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Irrigation during Flowering Improves Subsoil Water Uptake and Grain Yield in Rainfed Soybean

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Abstract: Water is the main factor limiting soybean yield and the timely supply of supplemental irrigation could increase the grain yield, but the effects of a supplemental water supply on soybean yields have not been well studied. Field and pot experiments were conducted to compare the grain yield, yield components, water use efficiency for grain yield (WUE_G), flower number, filled-pod number, soil water content, and root dry weight at different depths with and without supplemental irrigation at flowering. Field experiments showed that compared to rainfed conditions, 40 mm of water applied during flowering significantly increased grain yield by 26%, WUE_G by 12%, filled-pod number by 16%, grain number by 13.3%, and water uptake from soil by 11% in 2011, and increased grain yield by 22%, WUE_G by 7%, filled-pod number by 26%, grain number by 27%, and water uptake by 21% in 2012. The soil water content in the subsoil (1.2–2.0 m) layers under the irrigated treatment was lower, indicating greater water extraction, than in the rainfed treatment and water uptake was significantly and positively correlated with yield in both years. In a pot experiment, flower and filled-pod number, water use during flowering and podding were significantly higher in the well-watered (WW) treatment than cyclic water stress (WS) treatment. Flower number and filled-pod number were significantly and positively correlated with water use during flowering and podding, respectively, under both the WW and WS treatments. The root dry weight was higher in the 0.2–0.8 m soil layer in the WW treatment than the WS treatment. We conclude that supplementary water at flowering increased the water uptake from deeper soil layers by increasing the distribution of roots in the subsoil layers that resulted in the production of more flowers and filled pods and increased the WUE_G and grain yield.

Keywords: water supply; water uptake; root distribution; grain yield

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the 10 most widely grown crops and is a major source of protein for humans and animals. Drought stress is a major constraint on the production and yield stability of soybean [1], as shown by field and greenhouse studies in which soybean seed yield was reduced by 24–50% under drought stress [2,3]. A better understanding of the physiological basis for yield in relation to water supply will help identify targets for future soybean improvement in water-limited environments [4].

In arid and semi-arid areas, water is the main factor restricting grain yield. Previous studies in a range of crop species showed that water shortage during the flower and pod production stages led to significantly reduced grain yield by reducing the flower and pod number while increasing flower and pod abortion [5–7]. Irrigation is the most efficienct way to increase water use and irrigation at a strategic stage such as flowering can increase the grain yield by improving the grain number or grain size in wheat [8–11]. Irrigation significantly affected the root density, depth, and activity during the winter wheat growing season [8,12–15]. Irrigation can increase wheat root growth [9]; an increase in root weight density (RWD) in the 0–1 m soil layer facilitated root growth in deep soil layers [15]. While irrigation promoted root penetration, increased root density, increased the utilization of water from deep soil, and increased the yield of wheat [16,17], the effect of irrigation on root weight and soil water uptake from different soil depths in soybean grown in arid and semi-arid areas is not known.

The root is the main organ for water uptake. Faster water use induced by the increase in the growth rate of soybean roots had a negative effect on soybean yield under water stress due to the early extraction of water, leaving less for uptake during reproductive development and growth [18]. However, other studies have shown that extensive root development contributed to seed yield under terminal drought conditions by improving the extraction of available soil water [19–22]. Thus, the role of roots in water uptake and drought resistance is quite variable and requires clarification [23]. In the field, getting accurate water use data is difficult, but using large PVC tubes filled with undisturbed or repacked field soil to mimic a field soil profile can be used to determine the water use throughout the whole life cycle [24,25]. Data on the vertical distribution of roots at different soil depths can also be obtained by using large PVC tubes. We used large PVC tubes to determine water use from sowing to maturity, the distribution of roots and their role in water extraction in soybean.

In northwest China, rainfall is limited during the cropping season, and grain yield is dependent on the efficient use of precipitation and the water stored in the soil. This is true for soybean, but if available, one irrigation at flowering is imposed. In the present study, six soybean genotypes (four traditional landraces, and two cultivars released after 2000) were used to investigate the effect of irrigation during flowering on the water uptake from different soil depths and yield performances in the field during two growing seasons. Previous research had shown that the landraces yielded about 60% of that of the modern cultivars under rainfed conditions in the field in northwest China [7,26]. Additionally, four soybean genotypes (two traditional landraces, and two cultivars released after 2000) were used in a lysimeter system in a rainout shelter to investigate the effect at maturity of two water treatments, well-watered and cylic water stress, on root dry weight at different soil depths and the relationship between the flower number, filled-pod number, water use during flowering and during podding, and yield. The hypotheses tested were: (i) soybean yield is correlated with growing-season water use, (ii) water uptake is closely associated with root distribution in different soil layers, and (iii) irrigation at flowering will benefit yield more in modern genotypes than landraces by increasing the number of flowers and filled pods.

2. Materials and Methods

2.1. Field Experiment

Six soybean (*Glycine max* (L.) Merr.) genotypes [four landraces (Yuanhuangdou (YHD), Huangsedadou (HD), Longxixiaohuangpi (LX) and Yiwofeng (YWF)) grown by farmers in semi-arid areas of Gansu Province, China, for many generations, and two cultivars released in 2003 (Jindou 19 (JD)) and 2006 (Zhonghuang 30 (ZH)], were used in this study (Table 1). The Chinese Academy of Agricultural Sciences (YHD, HD, LX, and YWF) and Shanxi Academy of Agricultural Sciences (ZH and JD) provided the seeds.

Two field experiments were conducted during the growing season (April–October) of successive years, 2011 and 2012, at the Yuzhong Experiment Station of Lanzhou University in Yuzhong County, Gansu Province (35°51′ N, 104°07′ E, altitude 1620 m). The loess soil at the experimental site is classified as an Orthic Entisol with a field capacity of 27% by weight and a bulk density of 1.32 g cm^{-3} [27]. From 2003 to 2012, the average annual precipitation and temperature was 332 mm and 7.4 °C respectively;

the maximum and minimum mean temperatures were 20.7 °C in July and −7.2 °C in December, respectively. The two field experiments with the six cultivars and two water treatments, rainfed and irrigated with 40 mm of water at flowering, were conducted from 14 April to 13 October 2011 and 14 April to 6 October 2012 to determine the effects of irrigation with 40 mm of water at flowering on the grain yield, yield components and water uptake from different soil depths. The experiment had two blocks, irrigated and rainfed, separated by a distance of 2 m. The six cultivars were randomized within three replicate subplots within each block. In each water treatment (block) were 6 genotypes and three replicates ((6 × 3 = 18 plots) × 2 blocks = 36 plots). The plots were 4 m long and 5 m wide (0.5 m between plots), with 11 rows per plot (0.4 m between rows) and planted at a density of 18 plants m^{-2}. 50 kg N ha^{-1}, and 80 kg P ha^{-1} were applied before sowing. The water treatments were: (1) irrigated (IR): 40 mm water applied by flood irrigation when all genotypes had begun to flower [91 days after sowing (DAS) in 2011 and 95 DAS in 2012)]; (2) rainfed (RF): no water applied at flowering, dependent only on rainfall. The amount of water applied was measured with a flow meter. Weeds were controlled by hand; insecticides and fungicides were used to prevent insect and disease damage. Air temperature and precipitation were measured at the Yuzhong County National Meteorological Information Centre located 7.4 km from the experimental site.

