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A physiological Explanation of Drought Effect on Flag-Leaf Specific Weight and Chlorophyll Content of Barley

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Abstract

This study was carried out in Kalar technical institute, Sulaimani Polytechnic University in Garmian region, Iraq during 2016-17 and 2017-18 seasons. Five hybrid genotypes of barley were tested under drought and irrigated conditions to detect the flag-leaf specific weight, chlorophyll content index (SPAD; The Soil Plant Analysis Development chlorophyll meter) and the period from anthesis to physiological maturity. Across both years 2016-17 and 2017-18, there was no effect of drought on flag-leaf specific weight, however, SPAD was reduced by 4.77 and the period between anthesis and maturity was shortened by almost two days. Genotype 3//14 scored the highest values of flag-leaf specific weight (7.765 mg cm^{-2})and SPAD (25.56), and stayed green for the longest period (28.69 days) (P=0.05), showing its ability to be more tolerant to moisture reductionas compared to the other tested genotypes. In order to explain the physiological mechanisms among the assessed traits under both irrigated and drought conditions, linear regression analysis was applied for both seasons and averaged over seasons. A positive linear relationship was shownbetween flag-leaf specific weight and SPAD under both irrigated (R^2=0.83; P=0.03) and drought (R^2=0.76; P=0.05) conditions, explaining the high flag-leaf chlorophyll content resulting from high specific weight of leaves. The high flag-leaf specific weight was also associated with longer periods for leaves to stay-green after anthesis, under both irrigated (R^2=0.91; P=0.01) and drought (R^2=0.79; P=0.04) conditions, which provides a great chance to accumulate more resources of carbohydrates and protein in the grain and, consequently,a higher throughput of yield.

Keywords: Barley , drought, chlorophyll content, flag-leaf

التفسير الفسلجي لتأثير الجفاف على وزن ورقة العلم ومحتوى كلوروفيل الشعير

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الخلاصة

أجريت هذه الدراسات خلال موسمين 2016-2017 و 2017-2018 في حقول معهد كلار التقني – جامعة السليمانية التقنية. تم اختيار خمسة أنماط جينية مختلطة من الشعير من اجل اختبارها في ظروف الزراعة المروية والديمية من حيث صفتي الوزن المحدد لأوراق العلم و مؤشر محتوى الكلوروفيل والفترة المحددة من تخليق الازهار الى النضج الفسيولوجي(SPAD).على مدار الموسمين ، لم يكن هناك تأثير للجفاف على

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الوزن المحدد لأوراق العلم ,بينما محتوى الكلوروفيل (SPAD) انخفضت بمقدار 4.77 , وكذلك الفترة الزمنية بين تخليق الازهار والنضج نقصت بمعدل يومين.سجل النمط الجيني 14/3 أعلى قيمة للوزن المحدد لأوراق العلم (7.765 mg cm^{-2}) و SPAD(25.56) و بقيت خضراء لأطول فترة (P=0.05)(28.69 days) , أظهر هذا النمط الوراثي القدرة على أن يكون أكثر تحملا لنقص الرطوبة مقارنةً بالأنماط الوراثية الأخرى.من أجل شرح الآليات الفسيولوجية بين الصفات التي تم تقييمها في ظل ظروف الزراعة المروية و الديمية على حد سواء ,تم اجراء تحليل الانحدار الخطي لكل من المواسم والمتوسط على مدى المواسم.اظهرت التحليل علاقة خطية إيجابية بين الوزن المحدد لأوراق العلم و SPAD تحت كل من الظروف المروية (R2 = 0.83 ؛ P = 0.03) والجفاف (R2 = 0.76 ؛ P = 0.05) التي تفسر المحتوى العالي لكلوروفيل ورقة العلم والذي ياتي من الوزن العالي لاوراق العلم.ارتبط الوزن المحدد لاوراق العلم بطول فترة بقائها خضراء بعد التخليق تحت ظروف الري (R2 = 0.91 ؛ P = 0.01) والجفاف (R2 = 0.79 ؛ P = 0.04) و التي تعطي فرصة كبيرة لتجميع المزيد من المواد مثل الكاربوهايدرات والبروتينات في الحبوب و بالتالي الحصول على إنتاجية عالية .

