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Phenotyping winter wheat for early ground cover

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Abstract: The relationship between the early ground cover and the grain yield in winter wheat is not yet fully understood. In a winter wheat breeding programme, selection for early ground cover is traditionally made using visual scoring. Although visual scoring is preferred as a phenotypic screening tool by wheat breeders, its output may not be reliable, as it requires experience. A smartphone camera-based digital image technique can be recommended as a feasible, reliable, repeatable, affordable, and fast selection tool for early ground cover in wheat as an alternative to visual scoring. For this purpose, two wheat trials were conducted in the 2017–2018 and 2019–2020 seasons. In both seasons, 215 wheat genotypes in total, together with three checks from spring wheat, were tested under rain-fed conditions in the spring wheat zone in Turkey. All the tested wheat genotypes were grouped into spring, facultative, and winter growth habit using visual scoring. Simultaneously, photos were taken from each plot with a smartphone camera, and the early ground cover (%) was estimated using the smartphone camera-based digital image technique. The relationships between grain yield, visual scoring, and early ground cover could so be estimated. In both seasons, significant negative correlation between grain yield and visual scoring (r = −0.679** and r = −0.704**, respectively) and significant positive correlation between the grain yield and the early ground cover (r = 0.745** and r = 0.747**, respectively) were observed. The correlation between visual scoring and early ground cover were negative (r = −0.862** and r = −0.926**, respectively). The broad sense heritability estimates in both seasons were 0.51 and 0.85, respectively, for early ground cover, 0.91 and 0.94 for visual scoring, and 0.86 and 0.69 for grain yield. In this study, we revealed that testing winter wheat genotypes in the spring wheat zone rather than in the winter wheat zone could be a more effective way to unveil the positive relationship between the early ground cover and the grain yield. We have shown that the smartphone-based digital image technique is a useful selection tool for early ground cover in winter wheat.

Keywords: digital image; grain yield; growth type; visual scoring; wheat

Early ground cover (EGC) in wheat is one of the essential elements for drought tolerance and/or escape in countries such as Turkey, where storage-driven winter rain climates occurring in the Mediterranean ecosystems are dominant (Richards et al. 2007; Bodner et al. 2015). Typically, most precipitation in such climates is received in late autumn, winter, and early spring, when the wheat's early vegetative stages (e.g., germination, emergence, and tillering) occur. Precipitation decreases sharply during late spring and early summer when the flowering and grain filling phases occur. Thus, the reproductive period of wheat takes place under terminal stress (i.e., the combined effect of drought and heat stress). Wheat's EGC capacity plays a pivotal role in its adaption to Mediterranean ecosystems by benefiting more from the precipitation received during the vegetative period. The EGC provides remarkable advantages to wheat (e.g., increasing competition with weeds, enhancing the water use efficiency, and preventing water loss from the soil through evaporation) (Richards et al. 2007; Rebetzke et al. 2016; Ayalew et al. 2018). Also, it has been well documented that the EGC positively affects the biomass and, subsequently, the grain yield in wheat (Zhao et al. 2019; Hendriks et al. 2022).

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Many studies have been conducted on the EGC in spring wheat (SW) (Casadesus et al. 2007; Mullan & Reynolds 2010; Bellundagi et al. 2013; Rebetzke et al. 2016), while only two studies have been carried out on the EGC in winter wheat (Kipp et al. 2014; Li et al. 2014). Perhaps this is because the EGC has a low priority in the winter wheat breeding target. At this point, specific questions need addressing. First, it is assumed that the EGC is genetically suppressed in winter wheat (WW) by the vernalisation (*Vrn*) requirement and photoperiod (*Ppd*) sensitivity genes (Chen et al. 2018; Marone et al. 2020). WW can avoid cold injury only if it exhibits a slow growth and development rhythm during the winter season (Limin & Fowler 2000; Fowler et al. 2014). In other words, it has been presumed that the *Vrn* and *Ppd* genes slow down the EGC formation rate of WW (Chen et al. 2018; Mason et al. 2018; Marone et al. 2020; Capo-Chichi et al. 2021). Unlike WW, it has been suggested that the maximum EGC capacity of SW is limited by reduced height (*Rht*) genes (Zhao et al. 2019). However, the likely relationship of *Vrn* and *Ppd* genes, which differentiate WW from SW, with the EGC, has not been proven yet (Kosova et al. 2008). Second, contrary to what is assumed, there may be a wider genotypic variation for EGC within WW, which has not yet been detected. Thus, an effective phenotyping tool needs to be found to unveil the genetic potential for EGC in WW. A routine selection for EGC in WW breeding nurseries is made under winter wheat zone (WWZ) conditions, defined as Dsa, Dsb, and BSk according to the Köppen-Geiger climate classification (KGCC). Alternatively, WW breeding nurseries can be tested in an opposite environment (e.g., testing WW in a spring wheat zone (SWZ), defined as Csa in KGCC, rather than in WWZ) to uncover phenotypic variation for EGC (as undertaken in this study) (Peel et al. 2007).