Table 1. The principal characteristics and year when they were released of the six soybean genotypes used in this study.

Genotypes	Location	Year of Release	Still Grown by Farmers?
Yuanhuangdou (YHD)	Gansu	Landrace	No
Huangsedadou (HD)	Gansu	Landrace	No
Longxixiaohuangpi (LX)	Gansu	Landrace	No
Yiwofeng (YWF)	Gansu	Landrace	No
Jindou 19 (J19)	Shanxi	2003	Yes
Zhonghuang 30 (ZH)	Beijing	2006	Yes

At physiological maturity (165–182 DAS), the plants in a 2 m × 2 m area within each plot were harvested, the pods removed, dried at 80 °C for 72 h and weighed. The grain number and oven-dry grain weight were determined. Hundred grain weight (HGW) was calculated from grain weight/grain number. The soil water content of each plot was measured before sowing and at maturity. A drill was used to collect the soil from 0 to 2 m soil depth at 0.2 m intervals and the soil water content (SWC) was measured gravimetrically after oven drying at 80 °C for 72 h. The gravimetric SWC was converted to volumetric SWC using the bulk density.

There were no heavy rains, no run-off or run-on, no overland flow or water logging events during the growing season, and deep drainage and capillary rise were assumed to be insignificant. Thus, the followed equation was used to calculate the seasonal evapotranspiration (ET, mm):

$$ET = (P + I) - \Delta W \quad (1)$$

where P is precipitation, I is irrigation, and ΔW is the change in stored soil water in the soil profile which was calculated as the difference in volumetric SWC between sowing and maturity. Water use efficiency for grain yield (WUE_G, g mm^{-1}) was calculated as the ratio between the grain yield (GY, g m^{-2}) and evapotranspiration (mm) from sowing to maturity:

$$WUE_G = GY/ET \quad (2)$$

2.2. Pot Experiment

A pot experiment with four of the six soybean genotypes (two landraces, HD and LX, that showed similar and consistent differences in HGW and grain yield from the two cultivars, ZH and JD, in both field seasons), each with four replicates, was conducted to investigate the response to water shortage on grain yield, yield components, water use, WUE_G, root dry weight distribution and the relationship between water use during flowering and podding and the flower and pod number. The genotypes were grown from 17 April to 19 September 2014 in PVC cylinders (0.16 m diameter and 1.33 m long, the soil depth was 1.2 m) which contained 25.7 kg of sieved loess soil-based substrate (loess soil:vermiculite (*v*:*v*) = 3:1) in an open rainout shelter that was closed during rain events. The loess (Orthic Entisol) soil was obtained from the site of the field experiments. One transparent polyethylene sleeve (length: 1.4 m; width: 0.26 m; thickness: 101 μm) was placed inside each pot before filling to facilitate removing the contents of the cylinders at harvest. 27.9 μg K g^{-1}, 22.1 μg P g^{-1}, and 188 μg N g^{-1} dry soil was applied to each pot as NH_4NO_3 and KH_2PO_4 before sowing. Two seeds were sown in each pot, but reduced to one after germination. Soil evaporation was prevented by black plastic film placed on the top of the soil. A balance with 2 g accuracy (TCS-100, Jieli Weighing Apparatus Co., Ltd., Yongkang, China) was used to weigh and maintain the SWC between 85–100% pot capacity (PC). 100% PC was determined by fully wetting the soil profile until free draining and then weighing after being allowed to drain for 48 h [28].

The development stages of soybean were recorded according to Fehr et al. [29]. At the vegetative stage (5–6 trifoliate leaves, 40 DAS), two water treatments were imposed: (i) well-watered (WW) in which the SWC was maintained between 85% and 100% PC; and (ii) cyclical water stress (WS) in which water was withheld until the SWC decreased to 30% PC, re-watered to 100% PC and the SWC allowed to decrease again, as proposed by Turner [28]. This was repeated for 2–3 cycles until physiological maturity. The four genotypes and two water treatments were completely randomised (4 genotypes × 2 water treatments × 4 replicates = 32 cylinders). The following parameters were determined:

2.3. Water Use

In the WW treatment, all plants were weighed and watered every 4 days to maintain the SWC between 85% and 100% PC until physiology maturity (136–147 DAS) [29]. Every 4–8 days, the plants in the WS treatment were weighed as the SWC decreased and re-watered when the SWC reached 30% FC. This was repeated 2 to 3 times until maturity. Adding together the water used between sowing and physiological maturity gives the water use over the whole life cycle. Water use from the first flower to the last flower and from first the pod to the last pod was also calculated for each plant.

2.4. Tagging Flowers and Pods

The date that each plant produced a first flower was noted and then every 2 days each new flower was tagged with a dated tag and the date at which the flower developed into a visible pod was also written on the tag so that the flowering period and podding periods could be identified and the number of flowers and total number of pods produced during the reproductive stage could be measured. The tagged-pod number is the number of pods with a dated tag.

2.5. Grain Yield, Yield Components and Root Distribution with Soil Depth at Maturity

At physiology maturity, the whole plant in each cylinder was harvested at ~10 mm from the soil surface, and then divided into pods and stems and dried for 72 h in an oven at 80 °C before weighing. The pods with seeds were counted and defined as filled-pod number. The grain number was counted for each plant and weighed to determine the grain yield for each plant. The leaves were collected for each plant and dried for 72 h in an oven at 80 °C and weighed. The following variables were calculated: aboveground biomass (dry weight, g $plant^{-1}$) = leaf dry weight + pod (including seeds) dry weight + stem dry weight; hundred-grain weight (HGW, g) = (grain yield × 100)/grain number); water use efficiency for grain yield (WUE_G, g L^{-1}) = grain yield/water use from sowing to maturity.