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the important cereals in many dry areas of the world and it is necessary for the livelihoods of many farmers [1]. It is one of the major cereal crops that is primarily grown for its grain and used for animal feed [2]. Water stress is one of the vital limiting factors in crop production worldwide. In breeding programs, in order to enhance the drought resistance of a crop plant, it is necessary to have knowledge related to the physiology of drought tolerance mechanisms [3]. Drought is considered as one of the most effective abiotic stresses limiting agricultural production worldwide. Drought stress during the grain-filling period decreased the flag leaves' net photosynthetic rate of barley [4]. The sensitivity of drought effect 'was reported to occurjust before spike emergence stage'[5], particularly in environments where drought is encountered at the end of the plant'slife cycle [6]. Flag leaf is a primary source of carbohydrate production for grain filling and yield due to its short distance to the spike and the fact that it stays green for longer times than the rest of the leaves [7]. A previous study[8] found that some flag leaf traits, such as lengths and width, were inherited quantitatively. Understanding the role of physiological and morphological traits of flag leaf on yield will provide a new insight in crop growth and development [9].

Photosynthesis is the main source of grain yield and dry matter production in crop plants. It is also an essential process to maintain crop growth and development. Photosynthetic systems in higher plants are most sensitive to drought stress [10]. It was reported that measuring photosynthetic traits such as chlorophyll content might estimate the influence of environmental stress on crop growth and yield [11,12]. The objective of the present experiments was to study the effects of drought on flag leaf area and chlorophyll content in barley, and to physiologically explain the mechanism of their relationships under drought-prone environment.

2. Materials and Methods

Plant materials and environmental conditions

Two experiments were carried out for two seasons of 2016-17 (Feb 2017 – May 2017; referred to hereafter as 2017) and 2017-18 (Dec 2017 – May 2018; referred to hereafter as 2018) at Kalar technical institute (at longitude line 45º 22′ 681″ east, latitude line 34º 21′ 558″ north, and elevation level of 178 meters). Five introduced varieties were obtained from Kalar Agricultural Research Station, which were originally developedwith different sensitivities for drought conditions by the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria. Five hybrids of F_2 two-rowed barley (*Hordeum vulgare* L.) were then obtained from crossing a local variety in Garmian region with those developed by ICARDA, using a previously investigated full diallel cross[13].The hybrids were, namely, Local//Zanbaka (3//18), Local//ARTa/3/Avar (3//14), Local//Roho/Zanbaka (3//5), Local//Avar/H/Sout (3//1) and Local//Tadmor/Roho (3//4). The study region was of a semiarid climate [14] with anAridisols soil (characteristic of arid regions, containing typically saline or alkaline soils with low level of organic matter). Temperature was hyperthermic [15] based on day time temperature, and average daily temperatures (maximum + minimum temperature divided by 2) during the seasons were in the range 12.4-28.5 ^{o}C in 2017 and 6.5-31.3 ^{o}C in 2018. The soil was slightly moist or aridic (Torric) which requires irrigation for agricultural use [16]. The total rainfall in the region was 226.1 mm in 2017 and 287.4 mm in 2018. Figure-1 shows the distributions of total monthly rainfalls over both seasons (2016-17 and 2017-18). Complementary irrigation treatments were performed when required (four times in April and May in 2017 and six times in March and mid-late April in 2018), according to the severity of the drought in each season.