Traditionally, WW breeders prefer the visual scoring (VS) method when screening wheat breeding nurseries during early spring (UPOV 2012). Utilising this method, genotypes with winter, facultative, and spring growth types (WT, FT, and ST) are grouped in WW breeding nurseries (Chen et al. 2018; Jimenez-Berni et al. 2018). Generally, ST refers to a fast growth and development ability for EGC and FT refers to a medium growth and development ability for EGC, while WT refers to a slow growth and development ability for EGC (Chen et al. 2018). On the one hand, the rapid growth could signify the vulnerability to cold damage in WW. On the other hand, a high EGC capacity in WW may be prioritised as an adaptive trait to climate change (Cann et al. 2020). We hope that the studies to be carried out on the EGC in WW (especially determining the relationship of the EGC with the grain yield) will draw the attention of the WW breeder. Indeed, we believe that he or she is curious to know how the EGC will facilitate the WW adaptation to climate change (Bourgault et al. 2020; Kaya 2021).

It has been suggested that direct biomass measurements during the vegetative period to estimate the EGC capacity in wheat can be used as a surrogate for the VS method (Pietragalla et al. 2012). However, it is impossible to take thousands of biomass samples directly from wheat breeding nurseries in a narrow time window. Essentially, direct biomass measurements are not feasible because they are very costly, time-consuming, and labour-intensive. It has been suggested that a smartphone camera-based digital image technique (DIT) can be used as a breeder-friendly phenotyping tool in a wheat breeding programmes (Reynolds et al. 2020). As a matter of fact, it has been reported that DIT is an easy, affordable, repeatable, and reliable method to measure the EGC in wheat (Casadesus et al. 2007; Mullan & Reynolds 2010; Kipp et al. 2014; Ma et al. 2019). In wheat, DIT can be used not only to measure the EGC, but also to correlate other growth and development stages (i.e., booting, heading anthesis, and grain filling) with the grain yield (GY) (Morgounov et al. 2014; Shabannejad et al. 2020). At the same time, DIT helps, for example, select disease-resistant genotypes (Walter et al. 2019), detect the mineral deficiency or toxicity levels (Baresel et al. 2017), or phenotype the roots in wheat (Rosello et al. 2019; Středa et al. 2020). All things considered, wheat breeders, especially in the developing world, cannot afford sophisticated and expensive digital cameras for EGC measurements. Today, thankfully, an affordable smartphone enables the wheat breeder to take quality photos in high resolution. Moreover, open-source mobile applications that are adaptable to the camera of the smartphone have been developed to estimate the EGC more effectively (Confalonieri et al. 2013; Patrignani & Ochsner 2015; Tao et al. 2020; Yu et al. 2020).

In this study, we hypothesised that a smartphone-based DIT could dissect the wheat genotypes with the different EGC capacities. For this purpose, we observed a total of 215 wheat genotypes with different growth habits in the SWZ of Turkey during two

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seasons (2017–2018 and 2019–2020). By doing so, we tried to answer the following questions: (1) Can a smartphone-based DIT be used for EGC measurements in WW? (2) Are there relationships between the EGC, VS, and GY? (3) What are the broad sense heritability values for the EGC, VS, and GY? (4) Can the EGC be used as an indirect selection criterion for the GY? (5) Are there genotypes with different growth (winter, facultative and spring) types within WW? and (6) Can the SWZ be a suitable selection environment for unveiling genotypes with different EGC capacities within WW?

MATERIAL AND METHODS

Experimental design. During the 2017–2018 and 2019–2020 seasons, the trial was conducted under rain-fed conditions, at the experimental area (37°58'13''N, 41°50'43''E; 590 m a.s.l.) of the Faculty of Agriculture, Siirt University, located in the south-eastern Anatolia Region, representing the SWZ of Turkey. A total of 215 WW genotypes (110 in the first season and 105 in the second season) were tested across five blocks in an augmented randomised complete block design (ARCBD). All the WW genotypes were un-replicated, while three checks were replicated across five blocks in ARCBD during both seasons.

Genotypes. All the wheat genotypes tested in this experiment were obtained from the IWWIP (International Winter Wheat Improvement Program), coordinated by CIMMYT, ICARDA, and Turkey (IWWIP 2021). A total of 215 wheat genotypes were collected from the 25^{th} FAWWON-SA (Facultative and winter wheat observation nursery for semi-arid areas) tested in the first season (2017–2018) and from the 26^{th} FAWWON-SA in the second season (2019–2020) (Tables S1 and S2 in the Electronic Supplementary Material (ESM)). In this study, three cultivars from SW; namely Dinc, Tekin, and Kale were used as the checks.

