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'STAY GREEN' IN WHEAT: COMPARATIVE STUDY OF MODERN BREAD WHEAT AND ANCIENT WHEAT CULTIVARS

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ABSTRACT

Wheat production in arid and semi-arid environments can be limited by water stress which hastens premature senescence and consequently lowers yield. 'Stay green' is a vital characteristic associated with the capacity of the plant to maintain CO_2 assimilation and photosynthesis. Ancient wheat genotypes possessing the ability to maintain green leaf area duration (stay green traits) throughout grain filling are potential candidates for adapting and improving wheat for higher yield in arid and semi-arid regions. This study compared the 'stay green' of four cultivars of modern bread wheat and eight cultivars of three ancient wheat genotypes (*emmer, einkorn, spelt*). Values of stay green obtained through visual scoring and SPAD-502 chlorophyll content measurement were compared and explored for relationships at both the cultivar and genotype levels. The results show that spelt has superior stay green trait that can be explored for wheat improvement for arid and semi-arid environments. Linear relationship was also found between visual scoring stay green values and SPAD-502 values. Such a relationship would prove useful for research and crop management in resource-poor areas.

Keywords: bread wheat, stay green, ancient wheat cultivars, canopy persistence, SPAD, leaf senescence.

1. INTRODUCTION

Drought limits crop productivity particularly in Mediterranean and rain-fed regions (Mastrangelo *et al*., 2000). Thus the development of crop cultivars tolerant to the effects of drought is very imperative. Wheat (*Triticum* spp.) is a major crop of global significance. To meet the food needs of the current trend of population growth, improvement in wheat productivity and yield therefore plays a crucial role. Productivity in wheat and crop plants in general is mainly related to the acquisition and use of resources. The efficiency with which crops acquire and use such resources as water and radiation is subject to the photosynthetic activity, total leaf area and leaf green area duration of the crop. Stay green phenotypes maintain green leaf area for a longer period (Spano *et al*., 2003). This is a vital trait to incorporate into wheat in water stress environments as wheat yield is associated with the capacity of the plant to maintain CO_2 assimilation (Hafsi *et al*., 2007). Under drought conditions, photosynthetic duration is strongly limited by premature leaf senescence. Senescence rate in wheat is particularly sensitive to water and heat stress and like many other traits in crops, genetic variation for this trait has been reported in wheat (Falqueto *et al*., 2009; Hafsi *et al*., 2007; Srivalli, and Khanna-Chopra, 2009). Gregersen *et al*., (2008) reported a positive correlation between delayed crop senescence and grain yield and indicated that the potential for higher plant productivity from a 'stay green' phenotype may be due to a longer period of active photosynthesis. Senescence is regulated at the level of the individual leaf. Nutrients are thus mobilized from the older leaves to the younger leaves and eventually to the flag leaf, which contributes the majority of the nutrients and photo-assimilates used for grain-filling (Gregersen *et al*., 2008). During senescence, chlorophyll is lost thereby disassembling the photosynthetic apparatus and leads to decreases in the photosynthetic energy conversion capacity and efficiency. Both premature leaf senescence and overly delayed senescence are detrimental. Delayed senescence may interfere with nutrient remobilisation (Falqueto *et al*., 2009; Zhu *et al*., 2009). 'Stay-green' in the post-anthesis period is an efficient drought-tolerance trait in crops and can be studied at the physiological level. Genotypes possessing the ability to maintain green leaf area duration (stay green traits) throughout grain filling are potential candidates to assure yield in semi-arid regions (Hoang and Kobata, 2009; Larbi and Mekliche, 2004).