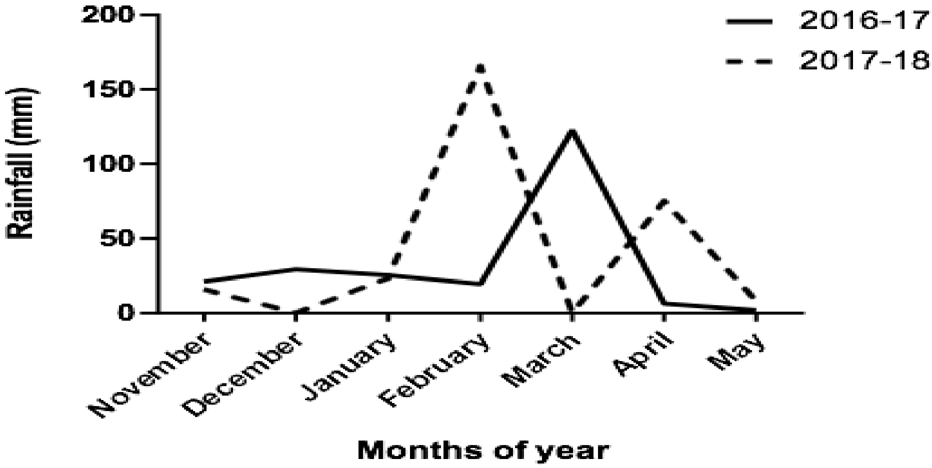

Figure 1-Total monthly rainfall for both seasons 2016-17 and 2017-18.

Experimental design and statistical analysis

Randomised block, split–plot design was used, including two main-plots and twenty sub-plots (5 rows x 4 columns) with four replicates (blocks) in each main plot. Irrigation treatments (fully irrigated and unirrigated) were randomised on main-plots. Genotypes were randomised on sub-plots (1 m^2). GenStat 19th Edition [17] was used for statistical analysis of variance (ANOVA) by applying a split-plot design for both years and cross-year mean data. Linear regression analysis and graphs were carried out using the GraphPad Prism 8.0.0 software package to calculate the relationships between all variables among years and for the cross-year mean [18].

Traits measurement

Number of days from anthesis to maturity dates (AD-MD; day)

Anthesis date (GS61; Mid-April in 2017 and Early-April in 2018) and maturity date (GS89; Mid-May in 2017 and Early-May in 2018) were measured based on the decimal code of growth stages (GS), as previously described[19]. Anthesis date was visually assessed for the whole plant in each sub-plot, and a growth stage was taken when more than 50 % of the main shoots were at the anthesis date.
Physiological maturity was also assessed based on the date when green area of the stem was less than 25%. Number of days from anthesis to maturity date (AD-MD) was then calculated by counting the total days from the date of anthesis till maturity date for each genotype.

Flag-leaf specific weight (FLSW; mg cm^{-2})

Five randomly selected flag-leaves in each sub-plot (40 plots) were hand-collected at anthesis date (GS61) in both years (Mid-April in 2017 and Early-April in 2018). The areas of collected leaves were measured by CI-202 LASER AREA METER, USA, and then the leaves were weighed after drying for 48 h at 80^{o}C to obtain the flag-leaf specific weight according to the equation below:

Flag-leaf specific weight = flag-leaves dried weight

Chlorophyll content index (SPAD)

Leaf chlorophyll content (SPAD), from GS61-14 days to GS61+14 days, was measured weekly on the main shoots for three plants in each plot for both years (2017-2018) using a chlorophyll content meter (CCM-200, OPTI-SCIENCES, Japan). The average chlorophyll content index was then used for data analysis. The readings were taken when the sky was clear and the leaves were well illuminated between 10.00h to 14.00h of daily hours [20].

3. Results

Flag-leaf specific weight (FLSW; mg cm^{-2})

There was no significant effect of drought on flag-leaf specific weight in both seasons 2016-17 and 2017-18 (P=0.55 and P=0.36, respectively; Table-1). In 2017, FLSW values for the genotypes under irrigated conditions ranged from 5.803 mg cm^{-2} for 3//5 to 8.279 mg cm^{-2} for 3//14, while under unirrigated conditions they ranged from 6.119mg $cm^{-2}$for 3//18 to 8.182 mg cm^{-2} for 3//14, with the differences being significant (P=0.05). In 2018, FLSW values for the genotypes did not differ significantly (P=0.52). However, for the cross year mean, genotypes were in the ranges of 5.803 mg cm^2 for 3//5 to 7.614 mg cm^{-2} for 3//14 under irrigated conditions, and 6.275 mg cm^{-2} for 3//18 to 7.917 mg cm^{-2} for 3//14 under unirrigated conditions, with the differences being significant (P=0.03). Results of FLSW for the interactions between irrigation and genotype showed no significant differences in both years (2017 and 2018) and averages over years (P=0.18, P=0.65 and P=0.39, respectively).