Climate data and evapotranspiration. For both seasons, the total precipitations (mm), minimum temperatures (Tmin, °C), and maximum temperatures (Tmax, °C) were obtained from the meteorological station at Siirt Airport, which was approximately 1 km away from the experimental field (TSMS 2021).

According to the Penman-Monteith method, the seasonal crop evapotranspiration values (ETc) for wheat were calculated using meteorological data (Allen et al. 1998) (Table 1).

Table 1. Climate data

Month	ETc	Precipitation (mm)			Minimum temperature (°C)			Maximum temperature (°C)		
		2017–2018	2019–2020	long term	2017–2018	2019–2020	long term	2017–2018	2019–2020	long term
November	36	85.6	51.4	82.2	0.8	7.3	6.3	23.8	17.6	15.4
December	15	47.4	75.8	95.8	−1.3	5.0	1.6	17.8	10.9	8.7
January	21	56.4	63.8	97.5	−0.9	−3.6	−0.5	13.4	11.6	6.6
February	31	75.6	137.2	97.6	0.7	−9.0	0.5	16.1	16.9	8.8
March	68	47.2	229.6	111.2	4.7	2.5	4.0	26.0	22.8	13.3
April	114	60.8	158.6	105.0	6.4	5.0	8.9	27.1	23.8	19.1
May	169	146.6	40.4	63.8	10.4	10.0	13.5	32.2	33.7	25.2
June	110	2.8	0.2	9.3	16.8	16.3	19.0	30.4	37.0	32.2
Total	564	522.4	757.0	660.4						
Mean					4.7	4.2	6.7	23.4	21.8	16.2

ETc – crop evapotranspiration

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The evapotranspiration demand of the wheat genotypes tested in both seasons during the germination, emergence, and tillering stages was adequately met by the precipitation (Table 1). However, the precipitation received in March and April of the first season, in which stem elongation and heading occurred, could not reach the level to meet the ETc values. On the other hand, the precipitation (40.4 mm) received in May of the second season, when the grain filling period in the spring-type wheat and flowering and fertilisation stages in the winter-type wheat occurred, was far behind the crop evapotranspiration demand (ETc = 169 mm). Furthermore, there was no precipitation in June in both seasons of the winter-type wheat in which the grain filling period took place. Therefore, the lack of seasonal precipitation in June deeply affected the winter-type wheat.

In mid-February of the second season, the temperature dropped to –9 °C (Table 1). Even though the spring-type wheat genotypes were damaged by the cold stress, they recovered rapidly during the spring season. On the other hand, the high temperatures recorded in May (32.2 °C and 33.7 °C) and June (30.4 °C and 37 °C) of both seasons negatively affected the flowering and grain filling stages of the WW genotypes.

Soil properties. The soil in the experimental site was clayey, calcareous, and slightly alkaline (pH = 7.9). The organic matter was low (14.2 g/kg). The extractable P and K levels were determined as 32 and 125 mg/kg, respectively.

Management. Each plot consisted of four rows in the trial, which were arranged 20 cm apart and were 4 m in length. The seeding rate was 500 seeds/m^2. Seeding was undertaken by hand on 10 December 2017, in the first season, and on 30 November 2019, in the second season. Fertilisers were applied at planting with diammonium phosphate (N 18% and P 46%), 180 kg/ha, and just before the stem elongation stage (Zadoks stage (ZS) 30) with urea (N 46%), 150 kg/ha. The weeds were controlled manually. No pesticides were applied for diseases and insect pests. Harvesting in both seasons was performed by hand (with a sickle) at the end of June.

Digital image technique. In this study, a mobile device compatible application developed by Patrignani and Ochsner (2015), CANOPEO, was used. CANOPEO uses the colour values in the red-green-blue system. It analyses and classifies all the pixels in the image and converts a processed photo into a binary image. White pixels represent the green canopy, and black pixels represent the background or a non-green canopy. The CANOPEO app for Matlab, iOS, and Android mobile devices can be downloaded (CANOPEO 2021).

Phenotyping. The EGC values (%) of 215 wheat genotypes and three checks tested in this experiment were estimated by photos (i.e., digital images) taken from a smartphone camera. The digital images were imported to the CANOPEO software for processing, and then the EGC values (%) were generated automatically (Patrignani & Ochsner 2015).

The growth types (or habits) of all the wheat genotypes tested were detected using the VS method proposed by the International Convention for the Protection of New Varieties of Plants (UPOV 2012). In this method, the visual scores (1, 3, 5, 7, and 9) stand for erect, semi-erect, intermediate, semi-prostrate, and prostrate, respectively. The EGC and VS measurements were made at Zadoks stage 30 in both seasons (Kipp et al. 2014).