Although many studies on the senescence of wheat have been reported (Gelang *et al*., 2000; Gregersen *et al*., 2008; Verma *et al*., 2004), historically, researchers have focused on the correlation of leaf senescence and yield or drought tolerance of modern bread wheat cultivars and literature on 'stay green' traits or evidence for differences in canopy persistence of ancient wheat is not readily available. Many studies have demonstrated a genetically determined correlation between yield and flag leaf area duration, explaining that delayed senescence holdup remobilization and leads to reduced grain weight (Gregersen *et al*., 2008; Rawson *et al*., 1983). With regards to rate of senescence in ancient wheat, Sparkes (2010) suggested that bread wheat senesces faster than both spelt and emmer which could indicate a 'stay green' trait in those wheat progenitors that could be useful for increased yield potential. Crop domestication and increased cultivation of varieties released by plant breeding programs have led to an alteration in the pattern of genetic variation in farmers' wheat fields. Widespread plant breeding and rigorous crop selection have increasingly narrowed the diversity among cultivars (Reif, 2005) and making wheat production overly susceptible to both biotic and abiotic stresses. Landraces and progenitors of modern bread wheat could offer greater source of genetic diversity. Progenitors of wheat offer the potential of identifying reliable physiological attributes to improve the productivity of modern bread wheat (Trethowan and Mujeeb-Kazi, 2008). To expand available genetic diversity and to maintain genetic progress it is crucial to determine the desirable physiological characteristics of wheat progenitors and breed them into modern cultivated genotypes.

The objectives for the present study is to determine whether canopy persistence (stay green) of ancient and bread making wheat differ such that ancient wheat could habour desirable stay green traits that could be exploited to improve modern bread wheat. Also, to determine the correlation if there is and therefore the validity of using flag leaf scoring for leaf senescence studies.

2. MATERIALS AND METHODS

This study compares the 'stay-green' characteristics of four bread wheat cultivars and eight cultivars of three ancient wheat genotypes (Table-1). The ancient wheat genotypes used in the study are *emmer*, *einkorn* and *spelt* which are among the first domesticated *Triticeae* by man. These are hulled ancient wheat species which form a transition between wild and cultivated wheat species and may harbour the genetic variation necessary for improvement for modern wheat cultivars. All these genotypes possess rich genetic resources potentially useful for wheat improvement. *Emmer* is said to have abundant genetic variation in resistance to physiological stresses including salinity and drought (Nevo *et al*., 2002). Einkorn accessions, on the other hand, are reported to exhibit adaptation to a wide range of environmental conditions, survival in marginal areas, resistant to diseases and drought-tolerant (Alvarez *et al*., 2006, Chen *et al*., 2009). Spelt also has the potential for low input production and adaptation to harsh ecological condition and resistance to diseases (Caballero, 2004).

Table-1. Genotypes and cultivars of bread wheat and ancient wheat studied.

Einkorn	Emmer	Bread wheat	Spelt
Einkorn	Emmer	Claire	Spelt (Oberkulmer)
Einkorn - Hungarian	Emmer - SB	Einstein	Spelt - SB
Einkorn - SB		JB Diego	Spelt (Tauro)
		Xi19	

2.1 Experimental design

A field experiment was established during the 2009/2010 winter wheat growing season at the University of Nottingham, Sutton Bonington Campus, near Loughborough, UK. The experimental site lies between latitude 52° 50' N and longitude 1° 15' W. The soil type is a clay loam with soil indices of P: 3, K: 3, Mg: 4 and pH: 6.9. The previous crop grown was winter oats and the soil had SNS index 5 with the N content measured at 174.1 kg ha^{-1} on November 12, 2009. A randomized complete block design was used for the 12 cultivars, with four replicates. Thus, the experimental field was divided into 48 plots, each measuring 24 m long and 1.625 m wide. The soil was ploughed and power harrowed in September 2009. Drilling was done with Ojyord drill on October 28, 2009 at 300 seeds m^{-2} after which post drilling soil rolling was done. Crop management followed standard and best agricultural practices for UK wheat production.

2.2 Growth stage determination

The cultivars were growth staged on plot basis on four dates namely: 2 June, 2010, 23 June, 2010, 29-2 June 2010 and 12 July, 2010. This was done to eliminate the possibility of confounding the results obtained from the senescence studies with probably the differential growth rate of the wheat species and cultivars. Growth staging of all field plots were performed according to the HGCA's benchmarks for wheat growth in the UK (HGCA, 2008[b]). Growth staging was carried out approximately once every 2 weeks during which depending on time constraints, five or ten plants were randomly selected from each plot. These were accordingly assessed non-destructively. Upon collation of growth stage data for all plots, an average growth stage was then obtained. Additionally, dates for the point at which each genotype reached vital growth stages (GS 31, 39, and 59) were pooled.

