thoroughly washed under tap water to completely remove the vermiculite and dried with paper towels. Shoot fresh weight (SFW) and root fresh weight (RFW) were measured on pooled samples of five plants per replicate. The shoot and root samples were then placed in paper bags and dried in an oven at 70°C for 48 h and the shoot dry weight (SDW) and root dry weight (RDW) were determined. Root dry matter (RDM) and shoot dry matter (SDM) were calculated from the fresh weight and dry weight of root and shoot and expressed as a percentage of RFW and SFW, respectively. Finally, the following derived traits (ratios) were calculated from the measured traits: root-to-shoot length ratio (RL/SL), root-to-shoot fresh weight ratio (RFW/SFW), root length-to-root fresh weight ratio RL/RFW and shoot length-to-shoot fresh weight ratio (SL/SFW). The experiment was set up as a completely randomized block design with three replicates (five plants/replicate) within each treatment (control and drought).

2.3. Statistical Analysis

An analysis of variance (ANOVA) across treatments (control and drought) and within treatments was performed using the GLM procedure of SAS/STAT version 9.4 [30]. Variance components for the traits were determined by equating the observed mean squares from the within-treatment ANOVA to their expectations and resolving for the desired variance components. Broad-sense heritability (h^2) was calculated using the following formula: $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_\epsilon / r)$, where σ^2_G and σ^2_ϵ are the genotypic and error variance, respectively, and r is the number of replicates [31]. Pearson correlation coefficients between traits were calculated using PROC CORR from the SAS/STAT version 9.4 [30].

3. Results

3.1. Analysis of Variance

The analysis of variance (ANOVA) combined across water treatments revealed significant effects of genotype (G) and treatment (T) for all traits and a significant $G \times T$ interaction for all traits except SDM, RFW/SFW and RL/RFW (Table 2). ANOVA by treatment revealed a significant effect of genotype for all traits under both control and drought conditions. Broad sense heritability ranged from 0.53 (RL) to 0.97 (SL) under control conditions, while it ranged from 0.56 (RL/RFW) to 0.96 (SL) under drought conditions. For most traits, heritability estimates were similar in the two treatments, except for RFW and RL/RFW for which it was much higher under control conditions, and for RL, for which it was much higher under induced drought. Generally, heritability was higher for the shoot traits than for the root traits.

Table 2. Analysis of variance (ANOVA) for 10 soybean seedling traits of 32 soybean genotypes grown under two treatments (control and drought).

Trait	Treatment	ANOVA across Treatments			ANOVA by Treatment	
		Genotype (G)	Treatment (T)	$G \times T$	Genotype	h^2
RL	Control	**	**	**	**	0.53
	Drought				**	0.83

Table 2. Cont.

Trait	Treatment	ANOVA across Treatments			ANOVA by Treatment	
		Genotype (G)	Treatment (T)	G × T	Genotype	h ²
SL	Control	**	**	**	**	0.97
	Drought				**	0.96
RFW	Control	**	**	**	**	0.85
	Drought				**	0.68
SFW	Control	**	**	**	**	0.84
	Drought				**	0.80
RDM	Control	**	**	*	**	0.67
	Drought				**	0.68
SDM	Control	**	**	NS	**	0.76
	Drought				**	0.80
RL/SL	Control	**	**	**	**	0.94
	Drought				**	0.96
RFW/SFW	Control	**	**	NS	**	0.72
	Drought				**	0.78
RL/RFW	Control	**	**	NS	**	0.80
	Drought				**	0.56
SL/SFW	Control	**	**	**	**	0.92
	Drought				**	0.94

*, ** F test of corresponding mean squares significant at the 0.05 and 0.01 probability levels, respectively. NS, F test of corresponding mean squares not significant. Traits: root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry matter (RDM), shoot dry matter (SDM), root-to-shoot length ratio (RL/SL), root-to-shoot fresh weight ratio (RFW/SFW), root length-to-root fresh weight ratio (RL/RFW), shoot length-to-shoot fresh weight ratio (SL/SFW). h² broad-sense heritability.