The plants in each plot were harvested with a sickle, threshed by hand, weighed, and the grain yield was expressed as kg/ha.

Statistical analysis. Since all wheat genotypes, except for the checks, had not been previously tested at the Siirt location, the genotypic effect was considered random in the analysis of variance (ANOVA) (Cook et al. 2021). The ANOVA, *t*-test, and descriptive statistics for each variable (EGC, VS, and GY) measured in this experiment, which was conducted based on the ARCBD, and the correlation analysis between the variables were performed using SAS software (Wolfinger et al. 1997; Kling & Merk 2021). As the genotypes tested in the first season were different from those tested in the second season, the significance levels of the differences between the variables measured over seasons were determined by the *t*-test. The broad sense heritability (H^2) values were predicted by the best linear unbiased predictor (BLUP) statistical method (Cullis et al. 2006; Cook et al. 2021; Kling & Merk 2021).

RESULTS

Analysis of variance. 215 wheat genotypes in total, consisting of 110 (the first set) wheat genotypes tested in the first season and 105 (the second set) in the second season, exhibited statistically significant differences ($P < 0.01$) in terms of the EGC, GY and VS (Table 2). Since a different set of wheat genotypes was tested for each season, a *t*-test was conducted

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Table 2. Analysis of variance for the variables

Source	SD	Early ground cover (%)	Grain yield (kg/ha)	Visual scoring (1–9)
Season 2017–2018				
Block	4	5.43	23 145.33	0.19
Check	2	12.06	40 532.47	0.88
Genotype	109	80.41**	405 833.58**	8.67**
Error	8	12.73	12 331.13	0.15
CV (%)		10.42	4.01	6.60
H^2		0.51	0.86	0.91
Season 2019–2020				
Block	4	14.26	27 941.83	0.22
Check	2	29.60	15 018.20	0.79
Genotype	104	260.71**	142 629.69**	9.31**
Error	8	8.76	11 605.78	0.11
CV (%)		5.85	3.63	6.18
H^2		0.85	0.69	0.94

SD – standard deviation; *CV* – coefficient of variation; H^2 – broad sense heritability; **significant at $P < 0.01$

to determine whether there was a statistically significant difference between the EGC values measured in the first season and those in the second season (Figure 1). Also, the *t*-test was conducted for the GY and VS to compare the two wheat sets. According to the *t*-test, it was determined that the two wheat sets were statistically different from each other for the EGC and GY ($P < 0.01$, *t*-test). However, there was no statistically significant difference for the VS between the two wheat sets ($P = 0.214$, *t*-test).

Visual scoring. According to the VS method, out of 113 wheat genotypes, including 110 from the 25th FAWWON-SA and the three checks, tested in the first season, 15 were categorised as ST, 67 were categorised as FT, and 31 were categorised as WT (Tables S1 and S2 in ESM and Figure 2). Among the 108 genotypes, consisting of 105 from the 26th FAWWON-SA and the three checks tested in the second season, 30 were categorised as ST, 32 were categorised as FT, and 46 were categorised as WT. The number of FT genotypes was higher in the first set, while the number of WT genotypes were higher in the second set. It was because the two wheat sets tested in both seasons were different. Meanwhile,

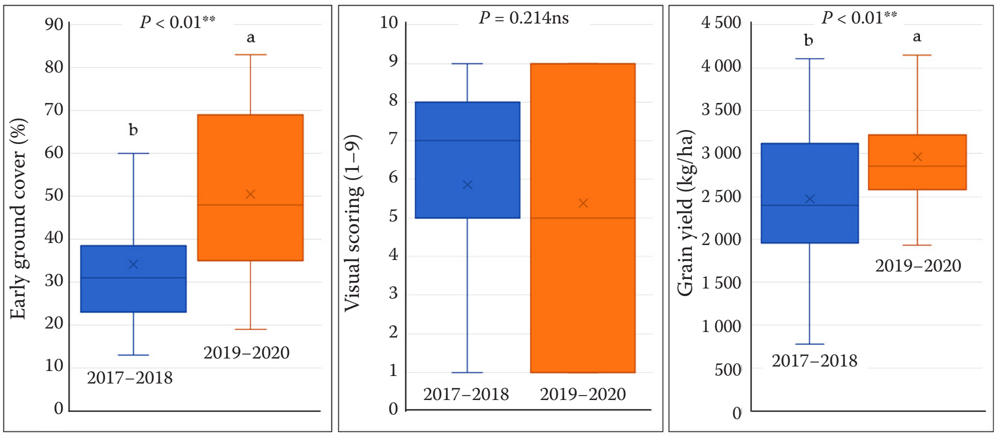

Figure 1. Comparing the first set of winter wheat (WW) genotypes tested in the first season (2017–2018) with the second set of WW genotypes tested in the second season (2019–2020)