The root dry weight at different soil depths was determined. After removing the shoot, the polyethylene sleeve from each cylinder was removed and divided into 0.2 m segments from the surface to 1.2 m soil depth. The root segments were washed over a 0.2-mm sieve, and the roots collected, placed in an envelope and dried for 72 h in an oven at 80 °C and weighed. Root dry weight was determined by adding up the root dry weight for each segment in each cylinder.

2.6. Statistical Analysis

The data are the means of three and four replicates in the field and pot experiments, respectively. In the field experiment, the data were analyzed in GenStat 17th Edition (VInternational Ltd., Rothamsted, England) using the general analysis of variance (ANOVA); the treatment structure consisted of main effects of water (W) treatment (rainfed and irrigated), year (Y), and genotype (G). The blocking structure was included in the analysis by accounting for the replication within each water treatment. In the pot experiment, the data were analyzed by a two-way analysis of variance (ANOVA) in GenStat 17th Edition, where water treatment (W) and genotype (G) were used as fixed factors and the random factor was block (replicate). Means were compared by LSD at $p = 0.05$.

3. Results

3.1. Weather Conditions for Field Experiments in 2011 and 2012

In 2011, the precipitation was 241 mm during the growing season (April–October), considerably below the long-term (2003–2012) average of 326 mm for the same period of the year, and the precipitation in 2012 was 343 mm, above the long-term average (Figure 1). The average air temperatures for the 2011 and 2012 growing seasons were 14.6 °C and 14.4 °C, respectively, both below the long-term (2003–2012) average of 16.9 °C (Figure 1).

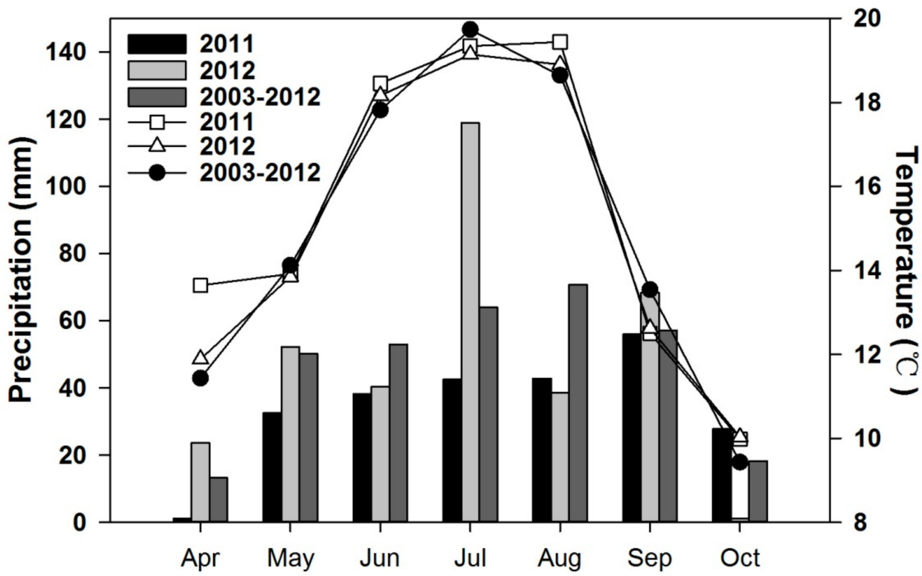

Figure 1. The monthly mean precipitation (mm) and mean temperature (°C) in 2011, 2012 and 2003–2012 during the growing season (April to October) at the National Meteorological Centre weather station in Yuzhong County, 7.4 km from the experimental site.

3.2. The Effect of Water Supply during Flowering on Grain Yield and Yield Components in the Field Experiment

Irrigation (IR, 40 mm supplied at flowering) significantly increased the grain yield, filled-pod number, grain number, hundred-grain weight and water use efficiency for grain yield (WUE_G) (Table 2). The grain yield of the six soybean genotypes varied between 135 and 228 g m^{-2} and 162 and 255 g m^{-2} in 2011 and 2012, respectively, under rainfed conditions, and between 180 and 298 g m^{-2} and 206 and 316 g m^{-2} with IR in 2011 and 2012, respectively; the soybean cultivar Jindou 19 (JD) had the highest, and landrace Yiwofeng (YWF) had the lowest grain yield in all treatment combinations in 2011, while the soybean cultivar JD had the highest, and the landrace Longxixiaohuangpi (LX) had the lowest grain yield in all treatment combinations in 2012 (Table 2). Irrigation increased the mean grain yield over all genotypes by 26% in 2011 and 22% in 2012. In 2011 IR increased the grain yield of the four landraces by an average of 21% and the two new cultivars by 36%, while in 2012 the irrigation increased the grain yield of the landraces by 19% and the cultivars by 27% (Table 2).

Table 2. Filled-pod number (m^{-2}), grain number (m^{-2}), hundred-grain weight (HGW, g), grain yield ($g\ m^{-2}$), seasonal evapotranspiration (ET, mm) and water use efficiency for grain yield (WUE_G, $g\ mm^{-1}$) for the four landraces (Yuanhuangdou (YHD), Huangsedadou (HD), Longxixiaohuangpi (LX) and Yiwofeng (YWF)) and two recent cultivars (Jindou 19 (JD) and Zhonghuang 30 (ZH) (**bold**)) with (i) 40 mm of irrigation at flowering, and (ii) rainfed conditions in the field in 2011 and 2012. The LSD values are given in parenthesis with level of significance: n.s not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Year (Y)	Water Treatment (W)	Genotype (G)	Filled-Pod Number	Grain Number	HGWx	Grain Yield	ET	WUE_G
2011	Irrigated	YHD	1111	1830	11.3	207	358	0.58
		HD	1299	2418	8.4	204	336	0.61
		LX	1331	2557	7.2	182	326	0.56
		YWF	754	1484	12.1	180	317	0.57
		ZH	**806**	**1708**	**17.3**	**296**	**322**	**0.92**
		JD	**795**	**1623**	**18.4**	**298**	**324**	**0.92**
	Rainfed	YHD	1075	1841	9.9	181	312	0.58
		HD	1150	2302	7.4	171	291	0.59
		LX	1095	2051	6.6	135	280	0.48
		YWF	740.	1545	10.2	158	273	0.46
		ZH	**610**	**1301**	**16.2**	**210**	**271**	**0.77**
		JD	**648**	**1369**	**16.7**	**228**	**268**	**0.85**
2012	Irrigated	YHD	1612	2157	10.4	225	458	0.49
		HD	1514	2556	8.9	228	449	0.51
		LX	1484	2975	6.9	206	447	0.46
		YWF	1070	1917	12.1	232	436	0.53
		ZH	**861**	**1861**	**17.0**	**307**	**429**	**0.74**
		JD	**839**	**1783**	**17.2**	**316**	**425**	**0.72**
	Rainfed	YHD	1467	1818	10.2	185	403	0.46
		HD	887	2152	9.1	196	402	0.49
		LX	983	1833	8.8	162	396	0.41
		YWF	872	1629	12.8	208	381	0.54
		ZH	**681**	**1465**	**16.2**	**237**	**363**	**0.65**
		JD	**636**	**1504**	**17.0**	**255**	**370**	**0.68**
LSD	W		*** (50)	*** (82)	** (0.4)	*** (13)	*** (2.3)	*** (0.0)2)
	Y		*** (50)	*** (82)	* (0.4)	*** (13)	*** (2.3)	*** (0.0)2)
	G		*** (86)	*** (143)	*** (0.7)	*** (23)	*** (4.1)	*** (0.0)3)
	Y × W		* (70)	** (117)	*** (0.5)	n.s	*** (3.3)	n.s
	Y × G		*** (121)	* (202)	** (0.9)	*** (31)	*** (5.8)	*** (0.0)5)
	W × G		*** (121)	*** (202)	n.s	*** (31)	*** (5.8)	* (0.05)
	Y × G × W		** (172)	n.s	n.s	n.s	n.s	n.s