3.2. Effect of Drought on Trait Means

Means of 10 seedling traits varied widely among soybean genotypes under both control and drought conditions (Tables 3 and S1). The lowest relative variation under control conditions was found for RL (CV = 3.3%) and under drought for RDM (CV = 7.4%), while the highest relative variation under both conditions was detected for SL (CV = 24.1% and 24.2% under control and drought conditions, respectively). For most traits, CVs were similar in the two treatments except for RL, for which it was much higher under drought conditions, and for RFW and RL/RFW for which it was higher in the control. Induced drought reduced RL and SL by 11 and 17%, respectively, compared to the control, with the magnitude of reduction varying over a wide range among genotypes (1 to 34% for RL and 5 to 35% for SL). The mean reduction in plant fresh weight due to drought was much higher reaching 38 and 34% for RFW and SFW, respectively, and also varied in a wide range among genotypes (24 to 50% for RFW and 16 to 50% for SFW). On the other hand, mean RDW and SDW increased by 11 and 8%, respectively under drought compared to the control. Changes under drought compared to the control ranged from a 6% decrease to a 33% increase in RDW, while SDW increased in a range of 3 to 22% for all genotypes.

Table 3. Means and descriptive statistics for 10 seedling traits in 32 soybean cultivars under control and drought conditions.

Trait	Treatment	Absolute Units				Change (% of Control)		
		Mean	Min	Max	CV (%)	Mean	Min	Max
RL (mm)	Control	362	329	381	3.3	−11	−1	−34
	Drought	324	231	359	8.3			
SL (mm)	Control	415	258	717	24.1	−17	−5	−35
	Drought	345	222	567	24.2			
RFW (g/plant)	Control	2.10	1.45	3.04	17.9	−38	−24	−50
	Drought	1.29	0.88	1.59	12.5			
SFW (g/plant)	Control	2.02	1.29	2.74	15.4	−34	−16	−50
	Drought	1.31	0.92	1.71	14.7			
RDM (%)	Control	6.85	5.71	8.34	8.9	13	−6	33
	Drought	7.72	6.77	9.12	7.4			
SDM (%)	Control	16.6	14.5	18.7	6.6	11	3	22
	Drought	18.4	15.6	20.8	7.6			
RL/SL	Control	0.92	0.51	1.39	20.3	8	−17	43
	Drought	0.98	0.59	1.53	21.4			
RFW/SFW	Control	1.05	0.85	1.33	11.5	−4	−21	25
	Drought	1.00	0.78	1.28	13.0			
RL/RFW	Control	179	129	241	15.7	45	9	79
	Drought	255	214	314	10.6			
SL/SFW	Control	208	142	301	18.9	28	5	55
	Drought	265	166	402	20.4			

Traits: root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry matter (RDM), shoot dry matter (SDM), root-to-shoot length ratio (RL/SL), root-to-shoot fresh weight ratio (RFW/SFW), root length-to-root fresh weight ratio (RL/RFW), shoot length-to-shoot fresh weight ratio (SL/SFW).

Among the four derived traits, the means of RL/SL, RL/RFW and SL/SFW increased by 7, 45 and 28%, respectively, under drought compared to the control, while the mean RFW/SFW decreased by 4% due to drought. However, the genotypes studied showed an opposite response to drought for RL/SL and RL/RFW, with some genotypes showing a decrease and some an increase in trait means compared to the control (Table S1). On the other hand, all genotypes consistently reduced the length-to-fresh weight ratio, which ranged from 9 to 79% for RL/RFW and from 5 to 55% for SL/SFW. Overall, of the 10 traits studied the highest relative change in trait mean due to drought was observed for RL/RFW.

Means for six directly measured seedling traits in 32 soybean genotypes under control and drought conditions are shown in Figure 2 and Table S1. The five genotypes with the highest RL under control conditions were ‘Toma’ (21), ‘OS-1’ (25), ‘OS-5’ (28), ‘OS-7’ (30) and ‘Merkur’ (31). The correlation between RL in control and drought was moderate positive ($r = 0.44$) and among the five highest-ranking genotypes only ‘Toma’ (21) and ‘OS-5’ (28) were common to both conditions. The three genotypes with the highest RL under drought conditions were ‘Tisa’ (12), ‘OS-8’ (14) and ‘OS-6’ (29) showing a negligible reduction in RL due to drought.