2.3 Stay green (Leaf Senescence)

Minolta Chlorophyll Meter, SPAD-502 (Minolta Camera Company Limited), estimates the amount of chlorophyll present by measuring the amount of light that is transmitted through a leaf. In essence, it determines "how green a plant is" and can be used to estimate the nitrogen requirements of the plant (Martínez and. Guiamet, 2004). The advantages of using the SPAD include easy and rapid measurement, non- destructive method and computation of the average value of several samples (Hoang and Kobata, 2009; Kashiwagi *et al*., 2006). However, the utility of SPAD may be affected by prevailing weather and localized environmental conditions (Martínez and. Guiamet, 2004). Studying senescence by flag leaf scoring may be as well uncomplicated and effortless technique suited to resource-poor regions but the inherent subjectivity in the technique renders synchronizing results between different researchers virtually impossible.

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Two methods were used to measure post-anthesis leaf senescence in this study. First, the stay green rating was visually scored on flag leaves at or soon after physiological maturity on a plot by plot basis. Ten plants were randomly selected per plot and the flag leaves scored twice per week during the later part of grain development. Scoring was done on a Lickert-type scale of 0 - 10 (0 = no leaf death; 1 = 10% dead leaf area; ... 10 = 100% dead leaf area) based on the proportion of leaf area of normal-sized leaves which had senesced and died. The scores of senescence were obtained by dividing the percentage of estimated total leaf area that is dead by 10. The post-anthesis leaf senescence visual scoring was performed on six occasions from 7th to 29th July 2010.

Second, total leaf chlorophyll content was measured with the SPAD. Values were taken from ten plants per plot at three positions on the flag leaf: the base, the middle, and the tip of the leaf lamina. The average per plot was computed and used in the analysis. It was not feasible to measure SPAD values on two occasions due to rain.

The data collected from these two methods were subjected to analysis of variance (ANOVA) and mean differences were tested using LSD at 5% probability level. Regression analysis was done to explore the relationship between the visual scores and the SPAD values. The statistical analysis was done using GENSTAT (version 12).

3. RESULTS

3.1 Developmental stages

Most of the plants were at different growth stages at any point in time. Table-2 shows an estimation of the dates on which different cultivars reached particular growth stages. The bread wheat species generally appear to have developed faster than the ancient species.

Table-2. Dates cultivars reached developmental stages.

Cultivar	GS 31	GS 39	GS 59	GS 61
Claire	27-Apr	02-Jun	10-Jun	23-Jun
Einkorn	02-Jun	10-Jun	23-Jun	23-Jun
Einkorn Hungarian	30-Apr	10-Jun	23-Jun	12-Jul
Einkorn SB	02-Jun	10-Jun	23-Jun	12-Jul
Einstein	27-Apr	02-Jun	10-Jun	23-Jun
Emmer	02-Jun	10-Jun	23-Jun	12-Jul
Emmer SB	27-Apr	10-Jun	23-Jun	12-Jul
JB Diego	27-Apr	02-Jun	10-Jun	23-Jun
Spelt Oberkulmer	30-Apr	10-Jun	23-Jun	12-Jul
Spelt SB	27-Apr	02-Jun	23-Jun	23-Jun
Spelt Tauro	27-Apr	02-Jun	23-Jun	12-Jul
Xi19	30-Apr	10-Jun	23-Jun	23-Jun

3.2 Stay green

3.2.1 Visual scoring

Figure-1 (a) and (b) shows the visual scoring for the cultivars. Significant differences (P<0.001) were found among the cultivars on all scoring dates. It was observed that a greater proportion of flag leaf (over 50%) for the einkorn cultivars had already initiated senescence before 7 July 2010 and it was therefore no surprise that einkorn (cv. Hungarian) recorded the highest mean ratings in the range of 6.2 to 9. 8 throughout the scoring period. On the other hand, spelt oberkulmer was the cultivar with longest ‘stay green’ duration and as at 29 July 2010 had only recorded mean ratings of approximately 6. In general all cultivars showed a consistent decline in flag leaf greenness over time.