Lower case letters (a, b) represent statistically significant differences between the seasons (i.e., WW genotype sets); **significant at $P < 0.01$ and ns – not significant at $P = 0.214$, based on the *t*-test

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Figure 2. Different growth types and their early ground covers in wheat

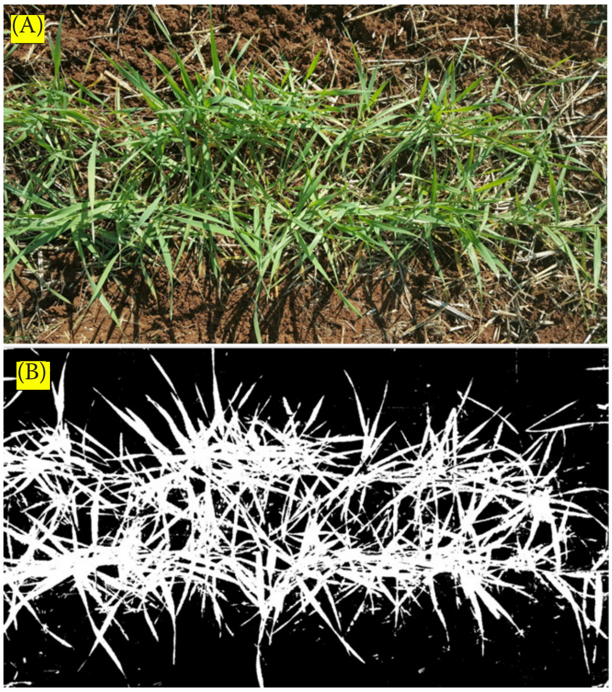

Figure 3. Wheat early ground cover: photo of a plot taken by smartphone (A) and a digitally processed image (digital early ground cover rate, 32.46%) (B)

it should also be considered that the season *per se* (i.e., environmental effect) may contribute to the involved genotypic differences (Hashjin 1992; Braun & Saulescu 2002).

Early ground cover. Among the 215 wheat genotypes tested in both seasons, a wide genotypic variation was detected for the EGC (Table 3, Tables S1, S2 in the ESM, Figures 2, 3). The highest EGC values (49 and 69% on average) were measured in the ST genotypes followed by the FT genotypes (30 and 50%). The lowest EGC values (22 and 32%) were measured in the WT genotypes. Our findings revealed that the ST genotypes' EGC values were more than twice as high as those of the WTs. Similarly, Mullan and Reynolds (2010) reported a large genotypic variation (55% to 100%) for the EGC in the SW.

Grain yield. The average grain yield (GY) of the ST, FT, and WT genotypes tested in the first sea-

Table 3. Descriptive statistics for the variables and wheat growth types

Growth type	No. of genotypes	Visual scoring (1–9)	Early ground cover (%)			Grain yield (kg/ha)		
			min	max	mean	min	max	mean
Season 2017–2018								
Spring	15	1–3	35	72	49	1 260	4 005	2 910
Facultative	67	5–7	13	50	30	780	3 342	2 370
Winter	31	9	13	31	22	1 080	3 138	1 916
Overall mean	110 (genotype) + 3 (check)	5.8	13	72	34	780	4 005	2 472
Variance		7.1			254			710 259
Season 2019–2020								
Spring	30	1–3	39	82	69	2 833	4 503	3 493
Facultative	32	5–7	28	64	50	2 743	3 695	3 166
Winter	46	9	19	40	32	2 235	3 661	2 881
Overall mean	105 (genotype) + 3 (check)	5.3	19	82	51	2 235	4 503	2 963
Variance		11.1			335			314 315

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son was recorded as 2910, 2370, and 1916 kg/ha, respectively (Table 3, Tables S1, and S2 in the ESM). Similarly, in the second season, the GY for the ST, FT, and WT genotypes were measured as 3 493, 3 166, and 2 881 kg/ha, respectively. Our findings showed that the ST group achieved the highest GY, while the WT group achieved the lowest GY. It was already expected that WT genotypes with longer phenological cycles would reach lower GYs, since the experiment was conducted in SWZ (Kaya 2021). Indeed, there were two factors causing the differences in the GYs between the two seasons: (a) different sets of genotypes tested (i.e., genotypic effects) (Table 3, Tables S1, S2 in the ESM) and (b) different seasonal climate patterns observed (i.e., environmental effects) (Table 1) (Baenziger et al. 2011; Gao et al. 2016).