Figure 2. Means for six directly measured seedling traits in 32 soybean genotypes in control and drought conditions and the Pearson correlation coefficients (r) between the two treatments. Traits: root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry matter (RDM), shoot dry matter (SDM). *, ** Correlation coefficient significant at the 0.05 and 0.01 probability levels, respectively.

The correlation between control and drought for SL was very strong and positive ($r = 0.93$), indicating that the value of this trait under control conditions is a good predictor of the trait value under drought. In fact, of the five genotypes with the highest SL, four were

common between the two conditions, namely ‘Ema’ (22), ‘OS-4’ (27), ‘OS-5’ (28) and ‘Xonia’ (32). Strong positive correlations were observed between control and drought for RFW ($r = 0.78$) and SFW ($r = 0.71$) and similar to SL, four of the five highest ranking genotypes were common in the two conditions for each trait. For RFW these were ‘Merkur’ (31), ‘OS-8’ (14), ‘Tisa’ (12) and ‘OS-4’ (27), and for SFW ‘Xonia’ (32), ‘OS-4’ (27), ‘OS-8’ (14) and ‘Tisa’ (12). ‘Tisa’ (12) and ‘OS-8’ (14) can be highlighted as genotypes that appeared among the top five ranked genotypes under drought conditions for the three traits RL, RFW and SFW. The correlation between RDM under control and drought conditions was moderate positive ($r = 0.50$) and only ‘Bahia’ (5) was among the five highest ranked genotypes under both control and drought conditions. The correlation between SDM under control and drought conditions was strong positive ($r = 0.80$) and three genotypes ‘Bahia’ (5), OS-2 (26) and ‘Merkur’ (31) were among the five genotypes with the highest dry matter concentration under both conditions.

Means for four derived seedling traits in 32 soybean genotypes under control and drought conditions are shown in Figure 3 and Table S1. Root-to-shoot length ratio (RL/SL) increased on average by 8% under drought conditions, but a high variation among genotypes was observed for this trait. The correlation for this trait between the control and drought conditions was very strong and positive (0.86^{**}). The genotypes ‘Gabriela’ (18) and ‘Korana’ (23) substantially increased this ratio by 43 and 32%, respectively, while for the genotype ‘Zlata’ (1) the highest reduction of this ratio (-17%) was observed under drought.