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A

B

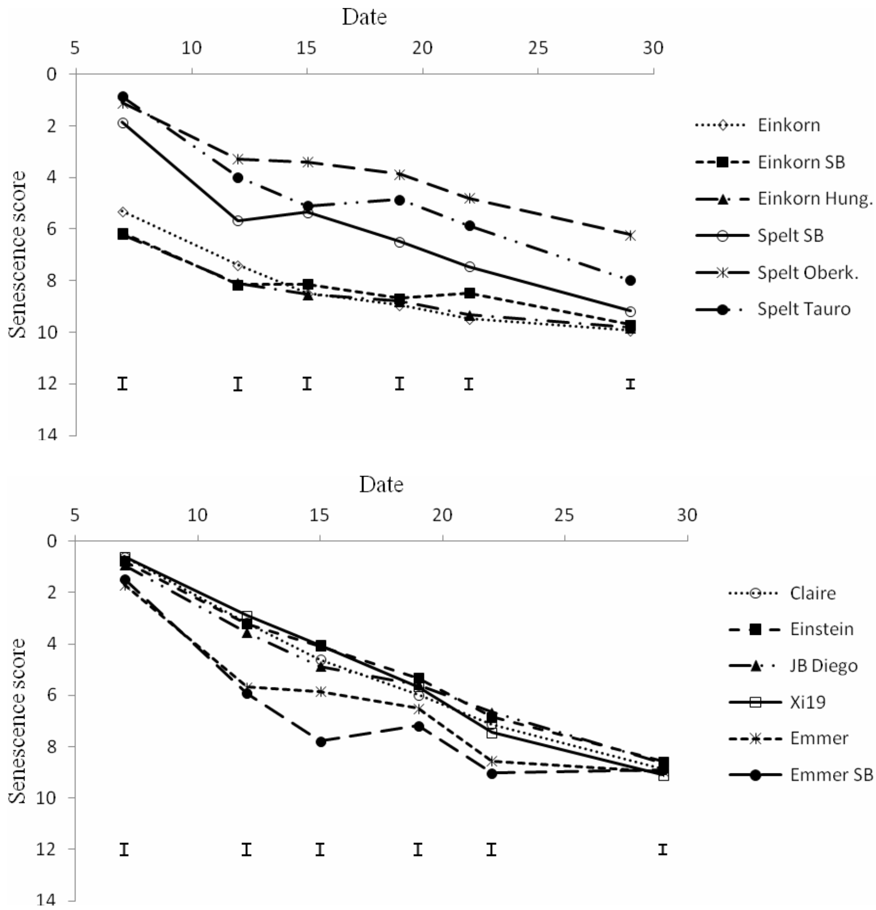

Figure-1. Visual scores of 'stay green' for (A) einkorn and spelt cultivars; (B) bread wheat and emmer cultivars. Bars show SED.

Averaging across the species, significant difference (P<0.00) was found between proportion of senesced flag leaf among the four species at all scoring dates. Although all the four species showed a consistent decline in flag leaf greenness, einkorn senesced faster. Bread wheat recorded initial low ratings indicating high proportion of greenness in the flag leaf than all the other species. However, this greenness declined rather sharply during later dates and it was observed that spelt had the longest stay green duration (Figure-2).