Table 1-Summary of analysis of variance for flag-leaf specific weight for 5 barley genotypes recorded under irrigation and unirrigated conditions in 2017, 2018 and cross-year mean.

Genotypes	Flag-leaf specific weight (FLSW; mg cm^{-2})					
	2017		2018		2017-18	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
3//18	7.103	6.119	6.168	6.430	6.636	6.275
3//14	8.279	8.182	6.949	7.652	7.614	7.917
3//5	5.803	7.409	5.803	5.976	5.803	6.693
3//1	6.701	6.615	5.094	7.377	5.898	6.996
3//4	7.831	6.681	6.691	6.681	7.261	6.681
Mean	7.143	7.001	6.141	6.823	6.642	6.912
SED (df)						
Year (1)					0.312^{ns}	
Irrigation (1)	0.214^{ns}		0.632^{ns}		0.334^{ns}	
Genotype (4)	0.595^{*}		0.835^{ns}		0.513^{*}	
Irrigation. x Gen. (4)	0.782^{ns}		1.230^{ns}		0.729^{ns}	
Year x Gen. (4)					0.719^{ns}	

(SED) Standard error of difference
(df) Degree of freedom
(***) P<0.001; (**) P<0.01 and (*) P<0.05 significance levels; (ns) not significant.

3.2. Leaf chlorophyll content index (SPAD)

Drought significantly reduced the SPAD value from 18.15 to 14.60 (P=0.05) in 2017, from 29.82 to 23.92 (P=0.02) in 2018, and from 23.99 to 19.26 (P=0.001) for the cross-year mean (Table-2). SPAD values for the genotypes significantly differed from 14.86 for 3//5 to 20.25 for 3//14 under irrigated, and from 13.13 for3//1 to 18.50 for 3//14 under unirrigated conditions (P=0.05) in 2017. In 2018, SPAD values for the genotypes ranged from 22.29 for 3//1 to 33.86 for (3//14) under irrigated, and from 19.28 for 3//5 to 28.22 for 3//14 under unirrigated conditions, with the differences being significant (P=0.04). For the cross-year mean, genotype 3//14showed the highest values of SPAD under both irrigated and unirrigated conditions (27.05 and 23.36, respectively), while genotype 3//1 under irrigated conditions and genotype 3//5 under unirrigated conditions showed the lowest values (20.11 and 17.01, respectively), with the differences being significant (P=0.002). The interaction between irrigation and genotype showed no significant differences for both years (2017 and 2018) and cross-year mean (P=0.21, P=0.32 and P=0.39, respectively).

Table 2-Summary of analysis of variance for chlorophyll content index (SPAD) for 5 barley genotypes recorded under irrigated and unirrigated conditions in 2017, 2018 and cross-year mean.

Genotypes	Chlorophyll content index (SPAD)					
	2017		2018		2017-18	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
3//18	18.94	11.58	32.57	24.40	25.75	17.99
3//14	20.25	18.50	33.86	28.22	27.05	23.36
3//5	14.86	14.74	28.40	19.28	21.63	17.01
3//1	17.92	13.13	22.29	23.71	20.11	18.42
3//4	18.78	15.07	32.00	24.02	25.39	19.54
Mean	18.15	14.60	29.82	23.92	23.99	19.26
SED (df)						
Year (1)					0.740 **	
Irrigation (1)	1.075 *		1.216 *		0.812 **	
Genotype (4)	1.564 *		2.718 *		1.568 **	
Irrigation x Gen. (4)	2.252 ns		3.646 ns		2.143 ns	
Year x Gen. (4)					2.117 ns	

(SED) Standard error of difference
(df) Degree of freedom
(***) P<0.001; (**) P<0.01 and (*) P<0.05 significance levels; (ns) not significant.