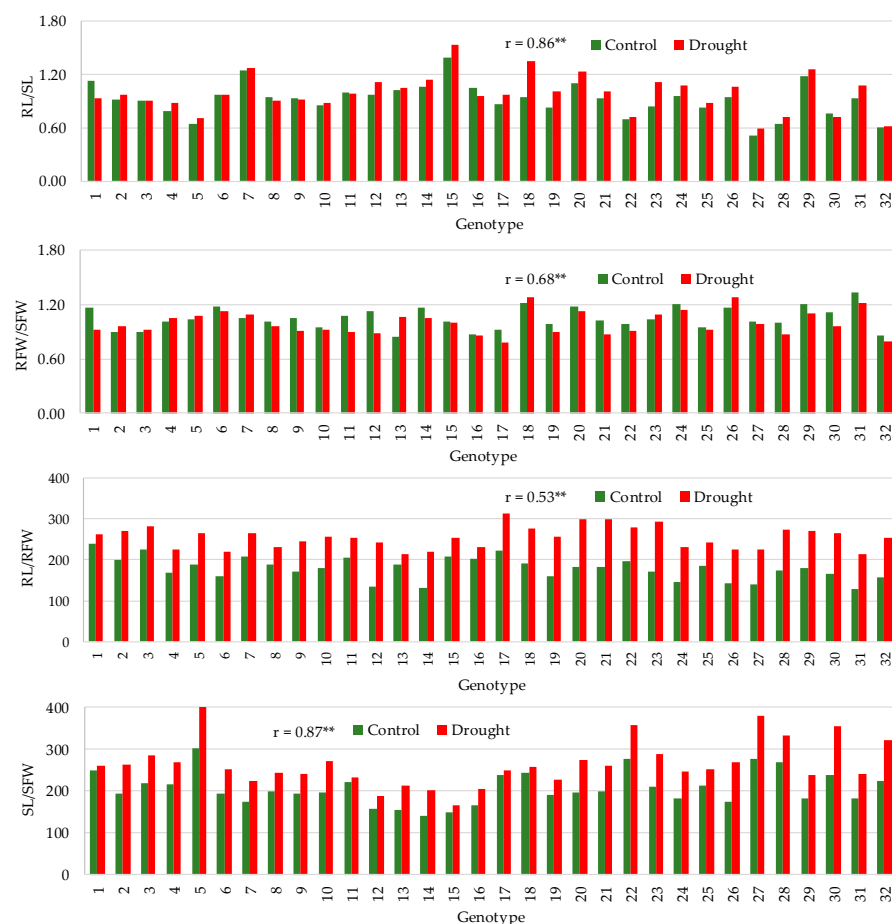


Figure 3. Means for four derived traits in 32 soybean genotypes in control and drought conditions and the Pearson correlation coefficients (r) between the two treatments. Traits: root-to-shoot length ratio (RL/SL), root-to-shoot fresh weight ratio (RFW/SFW), root length-to-root fresh weight ratio (RL/RFW), shoot length-to-shoot fresh weight ratio (SL/SFW). ** Correlation coefficient significant at the 0.01 probability level.

Root-to-shoot fresh weight (RFW/SFW) ratio between the control and the induced drought decreased on average by 4%. Some genotypes, like ‘Zlata’ (1) and ‘Tisa’ (12), responded with a strong reduction of this ratio by 21%, while the breeding lines ‘OS-2’ (26) and ‘OS-3’ (13) increased this ratio by 10 and 25%, respectively. For this trait, the correlation between the control and the drought conditions was also strong and positive (0.68 **). The root length-to-root fresh weight ratio (RL/RFW) and shoot length-to-shoot fresh weight ratio (SL/SFW) increased by 45 and 28%, respectively. The response of all genotypes for these two traits was positive, meaning that all genotypes extended the length of roots and shoots at the cost of the root and shoot weight, although the effect of change was stronger for the roots. This is also reflected in the strength of correlation coefficients observed for these two traits under control and drought conditions, 0.87 for SL/SFW and 0.53 for RL/RFW. The magnitude of response for the two traits differed among genotypes. The genotypes ‘Korana’ (23), ‘Merkur’ (31) and ‘Sonja’ (20) elongated their roots by 73%, 66% and 64%, respectively, at the expense of root fresh weight. The greatest elongation of the shoot at the expense of the shoot fresh weight was observed for the breeding lines ‘OS-2’ (26) and ‘OS-7’ (30), 55 and 48%, respectively.

3.3. Correlation between Traits

The correlations between the pairs of traits were generally low to moderate under both control and drought conditions (Table 4).

Table 4. Correlation coefficients between five directly measured seedling traits in 32 soybean genotypes under control and drought conditions.

Trait	Treatment	SL (mm)	RFW (g/Plant)	SFW (g)	RDM (%)	SDM (%)
RL (mm)	Control	0.37 *	0.49 **	0.38 *	0.41 *	0.49 **
	Drought	0.23	0.57 **	0.43 *	0.42 *	0.48 **
SL (mm)	Control		0.33	0.59 **	0.28	0.60 **
	Drought		0.16	0.49 **	0.08	0.61 **
RFW (g/plant)	Control			0.75 **	0.94 **	0.79 **
	Drought			0.57 **	0.88 **	0.49 **
SFW (g/plant)	Control				0.71 **	0.95 **
	Drought				0.43 *	0.86 **
RDM (%)	Control					0.80 **
	Drought					0.41 *

Traits: root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry matter (RDM), shoot dry matter (SDM). *, ** Correlation coefficient significant at the 0.05 and 0.01 probability levels, respectively.