The soybean cultivars had notably larger seeds at 170 mg $seed^{-1}$ (hundred grain weight (HGW) = 17 g) than the landraces at 95 mg $seed^{-1}$ (HGW = 9.5 g), but irrigation had minimal effect (3–6% increase) on seed size; JD had the highest and LX had the lowest HGW in all treatment combinations in 2011 and 2012 (Table 2). Irrigation increased the water use efficiency for grain (WUE_G) by 12.2% and 7.4% compared to the rainfed plots in 2011 and 2012, respectively; the two soybean cultivars ZH and JD had significantly higher WUE_G than the four landraces in all treatments in 2011and 2012 (Table 2). The filled-pod number and grain number with IR were 16% and 13% higher than in the rainfed plots in 2011, and 26% and 27% higher than in the rainfed plots in 2012, respectively; the landrace HD had the highest grain number in the rainfed treatment and LX in the irrigated treatment in 2011 and 2012 (Table 2).

3.3. The Effect of Water Supply at Flowering on Water Use and the Water Uptake at Different Soil Depths in the Field

The 40 mm of irrigation supplied at flowering significantly increased ET by 17% in 2011 and 14% in 2012, respectedly, even though seasonal ET was significantly higher in the wetter 2012 than drier 2011 (Table 2). The genotypes varied significantly in ET in both years; the four landraces had higher ET on average than the two cultivars in both years and both water treatments (Table 2).

The changes of SWC at different soil depths in both the rainfed and irrigated plots are shown in Figures 2 and 3. The SWC in the subsoil (1.2–2.0 m) was lower with irrigation than in the rainfed plots in both 2011 (Figure 2) and 2012 (Figure 3). Interestingly, the extraction of water from the soil below 1.2 m was greater in the wetter year (2012) than drier year (2011). As expected from the ET values, water uptake from the soil was higher in the four landrace genotypes than the two cultivars in both years (on average: 62.8 mm in the landraces vs. 53.3 mm in the cultivars), and irrigation increased the water uptake from the soil in all genotypes (Figures 2 and 3). This was particularly the case from depths below 1.0 to 1.2 m and particularly in the modern cultivars (Figures 2 and 3). For example, the two cultivars ZH and JD had the greatest difference in water uptake at depth between irrigated and rainfed conditions in both 2011 (Figure 2) and 2012 (Figure 3). The difference in water uptake between the irrigated and rainfed plants was positively correlated with the increase in yield between irrigated and rainfed conditions in both 2011 and 2012 (Figure 4); however, irrigation had a greater impact on yield (slope in Figure 4) in the drier year 2011 than the wetter year 2012 (Figure 4).