Broad sense heritability. The broad sense heritability (H^2) can be categorised into three levels: low H^2 (lower than 0.50), medium H^2 (from 0.50 to 0.70), and high H^2 (higher than 0.70) (Roy & Shil 2020). In this study, the H^2 values for the EGC ranged from medium (0.51 in 2017–2018) to high (0.85 in 2019–2020), ranged from medium (0.69) to high (0.86) for the GY, and was assessed as high (0.91 and 0.94) for VS in both seasons (Table 2). Bellundagi et al. (2013) in SW and Li et al. (2014) and Gao et al. (2016) in WW estimated the H^2 values between 0.40 and 0.81 for the EGCs. Their findings were in agreement with ours.

Correlations between variables. The correlation coefficients (CCs) estimated between the traits measured in this trial exhibited a similar pattern during both seasons (Figure 4). Significant negative CCs between the EGC and VS (r = –0.862** in the first season and r = –0.926** in the second season), significant positive CCs between the EGC and GY (r = 0.745** and r = 0.747**, respectively),

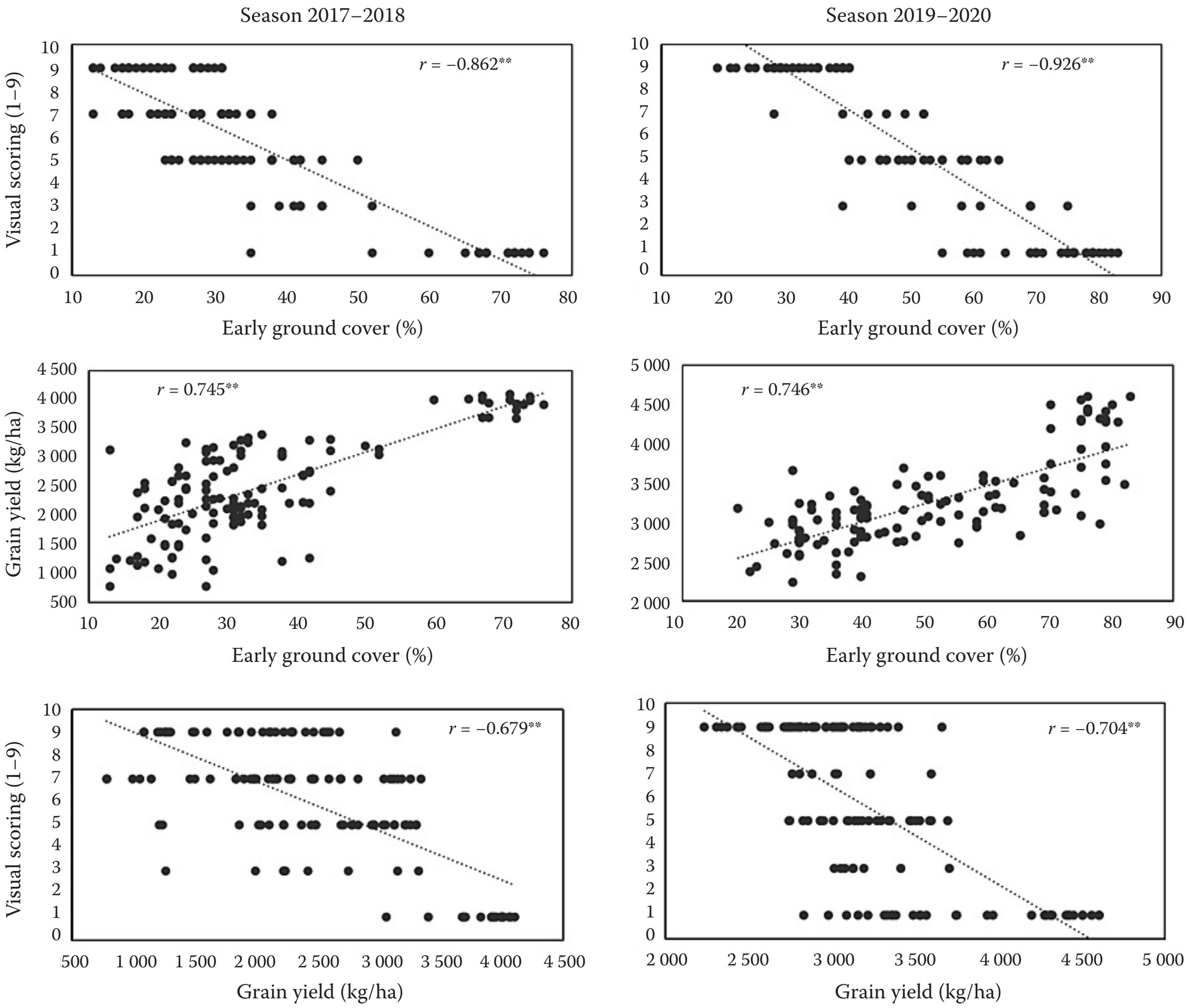

Figure 4. Correlations between the variables

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and significant negative CCs between the VS and GY (r = –0.679** and r = –0.704**) were estimated. Like our findings, Bellundagi et al. (2013), Li et al. (2014) and Gao et al. (2016) reported significant positive CCs between the EGC and GY (r = 0.270** and r = 0.662**) in the SW and WW, respectively.