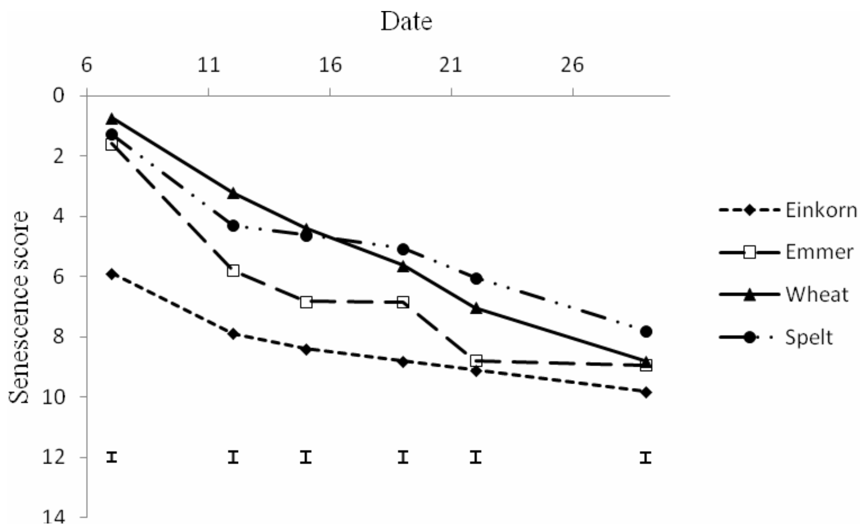

Figure-2. Visual scores of 'stay green' for Einkorn, Emmer, Spelt and modern bread wheat. Bars show SED between means of visual scores.

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3.2.2 SPAD values

Figure-3 (a) and (b) presents the SPAD-measured stay green. On all SPAD measurement dates, significant difference (P<0.001) were found among the twelve cultivars for flag leaf chlorophyll concentration. The first measurement on 7 July 2010, recorded relatively higher SPAD values for all cultivars in the range of 19 to 54, however, there appeared to be a sharp plummet in the values in subsequent measurements. Although Claire recorded 54.4 in the first measurement, Spelt oberkulmer generally had higher SPAD values on all subsequent measurements. The einkorn cultivars recorded the smallest chlorophyll content of 18.2, 19.0 and 19.9 for einkorn SB, einkorn and einkorn Hungarian, respectively on 7 July 2010, but spelt Tauro and emmer SB deteriorated quickly to catch up with the einkorns in subsequent measurement.