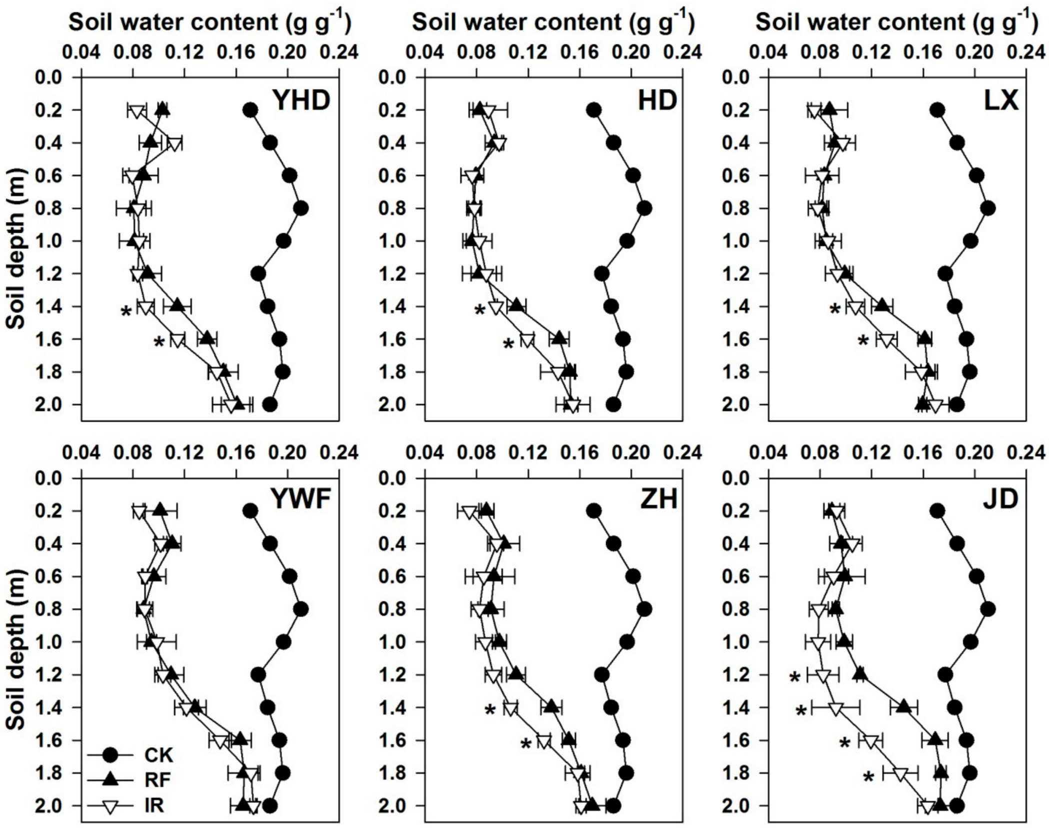

Figure 2. The soil profile of gravimetric soil water content (g g^{-1}) at 0.2 m increments in the upper 2 m of the soil profile of six soybean genotypes (four landraces: Yuanhuangdou (YHD), Huangsedadou (HD), Longxixiaohuangpi (LX) and Yiwofeng (YWF) and two recent cultivars: Jindou 19 (JD) and Zhonghuang 30 (ZH)) before sowing (CK) and at maturity under two water regimes (rainfed (RF): no water applied during flowering) and irrigated (IR): 40 mm water applied at flowering) in the field experiment in 2011. The bars indicate one standard error of the mean (n = 3). The * indicates that the SWC was significantly different (p = 0.05) between the IR and RF at the same soil depth.

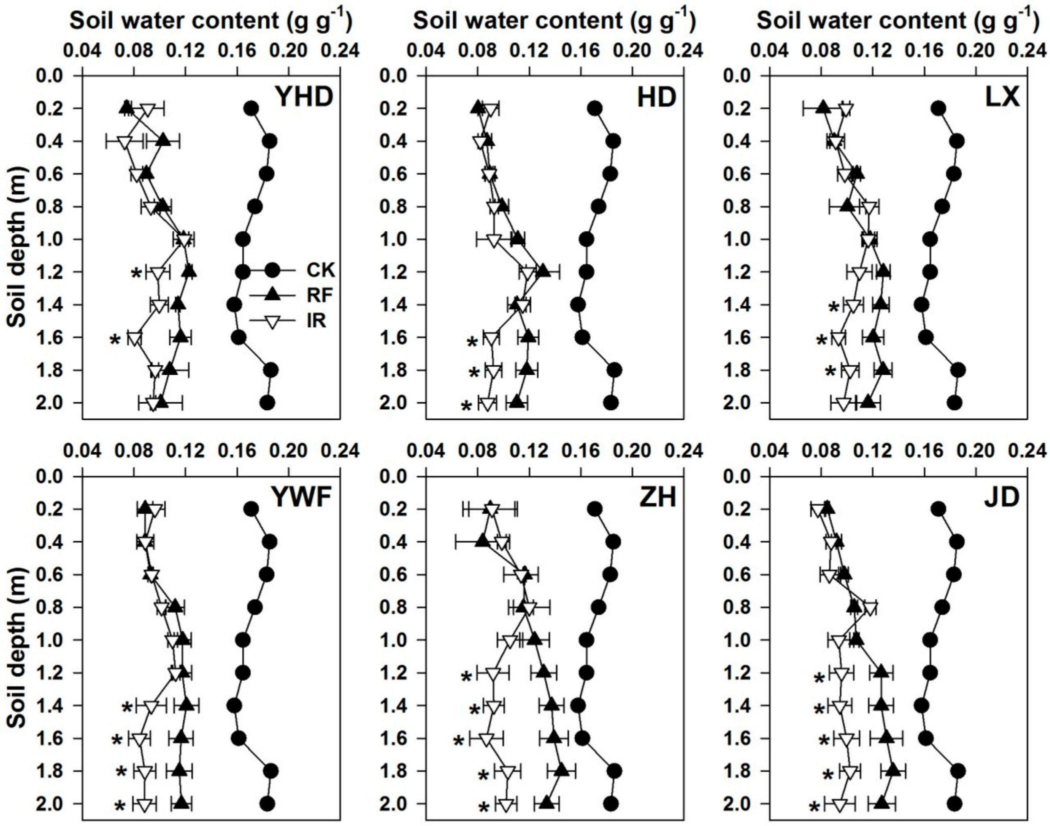

Figure 3. The soil profile of gravimetric soil water content (g g^{-1}) at 0.2 m increments in the upper 2 m of the soil profile of six soybean genotypes (four landraces: Yuanhuangdou (YHD), Huangsedadou (HD), Longxixiaohuangpi (LX) and Yiwofeng (YWF) and two recent cultivars: Jindou 19 (JD) and Zhonghuang 30 (ZH)) before sowing (CK) and at maturity under two water regimes (rainfed (RF): no water applied during flowering and irrigated (IR): 40 mm water applied during flowering) in the field experiment in 2012. The bars indicate one standard error of the mean (n = 3). The * indicates that the SWC was significantly different (p = 0.05) between the IR and RF at the same soil depth.

3.4. Grain Yield, Yield Components, Cumulative Biomass and Water Use, Flower and Tagged-Pod Number under WW and WS in the Pot Experiment

In the pot experiment, the cyclic water stress (WS) significantly reduced the root biomass, aboveground biomass, filled-pod number, HGW and grain yield (Table 3). The two new soybean cultivars had a significantly lower root dry weight, but had significantly higher HGW (seed size), than the landraces HD and LX in the WW and WS treatments (Table 3); for the aboveground biomass, filled-pod number, and grain yield, the effect of the water stress treatment was much larger in the landraces than in the new cultivars (Table 3). WS significantly reduced water use; the cumulative water use in the two soybean landraces, HD and LX, was higher than the two new soybean cultivars, ZH and JD, in both the WW and WS treatments (Figure 5).