The exceptions were very strong correlations between RFW and RDM, and SFW and SDM, which were expected since dry matter content directly influences the fresh weight of both roots and shoots. The correlation coefficients between most traits were of similar magnitude under control and drought, except between RFW and SFW, RFW and SDM, SFW and RDM, and RDM and SDM, which were much higher in control compared to drought.

4. Discussion

Although many previous studies have investigated the drought tolerance of soybean at an early vegetative stage, only a few of them deal with soybean germplasm bred in Europe. In the present study, 32 soybean genotypes adapted to southeast European growing conditions were grown under control and PEG-induced drought conditions to investigate the variability of root and shoot traits of soybean at an early vegetative/seedling growth

stage. Significant effects of genotype (G) and treatment (T) were found for all 10 traits studied, while the $G \times T$ interaction was significant for all but three traits, SDM, RFW/SFW and RL/RFW. In a similar study by Dayoub et al. [13] in which 21 seedling traits were examined in 10 European-grown soybean cultivars under well-watered and water-stressed treatments, genotype and treatment effects were significant for 16 and 10 traits, respectively, while the $G \times T$ interaction was not significant for any of the traits. The lack of a significant $G \times T$ interaction for soybean seedling root traits was also reported in the study by Kakati et al. [24], which involved 41 soybean plant introductions from South Korea, China, Japan, Russia and North Korea. The lack of a $G \times T$ interaction in these two studies may be because both studies were terminated at a much earlier time point than the present study (10 and 14 days after planting, respectively, vs. 26 days after transplanting). In the present study, a wide range of variation among genotypes was observed for all seedling traits examined under both control and drought conditions. Broad sense heritability ranged from 0.53 (RL) to 0.97 (SL) under control conditions, while it ranged from 0.56 (RL/RFW) to 0.96 (SL) under drought conditions. The moderate to high heritability of the investigated traits observed in the present study suggests that their improvement through selection could be possible. Zhang et al. [8] reported a broad-sense heritability for soybean seedling traits ranging from 0.19 to 0.70 in the association panel of 259 Chinese soybean cultivars studied under the conditions of repeated drought stress.

In the present study, induced drought resulted in a reduction of mean root and shoot length by 11 and 17%, respectively, compared to the control. Thu et al. [20] also reported a greater inhibition of shoot length than root length in their study, which included 13 Vietnamese soybean cultivars. Dayoub et al. [13] observed no significant effect of water stress on total root length, but shoot length decreased by 16% under water stress compared to well-watered conditions. In contrast, Umburanas et al. [11] observed no significant effect of water availability on main root length and shoot length in four Brazilian soybean cultivars. Inhibition of shoot growth in response to water deficits may prolong the period of soil water availability and plant survival and can be considered an adaptive response [32]. When drought occurs under field conditions, the water reserves may be increasingly situated in deeper soil layers and the onset of more severe water stress could be delayed and plant survival enhanced by an improved ability to maintain root growth towards water in the deeper soil layers [32]. Therefore, cultivars that have longer roots under dry conditions would have better tolerance to water deficit than those with shorter roots. In the present study, the greater reduction in shoot than root length under drought was also reflected in a significant increase of root-to-shoot length ratio by 8%.

Dayoub et al. [13] observed a 6% reduction in shoot dry weight, whereas Umburanas et al. [11] found no significant difference in shoot biomass under drought conditions compared to the control. In the present study, the mean reduction in plant fresh weight due to drought was much higher reaching 38 and 34% for RFW and SFW, respectively.

In the study by Du et al. [22] drought stress significantly increased the root-to-shoot fresh weight ratio in seedlings of two soybean cultivars. On the contrary, in the study by Dayoub et al. [13], induced drought did not affect the root-to-shoot dry weight ratio in seedlings of 10 European-grown soybean cultivars. Although in the present study mean RFW/SFW was significantly reduced under drought by 4%, some genotypes like breeding lines 'OS-2' (26) and 'OS-3' (13) showed an increase of this ratio by 10 and 25%, respectively (Figure 3 and Table S1), which, according to Boote [25] is another aspect to be considered when selecting genotypes with efficient water uptake capability.