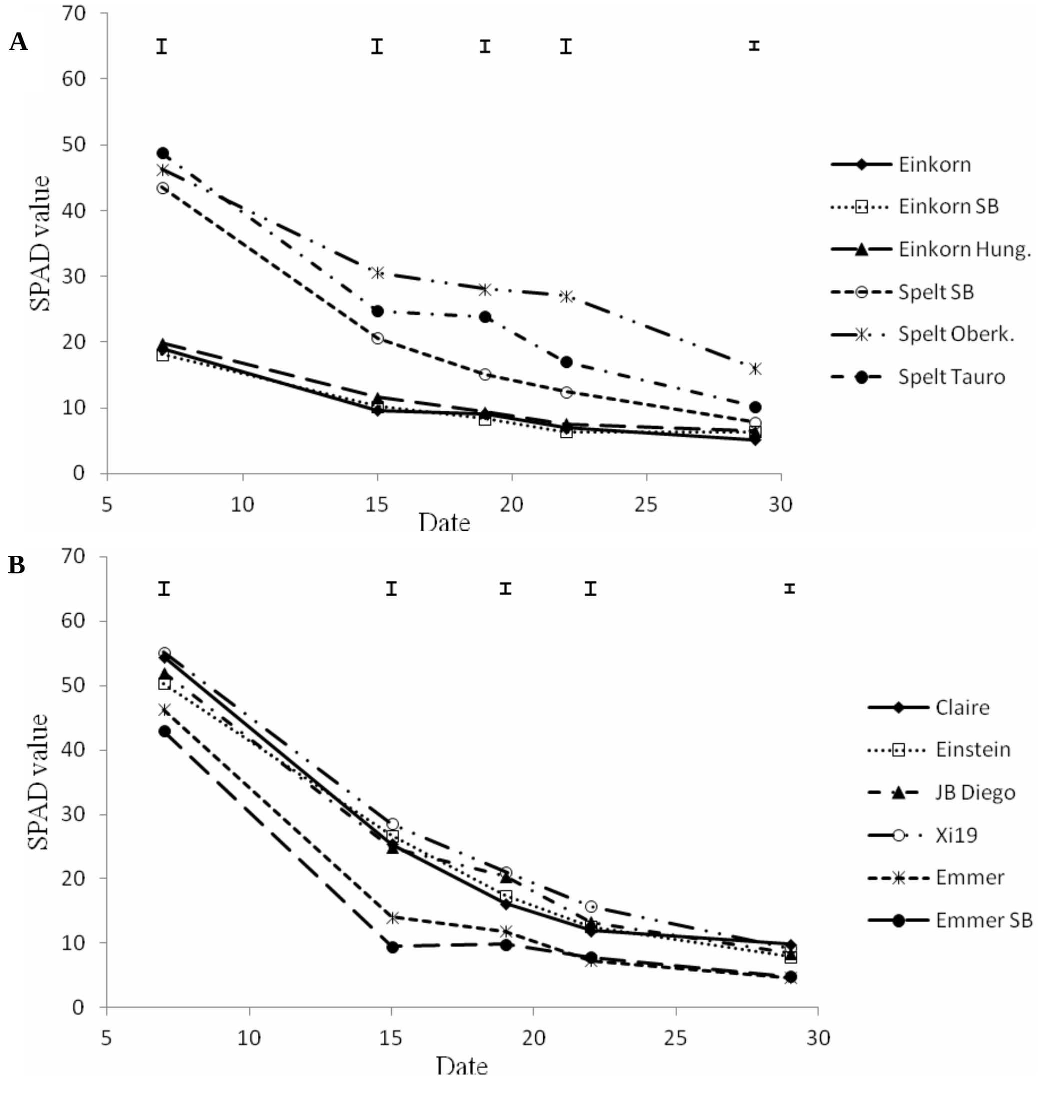

Figure-3. SPAD value plotted against date of SPAD measurements for (A) einkorn and spelt cultivars; (B) bread wheat and emmer cultivars. Bars indicate SED.

Significant differences (P<0.001) in canopy persistence at all measurement dates were found among the four wheat species when the SPAD values were averaged across all the species. Figure-4 shows that there was a wider variation in the chlorophyll content between the species in the early part of the post-anthesis period but the difference converged in later dates. Bread wheat initially recorded higher SPAD values but declined subsequently to be overtaken by Spelt. Einkorn consistently recorded the least SPAD values.

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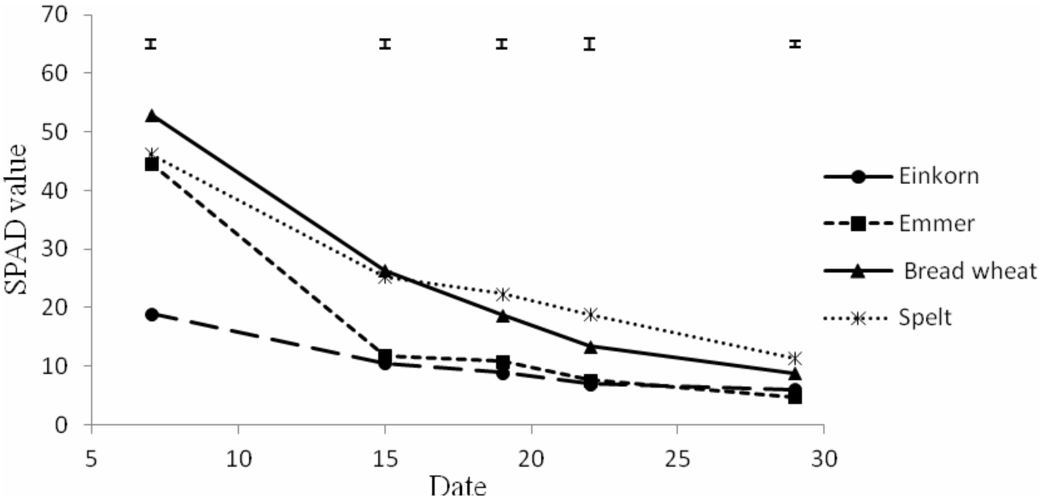

Figure-4. SPAD value plotted against date of SPAD measurements for einkorn, emmer, bread wheat and spelt. Bars indicate SED.