Regression analysis showed a significant positive relationship between flag-leaf specific weight and SPAD in 2017 under both irrigated and unirrigated conditions (R^2=0.87; P=0.02; Figure-2a). In 2018, the relationship was significant under irrigated conditions (R^2=0.88; P=0.02), but there was a trend for a positive correlation under unirrigated conditions (R^2=0.68; P=0.08; Figure-2b). For the cross-year mean, significant positive correlationswere also found between flag-leaf specific weight and SPAD under both irrigated and unirrigated conditions (R^2=0.83; P=0.03; R^2=0.76; P=0.05, respectively; Figure-2c).

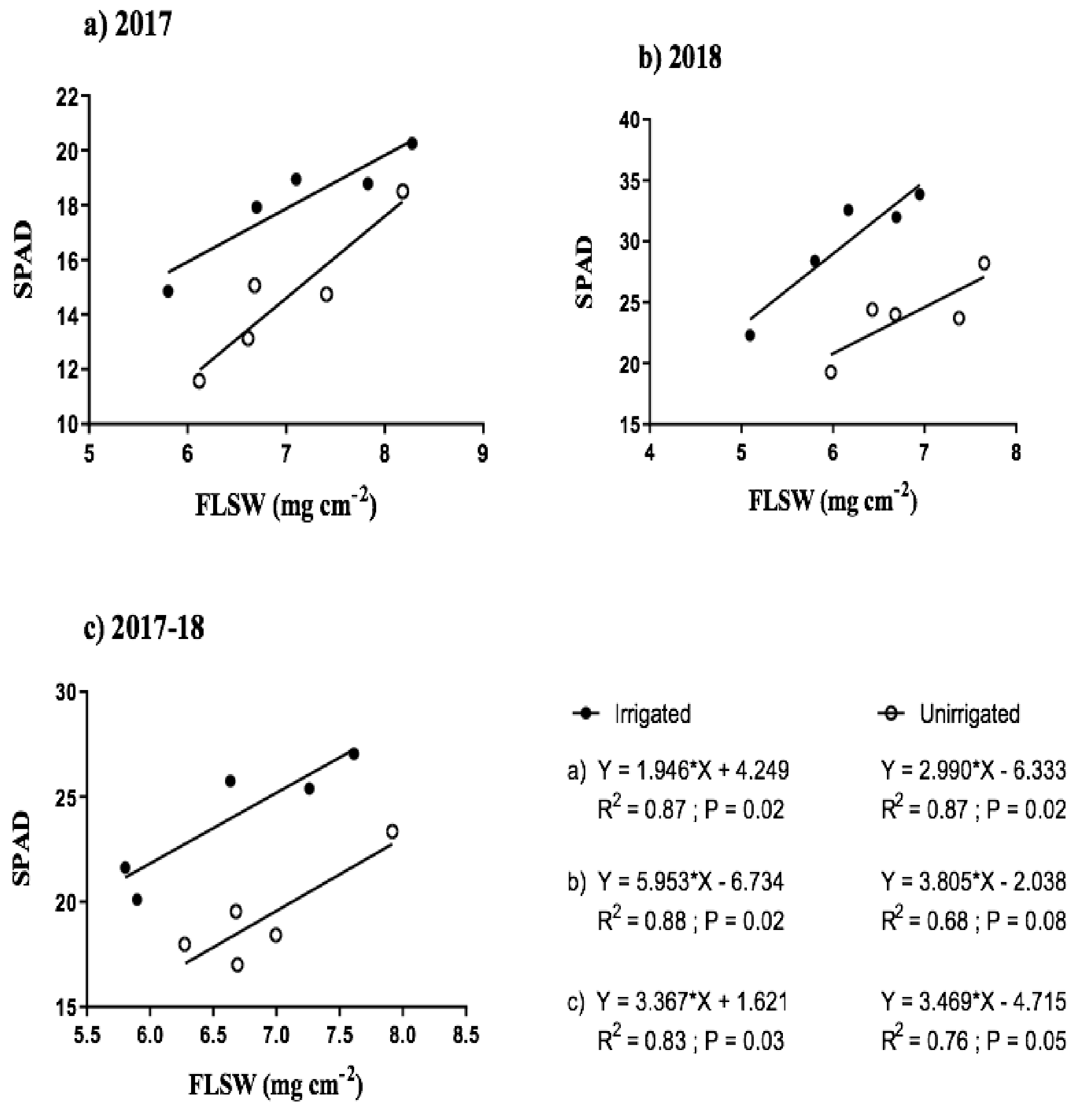

Figure 2-The Linear regressions of flag-leaf specific weight (FLSW; mg cm^{-2}) on chlorophyll content index (SPAD) for 5 genotypes of barley in (a) 2017, (b) 2018 and (c) cross-year mean under irrigated and unirrigated conditions.