The stronger effect of drought on R/S length ratio than R/S fresh weight ratio observed in our study is reflected in root and shoot length-to-fresh weight ratios (RL/RFW and SL/SFW), which were also significantly increased by 45 and 28%, respectively, under drought conditions, with corresponding ranges of 9 to 79% and 5 to 55%. This could mean that the partition of assimilates in the studied soybean genotypes under drought stress was in both upward and downward directions, in favor of axial growth at the expense of lateral branching and biomass production and was more pronounced in roots than in shoots.

Zhan et al. [33] compared two groups of maize inbreds with contrasting phenotypes in terms of lateral root number and length under water stress and found that reduced lateral root branching density will improve drought tolerance in maize by reducing the metabolic costs of soil exploration, permitting greater axial root elongation, greater rooting depth and thereby greater water acquisition from drying soil. This phenomenon was also observed in soybean by Dayoub et al. [13]. They reported an increase in the rate of root penetration in depth by 5%, and, at the same time, a decrease in the rate of lateral root expansion by 48% under water-stressed conditions compared to well-watered conditions, which was also reflected in a 12% increase in root length per unit of root dry matter. Lynch [34] reported that a shallow basal root growth angle would reduce nitrate and water uptake, while a deeper basal root growth angle would improve it.

In the present study, root and shoot lengths were weakly correlated under control conditions (0.37 *) and no correlation was observed under drought conditions. On the contrary, much stronger correlations between root length and shoot length were reported by Dayoub et al. [13] under both control and drought conditions (0.84 ** and 0.65 *, respectively) as well as by Zhang et al. [8] under repeated drought conditions (0.70 **). In our study, the correlation between root and shoot fresh weights was strong (0.75 **) under control and moderate (0.57 **) under drought conditions. A similar correlation between these two traits was found by Zhang et al. [8] under drought conditions (0.76 **), while Dayoub et al. [13] found no correlation between shoot and root dry weight independently of the soil moisture status. The correlations between the examined traits in our study were generally weak to moderate, especially under drought conditions, which indicates that the studied traits could be improved by selection independently.

The investigation of soybean root and shoot traits at an early vegetative growth stage in a growth chamber conducted in the present study was relatively quick and manageable, confirming the hypothesis of Zhang et al. [8] and Dayoub et al. [13]. On the other hand, the use of PEG to simulate the drying soil conditions may have advantages and disadvantages. The use of PEG decreases the osmotic potential of the water solution, making water less available to the soybean seedlings, but in the present study the water solution was available to seedling roots in full root profile within the vermiculite-filled plastic tubes. In actual field conditions, under drought, the availability of water is higher in the deeper layers of the soil. Therefore, genotypes with longer roots which suppress lateral root branching should be better adapted to the drought conditions in the field. Therefore, the final testing of drought resistance at the early vegetative stage of the soybean genotypes should be performed under the field conditions or under conditions that better simulate actual field conditions.

5. Conclusions

We have identified several soybean genotypes that might be considered resistant to drought stress at an early vegetative stage. Two genotypes, 'Tisa' (12) and 'OS-8' (14), can be highlighted as genotypes that appeared among the top five ranked genotypes under drought conditions for RL, RFW and SFW. The genotypes 'Gabriela' (18) and 'Korana' (23) substantially increased RL/SL ratio by 43 and 32%, respectively, and the breeding lines 'OS-2' (26) and 'OS-3' (13) showed an increase in the RFW/SFW ratio by 10 and 25%, respectively. All these genotypes could therefore be considered as desirable ideotypes for future phenotyping and breeding efforts. The weak to moderate correlations between root and shoot traits observed in this study, especially under drought conditions, suggest that the studied traits could be improved independently by breeding. The results of our study could contribute to filling the identified knowledge gaps on drought tolerance at the early vegetative stage in European soybean germplasm.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14061188/s1>, Table S1: Means of 10 seedling traits for 32 soybean genotypes under control and drought conditions.

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