3.3 Relationship between visual scoring and SPAD values

Linear regression was used to assess the relationships among SPAD-502 meter readings and visual scores and it was observed that 'stay green' visual scores were linearly related to SPAD values (P<0.001, R^2 = 0.93). The analysis derived parameter estimates for each cultivar and species based on the linear equation:

$$SPAD = A + BX \quad (1)$$

where X is the visual score.

Parameter estimates of relationship between SPAD and visual scores for cultivars and species are given in Tables 3 and 4, respectively, while Figure-5 shows the linear relationships for the species.

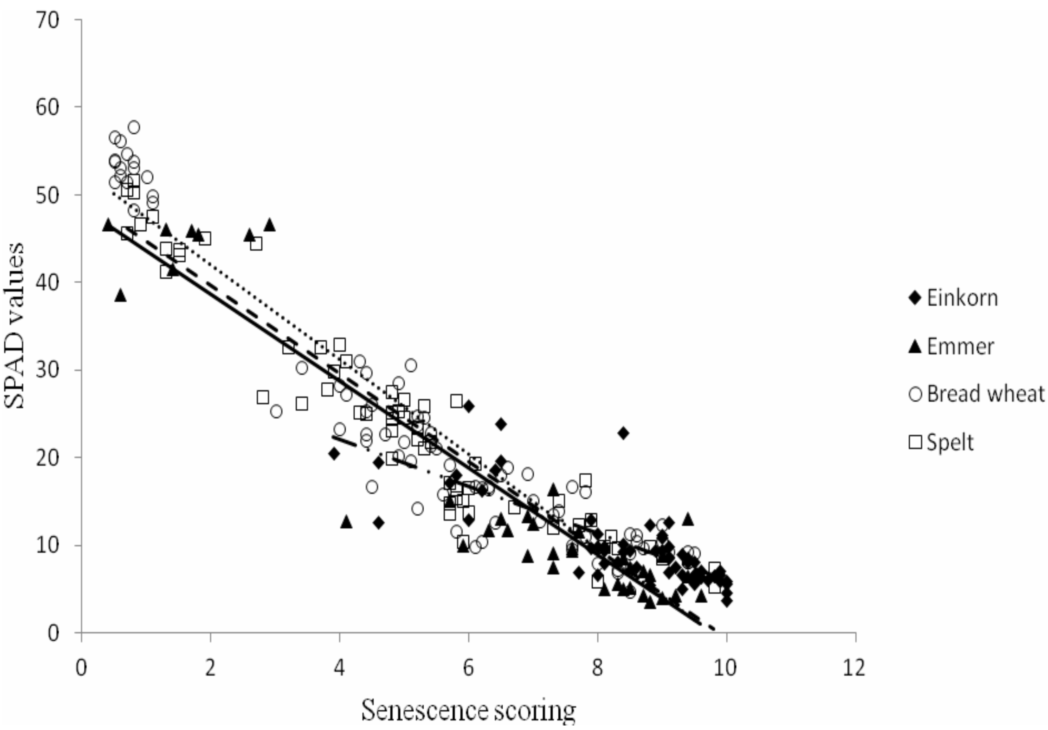

Figure-5. Linear relationship between visual ratings and SPAD-502 meter readings for einkorn, emmer, bread wheat and spelt.

Table-3. Parameter estimates for relationship between senescence scoring and SPAD meter readings for the twelve cultivars.

Cultivar	Parameter estimates	
	A	B
Claire	53.19	-5.449
Einkorn	32.42	-2.663
Einkorn Hungarian	31.9	-2.452
Einkorn SB	36.34	-3.216
Einstein	50.13	-5.298
Emmer	49.55	-5.181
Emmer SB	47.93	-4.792
JB Diego	54.2	-5.707
Spelt Oberkulmer	50.39	-5.359
Spelt SB	48.1	-4.652
Spelt Tauro	52	-5.501
Xi1	54.17	-5.254

Table-4. Parameter estimates for relationship between senescence scoring and SPAD meter readings for einkorn, emmer bread wheat and spelt.