Number of days from anthesis to maturity dates (AD-MD; day)

There was no significant drought effect on the duration between anthesis and maturity (P=0.10) in 2017 (Table-3). The duration for the genotypes ranged from 26.25 days for 3//5to 28.5 days for 3//14 under irrigated conditions, and from 24.25 days for 3//18 to 26.75 days for 3//14 and 3//1 under unirrigated conditions, with the differences being significant (P=0.05). The interaction between irrigation and genotype showed no significant duration differences (P=0.55; Table-3). Drought reduced the maturity date by 2.2 days (P=0.04) in 2018. There were no significant differences in the duration values between the genotypes under irrigated and unirrigated conditions (P=0.79; Table-3). Averaging over years, drought significantly reduced the period from anthesis to maturity from 28.68 to 26.90 days (P=0.005). Genotypes showed duration values that ranged from 27.75 days for 3//1 to 29.63 days for 3//14 and from 26.13 days for 3//18 to 27.75 days for 3//14 under irrigated and drought conditions, respectively, with the differences being significant (P=0.007). There were also significant differences between years (P=0.004), but not between genotypes (P=0.22).

Table 3-Summary of analysis of variance for number of days from anthesis to maturity date for 5 barley genotypes recorded under irrigation and unirrigated conditions in 2017, 2018 and cross-year mean.

Genotypes	Number of days from anthesis to maturity date (AD-MD; day)					
	2017		2018		2017-18	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
3//18	26.50	24.25	30.25	28.00	28.38	26.13
3//14	28.50	26.75	30.75	28.75	29.63	27.75
3//5	26.25	25.75	30.00	27.00	28.13	26.38
3//1	27.00	26.75	28.50	28.25	27.75	27.50
3//4	28.00	26.00	31.00	27.50	29.50	26.75
Mean	27.25	25.90	30.10	27.90	28.68	26.90
SED (df)						
Year (1)					0.531 **	
Irrigation (1)	0.585 ns		0.658 *		0.440 **	
Genotype (4)	0.735 *		1.232 ns		0.717 ns	
Irri. x Gen. (4)	1.098 ns		1.692 ns		1.009 ns	
Year x Gen. (4)					1.051 ns	

(SED) Standard error of difference
(df) Degree of freedom
(***) P<0.001; (**) P<0.01 and (*) P<0.05 significance levels; (ns) not significant.

In 2017, there was a positive correlation between flag-leaf specific weight and anthesis to maturity duration under irrigated conditions (R^2=0.83; P=0.03), but only a trend for a positive relationship was found under unirrigated conditions (R^2=0.38; P=0.27; Figure 3a). The duration between anthesis and maturity showed a strong positive linear relationship with flag-leaf specific weight amongst genotypes in 2018 under both irrigated and unirrigated conditions (R^2=0.90; P=0.01 and R^2=0.82; P=0.03, respectively; Figure-3b). Averaging across years, a positive linear relationship between number of days from anthesis to maturity and flag-leaf specific weight amongst genotypes was also found under both irrigated (R^2=0.91; P=0.01) and unirrigated conditions (R^2=0.79; P=0.04; Figure-3c).