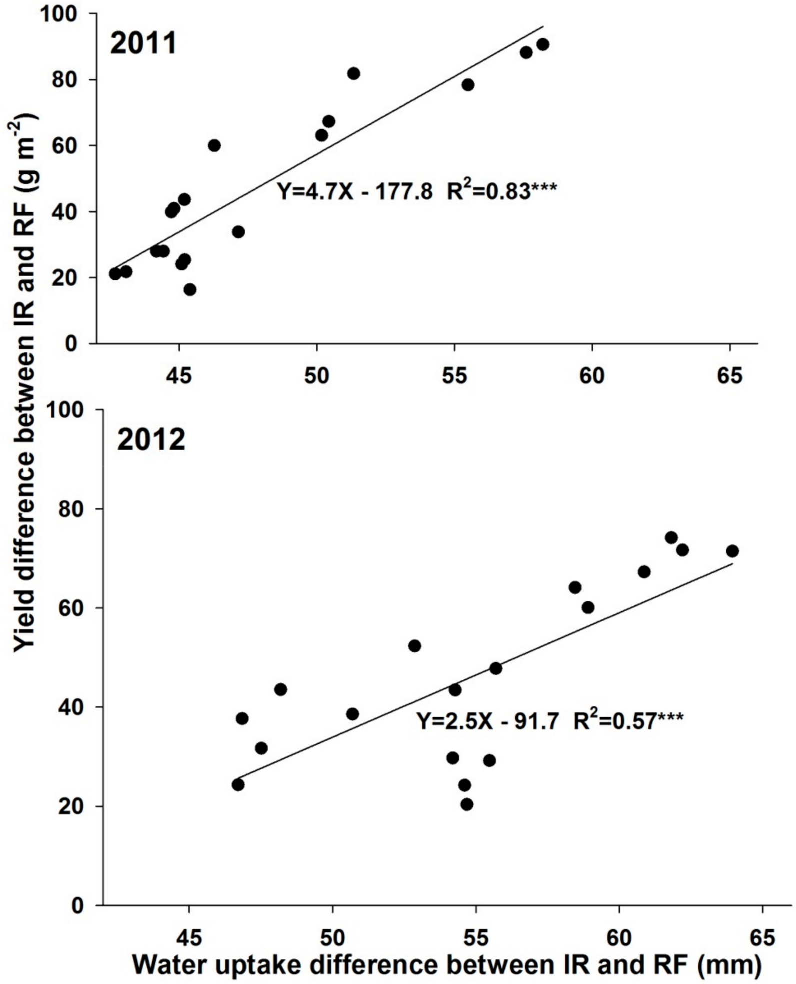

Figure 4. The relationship between the difference of yield and the difference in water uptake between the irrigated (IR: 40 mm water applied at flowering) and rainfed (RF: no water applied at flowering) in the field experiments in 2011 and 2012. The parameters of the fitted linear regression and its significance are shown: *** $p < 0.001$.

Table 3. The root biomass (g plant^{-1}), aboveground biomass (g plant^{-1}), filled-pod number (plant^{-1}), grain number (plant^{-1}), hundred-grain weight (HGW, g), grain yield (g plant^{-1}), and water use efficiency for grain yield (WUE_G, g L^{-1}) for the four soybean genotypes (two landraces: Huangsedadou (HD) and Longxixiaohuangpi (LX) and two recent cultivars: Zhonghuang 30 (ZH) and Jindou 19 (JD) (**bold**)) under well-watered (WW) and cyclical water stress (WS) in a pot experiment in 2014. The LSD values are given in parenthesis with level of significance: n.s not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Water Treatment (W)	Genotype (G)	Root Biomass	Aboveground Biomass	Filled-Pod Number	Grain Number	HGW	Grain Yield	WUE_G
WW	HD	20.6	83.7	128	186	8.2	15.2	0.31
	LX	16.6	80.1	147	217	7.3	16.0	0.32
	ZH	**8.2**	**58.6**	**52**	**111**	**15.1**	**16.6**	**0.70**
	JD	**11.1**	**60.5**	**50**	**112**	**17.6**	**19.6**	**0.63**
WS	HD	10.7	42.2	78	103	7.0	7.2	0.36
	LX	9.8	42.2	56	56	5.9	3.3	0.15
	ZH	**6.2**	**40.5**	**48**	**99**	**13.8**	**13.5**	**0.82**
	JD	**7.5**	**39.7**	**50**	**80**	**13.7**	**11.0**	**0.63**
LSD	W	*** (2.6)	*** (6.5)	*** (12)	*** (14)	*** (0.9)	*** (1.2)	n.s
	G	*** (3.7)	** (9.2)	*** (17)	*** (20)	*** (1.3)	*** (1.7)	*** (0.09)
	W × G	n.s	* (13.1)	*** (24)	*** (28)	n.s	***(2.4)	* (0.13)

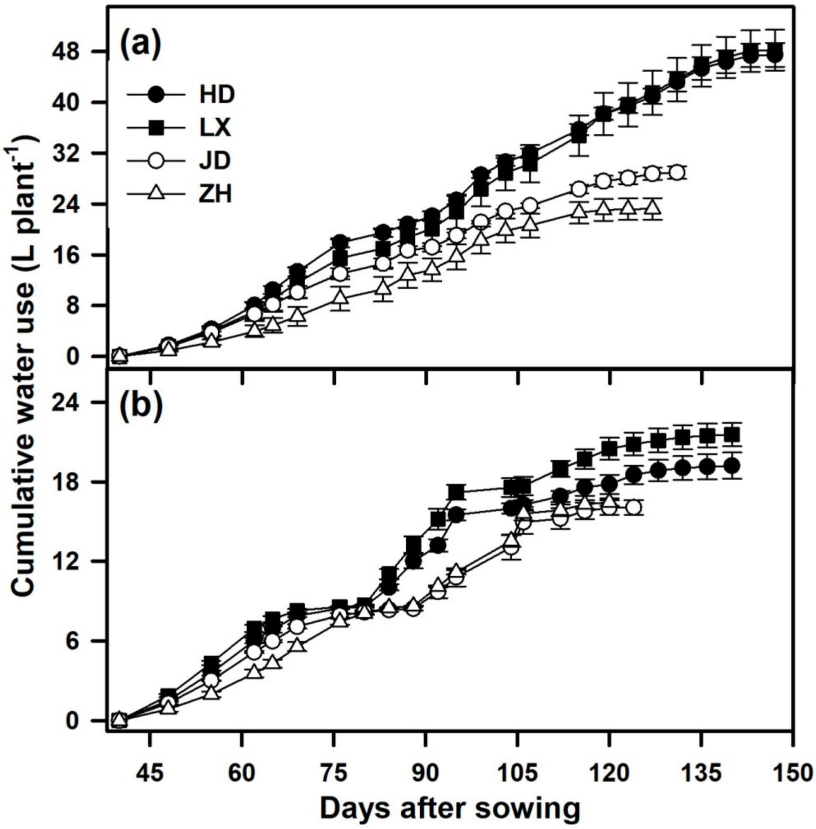

Figure 5. Cumulative water use with time in four soybean genotypes (two landraces: Huangsedadou (HD), Longxixiaohuangpi (LX), and two recent cultivars: Jindou 19 (JD) and Zhonghuang (ZH)) when (**a**) well-watered (WW) and (**b**) with cyclical water stress (WS) imposed from 40 days after sowing in the pot experiment in 2014. Note the differences in the *y*-axes of (**a**,**b**). The bars indicate one standard error of the mean ($n = 4$).