Species	Parameter estimates	
	A	B
Einkorn	32.79	-2.681
Emmer	48.62	-4.959
Bread wheat	52.76	-5.397
Spelt	49.65	-5.013

4. DISCUSSIONS

Abiotic stresses reduce wheat (*Triticum* spp. L.) yield in many environments and predicted changes in the physical climate are expected to alter wheat growth and yield. A sustained maintenance of genetic progress in the improvement of stress tolerance is therefore essential and this will require the identification and combination of new genetic variation (Ewert and Pleijel, 1999; Trethowan and Mujeeb-Kazi, 2008). Plants with the 'stay green' trait have been shown to resist drought induced premature plant senescence (Xu *et al*., 2000). Crops with 'stay green' phenotype such as sorghum has been reported to have increased yield in water limited conditions because they are able to keep their stalk transporting system functioning under severe drought conditions (Xu *et al*., 2000).

Generally, all cultivars studied showed a consistent decline in flag leaf greenness over time as assessed by senescence scoring. Einkorn senesced faster than the other species and this was reflected in its overall poor performance. Bread wheat recorded initial low ratings indicating high proportion of greenness in the flag leaf than all the other species, however, this greenness declined rather sharply during later dates and spelt (cv. Oberkulmer) was observed to show signs of stay green trait. These findings were supported by assessments of leaf greenness using a SPAD meter. Bread wheat initially recorded higher SPAD values but declined subsequently to be overtaken by spelt. This trend could be analogous to the trend in green leaf area index in the four species where bread wheat achieved overall initial higher GAI values but declined at GS61 (Data not shown). This suggests that the higher biomass accumulation by bread wheat may also be attributable to higher pre-anthesis photosynthetic ability and strong contribution of pre-anthesis assimilates to yield. The results are consistent with the findings of Sparkes (2010) that there may be a 'stay green' trait in spelt. Several reports (Gregersen *et al*., 2008; Hafsi *et al*., 2007) demonstrated a correlation between yield and flag leaf area duration. The leaf area duration is closely correlated with leaf size and potentially, a 'stay green' species, is thus in general believed to have higher plant productivity due to a longer period of active photosynthesis. Compared to bread wheat, spelt developed slowly and therefore the stay green trait identified could be confounded by its developmental stage, however, all the ancient wheat had similar developmental phases, yet einkorn senesced earlier. The superior stay green characteristics of spelt should be further explored because Larbi and Mekliche (2004) reported that genotypes possessing the ability to maintain 'stay green' and high relative water content traits throughout grain filling are potential candidates to assure yield in semi-arid regions.

SPAD-502 values were linearly related to visual scoring scheme but each cultivar/species was different. The prediction of SPAD values from senescence scores for different species will therefore require different correction factors. The SPAD measurements tended to be time consuming and quite limited by its dependency on the weather. Previous researchers have also suggested that the visual rating can be biased and subjective (Xu *et al*., 2000). The linear relationship between visual scoring and SPAD meter readings obtained in this study indicate that visual 'stay green' ratings were a reliable indicator of leaf senescence and could be useful for wheat breeders in evaluating physiological changes of wheat leaf canopy over time. However, senescence scoring could be recommended provided different regressions would be used for each species.

5. CONCLUSIONS

Significant differences in canopy persistence were found among the cultivars and across the wheat species when assessed with both senescence scoring and the SPAD meter. Signs of the stay green trait were found in spelt (cv. Oberkulmer) and could offer a potential source of this trait for improving resilience and hence yield of bread wheat in stressful environments such as arid and semi-arid regions. Significant linear relationship exists between the SPAD-502 canopy persistence and values obtained by visual scoring. Good relationships between visual scoring and SPAD values implies that senescence scoring could be recommended as a good technique provided different regressions would be used for each crop species or variety. Such a relationship is important for resource-poor regions as it simplifies the measurement of canopy persistence as part of scientific research or crop management.

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