DISCUSSION

The precision of the digital image technique can be improved. It is recommended that photos for estimating the EGC should be taken on cloudless and completely sunny days (Patrignani & Ochsner 2015; Shabannajed et al. 2020). However, Kipp et al. (2014) recommended taking photos on cloudy days. We observed that their suggestion might be correct. We tried to take the photos at noon on sunny days and noticed that the leaves shaded each other. In the Siirt location, where this study was conducted, the solar elevation angle (SEA) varied between 40° and 50° (from 15 February to 15 March) when we took the photos (Casio 2021). We believe that the lower SEA (< 70°) causes the leaves to shade each other. Because almost no shading was detected on the leaves on 15 May, when the SEA reached 70°. However, it was the wrong time to take the photos for the EGC, when the grain filling stages took place in the SW and the flowering stage in the WW. So, it is suggested that the photos for the EGC in February and March in the northern hemisphere should be taken on cloudy (not sunny) days or covered with a sheet of the area to be photographed in the plot (Kipp et al. 2014; Baresel et al. 2017).

Growth type affects the early ground cover capacity. It is known that the growth type in the wheat is expressed by the *Vrn* and *Ppd* genes (Stelmakh 1987; Kosova et al. 2008; Hyles et al. 2020). In other words, different levels (i.e., alleles) of the *Vrn* and *Ppd* genes determine the ST, FT, and WT in wheat (Limin & Fowler 2006; Kosova et al. 2008). Therefore, we believe that the differences in the EGC capacities between the tested ST and WT genotypes largely result from the *Vrn* and *Ppd* gene effects. It is already documented that the slower growth and development of the WT genotypes (due to effects of the *Vrn* and *Ppd* genes) during the vegetative period suppress their EGC capacity (Marone et al. 2020; Yang et al. 2020).

On the other hand, it was reported that the reduced height genes (*Rht-B1b* and *Rht-D1b*), also known as the magic genes of the green revolution, negatively altered the EGC capacity of the ST genotypes (Zhao et al. 2019). The plant height (PH) of the wheat genotypes tested in this study ranged from 85 cm to 115 cm in the first season and from 95 to 135 cm in the second season. In our experiment, the PH data demonstrated that *Rht* genes had a low level of influence on the EGC. The *Rht-B1b* allele was detected in 66, while *Rht-D1b* in only one out of the tested 110 wheat genotypes in the first season (IWWIP 2021). Interestingly, it has been suggested that even a single allele can reduce the EGC capacity by 10–15% (Jobson et al. 2019; Zhao et al. 2019). Detecting the *Rht-B1b* allele in more than half of the tested wheat genotypes in the first season may help explain why the EGC values are so low. However, since we do not know which *Rht* genes are present in the tested wheat genotypes in the second season, we cannot yet interpret the possible relationship between the EGC values measured in the second season and the *Rht* genes.

Spring wheat zone as a selection environment for early ground cover. *A priori*, we hypothesised that testing WW genotypes in the SWZ had its pros and cons. In other words, if we had conducted this study in a WWZ, we might not have observed the effect of the *Vrn* and *Ppd* genes on the EGC. We know that WT genotypes exhibit slower growth and development under the WWZ because of the *Vrn* and *Ppd* genes, while FT and ST genotypes are injured by the cold stress due to their faster growth and development (Hosseini et al. 2021). For this reason, the WW breeder routinely discards the FT and ST genotypes, which are susceptible to cold stress, from breeding nurseries (Beil et al. 2019). However, this type of selection strategy in WW unwittingly creates a dilemma. Unfortunately, there is no consensus on which genes (i.e., *Vrn* and *Ppd* genes vs *Egc* genes) the WW breeder should choose. At this point, specific questions need addressing. First, is there any interaction between the *Egc* genes and the *Vrn* and *Ppd* genes? Second, is there any relationship between the *Egc* genes and cold tolerance genes in WW? Third, how or in what ways can the WW's EGC capacity be increased? These questions underline that the EGC is regulated by a system that is not yet fully understood (Mason et al. 2018; Vukasovic et al. 2022).