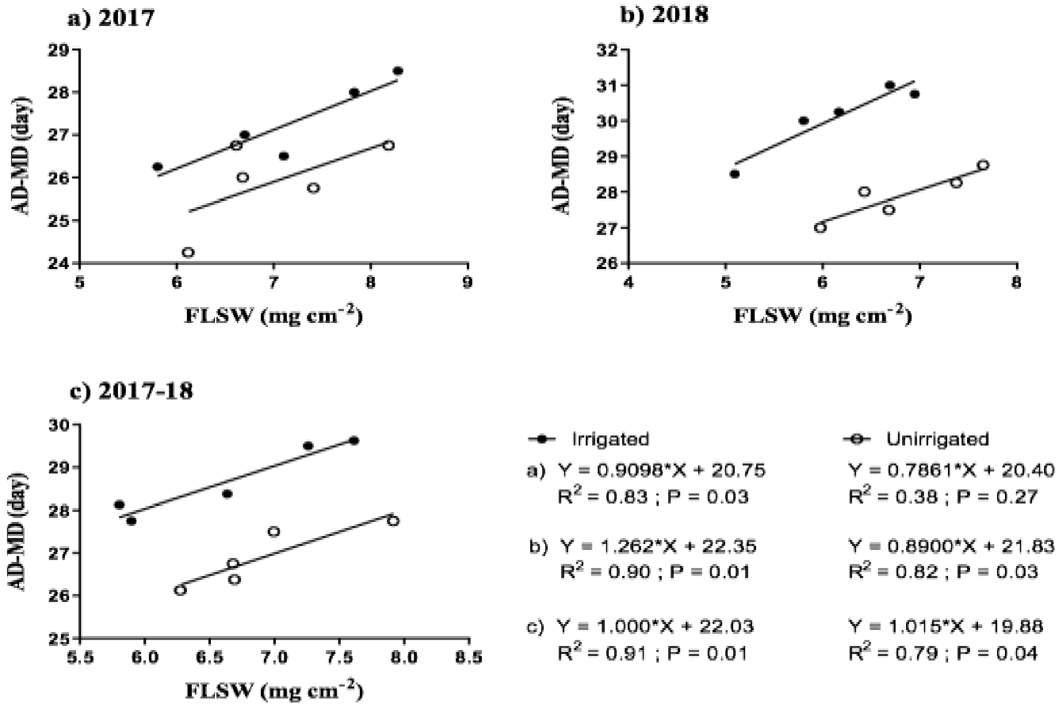

Figure 3-The Linear regressions of flag-leaf specific weight (FLSW; mg cm^{-2}) on number of days from anthesis to physiological maturity (AD-MD; day) for 5 genotypes of barley in (a) 2017, (b) 2018 and (c) cross-year mean under irrigated and unirrigated conditions.

4. Discussions

Environmental data in the studied region showed higher humidity condition in 2017 than in 2018 over the grain filling period (February to April). Averaging over seasons, leaf specific weight in both years was not affected by drought,which is expected when drought occurs in the late growing season when leaves are fully emerged[21]. However, relative chlorophyll content (SPAD) was significantly decreased in both years,which can be attributed to limited water availability after anthesis [22]. Genotype 3//14 had the highest value of SPAD in both seasons, whichmight be due to high flag-leaf specific weight which helps in increasing photosynthetic activity and higher grain yield. Drought shortened the cross year mean period between anthesis and maturity by almost two days, possibly through causing advanced physiological maturity [23].

Regression analysis revealed a positive association between flag-leaf specific weight and SPAD, which clarified the importance of leaf morphology and thickness in order to have a high rate of photosynthesis activity [24]. Averaging over seasons, the specific weight of flag-leaves was significantly correlated with the number of days between anthesis and physiological maturity under both irrigated and drought conditions. Although drought fastens leaf senescence and advances maturity, thegenotypes with higher flag-leaf specific weight had longer stay-green periods and were later senesced [25].

5. Conclusions

The physiological mechanisms behind the photosynthetic process under water stress play the main role for a better grain yield in barley. In this study, chlorophyll content index (SPAD) appeared to be positively associated with flag-leaf specific weight, indicating the importance of this trait in selecting superior genotypes inbreeding programs with respect to flag-leaf area. Flag-leaf senescence duration after anthesis was also extended by the effect of flag-leaf specific weight under both irrigated and drought conditions. For these reasons, flag-leaf specific weight can be recommended to be an indicator for the best yield under drought environments.

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