Water stress significantly reduced the flower number, tagged-pod number, filled-pod number and water use during flowering and podding; the two soybean landraces had a significantly higher flower number, tagged-pod number, filled-pod number and water use during flowering and podding than the two soybean cultivars ZH and JD under WW and WS conditions; moreover, the flower number was positively correlated with water use during flowering and filled-pod number was positively correlated with water use during podding in both water regimes, but all genotypes produced more flowers and filled pods per liter of water in the WS than WW treatment, but with low values of water use (Figure 6).

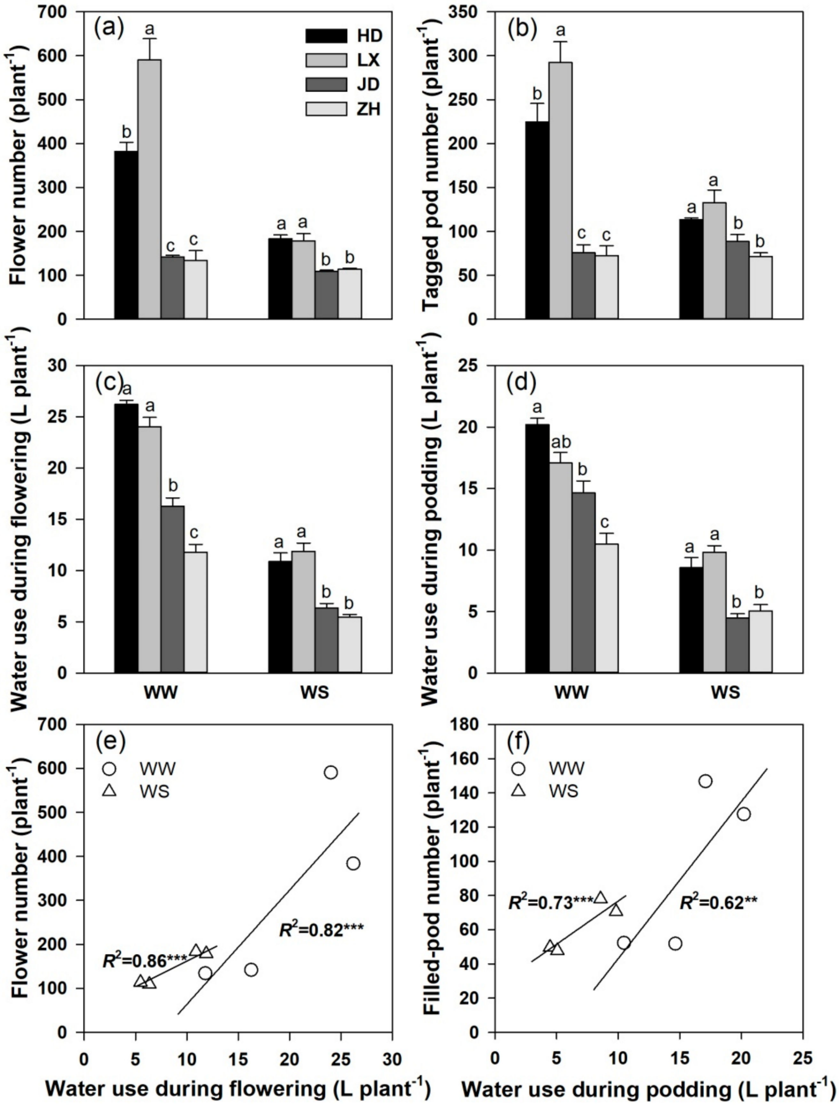

Figure 6. Flower number (**a**), tagged-pod number (**b**), water use during flowering (**c**), water use during podding (**d**), the relationship between water use during flowering and flower number (**e**), and the relationship between water use during podding and filled-pod number (**f**) for four soybean genotypes (two landraces: Huangsedadou (HD), Longxixiaohuangpi (LX), and two recent cultivars: Jindou 19 (JD) and Zhonghuang (ZH)) under well-watered (WW) and cyclical water stress (WS) imposed from 40 days after sowing in the pot experiment in 2014. Note changes in the *y*-axes in each part and the changes in the *x*-axes in (**e**,**f**). A number of tagged pods did not produce seeds so that filled-pod numbers were smaller than tagged-pod numbers. The bars indicate one standard error of the mean ($n = 4$). Means (**a**–**d**) in the same panel with different letters differ significantly at $p = 0.05$. The regression coefficients (R^2) of the fitted linear regressions in (**e**,**f**) are shown: ** $p < 0.01$, *** $p < 0.001$.

3.5. Root Dry Weight at Maturity under WW and WS in the Pot Experiment

The root dry weight at different soil depths was compared in the four soybean genotypes at maturity. Water stress reduced the root dry weight in all four soybean genotypes; the two landraces had significantly higher root dry weights in 0–0.6 m and 0–0.4 m soil depth than the two new cultivars in the WW and WS treatments, respectively (Figure 7).

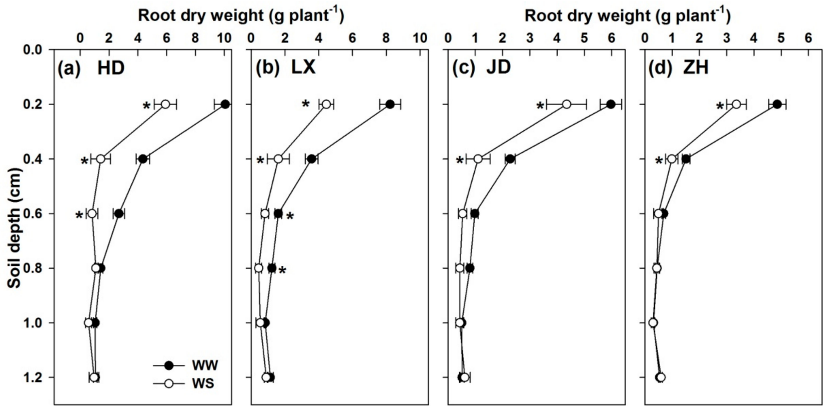

Figure 7. Root dry weight distribution at different soil depths in four soybean genotypes (two landraces: Huangsedadou (HD), Longxixiaohuangpi (LX), and two recent cultivars: Jindou 19 (JD) and Zhonghuang (ZH)) under well-watered (WW) and cyclical water stress (WS) imposed from 40 days after sowing in the pot experiment in 2014. Note the changes in the x-axes of (**a**,**b**) from (**c**,**d**). The bars indicate one standard error of the mean (n = 4). The * indicates that the root dry weights were significantly different (p = 0.05) between the WW and WS treatments at the same soil depth.