Digital image technique versus visual scoring. The strong correlations between the EGC and VS reveal that both can be used interchangeably as a selection criterion (i.e., surrogate). Moreover, the high correlation of both methods (VS and EGC) with

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the GY is desirable for indirect selection in wheat breeding (Kipp et al. 2014). Meanwhile, the H^2 values calculated for the EGC in this study were at a medium-high level, indicating that direct or indirect selection for the EGC could be successful (Li et al. 2014; Gao et al. 2016). However, a few points about VS and DIT need to be highlighted. In principle, VS requires experience gained by working for many years in wheat breeding nurseries. For this reason, it is not surprising there may be differences between the visual scores of even two experienced wheat breeders. Although the wheat breeder is experienced, his or her eye may fail to differentiate the wheat genotypes (Reynolds et al. 2020).

DIT does not require experience. Moreover, it is possible to accurately measure the EGC by using only a simple and affordable smartphone camera (i.e., DIT) without the need for expensive and complicated electronic devices (such as Li-DAR) (Yuan et al. 2018). Therefore, many wheat breeding trials (nurseries) can be phenotyped with a smartphone-based DIT effectively and quickly.

Patrignani and Ochsner (2015) and Tao et al. (2020) found high similarity (96%) between the DIT-based EGC and the direct sampling method-based EGC. Likewise, our findings showed that DIT could be substituted for both the VS and direct sampling methods. Measuring the EGC by direct sampling is a destructive method, damaging the plants and negatively affecting the measurements taken from a plot. However, DIT is a novel phenotypic tool that is non-destructive to plants, is easy, affordable, reproducible, reliable, and time and labour saving (Walter et al. 2019). We believe there is a prerequisite for the DIT we propose to work: only if wheat breeding nurseries are tested in a suitable selection environment, as was undertaken in this study, the DIT for EGC measurement can be expected to be successful.

Early ground cover as an adaptive trait to climate change. Our findings showed that the ST genotypes that produced more EGC produced a higher GY, while the WW genotypes with less EGC produced a lower GY (Yang et al. 2020). Accordingly, it was suggested that ST genotypes with a shorter phenological cycle (PC) could smoothly adapt to climate change, while WT genotypes with a longer PC could not sufficiently adapt (Fowler et al. 2014; Zheng et al. 2016; Bourgault et al. 2020). One of the morpho-physiological traits of WT genotypes that can affect adaptation to climate change is undoubtedly the leaf area (LA) (Capo-Chichi et al. 2021). Essentially, the LA is determined by the seed weight and size, embryo size, number of first leaves and their size, and coleoptile tiller. Effectively, LA encloses the EGC (Ayalew et al. 2018; Zhao et al. 2019; Vukasovic et al. 2022). In other words, the rapid LA formation during the early growth stages of ST genotypes indicates that their EGC capacity could be higher (Yang et al. 2020; Hendriks et al. 2022), as reaching a higher EGC capacity of the ST genotypes than the WT's can only be explained in this way (Rebetzke & Richards 1999).

Larger leaves are preferred for the ST genotypes, while smaller leaves are preferred for the WT genotypes (Lopez-Castaneda et al. 1996; Limin & Fowler 2000). It has been suggested that smaller leaves can protect WT genotypes from cold injury (Hyles et al. 2020). Furthermore, the formation ratio, duration, and speed of the smaller leaves are lower in the WT genotypes because of the effects of the *Vrn* and *Ppd* genes during the vegetative period (Fowler et al. 2014; Mason et al. 2018; Vukasovic et al. 2022). However, in our experiment, the EGC values of the WT genotypes were always behind those of the ST and FT genotypes. On the other hand, building the EGC capacity in WT genotypes can be related to the decrease in the duration and frequency of the cold stress. To reduce the effect of the *Vrn* and *Ppd* genes on the EGC and enhance the EGC capacity in WT genotypes, we suggest developing new WT varieties by crossing ST with WT genotypes using single, top, or backcrossing methods depending on the target environment (Kaya 2021). By doing so, the WW adaptation to climate change can be facilitated more easily.

CONCLUSIONS

The likely relationship between the EGC and the GY in WW has been well documented by this study. Unlike previous studies on the EGC, we think that testing WW genotypes in a SWZ (not in a WWZ) is more effective in uncovering the relationship between the EGC and GY. Of course, we should consider that the genotypic variation for the EGC within WW influences the emergence of such a relationship. All things considered, if the smartphone camera-based DIT did not accurately predict the EGC values of WW genotypes, we would not be able to prove the existence of a relationship between the EGC and the GY. Therefore, we demonstrated that DIT could be a feasible selection tool for the EGC in WW. The

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H^2 values, predicted from moderate to high levels for the EGC indicated substantially high genotypic variation within the WW. In this study, we revealed that the WW genotypes tested not only included winter types, but also facultative and spring types. In this regard, we believe that the WW genotypes with different growth types can be adapted to climate change more quickly. As a result, interactions between the *Vrn* and *Ppd* genes and the EGC capacity in WW should be investigated in detail.

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