4. Discussion

Timely irrigation is well known to increase yields in wheat [8,30–32] and cool-season legumes [33]. On average, each additional 1 mm of irrigation water increased the grain yield by 0.63, 0.83, 1.17, 0.55, 2.14 and 1.77 g m^{-2} in YHD, HD, LX, YWF, ZH and JD in the drier year of 2011, and 1.01, 0.78, 1.12, 0.61, 1.75 and 1.53 g m^{-2} in YHD, HD, LX, YWF, ZH and JD in the wetter year of 2012, respectively. In other grain legumes, Leport et al. [33] found that 1 mm of water applied increased the grain yield by 1.74, 0.84, 1.34, 0.28, 0.76, 0.98 g m^{-2} (on average) in faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Med.), grass pea (*Lathyrus sativus* L.), chickpea (*Cicer arietinum* L.) and white lupin (*Lupinus albus* L.), respectively. Thus the inter-species genetic variation of the grain yield response to water supply may be associated with different water use strategies [34], and we found that the two new soybean cultivars ZH and JD had a significantly higher response of grain yield per mm of water supplied. This may be the result of breeding for greater dry matter partitioning to grain in soybean [35]. Further, the greater increase of grain yield per mm of water use in the two new soybean cultivars indicated that these new soybean cultivars had higher efficiency of water use which is the main target for yield improvement in water-limited environments [36].

In both years, the increase in yield was linearly correlated with the increase in the water extracted from the soil (Figure 4), which supports hypothesis (i) that 'soybean yield is correlated with seasonal water use'. Increasing the subsoil water uptake from soil at the reproductive stage increases the grain yield in wheat [37,38]. Interestingly, the increased uptake (45–60 mm) was more than the water applied (40 mm), indicating that the irrigation stimulated greater water uptake from the profile, presumably as a result of more vigorous growth and greater podding. Moreover, the additional irrigation water and the additional water uptake that it stimulated was more efficiently used to increase the grain yield in the new cultivars (Table 2). Under rainfed conditions, WUE_G based on seasonal ET in the four landraces was 0.53 and 0.48 g mm^{-1} in 2011 and 2012, and in the two new cultivars, it was 0.81 and 0.67g mm^{-1} in 2011 and 2012, respectively. In the irrigated plots, the WUE_G increased slightly in all cases so that the values were 0.58, 0.50, 0.92 and 0.73 g mm^{-1} in the landraces in 2011 and 2012 and the new cultivars in 2011 and 2012, respectively. The mean efficiency of the additional water uptake as a result of irrigation in the production of grain yield was 0.70 and 0.68 g m^{-2} mm^{-1} in the four landraces in 2011 and 2012, respectively, and 1.44 and 1.08 g m^{-2} mm^{-1} in the two new cultivars in the same years. Clearly, the water uptake stimulated by the single irrigation at flowering was used more efficiently than that from natural precipitation, presumably because it stimulated uptake during the development of the reproductive organs and grain filling.

The supplemental water resulted in the plants extracting more water from the deeper soils layer below 1 m and this was also observed in wheat (30–32). While we did not measure the SWC immediately after irrigation, it is unlikely that the irrigation water penetrated below 1 m in the loess soil, so the water extracted from depth presumably was the result of greater root proliferation at depth to enable greater exploitation by the plants of the water in the deeper soil layers. It is notable that more soil water was extracted at depth in all genotypes in the wetter year of 2012 than the drier year of 2011. This suggests that not only was overall growth and ET higher in the wetter year (2012) than drier year (2011), but this was accompanied by greater root growth and activity at depth in the profile with the SWC below 1 m being lower at maturity, particularly with supplemental irrigation, in the wetter year than in the drier year (Figures 2 and 3). The pot experiment with 1.33 m PVC cylinders (1.2 m soil depth) were used to try and mimic the soil in field. The results from the pot experiment show that the root dry weight in the WW treatment was significantly higher than in the cyclical stress treatment (WS). HD had higher root biomass at 0.2–0.6 m soil depth, LX at 0.2–0.8 m soil depth, and JD and ZH at 0.2–0.4 m soil depth in the WW treatment than the WS treatment (Figure 7). While the watering regimes were different between the field and pot study, particularly in the WW treatment, the pot study suggests that the additional water from the irrigation resulted in greater root biomass in the field study, thereby enabling greater water extraction at depth. This supports the hypothesis (ii) that 'water uptake is closely associated with root distribution in different soil layers', but this needs to be verified by measuring the root distribution in the field with and without supplemental irrigation.

In the field and pot experiments, the increase in the grain yield in soybean was mainly the result of an increase of both grain size (hundred grain weight) and grain number (Tables 1 and 2) and the two new soybean cultivars had significantly higher grain yields in all treatment combinations than the landraces, as observed previously [7,35,39]. The small reduction of grain size under rainfed or water stress conditions may be caused by the lower photosynthesis rate during grain filling [30]. On the other hand, flower and filled-pod number were positively associated with water use during the flowering and podding stages (Figure 6), indicating that increased water availability during flowering and podding increases the flower and pod number and, in turn, grain number. In the pot experiment, the flower number in the WW treatment was 170% and 24% higher than in the WS treatment in the landrace and new soybean cultivars, respectively, indicating that the response of flower number to water supply was different between the landrace and the new soybean cultivars. On average, 40 mm of irrigation water increased filled-pod production by 10% and 28% in the landraces and new soybean cultivars in 2011, and 38% and 29% in 2012, respectively. In the drier year (2011), but not in the wetter year (2012), the results support the hypothesis (iii) that 'irrigation at flowering will benefit yield more in modern genotypes than landraces by increasing the number of flowers and filled pods.' This reflects the different responses of the landraces and new soybean cultivars to precipitation [34]; this needs to be evaluated over a greater range of environments.

5. Conclusions

One irrigation event of 40 mm at flowering increased the grain yield and WUE_G in the field in two consecutive years. The irrigation increased both the grain number and grain size. The additional water from the irrigation resulted in increased water uptake from subsoil layers at the reproductive stage and a greater number of flowers and pods associated with the increased grain yield and WUE_G. We conclude that supplemental irrigation, if available at flowering, will be beneficial for yield in water-limited environments, particularly in wetter years, as it induces greater root growth into and water extraction from deeper soil layers.

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